THE PREPARATION OF THERAGNOSTIC IMMUNOLIPOSOMES/IMMUNONIOSOMES FOR THE DIAGNOSIS AND THERAPY OF PARKINSON’S DISEASE

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THE PREPARATION OF THERAGNOSTIC IMMUNOLIPOSOMES/IMMUNONIOSOMES FOR THE DIAGNOSIS AND THERAPY OF PARKINSON’S DISEASE

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DOCTORAL THESIS

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ABSTRACT

M.Sc. Pharm. Mine Silindir Gunay, The Preparation of Theragnostic Immunoliposomes/Immunoniosomes for the Diagnosis and Therapy of Parkinson’s Disease, Hacettepe Üniversitesi – François Rabelais de Tours University, Health Sciences Institute, Radiopharmacy Doctoral Programme, UMR Inserm U 930, Team 3, Molecular Imaging and Brain Programme, Doctoral Thesis, Ankara-Tours, 2016. Parkinson’s Disease (PD) is degeneration of dopamine producing cells in substantia nigra. Blood-brain barrier (BBB) is a strong obstacle in PD therapy. More penetration and accumulation in the target tissue can be obtained by preventing RES uptake via “stealth effect”. Liposomes and niosomes are the promising systems for being biodegradable, bioavailable, non-toxic and targetable. Although CNS disorders are the first to endorse at their research in the diagnosis and therapy with several framework projects in Europe and over the world, there is still a huge gap in CNS drug delivery and the success of PD therapy. Although different studies have performed with pramipexole, evaluation of penetration and antiparkinsonian effect of pramipexole encapsulated liposomes and niosomes has never been studied before.

Among this thesis, nanosized, polyethylene glycol (PEG) coated, neutral and positively charged, pramipexole encapsulated liposomes and niosomes were formulated, characterized and release kinetics of the systems were evaluated. In vitro penetration of all formulations was evaluated in BBB cell co-culture model. Therapeutic efficacy of neutral, pramipexole encapsulated liposomes and niosomes were evaluated in 6-hydroxydopamine (6-OHDA) lesioned rats by rotometer test and autoradiography.

All formulations have approximately 10% encapsulation efficiency, around 100 nm particle sizes and fitted to first-order release kinetics. All formulations were found BBB permeable at in vitro cell culture studies. Nanosized, neutral niosomes designated similar but slightly better effect than pramipexole solution in autoradiography studies in 6-OHDA lesioned rats. This pramipexole dose is approximately 9 times lesser doses applied with conventional pramipexole tablets for humans in Neurology clinics. Nanosized, pramipexole encapsulated, neutral niosomes showed potential PD therapeutic effect in PD animal model depending on non-ionic surfactant properties of niosomes.

Key Words: Pramipexole Liposomes, Pramipexole Niosomes, Brain Targeting, Parkinson’s Disease Therapy, Dopamine Transporter Autoradiography.

Supporting Organizations: TUBITAK-SBAG (Project No: 112S244).
French Embassy-Service of Cooperation and Cultural Actions, Campus France.
RÉSUMÉ

M.Sci.Pharm. Mine Silindir Gunay, La Préparation de Theragnostic Immunoliposomes/Immunoniosomes Pour Le Diagnostic et Thérapie de Maladie de Parkinson, L'université de Hacettepe – François Rabelais de l’université de Tours, L’institut Scientifique de Santé, Programme de Radiopharmacie, Programme de UMR Inserm U 930, Equipe 3, Imagerie Moleculaire du Cerveau, Thèse de Doctorat, Ankara – Tours, 2016. La maladie de Parkinson (MP) provient de la dégénérescence des cellules du locus niger produisant de la dopamine. La barrière hémato-encéphalique (BHE) est un véritable obstacle pour le traitement de la MP car elle empêche ou réduit le passage d’un grand nombre de substances pharmacologiques vers le cerveau. L’encapsulation de ces substances dans des liposomes ou des niosomes avant leur libération intracérébrale représente une alternative de choix en raison de la biocompatibilité, la bio-fragmentation, la non-toxicité et les capacités de ciblage de ces systèmes. À l’heure actuelle le traitement de la MP reste un défi, malgré l’existence de nombreux projets de recherche dans ce domaine. Notre hypothèse est que l’administration de pramipexole encapsulé dans des liposomes et/ou des niosomes pourrait représenter une approche thérapeutique pertinente.

Dans le cadre de la thèse, la caractérisation et la cinétique de diffusion des liposomes et niosomes contenant du pramipexole ont été réalisées. La validation de différentes formulations a été réalisée sur un modèle de BHE constitué de co-cultures cellulaires. Les effets du pramipexole encapsulé dans des liposomes ou des niosomes sont ensuite été étudiés dans un modèle de MP chez le rat obtenu par lésion de la voie dopaminergique nigrostriée à l’aide de 6-hydroxydopamine (6-OHDA). Pour cela, nous avons évalué le comportement rotatoire induit par l’amphétamine et l’expression du transporteur de la dopamine (DAT) par autoradiographie quantitative chez des animaux lésés traités ou non par les nanocapsules.

Toutes les formulations que nous avons réalisées ont montré une capacité d’encapsulation d’environ 10% pour une taille de 100 nm, avec une cinétique de dispersion compatible avec une utilisation in vivo. Dans notre modèle de co-culture cellulaire, nous avons déterminé que nos formulations permettent le franchissement de la BHE.

Chez les animaux lésés à la 6-OHDA, la quantification du DAT indique que l’administration de pramipexole réduit l’intensité de la lésion, que la substance soit administrée seule ou encapsulée dans des niosomes.

Ces travaux montrent l’intérêt potentiel de l’administration de principe actif encapsulé pour le traitement de la MP, et devront être poursuivis afin d’optimiser cette approche thérapeutique, notamment au niveau des doses.

Mots-clés: autoradiographie ; BHE ; liposomes ; maladie de Parkinson ; niosomes ; pramipexole ; transporteur de la dopamine.

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ÖZET


Tez kapsamında, nanoboyutlu, PEG kaplı, nötral ve pozitif yükli lipozom ve niozomların formüle edilmiş, karakterizasyon ve salın kinetikleri değerlendirilmiştir. Tüm formülasyonların KBB geçirenliğini, hücre KBB ko-kültürü çalışmalarında incelenmiştir. Nötral, pramipeksol enkapsüle edilen lipozom ve niozomların tedavi etkinliği in vivo olarak 6-hidroksidopamin (6-OHDA) ile lezyon yapılarak PH modeli oluşturuldu ve旋转metre ve otoradyografi çalışmaları ile incelenmiştir. Tüm formülasyonlar yaklaşık %10 enkapsülasyon etkinliği ve 100 nm civarında partikül boyutu ile birincisi derece salım kinetiği göstermiştir. Hücre kültür çalışmaları, tüm formülasyonların KBB’den penetre olabilirdiği saptanmıştır.


Anahtar Sözcükler: Pramipeksol Hapsedilmiş Lipozomlar, Pramipeksol Hapsedilmiş Niozomlar, Beyne Hedeflendirme, Parkinson Hastalığı’nın Tedavisi, Dopamin Transporter Otoradyografi.

Destekleyen Kuruluşlar: TUBITAK-SBAG (Project No: 112S244).
Fransa Büyükelçiliği-Kültür Ataşeliği, Campus France.
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<td>AD</td>
<td>Alzheimer’s disease</td>
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<td>AET</td>
<td>Active efflux transport</td>
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<tr>
<td>AP</td>
<td>Anteroposterior</td>
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<td>BBB</td>
<td>Blood-brain barrier</td>
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<tr>
<td>BCSFB</td>
<td>Blood-cerebrospinal fluid barrier</td>
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<tr>
<td>BMVECs</td>
<td>Brain microvascular endothelial cells</td>
</tr>
<tr>
<td>Chol</td>
<td>Cholesterol</td>
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<tr>
<td>CMT</td>
<td>Carrier mediated transport</td>
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<tr>
<td>CNS</td>
<td>Central nervous system</td>
</tr>
<tr>
<td>COMT</td>
<td>Catechol-o-methyl transferase</td>
</tr>
<tr>
<td>CT</td>
<td>Computed tomography</td>
</tr>
<tr>
<td>DA</td>
<td>Dopamine</td>
</tr>
<tr>
<td>DAT</td>
<td>Dopamine transporter</td>
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<tr>
<td>DPPC</td>
<td>1,2-Dipalmitoyl-sn-glycero-3-phosphocholine</td>
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<tr>
<td>DSC</td>
<td>Differential scanning calorimetry</td>
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<tr>
<td>DTPA-PE</td>
<td>Diethylene triamine penta acetate-Phosphatidyl ethanolamine</td>
</tr>
<tr>
<td>EPR</td>
<td>Enhanced permeability and retention</td>
</tr>
<tr>
<td>FTIR</td>
<td>Fourier transform infrared</td>
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<tr>
<td>hCMEC/D3</td>
<td>Human brain endothelial cell line</td>
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<tr>
<td>HLB</td>
<td>Hydrophilic lipophilic balance</td>
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<tr>
<td>HPLC</td>
<td>High-performance liquid chromatography</td>
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<tr>
<td>L-DOPA</td>
<td>Levodopa</td>
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<tr>
<td>MAO-B</td>
<td>Monoamine oxidase B</td>
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<td>MPTP</td>
<td>1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine</td>
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<tr>
<td>MRI</td>
<td>Magnetic resonance imaging</td>
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<td>MRPs</td>
<td>Multidrug resistance proteins</td>
</tr>
<tr>
<td>MUVs</td>
<td>Multilamellar vesicles</td>
</tr>
<tr>
<td>NE</td>
<td>Norepinephrine</td>
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<tr>
<td>NMDA</td>
<td>N-methyl-D-aspartate</td>
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<td>Abbreviation</td>
<td>Definition</td>
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<tr>
<td>PD</td>
<td>Parkinson’s disease</td>
</tr>
<tr>
<td>PEG</td>
<td>Polyethylene glycole</td>
</tr>
<tr>
<td>PET</td>
<td>Positron emission tomography</td>
</tr>
<tr>
<td>Pgp</td>
<td>P-glycoprotein</td>
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<tr>
<td>PMAT</td>
<td>Plasma membrane monoamine transporter</td>
</tr>
<tr>
<td>PPX</td>
<td>Pramipexole</td>
</tr>
<tr>
<td>R²</td>
<td>Coefficient of determination</td>
</tr>
<tr>
<td>RES</td>
<td>Reticuloendothelial system</td>
</tr>
<tr>
<td>Rh</td>
<td>Rhodamine</td>
</tr>
<tr>
<td>RMT</td>
<td>Receptor mediated transport</td>
</tr>
<tr>
<td>ROI</td>
<td>Region of interest</td>
</tr>
<tr>
<td>SA</td>
<td>Stearylamine</td>
</tr>
<tr>
<td>SNc</td>
<td>Substantia nigra pars compacta</td>
</tr>
<tr>
<td>SPECT</td>
<td>Single-photon emission computed tomography</td>
</tr>
<tr>
<td>SURII</td>
<td>Non-ionic surfactant II (Alcool cetylique polyglycerole)</td>
</tr>
<tr>
<td>SUVs</td>
<td>Small unilamellar vesicles</td>
</tr>
<tr>
<td>TH</td>
<td>Tyrosine hydroxylase</td>
</tr>
<tr>
<td>TLC</td>
<td>Thin layer chromatography</td>
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<tr>
<td>TRIS</td>
<td>(Tris(hydroxymethyl)-aminomethan</td>
</tr>
<tr>
<td>UV</td>
<td>Ultraviolet</td>
</tr>
<tr>
<td>VTA</td>
<td>Ventral tegmental area</td>
</tr>
<tr>
<td>$[^{125}\text{I}]\text{PE2I}$</td>
<td>N-(3-iodoprop-2E-enyl)-2beta-carbomethoxy-3beta-(4'-methylphenyl) nortropane</td>
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<tr>
<td>6-OHDA</td>
<td>6-hydroxydopamine</td>
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1. INTRODUCTION and AIM

Parkinson’s disease (PD), the second most common age-related neurodegenerative disorder after Alzheimer’s disease (AD), is characterized by the relatively selective death of dopaminergic neurons in the substantia nigra pars compacta (1, 2). Generally, PD is diagnosed over age 50; however it is not only seen in middle to old people but also in young people (at the age below 20 years) as Juvenile PD 5% of all cases. While the disease incidence is 0.2-0.3 % among the community, it is 1% among the people who are over the age of 55. PD is classically described as a set of motor symptoms such as rest tremor, bradykinesia and rigidity due to the progressive deficit in dopamine in the caudate-putamen. However, it is now admitted that PD affects other neurotransmission systems leading to non-motor symptoms including sleep disturbance, fatigue, depression, cognitive impairment, dementia, and olfactory and autonomic dysfunctions. Current treatments of PD are efficient to improve several aspects of the disease, in particular motor symptoms, but are unable to slow down or stop its progression.

In addition, therapeutic approaches are often restricted due to the limited brain penetration of potential drug treatments. Brain is protected by tight, protective barriers composed of tight endothelial cells and tight junctions such as blood-brain barrier (BBB) and the blood-cerebrospinal fluid barrier (BCSFB) limiting delivery of drugs and other molecules for both diagnosis and treatment of brain diseases. These endothelial and epithelial structures comprise specialized and differentiated neurovascular system providing proper functioning of neuronal circuits, synaptic transmission and remodeling, neurogenesis and angiogenesis in the brain by separating and protecting neurons from blood circulation (3-5). Due to that, more than 98% of drugs cannot penetrate into the brain. Although there is a variety of approaches for BBB penetration, the utilization of drug delivery systems is one of the most frequently investigated one.

Levodopa (L-DOPA) administration as a gold standard in the treatment of PD is very valuable, however, its long-term use may cause some motor complications such as abnormal involuntary movements (dyskinesia) and shortening response to each dose (wearing off phenomenon). To reduce the duration of
immobile off periods and dependence to L-DOPA therapy for maintaining or improving motor impairment, dopamine agonists were developed (6). Pramipexole is one of these effective dopamine agonists with high relative in vitro specificity and full intrinsic activity at D2 subfamily of dopamine receptors and with a higher binding affinity to D3 than to D4 or D2 receptor subtypes (7). To obtain effective effect in brain it is required to increase the dose which results in the increase of side effects. Therefore, solutions are searching for increasing drug amount delivery without increasing drug dose. Nanosized drug delivery systems have important advantages in this issue. The term of Nano is called as manikin (dwarf, small man) in Greek and it is one in a million of a millimeter. Nanotechnology is first stated by a physicist, Richard Feynman, in 1959 about measuring nanomaterials by miniaturized devices and the use for identifying new scopes. According to USA National Health Institute, one of the most important areas in nanotechnology is nanomedicine and it comprises specific medical research in molecular level for the diagnosis and therapy of the diseases. Nanomedicine can be defined as the application of nanotechnology to health and can be described as a new field of science (8,9). Nanosystems that are used in nanomedicine comprise liposomes, niosomes, micelles, nanospheres, polymeric delivery systems, dendrimers, emulsions, nanoparticles and nanocapsules etc. Apart from other conventional drugs, the superiority of these drug delivery systems is their ability to accumulate in the desired tissue, increase bioavailability of drugs, increase effectiveness even in lower concentrations and decrease side effects (8-11).

Liposomes and niosomes are one of the most commonly investigated drug delivery systems used for delivering both drugs and diagnostic agents to the targeted area. Liposomes are formed by self-sustainable bilayered structure comprising phospholipids (12,13). Niosomes are non-ionic surfactant vesicles (14). The composition of niosomes is very similar to that of liposomes, however, the substances used for the preparation of niosomes, non-ionic surfactants, attain them a more stable structure and more ability to penetrate through BBB. Both liposomes and niosomes have proper characteristics such as being non-toxic, biocompatible and biodegradable. They can also carry a variety of drugs with different physicochemical properties such as hydrophilic, lipophilic and amphoteric drug
molecules by either entrapping inside hydrophilic core or anchoring on the lipid bilayer (15,16).

Passive targeting of liposomes and niosomes can be performed with the help of formation of a steric hindrance created by altering surface charge, surface properties, particle size, site membrane lipid packing, extent of steric hindrance and reducing particle size into nano-scale. These sterically stabilized long circulating delivery systems are distinguished from other conventional ones due to the altered pharmacokinetics and pharmacodynamics which enhances accumulation at diseased area (17). Coating was initially made by monosialogangliosides (18) to increase distribution half-life. The existence of steric hindrance achieved by coating with a hydrophilic biocompatible polymer such as PEG, propylene glycol, polypropylene oxide, polyethylene oxide or mannose coating for brain targeting prevents them from interaction between serum opsonins by lower contact angle between particles and phagocytic cells (15,16) and prevents them from opsonisation by tissue macrophages. Brain targeting can be obtained by this way rather than targeting to RES organs like liver, spleen, etc.

Although a variety of studies were performed about therapeutic efficacy of Pramipexole (6,7,19-22) and use of some delivery systems for the therapy of PD (23,24), the formulation of Pramipexole encapsulated liposomes and niosomes has not yet been studied before as an alternative to oral Pramipexole dosage.

Therefore, the aim of thesis was to formulate novel, nanosized for passive targeted, PEGylated for “stealth effect”, antiparkinsonian drug (Pramipexole Dihydrochloride Monohydrate) encapsulated, neutral and positively charged liposomes and niosomes for effective therapy of PD. Nanosized, pramipexole encapsulated, neutral and positively charged liposomes and niosomes were formulated and their release kinetics was characterized. BBB penetration of nanosized, neutral, pramipexole encapsulated liposomes and niosomes was evaluated in BBB cell co-culture model by using florescent intensity and fluorescence microscopy images. In vivo therapeutic efficacy of neutral liposomes and niosomes was monitored and compared by complementary methods such as rotational behavior and autoradiography in a rat model of PD obtained by a partial 6-hydroxypamine (6-OHDA) unilateral striatal lesion. It was expected to obtain
potential results in 6-OHDA lesioned rats which may also lead further studies with large number of animals leading to develop commercial preparations for also in PD patients at clinics in the future for effective therapy with decreased side effects and decreased frequency of administration which is very significant for patient’s compliance to the therapy.
2. GENERAL INFORMATION

2.1. Parkinson’s Disease

Parkinson’s disease (PD) also known as idiopathic or primary parkinsonism) is a degenerative disorder of the central nervous system. Although its mechanisms and reasons are not clearly defined, it is thought that its motor symptoms result from the death of dopamine-generating cells in the substantia nigra, a region of the midbrain. It is commonly seen in people in the middle or older ages. PD takes its name from James Parkinson, English physician, in 1817 as trembling stroke and now PD is in the beginning orders among geriatric neurodegenerative disorders. The main abnormality is generally seen in parts regulating coordinate and basic movements in brain. Formation of alpha-synuclein aggregation and Lewy bodies are hallmarks of PD and other related diseases (25).

According to the literature, environmental toxins, genetic factors and oxidative stress may cause PD onset. According to a study performed through the Europe, the minimal amount of pesticide exposure can increase the risk of PD in a large amount (26). Another factor is the genetic mutations. According to Kurosinski et al. (27), mutations in gene SYN (A30P and A53T) can cause PD in animal models by causing internal cellular α-synuclein aggregation and deposition to form Lewy bodies which are significant signs of PD. Dopaminergic loss in the basal ganglia and substantia nigra of several animal species such as mice and flies can be seen after these mutations (28). Another cause of PD is related to the formation of unstable free radicals which are by-products of oxidative stress contributing to nerve cell death. It was seen that MTH1 suppresses cell death depending on oxidative stress in human PD patients (28,29).

- **Dopaminergic Neurotransmission in PD**

Dopamine (3-hydroxytyramine; DA) is a catecholamine neurotransmitter which is a precursor for synthesis of the neurotransmitter norepinephrine (NE). DA in synthesized from tyrosine by a two step process, where tyrosine hydroxylase (TH) is the rate-limiting enzyme in the reaction (30). Tyrosine (L-Tyrosine) is a naturally occurring amino acid involved in the synthesis of neurotransmitters dopamine,
adrenaline and noradrenaline. Dopamine synthesis from tyrosine is given in Figure 2.1 (31).

![Dopamine synthesis from Tyrosine](image)

**Figure 2.1.** Dopamine synthesis from Tyrosine (31)

DA is a neurotransmitter that transmits messages from one nerve cell to another. Dopamine signals travel from the substantia nigra to brain regions including the corpus striatum, the globus pallidus and the thalamus in order to control movement and balance (32).

- **Dopaminergic Pathways and Nigrostriatal Dopamine Pathway Affected in PD**

There are four main dopaminergic pathways; the tuberoinfundibular pathway, the nigrostriatal pathway, the mesocortical pathway and the mesolimbic pathway (Fig. 2.2)
Figure 2.2. Pathways of dopamine signaling in the brain. (Illustration of major DA projections in the central nervous system. The nigrostriatal pathway originates in the substantia nigra and projects to the dorsal striatum. The mesolimbic and mesocortical projections originate in the ventral tegmental area and project both to ventral striatum and areas in the prefrontal cortex, respectively. The final system is the tuberoinfundibular system which projects from the hypothalamus to the pituitary). (30,33)

1. The tuberoinfundibular pathway, which refers to a group of DA neurons in the arcuate nucleus of the hypothalamus that project to the median eminence, controls prolactin secretion from the anterior pituitary gland (34) Hyperprolactinaemia is associated with a failure of this pathway (30).  
2. Dopaminergic neurons in the mesocortical pathway project from the ventral tegmental area (VTA) to the frontal lobes of the cerebrum, particularly the prefrontal cortex, and are involved in cognition and
emotion. Attention deficit hyperactivity disorder, addiction and schizophrenia can be seen in any failure of this pathway (30).

3. Neurons of the mesolimbic pathway also originate in the VTA but instead innervate the ventral striatum, also known as the nucleus accumbens. This pathway is implicated in reward and pleasure. Attention deficit hyperactivity disorder, addiction and schizophrenia can be seen in any failure of this pathway (30).

4. Nigrostriatal pathway is related with PD. The nigrostriatal pathway in the midbrain consists neurons whose cell bodies originate in the substantia nigra and terminate in the dorsal striatum. This area is implicated in movement since degeneration of these projections has been shown to cause Parkinson’s Disease; characterized by tremors, rigidity, and overall improper movement (35). This region is also important in feeding behavior (36). Addiction and chorea can also be seen in any failure of this pathway (30).

In PD, most of the dopamine signals from the substantia nigra are lost. Nigrostriatal pathway is the efferent connection between the substantia nigra and corpus striatum. Nigrostriatal pathway is particularly involved in the production of movement, as part of a system called the basal ganglia motor loop. Loss of dopamine neurons in the substantia nigra is one of the main pathological features of PD, leading to a marked reduction in dopamine function in nigrostriatal pathway. The symptoms of the disease typically do not show themselves until 80-90% of dopamine function has been lost.

The secretion of dopamine is actualized from membrane storage vesicles in the presynaptic neurons and binds to postsynaptic neurons and activates dopamine receptors to perform its physiologic effects (32). Afterwards, following dopamine signalization from one neuron to another at the synapse, it is removed via re-uptake back into the presynaptic cell by either the high-affinity DAT or the low-affinity Plasma membrane monoamine transporter (PMAT). Once it is taken back inside the cytosol, it is repackaged into vesicles at the end for new signaling. As an alternative way, dopamine is directly broken down into inactive metabolites by two enzymes.
called monoamine oxidase B (MAO) and catechol-o-methyl transferase (COMT). But enzymatic degradation does not account for inactivation of DA in the synapse. Instead, termination of DA neurotransmission is regulated by DAT. DAT allows DA to be cleared out of the synapse and taken up into the presynaptic bouton (30,37,38). Insufficient dopamine biosynthesis due to loss of the substantia nigra dopaminergic neurons causes PD. Figure 2.3 shows dopamine levels in a normal and Parkinson’s affected neurons. Figure 2.4 designates a horizontal section of substantia nigra in normal and PD patients.

![Diagram](image)

**Figure 2.3.** Dopamine levels in a normal and Parkinson’s affected neuron (39).
Consequences of Dopamine Reduction in PD

At the early phases of the disease, the most obvious symptoms are movement-related such as tremor (hands and head develop involuntary movements), muscle rigidity/rigour, slowness of movement/bradykinesia and postural instability (difficulty with walking and gait). At progressive stages, thinking and behavioral problems may arise and dementia can be occurred in the advanced stages of the disease generally (40). Additionally depression is the most common psychiatric symptom that can be observed in PD patients. The mechanism of depression in PD is not clear however, it may be due to the deficiency of multiple transmitters in mesocortical monoaminergic systems containing dopaminergic projections, noradrenergic and serotonergic projections (41-43). Other symptoms may comprise sensory, sleep and emotional problems such as hallucinations and sleep disturbance. Subtle cognitive deficits especially frontal lobe executive dysfunction may be seen in patients with early PD which can be detected with sensitive neuropsychological testing (44,45). Postural hypotension
can also be seen in some PD patients. It is more frequently seen in PD patients with dementia when compared with PD patients without dementia (46).

2.1.1. Therapeutic Approaches of Parkinson’s Disease

PD, which has a high incidence in neurodegenerative disorders, can be clinically diagnosed generally only when the symptoms are initiated. Early therapy can be managed by early diagnosis of PD such as the alterations are in molecular level before symptomatic changes are started. There is a balance in brain between acetylcholine and dopamine which increases and regulates the stimulation property. In PD, the balance is spoiled in favor of acetylcholine and dopamine should be substituted. Today, although the main therapy of PD comprises therapy with drugs, there are also other therapy approaches.

The patient should be protected to be withdrawn and separate from the public and stay physically active. Surgery can be used in some conditions which comprising the damage of diseased area. One method is the deep brain stimulation for the treatment (47). Deep brain stimulation involves the implantation of a medical device called a brain pacemaker sending electrical impulses composed of implanted electrodes, to specific parts of the brain (brain nucleus) for the treatment of movement and effective disorders. The use of deep brain stimulation as the approved procedure by US Food and Drug Administration in the treatment of PD initiated in 2002 and since then almost 80.00 applications were administered by physicians in all around the world (48). However, the most commonly, easily and practically applied therapy approach is the drug therapy for PD.

Treatment approaches may be grouped as dopamine prodrugs, dopamine agonists, COMT inhibitors, MAO-B inhibitors, anticholinergics and NMDA inhibitors. They are defined below very briefly.
- **Dopamine Prodrugs**

  L-DOPA is a prodrug and a dopamine precursor. Dopamine can not be used for PD therapy due to its absence of penetration into CNS. This problem was solved by the use of L-DOPA in 1960s which is thought as gold standart in PD therapy. It desigates its effects in CNS by turning into dopamine by the enzyme of Dopa-decarboxylase. A very low amount (such as 1-2%) of the given dose is only effective. Therefore, L-DOPA should be administered in high doses which may increase the side effects. The use of L-DOPA in some patients is effective, problem-free and successful for 2-4 years, however, after that the symptoms of PD can reappeared. The level of L-DOPA can be decreased at certain times which causes patients to designate PD symptoms (off period, or wearing off phenomenon). Dyskinesia is a common side effect of the long term use of L-DOPA and it is generaly observed in 40% of the patients suffering from PD after 5 years which can be assumed as the honeymoon period and in 80% of PD patients after 10 years of L-DOPA utilization (49,50). In the case of any contraindication or no therapy effect or resistance to L-DOPA, some other drugs such as dopamine receptor agonists, anticholinergics and other drugs can be chosen as single therapeutics or in conjugation with L-DOPA. Sometimes, L-DOPA can be given with a peripheral decarboxylase inhibitor like benserazide or carbidopa. Apart from this, amantadine or bromocriptine can be added to the therapy with L-DOPA to obtain synergistic effect (37,51).

- **Catechol O-MethylTransferase (COMT) Inhibitors**

  COMT enzyme is a peripheral metabolizer of L-DOPA. COMT Inhibition prevents L-DOPA metabolism into 3-O-methyldopa and prolongs L-DOPA's effect. Entacapone (Comtan, Stalevo) is COMT inhibitor. Wearing-off effect can be prevented and it can be given with dopamine precursor in PD's pharmacotherapy.

  Tolcapone (Tasmar) may cause some side effects related with liver functions so it has been taken off the market in many countries. Entacapone is
more safer. Headache, diarrhea, and abdominal pain are some other adverse effects (37,51).

- **MonoAmine Oxidase-B (MAO-B) Inhibitors**

MAO-B inhibitors are used as adjunctive therapy for PD because they inhibit the breakdown of dopamine by MAO-B and increase dopamine amount in the brain. Selegiline (deprenyl) blocks MAO-B which is a dopamine degrading enzyme and it may have some mild benefit as an initial therapy. It is generally used in early-onset disease. Rasagiline is used for PD treatment and for both early-onset or moderate to advanced disease with combination with L-DOPA. MAO-B inhibitors can cause some severe side effects. Orthostatic hypotension is one of the crucial one. Hypertension can be observed if combined with drugs inducing serotonin level enhancements like antidepressants and feeding with foods rich in the amino acid tyramine (37,51).

- **Anticholinergic**

These were the first drugs for PD in 1949 however they were replaced by dopamine drugs. Trihexyphenidyl is still used in several PD cases and especially for controlling tremor in early stages. It may cause dryness of the mouth. Nausea, dizziness, glaucoma, constipation, urinary retention, and some mental problems such as memory loss, confusion, and even hallucinations can also be observed (37,51).

- **N-Methyl-D-Aspartate (NMDA) Inhibitors**

Amantadine (Symadine, Symmetrel) is an antiviral compound. It stimulates dopamine release and designates its effect against dyskinesia in PD patients. Memantine is an uncompetitive antagonist of NMDA receptor, however it is not an option for PD therapy. NMDA inhibitors may cause swollen ankles and visual
hallucinations. Rarely, it may cause acute delirium or neuroleptic malignant syndrome (37,51).

The mechanism of action of antiparkinsonian drugs in dopaminergic synapse is given in Figure 2.5. (51). For the general therapy of PD; it is generally recommended to use dopamine precursors and dopamine agonists at early phase (37,51-55).

- **Dopamine Agonists**

  Dopamine agonists mimic dopamine for stimulating dopamine system in the brain and they induce dopamine receptors and currently being used as a treatment for improving many of the symptoms characterizing PD (28). Bromocriptine, pergolide, cabergoline and lisuride are specific dopamine agonists. Bromocriptine is the only ergot dopamine agonist approved for treatment of Parkinson in the U.S.A. Bromocriptine stimulates dopamine D2 receptors. Apomorphine is used as a very efficient drug in people having very severe on-off effects which may require going off L-DOPA for a few days. It is FDA-approved drug for the treatment off-time episodes of PD. Apomorphine has high affinity on D4 receptor. Adverse effects of these dopamine agonists are nausea, headache, vomiting, dizziness, constipation (37,51).

  Pramipexole is a non-ergot D2 and D3 receptor agonist. Pramipexole has similar selectivity with ropinirole. We selected pramipexole as antiparkinsonian agent to encapsulate in liposomes and niosomes.
The benefits of pramipexole selection in the treatment of Parkinson’s disease

Mine Silindir - A. Yekta Ozer

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Abstract Levodopa administration as a gold standard in Parkinson’s disease (PD) treatment is very valuable, however, long-term administration may cause some motor complications such as abnormal unintended movements and shortening response to each dose (wearing off phenomenon). Dopamine agonists were developed to reduce duration of inmobile off periods and dependence to levodopa for improving motor impairments (Claxton et al., Cochrane Libr 1:1–23, 2000). Pramipexole is one of these nonergot dopamine agonists with high relative in vivo specificity and full intrinsic activity at D2 subfamily of dopamine receptors, with a higher binding affinity to D3 than to D4 or D2 receptor subtypes (Porreca, Clin Neuropharmacol 21:141–151, 1998). It can be advantageously administered as monotherapy or adjunctive therapy to levodopa to decrease side effects and increase effectiveness in both early and advanced PD treatment.

Keywords Advantages of pramipexole - Parkinson’s disease

Introduction

Due to increase in lifetime, an increase is observed in the incidence of geriatric diseases, parallelly in research in this field across the world. One of the most recently observed neurodegenerative diseases in PD diagnosed over age 50 generally; however, it can also be seen in young people about 5% among all patients and is called juvenile PD. While disease incidence is 0.2–0.3% in the community, it is 1% among people over the age of 55. PD took its name from an English doctor James Parkinson in 1817, who published about the disease first in An Essay on Shaking Palsy. Its motor symptoms took its root from degeneration or death of dopamine-generating cells in substantia nigra of midbrain [3]. While movement-related symptoms are observed in earlier PD, cognitive, behavioral problems are generally observed in progressive levels of disease. In some cases of subsequent advanced stages, dementia may be occurred [4].

Antiparkinsonism drugs comprise dopaminergics and antimuscarinics. While dopaminergics are used for potentiating actions of dopamine, antimuscarinics are used for reducing excessive central cholinergic effects. Antimuscarinics are grouped as tertiary amines. Dopaminergics are grouped as levodopa, peripheral dopa-decarboxylase inhibitors, amantadine and antineutamine, ergot derivatives, various other nonergot dopamine agonists including pramipexole, specific monoamine oxidase type B inhibitors, and caged-G-methyltransferase inhibitors [5]. Pramipexole is developed as either an antiparkinsonian drug levodopa or monotherapy in PD treatment.

Physicochemical properties

Pramipexole dihydrochloride monohydrate (C13:2-aminocarbonyl-4,5,6,7-tetralinol-5-propylimino-benzothiazole dihydrochloride) is a white to off-white crystalline powder (302.27 g mol⁻¹) and stable under ordinary conditions. Its solubility is more than 20% in water, about 8% in methanol, 0.5% in ethyl alcohol and practically insoluble in dichloromethane [6]. Due to high solubility and high permeability
properties, pramipexole dihydrochloride monohydrate is classified as BCS C1 substance [7]. Due to proper solubility and properties, pramipexole dihydrochloride monohydrate is chosen instead of pramipexole for PD treatment. 87.50 μg of pramipexole is approximately equivalent to 125 μg of pramipexole dihydrochloride monohydrate [5]. Their molecular structures are given in Fig. 1a, b.

The word “pramipexole” is used in the meaning of “pramipexole dihydrochloride monohydrate” in this review.

Pharmacology

Precise mechanism of pramipexole is unknown; however, its effect is thought to be related to fully stimulate dopamine receptors in striatum which differs it from other dopamine agonists. Distinctively, ergot dopamine receptor agonists such as bromocriptine, lisuride, pergolide bind both dopamine and non-dopamine receptors. While all agents depressed dopamine neuron firing, only pramipexole, quinpirole completely silenced firing [6, 9]. Pramipexole is also used for treatment of restless legs syndrome. Although its mechanism of action is unclear, it is thought to be related with primary dopaminergic system [5, 10].

Due to high D3 receptor affinity, pramipexole takes role in treatment of both motor and psychiatric symptoms of PD. Its neuroprotective effect depends on its being dopamine receptor agonist [8]. The neuroprotective effect of pramipexole for both active S(+) and inactive R(−) enantiomers depends on suppressing dopaminergic neuronal death equally [11]. It also possessed excitotoxicity, autophagy, proteasome dysfunctioning, antioxidant effect, mitochondrial transition pore opening generally related with initiation of programmed cell death. It can be also used for major depression treatment [8].

Pharmacokinetics

Pramipexole is readily absorbed from gastrointestinal tract. While its peak concentrations have been obtained within 2 h fasting patients, it is within 3 h food taken patients. Absolute oral bioavailability found greater than 90% with little first pass metabolism. It exhibits linear pharmacokinetics. Its protein binding is less than 20%. Its distribution is extensive with a volume of distribution about 500 L [5, 8]. Metabolism is observed minimally and more than 90% of dose is eliminated unchanged, almost exclusively via renal tubular secretion into urine. Elimination half-life is 8–12 h requiring a dosage regime of three times a day (t.i.d.) [12].

Pharmacodynamics

Pramipexole simultaneously excites direct striatopallidal pathway, inhibits indirect striatopallidal pathway, and decreases PD symptoms by mimicking dopamine's effects in striatum via high relative in vitro specificity, and full intrinsic activity at D3 receptor subtype [6]. Binding affinities of dopamine D2L, D2S, D3, and D4 receptors for pramipexole using both [3H]pramipexole and [3H]sulpiride as radioligands at cloned, heterogeneously expressed receptors were evaluated in rat and human [13]. Pramipexole designated fivefold selectivity for D3 receptors than D2 and D4 receptors [13]. Since D3 receptors have greatest predominance in limbic system [14], pramipexole has potential effect on psychiatric symptoms in PD which can be used for depression treatment in PD patients [8].

Pramipexole designated weak to moderate binding affinity for adrenergic alpha-2, 5-HT1A, histamine-2 sites [15] and low binding affinity to 5-HT2A/B, D1 receptors having a potential role in cardiac valvulopathy, dyskinesias [16].

Drug–drug interactions

In contrast to other dopamine agonists, pramipexole exerts no strong cytochrome P450 inhibition in vitro minimizing drug–drug interaction risk [5, 6, 17]. Although some little alterations may be observed, pramipexole did not alter levodopa's bioavailability significantly [18]. It is recommended to take care of administration of other sedating drugs or alcohol [5].

Table 1 designates interactions of some other drugs with pramipexole combinations [6, 10, 19].

Dosage and administration

Pramipexole dose ranges between 1.5 and 6 mg day−1 to improve activities of daily living and motor symptoms in
Table 1. Interactions of some other drugs with pramipexole combination [9, 10, 30]

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbidopa and levodopa</td>
<td>May cause</td>
</tr>
<tr>
<td>combination</td>
<td>As increase in percent to reach peak levodopa plasma concentration by about 40%</td>
</tr>
<tr>
<td></td>
<td>A decrease in time to reach peak levodopa plasma concentration from 2.5 to 0.3 h</td>
</tr>
<tr>
<td>Selegline</td>
<td>Did not cause any alteration in pharmacokinetics of pramipexole in healthy volunteers</td>
</tr>
<tr>
<td>Carbimazole</td>
<td>May inhibit renal tubular secretion of organic bases via the canalicular transport system</td>
</tr>
<tr>
<td></td>
<td>May cause a 50% increase in AUC of pramipexole, as well as a 40% increase in the half-life of pramipexole in a small series of patients</td>
</tr>
<tr>
<td>Aminocarbamide</td>
<td>May interfere with oral clearance of pramipexole</td>
</tr>
<tr>
<td>Dopamine antagonists</td>
<td>May cause a decrease in pramipexole effect</td>
</tr>
<tr>
<td>(dopaminergic, norepinephrine)</td>
<td>Pharmacokinetics of agents that are secreted via</td>
</tr>
<tr>
<td></td>
<td>Catechol transport system, decreases clearance of pramipexole about 20%</td>
</tr>
<tr>
<td>Drugs excreted by renal secretion</td>
<td>Pharmacokinetics of agents that are secreted via</td>
</tr>
<tr>
<td>(dihydropyridines, quinolines, quinacetamide)</td>
<td>Catechol transport system, decreases clearance of pramipexole about 20%</td>
</tr>
<tr>
<td></td>
<td>Asthmatic transport system, has a little effect on clearance of pramipexole</td>
</tr>
<tr>
<td>Phenobarbital</td>
<td>Did not influence pharmacokinetics of pramipexole</td>
</tr>
</tbody>
</table>

observed in advanced PD patients. Dosage for patients with renal impairment should be arranged specially [10, 19].

Adverse reactions

Most commonly observed adverse effects are nausea, vomiting, dizziness and orthostatic hypotension. Some dopaminergic complications may comprise wearing-off dyskinesias, on-off fluctuations, freezing, changes in Unified Parkinson’s Disease Rating Scale (UPDRS) and scales of disability, tremor, instability, constipation, diarrhea, hallucinations, syncope, accidental injury, dream abnormalities, confusion, and nausea. 

Psychiatric adverse reactions are commonly seen with dopamine agonists rather than levodopa [23]. Most of the adverse reactions are predictable and may be related to dopaminergic hyperstimulation. These adverse reactions can be classified as central or peripheral, and may be related to gastrointestinal reactions, cardiovascular reactions or psychotic, behavioral syndromes, sedative reactions, respectively. Gastrointestinal and cardiovascular adverse reactions can be developed at the beginning of therapy. However, tolerance can be developed over time [24].

Acute dopaminergic administration was observed to increase the risk of a variable rate alternative significantly [27]. In 6-OH-Dopamine (6-OH-DA) -induced Parkinsonian models, pramipexole administration was associated with a decrease in the development and severity of motor and non-motor symptoms. In a comparison of levodopa and pramipexole as add-on therapy for advanced PD, the risk of a variable rate alternative was significantly higher in the pramipexole group compared to the levodopa group. However, the increase in risk was not associated with a decrease in the development and severity of motor and non-motor symptoms.

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Safety

It is recommended to perform ophthalmologic monitoring to observe for ocular toxicity with pramipexole.

Toxicity of
Pramipexole was investigated in juvenile rhesus monkeys administered as 0.1, 0.5, and 2 mg kg$^{-1}$ for 36 weeks, corresponding to doses approximately 25 times higher than recommended maximum dose in humans [30]. Although no significant toxicity was observed, a decrease in blood pressure, heart rate, and serum prolactin level was seen [30]. No carcinogenicity was observed after two-year experiments in primate-pooled administered mice and rats. In addition, pramipexole was not mutagenic or clastogenic depending on assays [30, 39].

### Use in special conditions

Pramipexole was not carcinogenic, mutagenic or clastogenic in rats and mice. Fertility studies performed on rats administered pramipexole (2.5 mg kg$^{-1}$ of body weight/day) prolonged estrus cycles and inhibited implantation [6, 10, 19]. Some dose-inhibited implantation throughout pregnancy period designated in rats. These results are due to prolactin lowering effect of pramipexole for rats but not for rabbits or humans. It is in pregnancy category C for FDA [6, 10, 19].

In a radiolabeled single-dose study in lactating rats, pramipexole was distributed into milk and radioactively amount in milk was 3–6 times greater than plasma. Due to some other reports, pramipexole induced inhibition of prolactin secretion in humans and rats [6, 10, 19].

Safety, efficacy, pharmacokinetics of pramipexole were not evaluated for pediatrics. Its clearance was approximately 30% lower in older people due to reduced renal function [6].

### Benefits of different pramipexole formulations and administration purposes in PD treatment

There are many arguments supporting benefits of dopamine agonist rather than levodopa in early PD treatment. Initiation to a treatment protocol with a dopamine agonist is better for slowing down initiation of dyskinesias, wearing off. It appears advantageous to use a dopamine agonist such as pramipexole in long-term treatment protocol as monotherapy or adjunctive therapy to levodopa for decreasing not only adverse effects but also treatment cost [31]. Other advantages of dopamine agonists are antioxidant effects and neuroprotection due to stimulation of dopamine autoreceptors, depressing dopamine neuron function and turnover. It has improving properties for its potential to delay neurodegeneration in PD, while other dopamine agonists designing autoreceptor stimulation [32]. Pramipexole has significant intrinsic antioxidant properties [32]. In rat brain, hydrox free radicals were scavenged by pramipexole in vivo when MPP$^+$ was perfused [33].

Pramipexole can inhibit neurotoxicity of levodopa [34] via neuroprotection effect toward nigral dopamine neurons in vivo and results in protection of dopamine neurons from toxicity of methamphetamine [32], 6-hydroxydopamine (6-OHDA), MPTP [35] and acute hypoxia or ischemia [32].

Pramipexole is FDA-approved nonergot dopamine agonist for treatment of motor dysfunction in PD and restless leg syndrome. NIRAPLEX$^5$ (SIRKIR$^5$ in Europe) is commercially available immediate release (IR) formulation [i.e.,] from Boehringer-Ingelheim, first approved for PD treatment in 1997 [36, 37] and idiopathic restless legs syndrome in 2006 [38, 39]. Generally short-acting dopamine agonists cause fluctuations in plasma, brain levels. Continuous dopaminergic stimulation induced higher therapeutic benefits such as alleviation of nocturnal disturbances, early morning akinesia [40]. NIRAPLEX$^5$ was then granted approval by European Commission for prolonged release, once daily formulation. Extended release (ER) formulation was developed afterward for eliminating pill burden, increasing patient compliance, decreasing fluctuations in plasma concentration and cost effectiveness [29, 37]. Pramipexole ER (0.5 mg t.i.d.) was found bioequivalent to 4.5 mg of pramipexole ER once daily over 24 h [29, 39]. No difference was seen in efficacy, safety, tolerability in patients switched overnight from pramipexole IR to ER formulation [41].

Some studies were carried out in different formulations of pramipexole. Alzet minipumps implants were compared with s.c. pramipexole injection for performing continuous release (CR) in rats [40]. While higher therapeutic benefit in early morning akinesia was obtained with pramipexole CR, motor impairment was reversed for several hours with pramipexole IR [40]. Pegylated, nanosized, $^{11}1$Te-labeled and pramipexole encapsulated, neutral and positive charged liposomes and niosomes were formulated for both diagnosis and therapy of PD. Characteristics of both tracers were observed proper and found potential for further in vivo brain targeting studies [42, 43]. Pramipexole microsphere formulation was prepared. Diffusion controlled, prolonged release about 24 h was obtained [44].

Effects of pramipexole were evaluated in a variety of studies on both animals and humans. MPTP, 6-OHDA are used as neurotoxins to produce PD model in small animals. Pramipexole 0.026 mg kg$^{-1}$ induced turning behavior for a period of 2 h in 6-OHDA model. Similar antiparkinsonian effect was obtained in MPTP model without initiation of any drug induced dyskinesias [45]. Pramipexole was found only effective in subacute model of MPTP administration due to another study [46]. Dopaminergic neurons were protected by pramipexole from glutamate neurotoxicity by reduction in intracellular dopamine content [11]. It completely antagonized neurotoxic effects of MPTP in PD mouse model in substantia nigra, ventral segmental area.
Pramipexole was observed effective in inhibiting free radical-mediated lipid peroxidation and protecting MPTP-induced nigral dopaminergic injury [48].

Pramipexole, pramipexole and bromocriptine were compared in MPTP PD model mice. Pramipexole and pramipexole found neuroprotective by suppressing reduction in dopamine amount after MPTP administration in striatum more significantly than bromocriptine [38]. Giutathione level was found higher with administration of pramipexole but not levodopa in MPTP PD model. Both designated compounds neuroprotective properties for dopaminergic neurons [49]. Their treatment effects were also compared by The Parkinson Study Group [50, 51]. Initial pramipexole treatment caused a reduction in development of wearing off, dyskinesias or on-off motor fluctuations rather than levodopa. Initial levodopa treatment was observed to cause sustained improvement in UPDRS total score. In early PD patients, percent loss in striatal uptake of 123I-labeled ICI was decreased by 40% at 22 and 46 months by SPECT. Significant reduction was observed in formation of dyskinesias, wearing off symptoms administered pramipexole rather than levodopa. Similar life quality was observed for both groups [21].

Distinctly, some controversial findings were also obtained. Effects of pramipexole, cabergoline and levodopa at similar doses inducing improvement purposeful forelimb use having greater or lesser effect on abnormal involuntary movements (AIMs) were compared in rats with severe nigrostriatal lesion. AIMs' development during subsequent levodopa monotherapy or dyskinesia was not prevented by D2 agonists in rats with nigrostriatal lesion [52]. Another similar study was performed in MPTP/parkin administered severe PD mice model. No improvement in neuroprotective effect was observed with pramipexole [53].

13C-FPL 457 was administered to quantitate D2/D3 receptor subtype and images were obtained by PET before and after receiving a single dose [54]. 0.25 mg pramipexole binds to D2/D3 receptors significantly in prefrontal cortex, amygdala, lateral, lateral thalamus related with depression. Pramipexole was observed to possess some antidepressive effects [54]. To evaluate BBB penetration in immortalized rat brain capillary endothelial cells were used as in vitro BBB model. Pramipexole was radiolabeled and penetration was found dependent to temperature, pH but not sodium ion concentration or membrane potential [55].

Conclusion

As an effective nonergot dopamine agonist, pramipexole can avoid formation of cardiac valvular disease and delay onset of dyskinesias by levodopa administration [15]. However, pramipexole may induce development of hallucinations, somnolence, impulse control disturbances, peripheral edema and gastrointestinal effects [15, 16]. Although pramipexole therapy seems more costly than levodopa, this change in favor of pramipexole over time due to life quality enhancement [16]. Apart from antiparkinsonian effects, it also possesses beneficial effects on nonmotor symptoms (depression) and improves treatment-resistant tremor and anhedonia [15]. A high rate of monotherapy survival was observed in PD up to 34 months and side effects can be prevented by proper dose acceleration [8]. It can be used as monotherapy or adjunctive therapy to levodopa to decrease side effects and increase effectiveness. In the future, novel formulations of pramipexole can be developed for both early and advanced PD treatment.

Conflict of interest Authors do not have a financial relationship with any organization and the authors declare that they have no conflict of interest.

Ethical standards The manuscript does not contain clinical studies or patient data. The editors reserve the right to reject manuscripts that do not comply with the above-mentioned requirements. The authors will be held responsible for false statements or failure to fulfill the above-mentioned requirements.

References

2.2. The Structure of Brain and Blood Brain Barrier

The brain is one of the most complex organs within the human body. It is made up of more than 100 billion nerves communicating lots of connections with synapses. It is thought that the cerebral cortex (the largest part) contains 15–33 billion neurons in a typical human (56). These neurons communicate with one another by axons carrying signal pulses called action potentials to every part of the body. The brain controls the rest of the body by generating patterns of muscle activity and driving the secretion of hormones. The lobes and general parts of the brain is given in Figure 2.5.

![Figure 2.5.](image)

**Figure 2.5.** The lobes and general parts of the brain (57).

There are 2 physical barriers separating brain extracellular fluid from the blood called BBB and BCSFB.

1. BBB is characterized by tight junctions between endothelial cells by the absence of fenestration (58). Brain microvascular endothelial cells (BMVEC) and neuroparenchymal cells such as pericytes, microglia, astrocytes and neurons surrounding the microvessels form BBB. This barrier regulates trafficking of ions, molecules and leukocytes into and out of the brain. It limits and prevents the penetration and entry of compounds from blood to the brain.
2. Blood-cerebrospinal fluid barrier (BCSFB) exists at the choroid plexus and separates the blood from the CSF (59). The epithelial cells exist on the choroid plexus forming BCSFB have complex tight junctions on CSF (apical) side of the cells. However, the tight junctions of the epithelial cells in the choroid plexus seems to be more permeable than the ones in the endothelial cells of BBB (60,61).

The schematic representation of two main barriers in CNS is given in Figure 2.6.

![Figure 2.6. Schematic representation of two main barriers in the CNS (61).](image)

### 2.2.1. Blood Brain Barrier Penetration Approaches

Drug and imaging agent delivery to the brain is still a kind of challenge for both the diagnosis and therapy of CNS disorders. Brain barriers can be spoiled and getting leaky in some conditions such as brain tumors, inflammation, etc. (62). This is because tumors develop their own vasculature to grow. The vessels within tumors have increased permeability depending on the presence of larger endothelial cell gaps when compared with the normal vessels and retention effect which is called EPR effect (62).
Formulation of new and effective drug delivery systems is crucial for brain delivery depending on the existence of barriers. BBB restricts the entry of compounds to the brain from the periphery (63,64). By this way brain entry of many low molecular weight drugs, compounds, biomacromolecules such as DNA and proteins are restricted. Some substances can penetrate to brain depending on the passive diffusion across BMVECs due to the lipophilicity and proper molecular weight of these substances. One of the drawback is the rapid efflux from the brain into the blood by extremely effective efflux pumps such as P-glycoprotein (Pgp) and Multidrug Resistance Proteins (MRPs) in BBB (65-68).

The molecules penetrating BBB by passive diffusion should have some properties because as mentioned above almost every large molecules and drugs can not penetrate BBB in which about 98% of the small molecules and drugs can not cross BBB (69,70). Small particle size, low molecular mass (< 400 Da), a log octanol/water partition coefficient between -0.5 and 6.0, lipid-solubility, being either neutral or significantly uncharged at physiological pH:7.4, and forming <8 H-bonds with water (71) are some important properties for formulating BBB penetrating drugs. BBB only permits the passage of some small lipid-soluble drugs through this barrier (72). It was observed that almost 98% of small molecules and nearly all large molecules such as recombinant proteins or gene-based medicines can not cross the BBB (73). Due to the presence of tight junctions of endothelial cells present in BBB, penetration pathways comprise paracellular aqueous pathway, transcellular lipophilic pathway, transcellular lipophilic pathway, transport proteins, receptor-mediated transcytosis or adsorptive-mediated transcytosis (74,75). These nanocarrier systems generally taken up by carrier-mediated transport, receptor-mediated endocytosis and adsorptive-mediated endocytosis and by this way reaching to the cerebral parenchyma or degraded within the lysosomes leading drug release (61).

In this section, different brain penetration mechanisms of drugs will be mentioned very briefly. A general table comprising drug delivery strategies to brain are summarized in Table 2.1.
There are 3 approaches to enhance brain penetration of drugs (61):

1. Invasive route is important to leave out the penetration through BBB and BCSFB by direct administration of the drug into the brain. Direct administration of drug into the brain can be applied via intracerebral, intracerebroventricular and intrathecal administration. By this way, a variety of compounds containing large and small molecules can be administered. However this method is impractical depending on being invasive and requires surgery and craniotomy. Another option for transporting through BBB is the intranasal administration to use the connection between nose and brain which is called the olfactory bulb (76). A variety of materials such as small molecules, proteins, viruses, pathogens and toxic materials can be administered by this way (77-80).

2. Pharmacological strategy depends on the increase of lyophilic solubility of drugs. By this way, drug molecules can be chemically modified or encapsulated in liposomal formulations. Generation of a transient disruption of BBB which allows the entrance of therapeutic agents into the brain from blood. The disruption of brain can be managed by pharmacological means. Many different endogeneous proinflammatory vasoactive agents like bradykinin, histamine, nitric oxide can induce an increment in the BBB permeability. Bradykinin was designated to increase the ionic permeability of the BBB reversibly (81,82). The transient disruption of BBB can also be observed by systemic administration of different molecules especially alkyl glycerols. BBB disruption depends on the length of the alkyl group and the number of glycerols exists in the structure (80,83).

3. Physical strategy depends on the penetration of nutrients, peptide and non-peptide hormones and transport proteins with the help of carrier mechanisms exist on BBB (80).
Table 2.1. General drug delivery strategies to brain (84).

<table>
<thead>
<tr>
<th>Invasive Techniques</th>
<th>Non-invasive Techniques</th>
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<tr>
<td></td>
<td>Chemical Way</td>
</tr>
<tr>
<td>-BBB disruption</td>
<td>-Prodrug design and lipidization</td>
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<td>-Direct drug injection</td>
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There are both chemistry-based and biology-based approaches for developing BBB drug-targeting strategies (Figure 2.7) (85,86). The chemistry-based strategies are the conventional approaches that rely on lipid-mediated drug transport across the BBB. The biology based strategies depend on numerous endogenous transport systems within the BBB. These transporters are conduits to the brain. The endogenous BBB transport systems may be broadly classified as carrier-mediated transport (CMT), active efflux transport (AET), and receptor-mediated transport (RMT). These BBB transport systems are situated on the luminal and abluminal membranes of the brain capillary endothelium.
**Figure 2.7.** Brain drug targeting strategies comprising biological and chemical bases (Chemistry-based strategies emphasize lipid solubility, hydrogen bonding, and molecular weight of the drug. Biology-based strategies emphasize endogenous BBB transporters. Small molecules can be transported across the BBB by either accessing certain carrier-mediated transport (CMT) systems or by inhibiting certain active efflux transporters (AET). Large-molecule drugs such as recombinant proteins or gene medicines can be delivered across the BBB via the receptor-mediated transport (RMT) systems. Reprinted with permission (85,86).

*(Pgp: P-glycoprotein, oatp2: organic anion transporter polypeptide 2, BSAT1: BBB specific anion transporter type-1, GLUT 1: glucose transporter isophorm-1, LAT 1: Large amino acid transporter-1, MCT 1: Monocarboxylate transporter 1, CNT 2: Concentrative nucleoside transporter 2).*

Drug delivery to the brain through the many endogenous transport systems within the BBB requires reformulation of the drug so that the drug can access the BBB transport system and enter the brain. Researchers within brain drug-discovery and brain drug-targeting should work together to formulate more effective formulations to enable BBB transport (85).
One of the most commonly researched approach is the formulation of effective drug delivery systems which can deliver to the brain which exists in pharmacological approach. Researchers work on improving drug delivery strategies to the brain to obtain an effective clinical outcome. This method is non-invasive for delivering drugs to the brain by the use of nanoparticular and nanovesicular delivery systems which will be explained in detail at the sections given below (76).

2.3. Nanotechnology and Nanomedicine

The major problem with drugs used for therapy of CNS diseases is the low amount of drug that penetrates from BBB and reaches the brain targeting. Dose increment to obtain the desired effect in brain may cause some side effects. Therefore, researchers are searching solutions for increasing drug amount without increasing drug dosage. Nanosized drug delivery systems have important advantages in this issue. The term nanotechnology is first stated by a physicist named Richard Feynman in 1959 about measuring nanomaterials by miniaturized devices and their use for identifying new scopes. Nanotechnology is generally related with materials and devices that are smaller than 100 nm. According to USA National Health Institute, one of the most important area in nanotechnology is nanomedicine and it comprises specific medical research in molecular level for the diagnosis and therapy of the diseases. Nanomedicine can be defined as the application of nanotechnology to the field of health and it can be described as a new field of science. Nano is one in a million of a milimeter (8,9).

Nanotechnology and nanomedicine are closely related research fields comprising a huge industry and projects with high budgets. Nature Materials Journal indicated that there was about 130 nanotechnology based drugs and drug delivery systems world-wide in 2006. Nanomedicine is a great field which comprising intense researchs every year with nanosized drug sales about 6.8 billion dollars, factory over 200, products over 38 and R&D budget higher than 3.8 billion dollars world-wide. While nanotechnology R&D investments reached about 1 billion dolars in USA in 2005, it reached about 1.3 billion euros in European Union in 2003-2006. As in general, there was a total nanotechnology investment about 3 billion dollars world-wide in 2003. The market related with nanosized devices and
molecular modelling designated 28% increase in one year and the income obtained from biomedical nanosized devices reached to 1.37 billion dollars in 2007. In the light of these datas, it is tought that in course of the development and improvement in this field, nanomedicine industry will bring significant benefits onto the economy of countries world-wide (87).

The potential applications of nanotechnology to drug field comprises subjects related with formulation, improvement and potential applications of drug delivery systems and diagnostic devices and gene therapy. So many benefits are obtained by the application of nanotechnology to pharmaceutical field also for brain delivery of drugs depending on very small particle size which can help passive targeting. Nanosystems that are used in nanomedicine as drug delivery systems comprise liposomes, niosomes, micelles, nanospheres, polymeric delivery systems, dendrimers, emulsions, nanoparticles and nanocapsules etc. and these systems are composed of an important part of nanomedicine.

2.4. Drug Delivery Systems

Apart from other conventional drugs, the superiority of drug delivery systems that use nanotechnology basically depends on small particle size. Nanosized drugs can be used in lower concentrations efficiently and faster effect can be obtained (8,10,11).

The significant properties that should be considered for a drug delivery system to become effective are high drug loading capacity, physical and chemical stability, low toxicity incidence of the carrier used, proper in vivo behavior of the carrier, the ability to scale up the producing process and the overall cost (88). Among drug delivery systems; polymeric or lipidic nanoparticles, liposomes, niosomes and polymeric micelles are mostly investigated for neuroprotection and facilitating the delivery of drugs and small molecules into the brain (89,90).

The essential reasons that directing formulation of drug delivery systems are their controlled drug delivery and release properties, improved pharmacokinetic and pharmacodynamic properties, enhanced blood circulation, effective and specific targeting potential, biocompatibility, biodegradability and nontoxicity. They can be both passively and actively targeted to the target tissue by altering
surface properties, surface charge and by specific ligand modification, respectively (70).

Nanoparticular and nanovesicular drug delivery systems are a reason of choice because of their sustained-release and controlled-release properties. These nanosized drug delivery systems are effectively used for diagnosis and therapy purposes in many diseases and although it is not very common, their use in neurodegenerative diseases such as PD, alzheimer’s disease and dementia are also investigated by researchers. For the therapy of PD, the observed frequency of dyskinesia depending on the use of L-DOPA can be diminished by the utilization of these nanoparticular drug delivery systems (50).
Drug Delivery Systems for Imaging and Therapy of Parkinson’s Disease

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Abstract: Background: Although a variety of therapeutic approaches are available for the treatment of Parkinson’s disease, challenges limit effective therapy. Among these challenges are delivery of drugs through the blood brain barrier to the target brain tissue and the side effects observed during long term administration of antiparkinsonian drugs. The use of drug delivery systems such as liposomes, microspheres, nanoparticles, nanocapsules, gold nanoparticles, microspheres, microbubbles, microbubbles and dendrimers is being investigated for diagnosis and therapy.

Methods: This review focuses on formulation, development and advantages of nanosized drug delivery systems which can penetrate the central nervous system for the therapy and/or diagnosis of PD, and highlights future nanotechnological approaches.

Results: It is essential to deliver a sufficient amount of either therapeutic or noninvasive agents to the brain in order to provide the best possible efficacy or imaging without undesired degradation of the agent. Current treatments focus on motor symptoms, but these treatments generally do not deal with modifying the course of Parkinson’s disease. Beyond pharmacological therapy, the identification of abnormal proteins such as α-synuclein, parkin and inducible repeat stretch/bronze protein kinase 2 could represent promising alternative targets for molecular imaging and therapy of Parkinson’s disease.

Conclusion: Nanotechnology and nanosized drug delivery systems are being investigated intensely and could have potential effect for Parkinson’s disease. The improvement of drug delivery systems could dramatically enhance the effectiveness of Parkinson’s Disease therapy and reduce its side effects.

Keywords: Advantages of drug delivery systems, drug delivery systems, imaging of Parkinson’s disease, liposomes, microbubbles, nanoparticles, therapy of Parkinson’s disease.

INTRODUCTION

Parkinson’s disease (PD), also known as idiopathic or primary parkinsonism, is a progressive degenerative disorder of the central nervous system [1, 2]. Although its mechanisms and causes are not clearly defined, it is thought that the motor symptoms of PD result from the death of dopamine-generating cells in the substantia nigra, a region of the midbrain, inducing a dopamine deficiency which is the hallmark feature of this disease. PD is most commonly seen in people who are middle aged or elderly [3]. Recent findings showed that about 1 in every 200 people between 60 and 69 years of age, about 1 in every 100 people between 70 and 79 years, and about 1 in every 35 people between 80 and 89 years of age suffer from PD in the United States [13] and Western Europe [4, 5].

PD takes its name from James Parkinson, an English physician who published a description of the disease in 1817. PD is among the most common genetic neurodegenerative disorders [6]. Environmental toxins, genetic factors, and oxidative stress may be involved in the development of PD. Pesticide exposure has been linked to a significantly increased risk of PD [7]. Mutations in the α-synuclein protein gene such as A30P and A53T can induce PD in animal models by causing intra-cellular α-synuclein aggregation and sequestration to form Lewy bodies of which are significant signs of PD [8]. Dopaminergic loss in the basal ganglia can be detected in animal models such as mice and flies that express these abnormal proteins [9]. Another mechanism of PD is suggested to be the formation of unreactable free radicals, which are byproducts of oxidative stress contributing to nerve cell death. MTII suppresses cell death due to oxidative stress in PD patients [9, 10].

In PD, most of the dopamine signals from the substantia nigra are lost. The nigro-striatal dopaminergic pathway travels from the substantia nigra to brain regions including the corpus striatum (caudate – putamen), globus pallidus and thalamus. It controls movement and balance, and is severely affected in PD. Dopamine is secreted from membrane storage vesicles in the presynaptic neurons and activates postsynaptic dopamine receptors to induce its physiologic effects [14]. Dopamine is directly broken down into inactive metabolites by the enzymes monoamine oxidase (MAO) and catechol-O-methyl transferase (COMT). In most areas of the brain, including the striatum and basal ganglia, dopamine is...
Drug Delivery Systems for Imaging and Therapy of Parkinson’s Disease

inactivated by re-uptake mechanisms via the dopamine transporter (DAT) and then enzymatically broken down by MAO [15].

Most of the symptoms of PD are related to motor functions because dopamine is essential for transmitting electrical signals for sustaining normal physical motion [16, 17]. At early stages of PD, the most commonly seen symptoms are movement-related such as tremor, rigidity, slowness of movement, and difficulty walking. As the disease progresses, thinking and behavioral problems may arise, and dementia can occur in the advanced stages of the disease [18]. Additionally, depression can be seen as a psychiatric symptom in PD patients. The mechanism of depression in PD is not clear, however it may be due to the deficiency of multiple transmitters in mesocortical monoaminergic systems containing dopaminergic, noradrenergic and serotonergic projections [19-21]. Other PD symptoms may include sensory and emotional issues such as hallucinations and sleep disturbances. Subtle cognitive deficits, especially frontal lobe executive dysfunction, may be detected in patients with early PD through sensitive neuropsychological testing [22, 23]. Postural hypotension can also be seen, particularly in PD patients with dementia [24].

One issue in the pharmacologic treatment of neurodegenerative diseases and brain related disorders is the difficulty for drugs to penetrate the blood brain barrier (BBB) and deliver a sufficient dose to the brain target tissues without metabolism [25, 26]. This difficulty stems from the protective barriers surrounding the brain such as the BBB and the blood-cerebrospinal fluid barrier (B-CSFB) formed by tight endothelial cells junctions [17, 26-29]. Passage of substances through the BBB can be increased in several cases such as inflammation and neoplasia due to increased vascular penetration and leakage. Because a limited number of substances can penetrate the BBB freely, it is very important to develop drug delivery systems with properties that allow effective treatment [25]. Factors that limit the ability to deliver therapeutic agents to the brain tissue of PD patients also limit the ability to deliver substances that would improve imaging and diagnosis of PD.

PD Diagnostics and Imaging

The ability to diagnose PD before the appearance of typical symptoms such as slowed movements, muscle rigidity, and deficits in posture could be an important step toward improving the quality of life for PD patients. Early diagnosis at the level of molecular alterations before the onset of symptoms can be approached by molecular imaging [31]. Molecular imaging can provide quantification of gene and protein functions, and thus provide better information about the molecular pathophysiology of a specific disease, on the protein-protein interactions and signal transduction pathways [30, 31]. Initially it is necessary to define molecular targets, such as receptors, transporters, enzymes or abnormal proteins in order to specifically study a disease. Generally, an age-dependent decrease is observed in the density of dopamine transporters in patients suffering from PD [32, 33]. A variety of specific imaging radioligands have been developed and investigated in order to improve the early diagnosis, follow-up and treatment of PD.

The dopamine D2 and D3 receptors have been proposed as useful targets in the field of PD. 18F-labeled precursors was synthesized for specific SPECT imaging of D2 receptor [34]. Other D2 receptor specific radioligands are -C(S)-)-N-(1-ethyl-2-pyrrolidinyl)-4-hydroxy-2-triphenylmethylbenzamide (1’[18F]FBZM) [37, 38]. 11C-F-2-[N-methyl-2-5-fluorophenyl]ethyl-l-5-methyl-2,3-dimethoxy-4-phenyl-1-adamantylamine (1’[11C]F-MPEP) [39]. 18F-fluoropropyl-2,3-dimethoxy-4-phenyl-1-adamantylamine (1’[18F]F-DA) [40]. [11C]1-[(2S)-1-(2-hydroxy-5-fluoropropyl)ethyl]-4-phenylbenzylamine ([11C]F-PE2I) [41]. [11C]F-3-(3-fluoropropyl)-3,4,5,6,10b-hexahydro-2H-naphtho[1,2-b] [1,4]oxazin-9-ol ([11C]F-89NHO) [42]. The name, molecular structure and synthesis process of some of D2/D3 receptor radioligands under research are provided in Table 1.

Dopamine transporter (DAT) imaging can be performed with radiolabeled cocaine derivatives such as 3-beta-(4-(4-fluorophenyl)prop-2-carboxylic acid methyl ester ([3H]RTI-55) [43] and (E)-3-[(2R,3S)-2-fluoroprop-2-enyl]-2-8-carboxybenzoyl-3,4-bis[2-carboxymethyl-3-4-tolyl]-nortropane ([3H]Br-PE2I) [45]. [125I]RTI-55 has demonstrated a high binding affinity to striatum and DAT. It also has a high binding affinity to cerebral cortex [44]. [125I]Br-PE2I was obtained by electrophilic substitution with a radiochemical yield of 10%. It demonstrated a high uptake in the striatum (2.2% ID/g tissue at 15 min post-injection). A specific accumulation ratio of 6 between the striatum and cerebellum was observed for [125I]Br-PE2I at 1 h p.i. [45]. Some tropine analogs such as 3-beta-[(4-fluorophenyl)1,2-dihydrobenzocycloheptene-3-carboxylic acid methyl ester ([3H]F-PE2I-CIT) and [125I]F-TRDAT-1 can also be used for DAT imaging [31]. The effectiveness of another DAT ligand, (E)-N-[(2S)-2-fluoropropyl]-2-methyl-3,4-bis[2-carboxymethyl-3-4-tolyl]-nortropane ([125I]F-PE2I), was investigated by Gulüten et al. [46]. PE2I demonstrated good affinity for the most selective DAT ligands. [125I]F-PE2I showed very intense and selective binding in the basal ganglia of postmortem human brains. It is a very efficient and specific radiotracer for DAT autoradiography studies [46]. Other DAT radioligands include (E)-N-(3-iodoprop-2-enyl)-2-carbocyclic-arboxylic acid methyl ester ([3H]F-PE2I) [47] and 2-[18F]fluoroethyl-(2-2-oxo-6-oxo-6-methyl-3,4-dihydrophenyl)-8-arylacyl[32.1]-octane-2-carboxylate ([18F]F-FECT) [48], which are potent DAT inhibitors. The inhibitory constant (K) of FECT for DAT was found to be 6 nM [48]. Chisen et al. investigated another marker for PET imaging of striatal as well as extrastriatal dopamine transporters: (E)-N-(4-fluorobut-2-enyl)-2-carbocyclic-arboxylic acid methyl ester ([11C]F-499) [49, 50]. [11C]F-499 bound to a single site with a Kd of 9 nM in vitro on rat striatal membrane and with a very high selectivity for the DAT [49]. This promising marker was radiolabeled with 18F or 125T for PET imaging [49, 51]. The name, molecular structure, and synthesis process of some of the DAT radioligands under investigation are provided in Table 2.

Other novel approaches have been taken for the diagnosis of PD. For example, Au-doped TiO2 nanotube arrays were
Table 1. Some of dopamine D2/D3 receptor radioligands under research [34-43].

<table>
<thead>
<tr>
<th>Radioligand</th>
<th>Molecular Structure</th>
<th>Availability</th>
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<tbody>
<tr>
<td>(^{33})C]Najopirolide</td>
<td><img src="image1" alt="Molecular Structure" /></td>
<td>Radiochemical synthesis</td>
</tr>
<tr>
<td>(^{11})RBM7M (65-N-{[(2S)-4-pyrimidinyl]methyl}-[1-ido-2]-methoxybenzamide)</td>
<td><img src="image2" alt="Molecular Structure" /></td>
<td>Commercially available</td>
</tr>
<tr>
<td>(^{99m})Tc-Fallypride</td>
<td><img src="image3" alt="Molecular Structure" /></td>
<td>Commercially available</td>
</tr>
<tr>
<td>(^{11})C-Fallypride</td>
<td><img src="image4" alt="Molecular Structure" /></td>
<td>Commercially available</td>
</tr>
<tr>
<td>(^{14})C-FLBM57</td>
<td><img src="image5" alt="Molecular Structure" /></td>
<td>Radiochemical synthesis</td>
</tr>
<tr>
<td>(^{123})I]Ipipropide</td>
<td><img src="image6" alt="Molecular Structure" /></td>
<td>Commercially available</td>
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Formulated for the purpose of producing a high sensitivity photoelectrochemical immunosensor for α-synuclein detection by An et al. [32]. Another tool based on nanomanipulation of a single molecule of α-synuclein was designed by Yu et al. for characterizing the misfolding and self-assembly of α-synuclein, which has a significant role in PD [53]. An in vitro quantitative assay for neurotransmitters involved in PD was performed by Baron et al. to investigate the plasma absorbance and Au nanoparticles, and very promising results were obtained [54].

PD Therapy

There are currently many proposed treatment regimens for PD patients. Most of the treatments are used to reduce the signs or symptoms of PD and enhance the overall quality of life for the patient [55, 56]. Surgical approaches can also be used to stimulate deep brain tissue or transplant of fetal neurons [57]. The use of deep brain stimulation was approved by the US Food and Drug Administration for the treatment of PD, in 2002, and since then almost 80,000 procedures have been administered by physicians around the world. However, this treatment is now very rare [58]. The most common, easiest, and most practical therapeutic approach for PD is drug therapy [28]. The conventional and current treatment approaches include carbidopa/ L-DOPA, dopamine agonists, monoamine oxidase type B (MAO-B) inhibitors, catechol-O-methyltransferase (COMT) inhibitors, amantadine and anticholinergics [59]. PD treatment with dopamine agonists like oral L-DOPA or pramipexole therapy is one of the most widely used approaches [60]. L-DOPA remains the most effective, and is considered as the gold standard for PD therapy. However, its chronic use is associated with potentially disabling motor complications and side effects.

The Use of Drug Delivery Systems for Diagnosis and Therapy of PD

It may be necessary to increase the dose of drugs administered to PD patients in order to obtain sufficient therapeutic effect in the brain. This increase in dose may
Table 1. Some DAT receptor radioligands under research [44-51].

<table>
<thead>
<tr>
<th>Radioligand</th>
<th>Molecular Structure</th>
<th>Availability</th>
</tr>
</thead>
<tbody>
<tr>
<td>(^{111})β-CIT</td>
<td><img src="image1" alt="Molecular Structure" /></td>
<td>Radiochemical synthesis</td>
</tr>
<tr>
<td>(^{123})I-FCIT (123I-[2-(\beta)-carboxy-3-(4-indolephenyl)-3-(3-fluorophenyl) nortropane])</td>
<td><img src="image2" alt="Molecular Structure" /></td>
<td>Radiochemical synthesis</td>
</tr>
<tr>
<td>(^{18})F-FECIT (2-beta-carboxymethyl-3-beta-4-chlorophenyl)6-C2-(18F)-fluorocetyl nortropane)</td>
<td><img src="image3" alt="Molecular Structure" /></td>
<td>Radiochemical synthesis</td>
</tr>
<tr>
<td>(^{11}C)Ccorate</td>
<td><img src="image4" alt="Molecular Structure" /></td>
<td>Radiochemical synthesis</td>
</tr>
<tr>
<td>(^{11}C)d-threo-methylphenidate</td>
<td><img src="image5" alt="Molecular Structure" /></td>
<td>Radiochemical synthesis</td>
</tr>
<tr>
<td>(^{11}C)PE2I (11C-[3-indolepropoxy]-5-(2-(\beta)-carboxy-3(4-methylphenyl)nortropane)</td>
<td><img src="image6" alt="Molecular Structure" /></td>
<td>PET tracer precursor is commercially available</td>
</tr>
<tr>
<td>(^{99m})Tc-TRODAT</td>
<td><img src="image7" alt="Molecular Structure" /></td>
<td>Easiest material is commercially available and radiochemical synthesis can be performed in some labs.</td>
</tr>
</tbody>
</table>
result in an increase in the frequency or severity of side effects. Researchers are searching for methods to increase the drug availability within the brain without increasing the drug dose. The use of nanosized drug delivery systems is a promising approach, and a number of different studies have been carried out on this issue. Advantages and disadvantages of some drug delivery systems are given in Table 3.

The superiority of these drug delivery systems lies in their ability to improve the undesired properties of drug molecules such as bad taste and odor, low bioavailability, adverse reactions, and insufficient targeting of the desired tissue or organ [76]. The drug or molecule can be protected from undesired metabolism and enzymatic degradation [77-79]. These systems have various advantages including increases in safety, efficacy, and bioavailability, with simultaneous reductions in dose requirement, toxicity, and adverse effect. However, significant drawbacks include their expense, and the increased difficulty of their manufacture [79-81]. Drugs, molecules, or contrast agents can be attached to these delivery systems and afterwards they can be actively delivered, localized, and targeted to the desired cell, tissue, or organ. Increased efficacy and safety can be achieved by controlling the release rate of the therapeutic agent, and a decreased volume of distribution can be obtained with active or passive targeted drug delivery systems by surface modification [82]. Additionally, bioavailability can be enhanced and drugs can be protected from enzymatic degradation by encapsulating in drug delivery systems [77-79].

Nanosized drug delivery systems have important advantages. The concept of nanotechnology is attributed to physicist Richard Feynman. Nanomedicine can be defined as the application of nanotechnology to health and is a relatively new field of science [83, 84]. Some examples of frequently used drug delivery systems include liposomes, siosomes, micelles, nanophores, nanocapsules, nanoparticles, microparticles, microspheres, microbubbles, polymeric systems, dendrimers, colloidal gold, gold nanoshells, quantum dots, superparamagnetic particles, carbon nanotubes, cycloexetrins, and sphingosines for the diagnosis and/or therapy of several diseases (Fig. 1) [85-101].

To allow BBB penetration of these nanosized drug delivery systems, many properties can be considered including surface functionalization for targeting [102], prolonged half-life in blood circulation and avoiding RES opsonization which is called “stealth” effect [26]. Unlike conventional drugs, nanosized and hydrophilic polymer coated stealth drug delivery systems tend to accumulate passively in diseased areas such as sites of inflammation, infection and neoplasm [86]. Drug delivery systems can also be delivered actively with the attachment or modification of targeted ligands like mAb, antibody fragments, small peptides, vectors, or avidin-biotin complexes [79, 103].

**Liposomes**

Liposomes are very promising systems in both diagnostic imaging and therapy [104-106]. These systems have been popular and important in research for over 50 years after they were recognized as mimicking the behavior of natural membranes due to their phospholipid structure [107, 108]. The utilization of phospholipids is mostly due to their biocompatibility, biodegradability, non-toxicity and non-immunogenicity. These properties give liposomes tremendous value [107, 109]. Modification of the phospholipids allow liposomes to be targeted either passively by surface coating with a hydrophilic polymer and reducing particle size to nanosizes, or actively by a specific ligand conjugation [77, 96, 110-112].

For treatment of PD, a variety of liposome formulations have been prepared and evaluated. A study was performed on the use of dopamine encapsulated within liposomes containing surfactants Span 20 (S20), Span 40 (S40), Spans 80 (S80), and a combination of Span 80 and Tween 80 (ST80) by Pichandy et al. [113]. The ST80 formulation was found to be more effective in the treatment of PD than the other formulations and a L-DOPA control solution (Syndopa), following i.p. injection in rats as determined by the reductions...
Table 3. Pros and cons of some different nanomedicine drug delivery systems for the diagnosis or therapy of a variety of diseases [61-75].

<table>
<thead>
<tr>
<th>Nanocarriers</th>
<th>Particle size (nm)</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liposomes, Nanotubes, Sphingosomes</td>
<td>20-3500</td>
<td>May be labeled with various radionuclides. Contrast enhancement for imaging in the urgent and timely enhancement.</td>
<td>The necessity of long time consuming radiolabeling process and careful control for reproducible efficiency.</td>
</tr>
<tr>
<td>Micelles</td>
<td>20-150 (very small)</td>
<td>Easily prepared and radiolabeled and removed by RES decoys depending on hydrophilic polymer shells.</td>
<td>The design of amphiphilic chelator should need attention.</td>
</tr>
<tr>
<td>Nanoparticles, Solid lipid nanoparticles</td>
<td>10-1000</td>
<td>These systems can be actively or passively targeted to the desired cell or tissue. They can escape from RES uptake. May be labeled with a variety of radionuclides.</td>
<td>Limited control of the site distribution and polydispersity.</td>
</tr>
<tr>
<td>Dendrimers</td>
<td>40 (very small)</td>
<td>Multivalent conjugation of radionuclides, biodistribution is enhanced depending on low polydispersity and spherical shape.</td>
<td>Toxicity may be formed depending on positive charge of dendrimer.</td>
</tr>
<tr>
<td>Gold Nanoparticles</td>
<td>1-100</td>
<td>They have unique optical properties which is affected from shape and size. They can be used for cancer diagnosis and photothermal therapy.</td>
<td>It is important to discern the toxicity of the nanoparticle core and that of its capping ligands.</td>
</tr>
<tr>
<td>Microbubbles</td>
<td>≤10000</td>
<td>Act like red blood cells within the capillaries. Safer than molecular imaging modalities such as radionuclide imaging.</td>
<td>Have low circulation time due to rapid RES uptake. Heat increase can be formed due to increase in frequency which should be carefully monitored.</td>
</tr>
<tr>
<td>Magnetic Nanoparticles</td>
<td>10-50</td>
<td>They can be functionalized and manipulated with a selective molecule and magnetic properties can be controlled with a magnetic field produced by an electromagnet or permanent magnet.</td>
<td>Metallic magnetic materials such as iron, cobalt and nickel are toxic and generally susceptible to oxidation. Uncoated magnetic nanoparticles should be chemically stabilized against degradation.</td>
</tr>
<tr>
<td>Quantum dots (typically &lt;10)</td>
<td></td>
<td>Possess long-term, multiplexed, and quantitative imaging and detection. They can be used as alternative and multimeric imaging of molecular targets.</td>
<td>Their delivery process across cells may be dangerous for the cell and may destroy it. In other cases a QD may be toxic for cells.</td>
</tr>
</tbody>
</table>

The use of Parkinson’s extrapyramidal side effects using an actophotometer and a rotarod. The encapsulation of L-DOPA derivatives in liposome formulation as potential prodrugs was performed by Di Stefano et al. for PD treatment in an attempt to decrease side effects [114]. Striatal levels of L-DOPA and dopamine demonstrated a 2.5-fold increase after i.p. administration of the liposomal formulation of the prodrug when compared with L-DOPA itself or free prodrug. In another study performed by Xurasov et al., a 10-fold smaller dose of L-DOPA provided effective treatment after the L-DOPA was encapsulated in nanaized, unilamellar liposomes when compared with free L-DOPA [115]. A significant decrease in the occurrence of side effects was also observed with these liposomal formulations. In a rat model of PD, liposomes containing dopamine were formulated and stereotactically implanted into the corpus striatum. These liposomes resulted in a sustained release of dopamine for 40 days. Higher extracellular dopamine levels and partial behavioral recovery were achieved with dopamine liposomes when compared with control liposomes [116]. Another direct brain administration was performed by Alemddar et al. by formulating liposomes of immunosuppressive drugs tacrolimus and rapamycin [117]. Higher neuroprotective effect on dopaminergic neurons was obtained with liposomes when compared with the control group. It was reported that the combination of both liposomal formulations produced a synergistic effect [117].

Jain et al. developed charged liposomes containing dopamine HCl for the therapy of PD [118]. It was observed that dopamine was effectively delivered to the brain by passive targeting and was protected from degradation by incorporation into liposomes when compared with plain dopamine HCl. L-DOPA preparations and marketed formulation of L-DOPA containing carbidopa (Syndopa®) [118]. Other studies have found that cationic liposomes can easily cross the BBB by the mechanism of absorption mediated transcytosis [119]. Another study was performed by Amicarelli et al. in 1999 focussed on stereotactic injection
of tyrosinase encapsulated within liposomes [120]. A significant increase in dopamine level was observed in rat brain by providing L-tyrosine after correcting of the lack of tyrosine hydroxylase (TH) with the tyrosinase [120].

Liposomes containing glutathione were prepared for PD treatment and found to be effective in maintenance of intracellular glutathione and neuroprotection in mesencephalic neuronal cells. It was observed that glutathione encapsulated within liposomes could be a promising alternative when compared with non-encapsulated glutathione [121]. In a novel approach, L-DOPA was encapsulated in a stealth liposome modified with cholesteryl, a 36-amino acid peptide. These liposomes were intended to bind specifically to glia, and proliferating vascular endothelial cells for PD treatment. This targeted delivery system produced successful results both in vitro and in vivo. These liposomes diminished serious behavioral disorders and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) induced loss of TH-positive dopaminergic neurons [122]. To overcome the oxidative stress that plays a significant role in PD, resveratrol derived from Polygonum cuspidatum was encapsulated in liposomes. When compared with resveratrol, liposome-encapsulated resveratrol produced a remarkable increase in the antioxidative capacity of nigral tissues and a remarkable decrease in the loss of nigral cells, abnormal rotational behavior and the level of total reactive oxygen species after two weeks of oral treatment [123].

Glia cell line-derived neurotrophic factor (GDNF) encapsulated liposomes for intranasal administration were found to produce neurotrophic effects in the intact substantia nigra and neuroprotective effects in the unilateral 6-hydroxydopamine (6-OHDA) model of PD [124]. Kintzibl et al. prepared naxolone, PEGylated, wramamine, which is a reactive oxygen species, encapsulated liposomes for the therapy of neurodegenerative diseases including PD and MS. These liposomal systems demonstrated higher brain accumulation when compared with free amipazine and inhibited experimental autoimmune encephalomyelitis (EAE) in mice [125].

A patent was granted in 2002 for a liposome formulation which was actively targeted to a BBB receptor by a modified antibody. It was observed that this antibody fragment allowed attachment of the liposome to the wall of the endothelial cells of the BBB and release of the drug just in proximity to the receptors to provide brain penetration. Transferrin receptor, insulin receptor, insulin-like growth factor (IGF)-I receptor, IGF-II receptor [27, 126] or glucose transporter [127] were used for the formulation of these liposomes.

Gene therapy with chromosomal-derived forms of genes with Trojan horse liposomes was performed by Xia et al. [128]. It was observed that sustained replacement of striatal TH enzyme activity was produced by combination gene therapy with both cDNA and genomic forms of the TH gene administered simultaneously in experimental PD. Therapeutic genes can be successfully incorporated in Trojan horse liposomes and delivered to the brain after i.v. administration [128]. Another non-vital gene therapy study was performed by the same group related to the formulation of active targeting of rat transferrin receptor specific mAb conjugated, pH-activated-GDNF plasmid DNA encapsulated Trojan horse liposomes for PD treatment [129]. Effective and sustained therapeutic effect was achieved in 6-OHDA model of PD.
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lesioned rats [129]. Similarly, DNA encapsulated, nanorized, PE2Glylated immunoliposomes were formulated for transvascular gene therapy of PD. These liposomes can be actively targeted to the brain with the attachment of mAb specific to BBB receptors, such as the insulin receptor or transferrin receptor. It was observed that striatal TH activity was completely normalized after i.v. administration of actively targeted DNA encapsulated liposomes in 6-OHDA lesioned rats [130].

Nanoparticles

Nanoparticles are solid colloidal drug delivery systems with a particle size of 1-100 nm. They are composed of natural and synthetic polymers and ceramic or inorganic elements. Drugs can be dissolved in the composition of the nanoparticle or attached, or adsorbed, or linked on the surface of the nanoparticle [131]. Nanoparticles are one of the most commonly researched drug delivery systems for many diseases including neurodegenerative diseases. Polymeric nanoparticles can be used for BBB penetration either passively or by actively targeting molecule in the desired brain site and delivering sufficient amounts of drug [17, 132, 133]. Receptor-mediated nanoparticle delivery to the brain needs chimeric peptide technology. Generally, a BBB impermeable drug can be combined with a BBB transport vector [134, 135].

Novel α-synuclein specific mAb modified poly(butylcyanoacrylate) nanoparticles were prepared by Haasad et al. [136]. The particles were found to be very effective in treating neuronal disorders in vivo in cultured neurons and neuronal cell lines with an endocytosis uptake mechanism for proteins [136]. Chitosan nanoparticles with dopamine modification onto the external surface were prepared by Trapani et al. [137]. It was observed that dopamine loaded nanoparticles were less cytotoxic, and produced more brain accumulation and increased uptake in the striatum than DA alone after i.p. administration. Nanoparticles were also studied for their gene therapy efficacy as transfection vehicles for PD treatment. This was found to be significant for limiting the risk of excessive immune response and mutagenesis. Huang et al. formulated human neurotrophic factor gene encapsulated, lactoferrin-modified nanoparticles [138]. Significant improvement was observed in locomotor activity and dopaminergic neuronal decline. Lactoferrin conjugated polyethylene glycolpolylactide-polyglycolide (PEG-PCLA) nanoparticles were formulated by Hs et al. [139]. These brain-targeted nanoparticles were found to be more effective when compared with non-conjugated nanoparticles after i.v. administration, with almost 3 times higher accumulation observed by a fluorescent probe compared 6 h in the striatum of 6-OHDA administrated rats [139]. Another study related to lactoferrin modified nanoparticles was performed by the same group in which these liposomes were loaded with human glial cell line-derived neurotrophic factor gene (hGDNF) [140]. The lactoferrin modified nanoparticles may have potential as a nonviral gene therapy of chronic brain disorders in unilateral 6-OHDA lesioned PD model [140]. Afterwards same group developed a novel non-viral gene vector: brain targeted, lactoferrin modified hGDNF encapsulated nanoparticles for PD therapy. It was observed that multiple i.v. administration demonstrated potential results for neuroprotective effects in a rotenone-induced chronic rat model of PD [138]. The same group developed a gene delivery system comprising an angiopoiet, which is a specific ligand having an affinity to the low density lipoprotein receptor, conjugated to demigranated poly-L-lysine. Neuroprotective effects as evidenced by improved locomotor activity and apparent recovery of dopaminergic neurons in rats was achieved in rotenone-induced chronic model of PD [141].

Bromocriptine encapsulated solid lipid nanoparticles were formulated and evaluated for their anti-parkinsonian effect. When compared with free bromocriptine, bromocriptine encapsulated solid lipid nanoparticles showed a prolonged release for 48 h and were found to be very effective in 6-OHDA lesioned hemiparkinsonian rats [142]. Other bromocriptine encapsulated chitosan nanoparticles were formulated by Md et al. by ion exchange method for intranasal application [143]. It was observed that selective degeneration of the dopaminergic neurons in haloperidol-treated mice was reverted by bromocriptine loaded chitosan nanoparticles and it was found effective in PD therapy [143]. Odontorhadin conjugated poly(ethylene glycol)-poly(lectic-co-glycolic acid) (PEG-PGLA) nanoparticles were developed to reduce the immunogenicity of lectine and to improve the nose to brain ratio of drug delivery. It was observed that the brain delivery of these nanoparticles was enhanced after nanoparticle conjugation. The therapeutic effect of nasal delivery of urecton peptide conjugated nanoparticles was demonstrated by increased brain delivery in hemiparkinsonian rats [144].

Tani et al. developed solid lipid nanoparticles emulsified with glyceryl monostearate or polyethylene glycol monostearate which encapsulated apomorphine for oral administration to increase its oral bioavailability [145]. It was observed that the number of rotations increased significantly and the bioavailability of apomorphine was increased 12-13 times higher with the use of these solid lipid nanoparticles in 6-OHDA lesioned PD rats [145].

BBB delivery of nanoparticles can also be investigated by intranasal administration. Rotigotine hydrochloride-loaded chitosan mucoadhesive nanoparticles were formulated for intranasal drug delivery for PD therapy. After intranasal administration of rotigotine hydrochloride loaded chitosan nanoparticles, a sustained release profile was obtained for about 18 h and enhanced brain accumulation was observed compared to that seen with rotigotine hydrochloride solution by gamma scintigraphy after 125I-radiolabeling [146]. L-DOPA encapsulated nanoparticles were formulated for intranasal administration and their effectiveness was compared with the standard form for PD treatment. L-DOPA encapsulated nanoparticles demonstrated higher effective half-life, bioavailability, brain delivery, and efficacy according to motor movements and behavior [147].

VP025 (Vasogum Inc.) was prepared as a novel phospholipid nanoparticle incorporating phosphatidylglycerol in order to obtain neuroprotective effect. Fitzgerald et al. showed that VP025 has a potential therapeutic effect on the impairment of dopaminergic motor activity and dopamine deficit induced by protease inhibition in rat model of PD [131, 148]. The effect of VP025 was also evaluated in 6-OHDA administered
PD rats by Croty et al. [149]. It was observed that VP02 inhibited dopaminergic neuron loss in 6-OHDA administered rats and it has a potential neuroprotective effect [149]. Nicotine encapsulated poly(lactic-co-glycolic)acid (PLGA) nanoparticles were prepared by Tiwari et al. [150]. It was reported that the neuroprotective effect of nicotine was enhanced by preparation of its nanoparticles depending on the results of oxidative stress and apoptosis in MPTP-treated mice.

Another study was performed by Liu et al. with the formulation of a brain targeted gene delivery system based on a non-viral gene vector: a rabies virus glycoprotein peptide with 29 amino-acid linked to dodecylpoly-L-lysines which can pass the BBB by specific receptor mediated transcytosis [151]. The effect of silencing caspase-3 genes by RNA interference in inhibiting the activation of caspase-3, which has a significant role in PD treatment was investigated. This nanoparticle was found to be promising when applied i.v. in a multiple dose regimen because it reduced the activated caspase-3 levels, causing a significant improvement in locomotor activity and rescue of dopaminergic neuronal loss in PD treatment of rats [151].

Dopamine-loaded chitosan nanoparticles were formulated for the therapy of PD [152]. Brain delivery of dopamine in different concentrations after ip. administration was accomplished by formulating chitosan nanoparticles with varying concentrations of dopamine. It was found that chitosan nanoparticles with dopamine at a concentration of 6-12 mg.kg⁻¹ produced a dose dependent increase in dopamine in the tissue [137].

Rhodamine-B conjugated multimodal iron oxide nanoparticles were formulated by Sibox et al. to label mononuclear stem cells [153]. It was observed that about 5 x 10⁴ labeled cells were efficiently imaged with MRI shortly after infusion in the brain striatum of a rat model of PD [153]. A fluorescent nanoparticle with multiple functionalization of highly fluorescent cerium oxide Ce50Ce50 quantum rods for specific targeting and controlled release of dopamine was prepared for both diagnosis and therapy. It was reported that PEUylation provided biocompatibility, a carbohydrate shell covering, and specific GLUT-1 recognition which is essential for both diagnosis and therapy of neurodegenerative diseases such as PD [154].

Gold Nanoparticles

Gold nanoparticles (Au nanoparticles) are used less frequently than other drug delivery systems. However, a significant amount of research has been performed on these systems. Gold nanoparticles (colloidal gold) have applications both in biology (e.g. bio-imaging) and technology (e.g. Phonics) due to their unique optical properties arising from the interaction of light with electrons on the nanoparticle surface [155]. These systems were investigated as drug carriers, photothermal agents, contrast agents and radioimnuosensors for both diagnosis and therapy of disease. Their use in a variety of diseases, including cancer, has been investigated [156-158].

The use of gold nanoparticles was investigated for the diagnosis of PD. As described previously, the aggregation of α-synuclein is a potential cause of PD. The use of gold nanoparticles provides quantitative colorimetric detection of neurotransmitters such as dopamine, L-DOPA, epinephrine and norepinephrine by plasmon absorptivity. Tyrosinase oxidizes tyrosine to L-DOPA and its activity can be probed by gold nanoparticles. The activity of tyrosinase is crucial for PD detection [54]. A study was performed by Yang et al. to investigate the ability to adsorb α-synuclein onto positively charged poly(allylamino hydrochloride) coated gold nanoparticles [159]. It was observed that the access of α-synuclein to enzymatic attack was altered and the conformation was changed after adsorbed onto gold nanoparticles [159].

Gold-doped TiO₂ nanotube arrays were prepared by Au et al. to design a phototocerochemical immunosensor for the detection of α-synuclein which is important in PD diagnosis [160]. They produced highly ordered TiO₂ nanotubes by using an electrochemical anodisation technique on pure Ti foil [160].

Microbubbles, Microbubbles and Nanobubbles

Microbubbles can be defined as small spherical particles with a diameter ranging from 1 μm to 1000 μm. Sometimes microbubbles are called microbubbles [161]. The neuroprotective effect of lipoid-coated glial cell derived neuroprotector factor (GDNF) microbubbles was investigated in a 6-OHDA injected rat model of PD [145]. It was observed that after intrastriatal administration of lipid coated GDNF microbubbles with a low frequency ultrasound stimulation, GDNF levels were increased. A neuroprotective effect was obtained with intrastriatal administration of lipid coated GDNF microbubbles as evidenced by reduced apomorphine induced rotations and an increase in striatal dopamine and nigral TH levels in PD rats [162]. Another similar study was performed by Garbayo et al. related to the formulation of N-glycolylated recombinant GDNF encapsulated microbubbles for PD treatment [163]. This formulation was found to have suitable release kinetics over up to 5 weeks in vivo and a neurorestorative effect in the rotational behaviour test and increased TH+ fiber density at the striatal level [163]. Another study was performed by Herranz et al. evaluating the neuroregenerative effect of bubble endothelial growth factor (VEGF), GDNF encapsulated polymeric microbubbles, and their combination in rats representing a severe stage of PD [164]. Higher levels of neuroregeneration/neuroprotection in the substantia nigra were obtained with the treatment of GDNF microbubbles and with both VEGF and GDNF microbubbles when compared with control group, evidenced by the rotation behavior test and surviving TH+ cells [164]. The effect of intrastriatal administration of GDNF encapsulated microbubbles was also evaluated by Gouhier et al. by using ramaneter, TH immunohistochemistry, and PEU50-labeled DAT density [165]. GDNF encapsulated microbubbles were found to be neuroprotective, and PEU50 was also found to be an effective SPECT and PET imaging radiotracer for both the diagnosis and follow up of PD [165].

Rotigotine is a non-ergoline [3,3/2/D] dopamine agonist. Rotigotine loaded poly(lactic-co-glycolide) containing microspheres were prepared and their subchronic toxicity was
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were formulated for the therapy of PD. Both apomorphine HCl and base encapsulated perfluorocarbon nanobubbles demonstrated delayed and sustained release profiles. While a 2-4 fold enhancement was observed in apomorphine HCl release, a decrease was observed in apomorphine base release with ultrasound application when compared with the non-ultrasound group [179].

Dendrimers

Dendrimers are repetitively branched molecules [180, 181]. Dendrimers are composed of components having unique properties: they are typically monodisperse and symmetric around the core, and generally have a three dimensional structure. Their size ranges from 1 - 100 nm. Dendron is the small single structure of a dendrimer, comprising only a single, chemically addressable group called a focal point. Dendrimers are simply composed of the addition of layers of branching groups in which each new layer is defined as the generation [182]. Due to the unique characteristics, dendrimers can act as a particular system while retaining its polymeric properties [183]. Bioactive agents such as antioxidants, alkylating, analgesics, antibiotics, or anti-inflammatory drugs can be released from dendrimers through the release of the core by recognition of the core by the target [184].

Dendrimers have a variety of applications in drug delivery and imaging. A study was performed by Keskes et al. related to the formulation of G protein coupled receptor ligand-dendrimer (GLIDE) conjugates from an adenosine receptor antagonist for the diagnosis or therapy of PD and some other diseases [185]. It was reported that effective multivalent dendrimeric derivatives of adenosine receptor antagonists were synthesized and very promising results were obtained [185].

Some disorders are believed to be related to fibrillar aggregation of proteins. These proteins are Aβ peptides in Alzheimer’s Disease, α-synuclein in PD, amylin in type II diabetes, α-microglobulin in dialysis-related amyloidosis, and prion proteins in Creutzfeldt-Jakob disease (CJD). A study was published for Razek et al. to determine the effect of polyamidodiamine (PAMAM) dendrimers (generations G3, G4 and G5) on the fibrillation of α-synuclein, which is related with neurodegenerative disease formation [184]. It was observed that PAMAM significantly reduced the breakdown of pre-existing α-synuclein fibrils and inhibits formations of β-sheets [186]. Another study related to the evaluation of the effect of viologen-phosphorus dendrimers in the fibrillation process of α-synuclein was performed by Milowska et al. [187]. Vsg-I dendrimer comprising phospholipid groups on the surface were found very effective. It was observed that viologen-phosphorus dendrimers can inhibit a-synuclein formation and this may be potentially used as regulating agent in neurodegenerative diseases such as PD [187].

CONCLUSION

The use of drug delivery systems such as liposomes, ionones, nanoparticles, microbubbles, micelles, nanobubbles and dendrimers has the potential to have significant effects for drug delivery in PD. A large number of papers have highlighted the potential importance and...
effectiveness of colloidal drug delivery systems in both imaging and therapy of PD. Active targeting by ligand modification, enhanced BBB penetration, increased accumulation by passive targeting, controlled delivery, and sustained release can be achieved with the use of these drug delivery systems in PD therapy or diagnosis. A significant decrease in the frequency of side effects was also observed with the use of these drug delivery systems, such as parkinsonian's extrapyramidal side effects. Nanotechnology and nanosized drug delivery systems are being investigated intensively and could have potential effect in the field of neuroprotection [18]. With improvements in molecular imaging, very early diagnosis can be achieved before the initiation of motor symptoms with the help of special ligands specific to molecular targets such as receptor and transmitters. The progress in medicine, pharmacology, biology, and chemistry will bring the direction to the future development of a variety of drug delivery systems in BBB penetration in brain delivery for either imaging or the therapy of various central nervous system disorders, including PD [59, 189].

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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Declared none.

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2.4.1. Liposomes and Niosomes

Liposomes and niosomes are one of the mostly studied formulations among drug delivery systems depending on being in phospholipid structure, biocompatible and biodisintegrable and ability to target drugs. Niosomes are very similar systems to liposomes except comprising nonionic surfactants instead of phospholipids which may attain them a superiority for passive targeting through very tight endothelial junctions such as BBB.

**Liposomes** were first studied in 1960s by Bangham et al (91). They are synthetic analogous of natural membranes. These are generally composed of one or more consentric vesicles containing lipid bilayers that are seperated by aqueous buffer compartments. Their size distribution changes between 80nm-100 μm and they contain phospholipids, cholesterol and sometimes charge inducer substances in their structure. The encapsulation behaviour of drug molecules depends on the physicochemical behaviour of drug molecules and lipid composition. While hydrophilic drug molecules are generally encapsulated in the aqueous compartment, hydrophobic drugs can be carried within lipid vesicles (92-94). The schematic representation of liposome and its formulation is given in Figure 2.8.

![Figure 2.8. Structure of a liposome and its formation (95).](image)

Liposomes are one of the most commonly investigated drug delivery systems used for delivering both drugs and diagnostic agents to the targeted area.
**Niosomes** are nonionic surfactant vesicles in which hydrophobic and hydrophilic active pharmaceutical ingredients can be encapsulated (14). The composition of niosomes are very similar to that of liposomes however the substances used for the preparation of niosomes, non-ionic surfactants, give them a more stable structure and more ability to penetrate through BBB (Figure 2.9.).

![Figure 2.9. The structure of niosomes (96).](image)

Therefore, passive and active targeted liposomal and niosomal delivery systems can be successfully used for the purpose of BBB penetration and either diagnosis or therapy of different central nervous system disorders.
Liposomes and their applications in molecular imaging

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Abstract

Molecular imaging is a relatively new discipline with a crucial role in diagnosis and treatment tracing of diseases through characterization and quantification of biological processes at cellular and sub-cellular levels of living organisms. These molecular targeted systems can be conjugated with contrast agents or drug ligands to obtain specific molecular probes for the purpose of diagnosis of diseases more accurately by different imaging modalities. Nowadays, an interesting new approach to molecular imaging is the use of stealth nanoparticulate drug delivery systems such as liposomes having convenient properties such as biodegradability, biocompatibility, and low toxicity and they can specifically be targeted to desired disease tissues by combining with specific targeting ligands and probes. The targeted liposomes as molecular probes in molecular imaging have been evaluated in this review. Therefore, the essential point is detection of molecular target of the disease which is different from normal conditions such as increase or decrease of a receptor, transporter, hormone, enzyme etc. Or formation of a novel target. Transport of the diagnostic probe specifically to targeted cellular, sub-cellular or even to molecular entities can be performed by molecular imaging probes. This may lead to produce personalized medicine for imaging and/or therapy of diseases at earlier stages.

Keywords: Molecular imaging, molecular targeting, drug delivery systems, targeted liposomes, targeted delivery systems, liposomes for imaging

Introduction

Considerable progress has been achieved in the field of molecular imaging due to recent advances in imaging modalities, molecular biology and chemistry (Ishii and Wang, 2010). Molecular imaging is defined as the measurement, characterization and visualization of biological processes in cellular and molecular level in human and other living systems (Blasberg, 2003; Cai and Chen, 2008). Imaging of multiparametric processes performing in the same time interval, gene expressions or protein-protein interactions, monitoring of cell-targeting, drug and gene therapy optimization, determination of the progress of molecular pathologic diseases, imaging of drug effect at molecular and cellular level, supplying rapid, reproducible and three dimensional computerized images with quantitative results of therapeutic effects of gene products on animals or patients, therapy tracing are some significant application fields of molecular imaging (Blasberg, 2003; Massoud and Gambhir, 2003; Saha, 2004).

Many branches like molecular biology, cellular biology and imaging technology are related with molecular imaging (Massoud and Gambhir, 2003). Table 1 represents significant properties of some imaging modalities and molecular probes (Massoud and Gambhir, 2003). The basic principle of molecular imaging depends on obtaining significantly high signal intensity by the use of minimal amount of molecular probe. Studies on the development of special reagents, ligands, protocols and devices for molecular imaging have been carried out over the past two decades. Better biocompatible probes/ligands for selecting appropriate cellular and sub-cellular targets for imaging were developed. The delivery of these probes by overcoming biological barriers and image amplification for detection of trace amounts of target concentrations such as plasmolympho-molecular (pL-M) and development of imaging systems to a level of higher spatial/temporal
resolution and sensitivity are crucial issues for molecular imaging (Massoud and Gambhir, 2003).

As a common approach, molecular imaging can be classified as direct and indirect method. In direct method, molecular probes are needed for directing to specific molecular markers/targets like transporters or enzymes. Indirect method is generally related with imaging of specific molecules and cellular processes. In this method, pre-targeting molecules are needed for activating the formation of a specific molecular process. Specific molecular probes, can be modified with a contrast/radiocontrast agent, and are generally used as contrast source in molecular imaging. Small molecules, aptamers, peptides, antibodies, nanoparticles, nanovessels, and quantum dots are some instances of molecular probes that have been used in research for imaging of diseases (Saha, 2004; Iohit and Wang, 2010).

Nanoparticles for drug delivery systems are promising for molecular imaging. Although their development is expensive and time consuming, different type of molecular imaging probes may be delivered with increased safety and efficacy to the target organ, tissue or even to cells by the use of targeted nanoparticles. Targeted drug delivery systems can be used in molecular imaging by the conjugation of a specific imaging probe. Molecular imaging probes are mainly used for molecular imaging that supply significant signal and they can be called with different names like molecular beacon, activatable probes, reporter probes, smart probes, tracers or contrast agents (Massoud and Gambhir, 2003; Saha, 2004; Wesseler and Mahmoud, 2001; Kamaly et al., 2008).

Probes for molecular imaging

Targeted molecular imaging probes are typically composed of an affinity component for interacting with its target and a signaling component supplying significant signal intensity for imaging. For instance, radiolabeled was used as signaling component and oligonucleotides as affinity component in radiolabeled oligonucleotide antisense probes for imaging messenger RNA (mRNA) by blocking protein synthesis (Massoud and Gambhir, 2003; Saha, 2004; Wesseler and Mahmoud, 2001; Kamaly et al., 2008). Molecular imaging can be achieved by targeting DNA, mRNA, peptides, proteins or receptors located on the surface of cell membranes or enzymes in cytoplasms (Saha, 2004).

Table 1: Properties of some imaging modalities and molecular probes (Massoud and Gambhir, 2003).

<table>
<thead>
<tr>
<th>Imaging modality</th>
<th>Reporter molecule</th>
<th>Spatial resolution</th>
<th>Sensitivity (mole L⁻¹)</th>
<th>Molecular probe type</th>
<th>Molecular probe amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRI</td>
<td>Paramagnetic ions (Gd, Mn)</td>
<td>0.2-1.0 mm</td>
<td>10⁻¹⁴ - 10⁻¹³</td>
<td>Activatable (direct/indirect)</td>
<td>1-2 mg</td>
</tr>
<tr>
<td>PET</td>
<td>Radionuclides (18F, 11C)</td>
<td>1-2 mm</td>
<td>10⁻⁶ - 10⁻¹</td>
<td>Radiolabeled (direct/indirect)</td>
<td>not defined</td>
</tr>
<tr>
<td>SPECT</td>
<td>Radionuclides (111In, 76Br)</td>
<td>1-2 mm</td>
<td>10⁻⁶ - 10⁻¹</td>
<td>Radiolabeled (direct/indirect)</td>
<td>not defined</td>
</tr>
<tr>
<td>Optical fluorescence imaging</td>
<td>Visible light or near infrared</td>
<td>2-3 mm</td>
<td>Not clearly defined</td>
<td>Activatable (direct/indirect)</td>
<td>1-2 µg</td>
</tr>
</tbody>
</table>

An ideal gene/probe should have some specific properties such as (Massoud and Gambhir, 2003):

- It should have high intracellular stability and should not be metabolized before reaching to its target.
- If the reporter gene is not expressed, then reporter probe should not be accumulated.
- The size of reporter gene should be small enough to be a part of delivery vehicle.
- It should not cause immunogenic reactions within the body.
- The reporter probe should not interfere with detection of specific signal and should be rapidly cleared from the circulation after the completion of its process.
- The reporter probe and its metabolites should not be cytotoxic.
- Natural biological barriers must not prevent the interaction of reporter probe with its target.
- The image signal intensity should be directly proportional with the amount of reporter gene/probe.

Although, these properties are ideal for a single reporter gene/probe mostly it is not possible for a reporter gene/probe to have all these criteria (Massoud and Gambhir, 2003).

Reporter gene imaging can be performed by protein-protein interactions and it may supply gene therapy monitoring following gene labeling. The mechanism of reporter gene imaging depends on disease formation results, from alterations in normal regulation of gene expressions and diseases can be monitored by this way (Massoud and Gambhir, 2003; Kamaly et al., 2008). Radiolabeled substrates can be used for imaging receptors by interacting with proteins produced from specific genes. For brain imaging, dopamine-2-receptors have significant role in diagnosis of Parkinson's disease, are targets for brain imaging with ¹¹C-methylprenicoline (Saha 2004).

Antibodies have been developed against many antigens of specific genes and they have been radiolabeled for imaging of various diseases. In recent times, although the use of proteins and peptides as specific targeting ligands have become popular, high molecular weight and short half-life depending on the enzymatic degradation or immune response have become a limiting point for transportation through the biological membranes. These
drawbacks can significantly be reduced by designing proper protein/peptide conjugated nanocarrier systems (Solorio et al., 2010).

Different types of imaging have been used for different imaging modalities. Some atoms such as fluorochrome and gadolinium can be used in optical imaging and magnetic resonance imaging (MRI), respectively. Some other imaging modalities such as positron emission tomography (PET) and single photon emission tomography (SPECT) are performed by the use of radionuclide probes producing signal by radioisotopic decay. The signal is formed by interaction of active probes with its targets. Although some nuclear and fluorochrome tracers have been utilized in physiological process imaging like alterations in blood volume, blood flow and porosification, they cannot be targeted to some specific biological processes in cellular or sub-cellular levels and they also can not identify the diseases in early stages. Novel molecular imaging probes are designed or improved by significant relationship between imaging investigations and drug industry. Molecular imaging probes are classified into nonspecific or specific. Although nonspecific probes do not have a definite target, they can produce signals depending on a set of biological processes. Specific radionuclide ligands having significant affinity to the targeted probes and proteins of a specific gene are used for imaging of the final product in the gene expression. These interactions are generally receptor-radionuclide binding or enzyme-radionuclide substrate reaction (Mansoori and Cambhir, 2003; Weisssleder and Mahmood, 2001; Kamaly et al., 2008).

Improvements in molecular imaging and validation on gene or probe systems in living subjects were investigated by and Adams et al. (2002). Important molecular signs in nucleic acid hybridization were observed in living subjects that are especially used in polymerase chain reactions (PCR). In a research in 2002 (Perez et al., 2002), magnetic nanosensors having an affinity to DNA or RNA sequences were investigated and these nanosensors were found to be polymerized by peroxidases and tyrosinases. Another research was performed by Li et al. (2002) related with the production of molecular aptamer probes with smart probes which are DNA or RNA oligonucleotides isolated for binding to different biomolecules specifically. Nowadays utilization of aptamers for in vitro specific protein-binding experiment becomes widespread (Li et al., 2002). Couteg et al. (1999) used molecular imaging reporter genes or probes which are targeted and activated with an aptic reporter for an enzyme (such as luciferase enzyme) supplying the identification, quantification and quantitative specification of the release of light photons.

A specific molecular imaging probe should reach to target tissue at sufficient concentration and remain there as long as possible to image the tissue. Drug concentration in plasma, absorption, distribution, metabolism and excretion (ADME), plasma half-life, distribution volume, protein-binding and toxicity are some of essential issues to consider. The penetration of large probes through the barriers are one of the most challenging processes. Several strategies have been developed for overcoming this problem. Research has been made by Tosbjergen et al. (1999) by using peptide-mediated translocation signals making active shuttling of imaging probes into cells through lipid bilayer membrane. Hydrophilic polymer such as polystyrene glycol (PEG) modification of biomolecules has been performed for decreasing immunogenicity and opsonization by RES organs and enhancing targeting efficiency. Although targeted probes are specific, sometimes target-to-background ratio may be decreased depending on the receptor density and availability, binding affinity, rapid efflux from cells, altered kinetics and non-specific cellular uptake or adhesion of probes. More sensitive and effective imaging can be obtained with signal amplification in molecular imaging modalities (Blasberg, 2003; Mansoori and Gambhir, 2003; Saha, 2004).

**Targeted nanosystems as drug carriers**

Drug delivery systems enable the delivery of drugs or radioactive agents to the target tissue/organ within the body and improve their efficacy and safety by controlling the release rate and special design for diagnosis and/or therapy of the diseases. Mainly they should have some essential properties such as safety, efficacy and quality. Drug properties have been improved by developing targeting localization of drug delivery systems and by this way personalized medicine may become true by altering the pharmacokinetics and pharmacodynamics of the drug (Kishisaga, 2000; Jani, 2000).

Nanospheres, nanospheres, nanoparticles, polymeric systems, dendrimers, colloidal gold, gold nanoshells, quantum dots, superparamagnetic particles, micelles, liposomes, niosomes and sphingosomes are some of drug delivery systems for diagnosis and/or therapy of several diseases (Mitra et al., 2006; Chan et al., 2002; Arya et al., 2006; Tong et al., 2010). A schematic expression of the advantages and disadvantages of a variety of different nanocarriers radiolabeled for diagnosis or therapy of diseases are given in Table 2 (Mitra et al., 2006).

Among different delivery systems, liposomes draw more attention in both diagnostic imaging (Gosnold et al., 2011; Selim, 1983) and therapy (Vennir and Rhodes, 1995) due to the suitable properties such as encapsulation of drugs having different physicochemical properties and modification of lipid bilayer to achieve desired aim. Liposomes can either be passive targeted by surface coating with a hydrophilic polymer or active targeted by specific ligand conjugation or both (Kishisaga, 2000; Zhuang et al., 2013; Chai et al., 2001).

**Liposomes: attracting delivery system for targeting, therapy and diagnostic imaging**

The term of liposome was first defined in the year 1935 and first it was used as a model of the biological membranes. One of the most important advantages of
liposomes is the utilization of phospholipids which are the natural components of cell membrane. Therefore, they can be eliminated from the body by simple degradation pathways without causing any toxic effect. These properties attain liposomes a superior value than some other drug delivery systems such as polymeric nanoparticles and carbon nanotubes. The use of liposomes as drug carrier systems has been an important and a popular issue for research in improving better targeted formulations in the last 20 years (Bangham et al., 1965; Beuggeri et al., 1993).

Liposomes are simply defined as the concentric vesicles composed of phospholipids containing an aqueous volume inside and within the lipid bilayers (Nose, 1986; Lipowsky and Sackmann, 1985). Among a variety of drug delivery systems, liposomes have drawn a special attention because of their easily controlled properties and suitable pharmacological characteristics such as excellent biocompatibility and biodegradability. Liposomes provide enhanced bio-availability of drugs with reduced adverse side effects and toxicity. Phospholipid composition, surface charge, particle size and zeta potential comprise physical properties of liposomes affecting their vascular circulation time after IV administration and these factors are important for improving better liposomal formulations, which are not rapidly removed from vascular circulation by RES and by this way better accumulation in the target tissue can be achieved. These desired improved properties cause better diagnosis and therapy eventually (Goltiz et al., 1994).

Although, the use of new liposomal formulations are still in the research level, the previous ones have entered to the market depending on the necessity to produce more personalized, biodegradable, safe and nontoxic agents. A number of different researches have been performed about liposomes in therapy of several diseases such as cancer, infection (Panheo et al., 2011) and inflammation (Hegeman et al., 2011), skin (Pierre and Dos Santos Minicola Costa, 2011) and eye diseases (Bochoi and Fatil, 2012), antimarial (Baeschi et al., 2011), osteosarcoma (Wu and Wen, 2012) and for different purposes such as enzyme, gene therapy (Ach-Uskud et al., 2011) and hormone replacement therapy and as metal chelation, vaccines (Gordon et al., 2011).

Commercially available liposomes

While most of the liposomal formulations used for therapy are on the market, only a very small number of diagnostic liposomes are currently commercially available. Doxorubicin containing Doxil®/Caelyx®, was the first stealth liposome for the treatment of Kaposi's sarcoma and recurrent ovarian cancer, and was approved in USA and Europe. Lipobex® is another commercial preparation comprising PECLipidated liposomal doxorubicin formulation. Donorubin including Thermolix® is a triggered release formulation in phase III of clinical trial and releases its content upon heat produced by using radio frequency ablation. Antifungal amphotericin is encapsulated Ambisome® is another instance of commercially used liposomal formulation. Some other instances that are used for anticancer purposes are dexamethasone entrapped Dassomil® for the treatment of Kaposi's sarcoma, doxorubicin encapsulated Mycex® for the treatment of recurrent breast cancer, paclitaxel containing LEP-ET® is completing phase I clinical trials, for the treatment of ovarian, breast and lung cancer, vincristine comprising Marquibo® for metastatic melanoma used melanoma and cisplatin including Lipoplatin® for various tumors and SPH-0779 for head and neck cancer. Vepirepar comprising Vistaxyn® is used for the treatment of age-related macular degeneration, pathologic myopia and ocular histoplasmosis. Cytarabine containing DepoCyt® utilizes Depo-Foam platform and was used for the treatment of neoplastic meningitis and lymphomatous meningitis by intrathecal administration. Mophene sulfate containing DepoDura® can be administered epidurally for treatment

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of postoperative pain following major surgery. Amikacin comprising Anklace® is used for treatment of lung infections due to susceptible pathogens. Anklace® is used as a nebulized form for inhalation and it is in phase III of clinical trial (Hibayumi and Turchin, 2010; Ceyal et al., 2005; Mirafzali). Liposomes are also used as adjuvants in vaccines such as Epxaxide for Hepatitis A, Hexapelle for Hepatitis B and Infexus V® for influenza. It is crucially important that the vesicular formulations are more commonly used for the delivery of conventional drugs and for the delivery of novel biotechnology therapeutics such as cloned genes, recombinant proteins and antisense oligonucleotides (Veerm and Rhodes, 1995; Lipowsky and Sackmann, 1995; Mirafzali). Vescan® is an example of commercially available diagnostic liposomes. Small neutral cholesterol rich liposomes labeled with 111In, having a brandname Vescan®, was developed for imaging of tumors in melanoma, sarcoma and lymphoma patients. Clinical phase I/II trials were performed and with this formulation cucinomas, melanomas, sarcomas and lymphomas can be imaged with high sensitivity (>60%) and specificity (>96%) (Turner et al., 1988; Kubo et al., 1993).

Passive and active targeting of liposomes

Surface modification different hydrophilic polymers, ligands or proteins has been applied as a significant procedure in liposome formulations in order to target them to the cell of interest, tissue or organ by passive or active targeting. The principle of targeting drugs to a specific site of the disease while protecting normal healthy tissues was firstly described by Paul D. Boyer as the "magic bullet". Liposomes are one of the most commonly used drug delivery systems for adapting this targeting principle in order to protect both normal healthy tissues and drug properties until it reaches to its target (Bogert et al., 1985; Weiner, 1989).

The nuclear imaging of liposomes for medical research can generally be performed by conjugation of radionuclides such as 99mTc, 67Ga and 111In. Proper anchoring molecules are essentially needed for conjugation of these radionuclides with liposomes which are soluble in the internal aqueous core such as decane, nitrotriacetic acid (NTA) in case of encapsulation or has a lysophospholipid for anchoring on the lipid membrane such as phosphatidylethanolamine (PE) or steroylamine (SA) conjugated to diethylene triamine penta acetie acid (DTPA) (Hameed and Do, 2008). For increasing the signal intensity in the desired tissue and to obtain better images eventually (Torchilin, 2000; Tubatsky and Torchilin, 1994; Torchilin, 2007).

Liposomal vesicles can be produced at different size ranges. Although particle size has not a direct effect on targeting, nanosized production is crucial for increasing interaction probability with desired tissue or organ depending on longer duration time in circulation. Nanosized drug delivery systems are popularly investigated nowadays for their advantages such as increased absorption, decreased excretion, decreased uptake and removal from circulation by RES, longer half-life within blood circulation, lower toxicity and ability to cross various biological barriers that are more in tumor tissue or inflammation by enhanced permeability and retention (EPR) effect (Mironida and Tsatsatilis, 2010).

Importance of radiolabeling efficiency and stability of liposomes

One of the most important issues in obtaining better images and by this way more accurate diagnosis is the stability of the radionuclide after radiolabeling to the delivery system. A number of different researches were performed for investigating better and stable labeling method of drug carrier systems with a variety of radionuclides such as 67Ga (Phillips et al., 2009; See et al., 2008), 111In (Phillips et al., 2009), 125I (Phillips et al., 2009; Urakami et al., 2007; Mark et al., 2007; Ferrara et al., 2009) and 123I (Hwang et al., 2010; Urakami et al., 2009). The choice of radiotracers agents depends on their physicalchemical properties that change the imaging modality. Iodine containing contrast agents can be used for CT imaging, paramagnetic contrast agents like gadolinium, superparamagnetic iron oxide for MRI, microparticles comprising perfluorocarbon gases for ultrasoundography (US), medically used gas emitting radioactive like 85Kr, 111In, 133Xe for gamma scintigraphy, SPECT, PET/CT and some positron emitting radionuclides like 18F, 15O, 124I can be utilized for PET/PET/CT imaging. The common use of 67Ga as radionuclide depends on its less cost, better imaging properties and easier manipulation than 111In and 125I. Labeling with 111In is needed an incubation process at high temperatures which is not necessary for radiolabeling with 67Ga. Several methods are available for radiolabeling of liposomes having different mechanisms. Encapsulation of the radiolabel is one of the first used method in the aqueous core of the liposome during the manufacturing process. In this method, radioactivity is encapsulated into the aqueous core which is not found as an efficient method depending on short half-life of radionuclides and on the contrary long time for the preparation of liposomes. Additionally, although the encapsulation method is efficient enough, the amount of contrast agent that is encapsulated inside the aqueous core is limited. A relatively simple second method is the reduction of the radiolabel with liposome that causes conjugation of radiolabeled with the lipid bilayer. This method can also be defined as the run through method reduction method. For 67Ga in radiolabeling of liposomes, reduction should commonly be maintained by SnCl2 (New, 1990). The disadvantage of this method is less in vivo stability of.
radiolabeled liposomes. Therefore, some chelator agents such as DTPA may be used for increasing in vivo stability of these systems. Erdogan, 2009; Torchilin, 2006. The third method can be defined as to trap the radiolabel in the aqueous core of the liposome after the preparation. In this method, radiolabel passes bilayer with a variety of different mechanisms. As being another method, radiolabel may be chelated with a lipid-chelator that is incorporated in the lipid bilayer of the preformed liposomes. In vivo stability of radiolabel is essential for liposomes if non-free radiolabel can accumulate in non-target tissues such as the thyroid gland, stomach, kidney and urine that may cause poor image quality and radioactivity dose exposure to normal tissues depending on uptake of the free radionucide by untargeted tissue (Evetts, 1990; Erdogan, 2009).

Radiolabeling of liposomes with \(^{99m}\text{Tc}\) can be effectively performed by a lymphoid chelator hexamethylene-pyridylacetamide (HMPAO) which can be obtained as a commercial kit. HMPAO has been used for encapsulating \(^{99m}\text{Tc}\) into the aqueous core of reduced glutathione containing liposomes. This easy and stable \(^{99m}\text{Tc}\)-radio-labeling method was performed by Erdogan et al. (2006). Injection imaging was obtained considerably with \(^{99m}\text{Tc}\)-labeled HMPAO liposomes having high radiolabeling efficiency (80%). Reduced glutathione containing liposomes were prepared and radiolabeled with \(^{99m}\text{Tc}\) using HMPAO by Gouil et al. (1994) for tumor imaging. Better tumor imaging was obtained with neutral liposomes than liposomes having negative surface charge. A different usage of HMPAO is infection and inflammation scintigraphy in clinics by \(^{99m}\text{Tc}\)-Di-2-ethylaminoethane (HMPAO)-labeled leukocytes.

Another simple chelation procedure was developed by Sven et al., 2000 at low temperature and mild condition for post labeling of preformed liposomes with \(^{52}\text{Ca}\). High radiolabeling efficiency (65%) was achieved by using a specific chelator 6-[1-14C]-benzyl-1,4,2,1-tetracyclic-1,4-benzene-N,N,N'-tetraacetic acid (HAT). The labeled liposomal formulation and free \(^{52}\text{Ca}\) were injected separately to mice for comparison. Liposomal formulation was observed to accumulate seven times more than free \(^{52}\text{Ca}\) in the target tissue.

Researches concerning liposomes for different applications

A number of different researches have been performed about liposomes for many years in both diagnosis and therapy of several diseases. Some instances of recently investigated liposomes for imaging and/or therapy of various diseases are given in Table 3 (Erdogan, 2000; Erdogan et al., 2002; Erdogan et al., 2004; Erdogan et al., 2005; Elhayouni and Torchilin, 2007; Elhayouni and Torchilin, 2007; Siliander et al., 2008; Siliander et al., 2010; Dugar et al., 2003; Demos et al., 1997; Demos et al., 2001; Danilo et al., 2009; Hellweyler et al., 1957; Turker et al., 2005; Farooq et al., 1996; Al Muhammed et al., 1996a; Oku et al., 1996; Carni et al., 2008; Ogihara-Umeda et al., 1995; Ogihara et al., 1995; Leung et al., 2004; Zheng et al., 2006; Zheng et al., 2010; Zavatska et al., 2008; Smith et al., 2010; Kawaguchi et al., 2010; Chow et al., 2009; Grange et al., 2010; Barnett et al., 2010).

A research was performed by Harrington et al. (2001) about the preparation, biodistribution and pharmacokinetics of \(^{99m}\text{Tc}\)-HMPAO-labeled PE-Glylated liposomes in advanced cancer patients. Sufficient tumor images were obtained in patients having breast, head and neck, bronchus, glesia and cervix cancer by gamma camera. The maximum uptake levels were observed in patients having solid head and neck cancer. Therapeutic efficacy of \(^{99m}\text{Tc}\)-HMPAO-labeled liposomal vinorelbine in murine colon carcinoma was investigated by Chow et al. (2009) with molecular imaging. Positron emission imaging and \(^{18}\text{F}\)-FDG small animal PET were performed for monitoring the therapeutic response. 5 mole\% FEG containing \(^{99m}\text{Tc}\)-vinorelbine liposomes were found to help in increasing the therapeutic efficacy and reducing toxicity of chemotherapy. Another study was made by Chang et al. (2001) about developing \(^{15}\text{N}\)-N,N,N,N-dieethylthiylamidamine (BEMA)-labeled FEGylated liposomes. Biodistribution and pharmacokinetic parameters of radiolabeled liposomal formulation and free \(^{15}\text{N}\)-BEMA were investigated. The highest tumor uptake was observed after i.v. administration of radiolabeled liposomal formulation at 24 h by microPET/CT which is almost seven times higher than the muscle uptake. The area under the tissue concentration-time curve of radiolabeled liposomes was found almost five times higher than free \(^{15}\text{N}\)-BEMA depending on pharmacokinetic studies.

CT imaging has been performed by iodine containing contrast agents such as topiemic, ioversol and their conjugation to liposomes generally performed by encapsulation in the aqueous core. Iodine containing liposomes with ethanol evaporation method was prepared by Sachse et al. (1999) and it was applied to rats and rabbits for monitoring biodistribution by CT. Iopromide containing multimamellar vesicular (MLV) type liposomes were administered to rats and rabbits. The contrast enhancement in the liver was observed to begin immediately after i.v. injection and continued for 90 min. The maximum CT contrast was found in kidney parenchyma and urethra after 3-5 min. Increasing availability of short imaging time course of iopromide by encapsulating in liposomal formulation was observed by Erdogan et al. (1999). The time interval of iopromide was found to reach to 24 h with its liposomal formulation enabling more comfortable imaging procedure for both the physicians and especially child patients with faster metabolic ability.

The use of liposomes in molecular imaging

It is possible to image the alterations of different disease symptoms at molecular level with the help of different imaging modalities by development of surface modification of drug delivery systems especially liposomes, and
Table 3. Some instances of recently investigated liposomes for imaging and/or therapy of various diseases (Erdogan et al., 2009; Erdogan et al., 2005; Erdogan et al., 2006; Erdogan et al., 2008; Elbayoumi et al., 2006; Elbayoumi et al., 2007; Sillindir et al., 2009; Sillindir et al., 2010; Deger et al., 2010; Demos et al., 1997; Demos et al., 1998; Damke et al., 2005; Hawryluk et al., 1997; Turner et al., 2005; Farshid et al., 1996; Al-Mohammed et al., 1996; Okita et al., 1996; Caruso et al., 2006; Ogihara-Kimura et al., 1996; Ogihara et al., 1996; Leung, 2006; Zheng et al., 2006; Zhang et al., 2016; Zamuro et al., 2008; Smith et al., 2016; Kawaguchi et al., 2010; Chow et al., 2006; Grunere et al., 2010; Humana et al., 2010).

<table>
<thead>
<tr>
<th>Type</th>
<th>Size (nm)</th>
<th>Active Substance</th>
<th>Area</th>
<th>Targeting (**)</th>
<th>Molecular Composition</th>
<th>Labelling</th>
<th>Imaging Modality</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>MIV</td>
<td>150</td>
<td>Empty</td>
<td>Tumor imaging</td>
<td>AT with mAb 2G5</td>
<td>PC-Chol (21%) + PEG2000–PE (5 mol %) + 111In-DOTA-PLEX(NGPE) (1.75 mol %)</td>
<td>111In</td>
<td>SPECT/CT</td>
<td>Erdogan et al. (2008); Erdogan et al. (2000)</td>
</tr>
<tr>
<td>MIV</td>
<td>200-150</td>
<td>Empty</td>
<td>Tumor imaging</td>
<td>AT with mAb 2G5</td>
<td>PC-Chol (21%) + PEG2000–PE (5 mol %) + 111In-DOTA-PLEX(NGPE) (1.75 mol %)</td>
<td>111In</td>
<td>SPECT/CT</td>
<td>Erdogan et al. (2008); Erdogan et al. (2000)</td>
</tr>
<tr>
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<td>300-100</td>
<td>Intrapleural</td>
<td>Tumor imaging</td>
<td>AT with mAb 2G5</td>
<td>DPPC-PEG4000–PE-Chol:DTA-PEG1000–PE-Chol:DOPE (60:35:5)</td>
<td>125I</td>
<td>SPECT/CT</td>
<td>Sillindir et al. (2009); Sillindir et al. (2010)</td>
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<tr>
<td>MIV</td>
<td>800</td>
<td>Air</td>
<td>Imaging of atherosclerosis plaques</td>
<td>AT with anti-ICAM-1 antibody or anti-endothelin</td>
<td>PC-MIP-PEG2000–PE-Chol:DOPE (60:35:5)</td>
<td>125I</td>
<td>SPECT/CT</td>
<td>Demos et al. (1997)</td>
</tr>
<tr>
<td>MIV</td>
<td>130</td>
<td>Inoperable</td>
<td>Imaging of atherosclerotic plaques</td>
<td>AT with anti-ICAM-1 antibody</td>
<td>PC-Chol:PEG2000–PE-Chol:DOPE (7:10:3)</td>
<td>125I</td>
<td>CT</td>
<td>Dardas et al. (2009)</td>
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<tr>
<td>MIV</td>
<td>163</td>
<td>Urokinase</td>
<td>Deep vein thrombolysis (DVT) imaging</td>
<td>AT with anti-PT</td>
<td>DPPC-DC-Chol:DOPE-Chol (10:14)</td>
<td>111In</td>
<td>SPECT/CT</td>
<td>Erdogan et al. (2003)</td>
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<tr>
<td>MIV</td>
<td>365</td>
<td>Streptokinase</td>
<td>DVT imaging</td>
<td>PT</td>
<td>DPPC-DC-Chol:DOPE-Chol (10:14)</td>
<td>111In</td>
<td>SPECT/CT</td>
<td>Erdogan et al. (2003)</td>
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<tr>
<td>MIV</td>
<td>275</td>
<td>Dichloroacetic</td>
<td>Therapy of inflammation</td>
<td>AT with OX25 mAb</td>
<td>DPPC-DC-Chol:DOPE-Chol:PEG-PE (5:5:30:15)</td>
<td>111In</td>
<td>SPECT/CT</td>
<td>Turner et al. (2005)</td>
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<tr>
<td>MIV</td>
<td>150-230</td>
<td>Sodium Phosphate</td>
<td>Therapy of renal tumors</td>
<td>AT with OX25 mAb</td>
<td>DPPC-DC-Chol:DOPE-Chol:PEG-PE (5:5:30:15)</td>
<td>111In</td>
<td>SPECT/CT</td>
<td>Farshid et al. (1996)</td>
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<th>Area</th>
<th>Targeting (**)</th>
<th>Nuclei Composition</th>
<th>Labeling</th>
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<td>PT</td>
<td>DPPC-Chol-modifier (4:1) DPPC-Chol, PE / cholesterol</td>
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<tr>
<td>S. V.</td>
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<td>Indole and Carbonate</td>
<td>Therapy for advanced AML with bortezomib</td>
<td>-</td>
<td>DPPC-Chol (1:1:1) DPPC-Chol, PE / cholesterol</td>
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<td>230</td>
<td>Liposome-formulated TPA</td>
<td>Therapy for acute stroke</td>
<td>-</td>
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<td>Doxorubicin</td>
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<td>-</td>
<td>DPPC-Chol (1:1:1) DPPC-Chol, PE / cholesterol</td>
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<td>Neutrophil</td>
<td>Therapy for acute stroke</td>
<td>-</td>
<td>DPPC-Chol (1:1:1) DPPC-Chol, PE / cholesterol</td>
<td>¹⁸F-FDG</td>
<td>PET</td>
<td>Oghara et al. (2001)</td>
</tr>
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</table>

*MLV: multilamellar vesicles, SLV: small lamellar vesicles; **MLV: active targeting, PT: passive targeting.
by this way therapy is achieved in early stages of diseases. A number of studies for the use of liposomes in molecular imaging of different diseases such as tumor, brain disorders, atherosclerosis and blood perfusion are described further in this review.

**Tumor imaging**

Cancer is one of the most studied diseases which causes some alterations within the tissues and vasculatization such as EPR effect and angiogenesis providing more localization of delivery systems in pathologic organs, tissues, cells than the normal ones. By this way, the penetration of some molecules such as bradykinin, nitric oxide/peroxynitrite, prostaglandin, vascular endothelial growth factor (VEGF), tumor necrosis factor and other pathophysiological factors increases out of vasculature (Maeda et al., 2000; Lukyanov and Torchilin, 2004).

Surface modification of liposomes with some hydrophilic polymers like PEG helps them to stay longer in blood circulation and eventually localizes in the tumor site in higher concentrations due to EPR effect. Specifically targeted molecular imaging of drugs or delivery systems can only be achieved by active targeting them to the desired specific cell, sub-cell, molecule, receptor, protein, peptide, hormone or enzyme related with the disease (Park, 2002). This targeting can be accomplished by conjugation of target specific ligand on lipid layer of the liposomes (Torchilin 2006; Libyayani and Torchilin, 2006) by magnetic targeting (Uzundzhov et al., 2009) or by ultrasonic targeting (Hu et al., 2009; Ren et al., 2010). In order to achieve these purposes, different kinds of liposomes can be used for noninvasive imaging of tumor angiogenesis and for evaluation efficacy of angiogenesis therapy of tumor for specific molecular targeting with paramagnetic and fluorescent liposomes (Stuifers et al., 2010). Formation of a new receptor or antigen selectively expressed or over expressed on malignant tumor cells and altered receptor density are some of quite significant targets in specific targeting. An effective specific imaging and/or therapy can be performed in the case of existence of 16β receptor density above tumor tissue. Target receptors should have minimum heterogeneity in antigens and receptors but, generally tumors have heterogeneous antigens. For overcoming this problem, a mixture of antibodies or antibody fragments can be utilized (Sapra and Allen, 2003).

Nowadays, monoclonal antibodies (mAbs), specific to a variety of antigen of tumors, are generally used for active targeting. Between the years 1990–2005, almost 200 mAbs were studied in clinics worldwide. The first approved mAb by Food and Drug Administration (FDA) was pan-CD20 mAb (Rituximab) in 1997 for the treatment of non-Hodgkin's lymphoma. Nowadays, the number is almost reached to eight mAb approved by FDA and apart from that, four mAbs are approved in other countries apart from USA that can be used for targeting nanosized diagnostic or therapeutic delivery systems (Ting et al., 2010; Adams and Weiner, 2005; Newman and Ernstoff, 2000; Reichert and Voge-Archer, 2007; Carter, 2008). mAb 2C5 modified nanocarriers for diagnosis and/or therapy of different kinds of tumors by active targeting was performed by Torchilin (2007) and Libyayani and Torchilin (2006). mAb 2C5, one member of natural antinuclear autoantibodies (ANA), is specific to nucleosome molecules that exist on a large variety of tumor types after it is released from dead tumor cells and bound on the surface of neighboring tumor cells. By this way, molecular imaging of tumor was performed by different studies related with the conjugation of mAb 2C5 on liposomes. By radiolabeling of these immunoliposomes with 111In and iodopride encapsulation, SPECT/CT images of tumor can be evaluated (Silindir et al., 2009; Silindir et al., 2010). Tumor accumulation was performed in tumor bearing mice with the use of 111In labeled immunoliposomes by γ-scintigraphy (Irodoglu et al., 2006; Libyayani and Torchilin, 2006; Irodog˘lu and Torchilin, 2007). By the use of 68Ga containing immunoliposomes, MRI imaging of tumor was done successfully in tumor bearing mice (Irodog˘lu et al., 2006; Irodog˘lu et al., 2008).

Vasoactive intestinal peptide (VIP) is one of the most important markers to use for molecular imaging or therapy of breast cancer. VIP receptors are expressed approximately five times more in human breast cancer than normal breast tissue. VIP conjugated 111In-HMPAO encapsulated PEGylated liposomes were prepared by Dugat et al. (Dugat et al., 2005) and administered to rats bearing breast cancer for the purpose of breast cancer diagnosis. The particle size, encapsulation, pharmacokinetics and biodistribution of liposomes were observed similar before and after VIP conjugation. 111In-HMPAO encapsulated PEGylated VIP conjugated liposomes were observed to accumulate more in rat bearing breast cancer than nonconjugated liposomes. VIP has also been used for its vasodilatory effect due to being an active potent vasodilatory neuropeptide. Polymer-grafted liposomal formulation for aerosol pulmonary delivery of VIP was prepared by Stark et al. (2007). VIP grafted liposomes were found to induce vasodilatory effects more than free VIP in an ex vivo lung arterial model system. A study related with molecular imaging of angiogenesis was performed by Winter et al. (2005) in nascent Vc2 rabbit tumors using a novel cβPβ-targeted nanoparticle by MRI. After 2 h of postinjection of cβPβ-targeted paramagnetic nanoparticles to tumor bearing New Zealand White rabbits, increased MRI signal by 126% was in the periphery of the tumor and within the walls of some vessels proximate to tumor similarly.

Not only tumors but also different kinds of diseases can be diagnosed, characterized or monitored with molecular imaging by multifunctional imaging techniques like MRI, US, CT, PET/CT or SPECT/CT.

**Imaging of brain disorders, neurodegenerative diseases and brain tumors**

Brain imaging is harder than imaging of other tissues and organs within the body depending on the very tight junctions. One of the most commonly seen problem in
drug delivery to brain is the blood-brain barrier (BBB) that is a barrier formed by blood vessel endothelium and a dynamic block effect by physical, humoral and neural stimuli. In blood vessel endothelium, there are tight junctions which prevent the passage of solid and aqueous substances freely on the contrary to peripheral tissue endothelium. In the presence of BBB, most of the active ingredients cannot penetrate into brain. The penetration degree of BBB depends on several properties such as drug dissolution, molecular weight, charge and the degree of binding to serum proteins.

Another way of penetration of BBB is the use of nanoparticles, nanovaccines as drug carrying systems producing in nanoscale. In recent years, the studies are majorized in the subject of multifunctional drug delivery systems. Convection-enhanced delivery (CED) can be chosen as an alternative way of continuous injection under positive pressure of a therapeutic and diagnostic agent in a fluid. CED technique was first proposed in early 1990s to deliver drugs to the brain and transport them into the parenchyma. Liposomes can also be administered locally by CED technique for enhancing accumulation of diagnostic or therapeutic agents in brain tumors or some parts of brain related with neurodegenerative diseases such as Alzheimer's disease or Parkinson's disease (Bobo et al., 1994; Schwyzer and Hawley, 2005).

MRI was performed to evaluate the distribution of liposomes co-loaded with Gd and a fluorescent indicator, 1,1'-dioctadecyl-3,3',3'-tetramethylindocarbocyanine-5,5'-disulfonic acid [DiI-DS; formerly DMPC/15%DS] within target regions in normal and Gd and 9L-2 glioma model rat brains by Saito et al. (2004). Liposomes were administered by CED into striatal regions and distribution of Lip/Gd/DI-DS was found rather high in both tumor models. A treatment strategy was designed in the 9L-2 model by infusing liposomes containing Gh [Lip/Gd], prepared in the same size with Lip/Gd/DI-DS and Dox. Optimum CED parameters were observed with Lip/Gd co-administered with Dox by MRI. In another study made by the same research group (Saito et al., 2005), liposomes containing Gd, Cisplatine, DII-DS, and rhodamine were infused by CED in corona radiata, putamen nucleus, and brain stem in non-human primate brain. The volume of distribution was evaluated by histology and MRI. The tissue distribution of Gd containing liposomes was found highly accurate as confirmed by histological results obtained from simultaneously administration of fluoresein liposomes.

Neutral cell adhesion molecule (NCAM)-targeted doxorubicin-loaded and Gd containing liposomes were formulated by Grando et al. (2010) and liposomes were investigated for their delivery in Kaposi's sarcoma by MRI. Enhanced doxorubicin internalization was observed by NCAM-targeted liposomes within Kaposi's cells and by this way tumor mass and vascularization reduction, cell necrosis and apoptosis were achieved when compared with non-targeted ones.

The macrophage mediated tumor uptake of paramagnetic/fluorescent liposomes were performed by Castelli et al. (2009). Dyasposum (Dy) was encapsulated in liposomes and fine T2 susceptibility agents were sustained at high magnetic field strength. After the administration of Dy-loaded liposomes to tumor tissue culture, liposomes were found to attach to highly expressed glutamine receptors of tumor depending on glutamine (Gln) residues and by this way lesser tumor specificity of Gln-functionalized liposomes was seen depending on their removal by RES or macrophages. Another study was also performed by the same group (Castelli et al., 2010) with paramagnetically labeled liposomes for imaging their uptake and intracellular trafficking in tumor bearing mice by MRI. Stealth liposomes were loaded separately with paramagnetic complexes such as Gd-III-(2-hydroxypropyl)-1,4,7-tetrazacyclododecane-l,4,7-triacetic acid (HPDO3A), [Ir1-N-(1,2-dicyanobenzyl)pyridyl-N,N,N-trimethylammonium chloride (IOBMA)][Na] for evaluating T1/T2 characteristic properties and another agent for chemical exchange saturation transfer (CEST)/T2 for MRI. Apart from liposomes, dextran coated iron oxide nanoparticles can be used for brain imaging as superparamagnetic MRI contrast agents. Depending on the lipophilic property of iron oxide, it should be coated with a hydrophilic polymer. This property provides them longer half-life than conventional MRI contrast agents; as a result, imaging can be performed many hours after infusion (Newhall et al., 2004).

A research was made by Burks et al. (2010) about anti-Human Epidermal Growth Factor Receptor 2 (HER2) immuno-liposomes for selective delivery of Electron Paramagnetic Resonance (EPR) imaging prebeeho HER2-overexpressing breast tumor cells. After encapsulating 750 mM concentration of nitrooxide, highly attenuated EPR spectral signals were obtained and a cell-activated contrast-generating mechanism was achieved by endocytosis of liposomes. Nitroxide encapsulated and HER2 immuno-liposomes were observed to target 1067 cells in larger amounts.

Another study was performed with [19F]Fluorodipalmitin ([19F]FDP)-labeled liposomes by incorporating [19F]FDP after synthesis by nucleophilic substitution of the p-vanilinoligorylic moiety of 3-tosyl-1,2-dipalmitoyl glycerol by Leung (2000). The images obtained from [19F]FDP and [19F]FDP labeled liposomes were compared in normal rats. The accumulation of [19F]FDP was mainly seen in liver and spleen; however, almost no accumulation was observed in the bones and heart. On the other hand, the accumulation that was seen with [19F]FDP-liposomes stayed longer in blood with higher contrast levels and slowly metabolized and by this way they can be used for imaging tumors. Different from liposomes a study about peptide radiotherapeutics was made by Wu et al. (1997) for brain imaging. A1-F2 peptide was labeled with 19F and monochromatized for conjugation with a complex of 40–14 nM specific to human insulin receptor, tagged with streptavidin (SA). A significant

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brain imaging was obtained in the mouse brain after 3 h of iv injection of the radiopharmaceutical peptide. It was observed that 90% of the radioactivity was cleared from the brain after 48 h. Brain targeting can be achieved by developing Ox26 monoclonal antibody conjugated streptavidin (SA) that is specific to biotin conjugated PEGylated drug delivery system. Ox26 is anti-transferrin receptor antibody Ig2a which is specific to transferring receptors that are expressed by brain capillary endothelial cells (BCECs). In a study performed by Kunitake et al. (1999) about brain tumor imaging by epidermal growth factor (EGF), a potential peptide specific to EGF receptor (EGFR) overexpression of many human gliomas, monoclonalized with NHS-PEG2000-biotin and it was radiolabeled with either 111In or 192Ir through DTPA. Both In-DTPA-EGF-PEG2000-biotin and Ir-DTPA-EGF-PEG2000-biotin were conjugated with monoclonal antibody Ox26-SA for specific targeting. However, only In-DTPA-EGF-PEG2000-biotin conjugated to Ox26-SA was found metabolically stable after iv injection in rats. In vivo studies of C6-EGFR experimental tumors in Fischer 344 rats demonstrated successful brain imaging. Another brain imaging study was made by Suzuki et al. (2004) with radiolabeled peptide nucleic acids (PNA) that are biodegradable. Imaging of in vivo gene expression in brain tumors was achieved by 111In-labeled and rat transferrin receptor specific SA-murine Ox26 monoclonal antibody-modified for specific targeting of PNA antisense agents. PNA were designed that were antisense to either the rat gliarial fibrillary acidic protein (GFAP) mRNA or the rat caveolin 1 mRNA. According to the in vivo results, although no brain cancer imaging was performed by In-GFAP-PNA conjugated to SA-Ox26 delivery systems, a significant cancer imaging was performed by 111In-Cav-PNA conjugated to SA-Ox26 due to Cav gene expression upregulation in brain cancer. A study about dopaminergic degeneration analysis in animal models of Parkinson’s disease (PD) was performed by Andringa et al. (2005) using in vivo SPECT with the DAT probe [123I] N-o-fluorobenzyl-2I-carboxymethoxy-3β-(4-iodophenyl) nitroscyanine (FP-GTS). It was found that accurate detection of ventral DAT levels in C57Bl/6J mice and can be used for analyzing more subtle effects of neuroprotective treatment.

Atherosclerosis and blood perfusion imaging

Liposomes have also been designed as contrast agent for imaging localization or distribution in target tissues or organs by US with the help of ultrasound pressure waves and by the use of gas-filled microspheres (microbubbles). These microbubbles are currently used in clinics and approved by FDA as a novel contrast agent for imaging the opacification of left ventricle in echocardiography by US. Apart from molecular imaging, therapy can also be performed by controlling drug or gene release site by loaded liposomes or microbubbles. The stability in circulation of both systems can be enhanced by the utilization of long acyl chains and PEGylation. They can be radiolabeled with 111In or 192I for tracing of its biodistribution. Microbubbles and liposomes generally release drugs with different mechanisms thus, while microbubbles are activated mechanically, liposomes are activated thermally. Mild heating by the effect of ultrasound either before or after the injection of the drug can induce easy transport of liposomes from blood vessels to the tissue to increase accumulation in the target site (Ferrare et al. 2003, Deloage, 2008, Koning and Krijger. 2007).

Molecular imaging with contrast-enhanced ultrasound (CFU) relies on the detection of the acoustic signal produced by either acoustically active microbubbles or other acoustically active particulate agents that are targeted to the disease site, biological processes leading to unidirectional plaque growth or active atherothrombotic events can be monitored by molecular imaging. CEU molecular imaging of atherosclerosis is related with the detection of endothelial cell activation on the plaque surface or in the underlying vasa vasorum; or detection of a prothrombotic environment. Endothelial cell adhesion molecules (ECAMs) are main targets for inflammation imaging such as vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1) or selectins that are important in the process of atherosclerosis and plaque instability (Yllanueva et al. 2004).

Functional ultrasound imaging of tissues by targeted microbubbles is a relatively new approach that is different from microbubbles passively transit circulation like red blood cells. Targeted microbubbles can be used for: (i) inflammation imaging by targeting to adhesion molecules such as ICAM, VCAM, (ii) angiogenesis imaging by markers including αv integrins increased during neovessel formation and angiogenic growth factor receptors increased during ischemia and VEGF that increases in tumor formation, (iii) targeted imaging of atherosclerotic plaque with ICAM-1, VCAM-1 targeted microbubbles, and (iv) Thrombus-targeted imaging associated with myocardial infarction, stroke and deep venous thrombosis (Yllanueva et al. 2004; Lanza and Wickline, 2001).

There are several advantages of using a CEU in molecular imaging for both clinical and research: approaches such as cost effectiveness, easy and speed process, the protocol of CEU can be completed in 5–10 min and both structural and functional information can be obtained by this way. However, CEU has a limitation such as inability to target events occurring outside of the vascular compartment (Jonathan et al., 2010). Activated human coronary artery endothelial cells (HCAEC) specific anti-ICAM-1 conjugated immunoliposomes were prepared by Danila et al. (2009) for the purpose of designing a CE imaging agent for early detection of atherosclerotic plaques. Anti-ICAM-1 mAb was covalently attached to PEGylated liposomes. The encapsulation of techoxin in liposomes was determined as 18% (30–35 mg ml−1). Liposomal dispersion and the average diameter was found as 1.36 nm. According to the immunostaining assays and flow cytometry, techoxin encapsulated immunoliposomes have been found as the potential for implementation.
in CT to detect atherosclerotic plaques. Another study was made by Demos et al. (1997; 1999) about endothelial adherence of immunoliposomes conjugated with anti-fibrinogen or anti-ICAM-1 antibodies. These formulations were injected into an atherosclerotic pig model and imaged by US for comparison. It was observed that while anti-fibrinogen antibody conjugated liposomes were accumulated more in fibrous and thrombotic portions of atherosclerotic plaque, anti-ICAM-1 conjugated liposomes were found to accumulate more in areas of early atherosomatous lesions. A study was performed by Alkam-Omaryek et al. (1996) for developing acoustically reflective echogenic liposomes with dephogulation/depolymerization method. The formulations were optimized by varying lipid concentrations. Their acoustic reflectivity was found sufficient both in intravascular and in vivo. The acoustic behaviour of liposomes were found same when coated with blood at RT and at 27°C under in vitro conditions. Liposomes were also injected into a mini-swine model and they were also found acoustically reflective.

Modern ultrasound contrast media is defined as intravenously injectable, stabilized, gas-containing microbubbles increasing signal intensity by improving signal to noise ratio. The microbubbles of Levovist® and Sonovue® (Bracco et al. 2000) are mostly commonly used contrast media in the world that is available in more than 60 countries. Optimus® and Sonovue® (Fonar et al. 2000) are recently developed ultrasound contrast media. The biologic effects and imaging features of all of them are similar due to stable structure and small size of gas-containing microbubbles. These are bubbles of perfluorcarbon gas along with human albumin shell. They can be used for passing through the pulmonary capillary bed and remain intact in the circulation for 2-5 min. Any adverse effects such as air emboli or allergic reactions have not been reported. The mechanism of uptake of microbubbles in RES organs depends on their phagocytosis by RES cells (Sztainberg et al., 2003).

Results and conclusion

Identification of novel targets such as specific molecules, markers, genes, receptors or transporters provides detection and diagnosis of diseases and by this way earlier therapy may be achieved. Further improvements in molecular imaging will take place by perfect harmony and collaboration of imaging modalities, medicine, pharmacy, molecular biology, chemistry and computer engineering. Utilization of hybrid systems for molecular imaging of living subjects is a promising improvement in both nuclear medicine and radiology for diagnosis of the diseases in early stages. Depending on the developments, there is an essential need for developing novel contrast agents that can specifically deliver to the target tissue or cell, give sufficient signal/contrast intensity with less target/background contrast ratio for obtaining better images in shorter times. For this purpose, nanoparticles and nanovesicle drug delivery systems such as liposomes can be used for in vivo cellular imaging by conjugating with a radionuclide or another contrast agent. Therapy can also be performed by encapsulation of a drug into diagnostic liposomes for producing therapeutic liposomes. In the light of novel investigations, more specific and effective new generation probes may be developed to provide better imaging quality, higher image resolution and cost effectiveness in the future.

Declaration of interest

The authors of this review article explicitly state that there are none declaration of interest.

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Journal of Drug Targeting.


Liposomes and their applications

Freiberg UK, Ltd.

Liposomes for drug delivery: An overview.


Liposomes for targeted drug delivery.


Liposomes for targeted drug delivery.


Liposomes for targeted drug delivery.


Liposomes for targeted drug delivery.


Liposomes for targeted drug delivery.

2.5. In Vitro BBB Cell Co-Culture Model

Cell culture is the process in which cells are grown under controlled conditions, generally outside of their natural environment. These cells may be removed from the tissue directly and disaggregated by an enzymatic or mechanical means before cultivation, or they may be derived from a cell line or cell strain (97). The historical development and methods of cell culture depends on tissue culture and organ culture. English physiologist Sydney Ringer developed salt solutions containing the chlorides of sodium, potassium, calcium and magnesium suitable for maintaining the beating of an isolated animal heart outside of the body in 19th-century (98). In 1885, Wilhelm Roux removed a portion of the medullary plate of an embryonic chicken and maintained it in a warm saline solution for several days and established the principle of tissue culture (99). In 1907, Ross Harrison succeeded to grow the frog neurons in a lymph liquid medium (100,101).

Primary culture is the stage of the culture after the cells are isolated from the tissue and proliferated under the appropriate conditions until they occupy all of the available substrate called as rich confluence. Cells have to be subcultured by transferring them into a new vessel with fresh growth medium to provide enough space to continue growing (97).

Cell line or subclone term is called after the first subculture. This subclones have a limited life span and highest growth capacity due to the passaging process (97).

The use of cell culture studies became popular nowadays due to its significant role in enlighting molecular genetic, immunology, surgery, bioengineering and monoclonal antibody, drug development and vaccine formulation. Recently use of in vitro cell culture techniques are taking place of animal experiments due to its being cost effective and improved ethic rules in drug therapy, toxicity and permeability studies.

The conditions such as special medium and substrate carry a significant role in performing a proper culture medium. Cell confluent, cell-matrix interactions and nutritional factors are also essential issues for cell differentation and cell proliferation. It is essential for supporting the cellular functions and production of new cells. However, it is obvious that different cells have different neccessities.
Cell/tissue necessities and selection depends on the structure of the planned study. Embryonic tissues or cells proliferate faster than mature tissues or cells. Mature cells comprise less proliferating and non-splitting cells but more specialized cells. The proliferation speed is limited in normal cells when compared with neoplastic cells. There are some special cell cultures that can be used as in vitro models of brain uptake. To compose a human BBB cell culture model, cerebral microvessel endothelial cells were first isolated in 1970s (102). In order to perform quantitative permeability studies and drug discovery, brain microvessel endothelial cell (BMEC) culture can be used (103,104).

Transwell containing immortalized human brain endothelial cell line (hCMEC)/D3 BBB model is the most commonly used BBB model to evaluate the transport and penetration studies of drugs and drug delivery systems. A schematic in vitro BBB model in a Transwell comprising hCMEC/D3-NHA-BBB system including hCMEC/D3 as endothelium and NHA as astrocytes in co-culture is illustrated in Figure 2.10.

![Figure 2.10. In vitro model of BBB (105).](image)

The hCMEC/D3 cell line was derived from human temporal lobe microvessels isolated from tissue excised during surgery for control of epilepsy. The primary isolate was enriched in CECs. The hCMEC/D3 cell line as human BBB model which is the closest human BBB model for evaluating nanoparticle interaction with a cellular barrier (102,106). To perform a better a BBB model, it then supported
by direct/indirect co-culture with human astrocytes (107) which provides evaluating of cell–cell signalling and other physiological effects.

T dos Santos et al designated the interactions of this model, uptake into and transport across the BBB (108). In the concept of continuing studies, with the imaging modalities, very small numbers of nanoparticles can cross in vitro BBB. According to another study performed by Raghnaill et al that the effect of nanoparticle accumulation in the BBB on lysosome health and paracrine signalling affecting BBB cells. This effect can be essential for the protection of vulnerable tissues by the biological barriers (105). Raghnaill et al investigated the behaviour of carboxylated polystyrene nanoparticles in vitro BBB model. It was observed by TEM imaging that nanosized nanoparticles accumulate but not degraded within the lysosomes over time. It was observed that in vitro BBB does not restrict the uptake of NPs completely. However, the uptake of these nanoparticles was observed to slow down in a large amount in BBB monolayer compared to that of in single hCMEC/D3 cells (105).

Among this thesis, we chose and used a non-contact BBB co-culture model. Endothelial cell layer divides this system as apical (blood side) and basolateral side (brain side). This BBB co-culture model comprise Human brain microvascular endothelial cell and astrocytes.

2.6. Parkinson’s Model in Small Animals

To investigate the pathogenesis and patophysiology of PD, PD animal models have been widely used. To perform PD animal models, neurotoxins can be applied either by systemic or local (intracerebral) administration in mammals like rodents or primates (109). Toxic and transgenic animal PD models have their own properties and limitations which should be kept in mind when choosing the appropriate model specific to the purpose of the research. In case of obtaining a substantial and reproducible nigrostriatal lesion to test therapeutic interventions aimed at counteracting PD-related cell death, a classical toxin application model such as MPTP for mice or 6-OHDA for rats can be used. If the purpose is to investigate the selected molecular mechanisms of PD pathogenesis, transgenic models can be used
however, this is not the issue of this thesis. Therefore, these models are valuable till obtaining the ‘perfect’ model in the future (109).

2.6.1. 6-OHDA

6-OHDA is a hydroxylated analog of dopamine (Fig. 2.11.) (110,111). The mechanism of 6-OHDA induced neurotoxicity was given in Figure 2.13. Briefly, 6-OHDA is taken up from the extracellular space by DAT or noradrenaline membrane transporter (NAT). Then, 6-OHDA is stored in catecholaminergic neurons. 6-OHDA undergoes both enzymatic degradation by monoamine oxidase (MAO-A) and autoxidation inside neurons, generating several cytotoxic species which, by damaging endocellular proteins and nucleus, produce neuronal damage. Moreover, 6-OHDA might induce neurotoxicity by impairing the activity of mitochondrial complex I. In experimental animals, 6-OHDA is usually administered in association with NAT blockers, such as desipramine (DMI), to prevent its uptake by noradrenergic terminals and to selectively target dopaminergic neurons (112). The mechanism of 6-OHDA induced neurotoxicity was given in Figure 2.12.

Figure 2.11. The molecular structure of 6-OHDA (113).
Figure 2.12. Mechanisms of 6-OHDA-induced neurotoxicity (112).

*Malondialdehyde (MDA) level, the activity of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx).

It was reported at the beginning that 6-OHDA caused depletion of noradrenaline in the mouse heart (114,115). 6-OHDA was also used for inducing selective degeneration in sympathetic adrenergic nerve terminals (116) a specific cell population (117). The neurotoxin 6-OHDA has been widely used as a model of PD through the lesion of nigrostriatal dopaminergic system. 6-OHDA is responsible from the degeneration of both dopaminergic and noradrenergic neurons (118). 6-OHDA accumulates in the cytosol of neurons and there to generate reactive oxygen species it is oxidized and afterwards oxidative stress-related cytotoxicity is formed (119,120). To degenerate specific neurons after located stereotactically, 6-OHDA is carefully applied in specific regions in brain to target specific neurons and region of interest. It is preferred to administer 6-OHDA to rats when compared with mice to perform PD model due to larger body size when compared with mice (121). 6-OHDA can be also used in cats, guinea pigs, dogs, and monkeys (113,122,123).
The importance of the site of injection and the exact coordinate within the brain depends on magnitude and characteristics formed by neurotoxin 6-OHDA (124-126). The degree of neurodegeneration affects the therapy effectiveness of drugs or drug delivery systems. It is commonly preferred to administer 6-OHDA unilaterally to the substantia nigra, medial forebrain bundle or striatum (127,128). When delivered to the striatum, 6-OHDA induces slow, progressive, and partial damage to the nigrostriatal structure in a retrograde level over a period of up to 3 weeks (125,129). The administration to the striatum has three advantages: First, the progressive and less extensive lesion model is more proper to early PD. Second, this model generally produces PD nonmotor symptoms with cognitive, psychiatric, and gastrointestinal dysfunction (130-132). Third, the simplicity in administration to stereotactically located small animals with large structure such as the striatum increases the success of the model (113).

The main benefit of 6-OHDA model in animals is its specific effect on quantifiable circling motor abnormality (133). It is generally administered to one hemisphere (hemiparkinsonian model) unilaterally which gives the chance to have a contact unlesioned side as control. To evaluate the success and performance of the lesion, some drugs such as dopamine receptor agonists (apomorphine), L-DOPA (the dopamine precursor), or dopamine releasing compounds (amphetamine) can be systemically administered inducing asymmetrical rotation (133,134). This circling motor behaviour of animals depends and correlates with the magnitude of nigrostriatal lesions (118,125,133,134). Briefly, unilateral 6-OHDA rat model is generally used for evaluating antiparkinsonian effects of drugs and neuroprotection effects of PD (135-137). Unilateral 6-OHDA rat model can also be used to evaluate the clinical improvement of cell transplantation (138-140). This model has also some deficiencies such as lacking of progressive and age-dependent effects of PD (113).

2.6.2. MPTP

There is also another neurotoxin model to produce PD model in mice. However, this model is given very briefly because it was not used within the concept of our thesis. We used Wistar rats among our thesis depending on that we had chose 6-OHDA lesion for PD formation.
The first study about 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) model was performed in 1980s (141,142). The use of dopaminergic neurotoxin, MPTP, induces replication of pathological signs and motoric signs related with PD in primates and rodents by selective destruction of dopamine (DA) neurons of substantia nigra (25). It was reported that MPTP induces loss of nigrostriatal structures in postmortem parts of patients (141,142). The use of MPTP significantly inceased the number of PD research to provide information about pathogenesis and mechanisms for cell death in PD. Additionally, work with this model provides development of some current treatments for PD (143). MPTP toxicity was studied and characterized extensively (144,145). Because, MPTP can easily cross BBB due to its lipophilic property. In astrocytes, MPTP is metabolized by monoamine oxidase-B, and the active toxic cation form 1-methyl-4-phenylpyridinium (MPP+) is formed (Fig. 2.13.).

![Figure 2.13. The molecular structures of MPTP and MPP+](image)

MPP+ is released into the extracellular space from the nigral and striatal astrocytes through organic cation transporter 3 (145,147). Afterwards, it is taken up by neighboring dopaminergic neurons and terminals through the DAT. MPP+ induces neurotoxicity mainly by inhibiting complex I of the mitochondrial electrontransport chain after accumulated in dopaminergic neurons. This causes ATP depletion and oxidative stress increment (148,149). MPTP is widely used for preclinical testing of therapeutic strategies for PD especially in mice however, it can also be used for various mammalian species, including sheeps, dogs, guinea pigs,
cats, mice, rats and monkeys (122,150,151). The reason of broad usage of MPTP in mice rather than in rats depends on lesser sensitive of rats to MPTP toxicity (152). MPTP primarily causes damage to nigrostriatal dopaminergic pathways in both mice and monkeys (143,144,153). The advantage of MPTP model is its specific and reproducible neurotoxic effect to the nigrostriatal system (153). Another advantage is that the motor deficits induced by MPTP can be characterized in both monkeys and mice. These abnormal phenotypes can be reversible by L-DOPA or a dopamine agonist, confirming a connection between these symptoms and damage in the nigrostriatal system (154,155). MPTP neurotoxin can be used in PD research based on its ability to produce PD-like effects in humans and nonhuman primates, its reproducible L-DOPA responsive lesion on nigrostriatal system and the simplicity of administration (typical intraperitoneal injection) as a commonly used PD model (113).

2.7. Monitoring Therapeutic Efficacy in PD by Rotational Behaviour and Imaging

Therapeutic efficacy can be observed by some tests. In case of neurodegenerative diseases such as PD, AD, dementia and epilepsy some tests comprising rotational behavior for unilateral lesions and movement tests can be used to evaluate therapeutic efficacy. Imaging can be used for different purposes to improve various different aspects of drug delivery and drug therapy of novel drug molecules or drug delivery systems. This can be used for visualizing and quantifying the site of drug and drug delivery system accumulation and to assess their efficacy by this way non-invasively. Furthermore, image-guidance is highly useful for monitoring the success of targeting and release of drug delivery systems such as liposomes, niosomes, micelles, nanoparticles, microbubbles, nanocapsules etc. This can be performed by two ways. One is the radiolabeling of the outer shell or encapsulating the desired radioligand within the core of drug delivery systems and then imaging targetability of drug encapsulated drug delivery system. Second way is the imaging of therapy efficacy of drug encapsulated drug delivery system by administering target specific radioligand or radionuclide separately, afterwards. With the combination of drug targeting and imaging protocol, biodistribution and drug
release kinetics can be visualized, patients can be pre-selected, proper and minimum effective dose can be selected and personalized therapy protocol can be administered (156). Medical imaging has an enlarging role in new drug development.

It is anticipated that greater use of imaging during pre-clinical stages will facilitate better translation from animal models to human subjects. The main emphasis is on the application of medical imaging in therapeutic drug trials is very similar to the development of novel imaging contrast agents and radiopharmaceuticals (157). The use of pre-clinical imaging with a proper imaging instrument such as micro-PET, SPECT, CT, MRI, optical imaging and autoradiography in neurodegenerative diseases in PD animal models can contribute to the identification of new imaging biomarkers and also evaluation the therapeutic efficacy of active drug encapsulated drug delivery systems. It is important to use a specific molecular imaging ligand.

2.7.1. Rotational Movements

This is another method than imaging for evaluation of PD lesion and potential efficacy of therapeutic efficacy of new drug delivery systems. Rotational behaviour is one of the generally used measurement tests to evaluate the efficacy of drugs for the treatment of neurodegenerative diseases such as PD, AD, dementia and epilepsy in unilateral lesined animal models. Rotational movement test is used in 6-OHDA rats evaluating lesion-induced motor impairment. Among other rotational behaviour and movement tests, rotameter test can be used for the evaluation of measuring skilled motor performance. 6-OHDA neurotoxin is generally administered to one hemisphere (hemiparkinsonian model) unilaterally which gives the chance to have a contact unlesioned side as control. Systemic administration of some drugs such as apomorphine and amphetamine induces asymmetrical rotation to evaluate lesion performance (133,134). This circling motor behavior of animals increases with the enhanced magnitude of nigrostriatal lesions (118,125,133,134). This behavior can be used for the evaluation of the efficacy of some new antiparkinsonian drug molecules and drug delivery systems. Antiparkinsonian therapeutic efficacy of drug molecules and drug delivery systems is inversly proportional with the number of ipsilateral turns in rotameter test of lesioned PD model animals after dopamine
agonist apomorphine or amphetamine injection (158). Apomorphine induced rotation test is a useful tool for primary screening of 6-OHDA lesion (159). Although 6-OHDA induced rotameter test has some drawbacks with the use of apomorphine test such as its use in the prediction of rotational performance of 6-OHDA rats to L-DOPA, (158) it is very frequently used in rats performed PD for assessing both the success of neurodegeneration and the efficacy of drug therapy.

2.7.2. Imaging Modalities

The use of imaging and drug monitoring or following up with a proper imaging modality such as There are many methods to monitor PD as other CNS neurodegenerative diseases like AD, multiple sclerosis (MS), dementia and epilepsy. CT, MRI, PET, SPECT, PET/CT, SPECT/CT, PET/MRI, optical imaging and autoradiography are some of the commonly used and desired imaging techniques and instruments. All of these techniques have some pros and cons however they can all be used for evaluating different aspects of brain structures or functions (160-162). These modalities can also contribute to the identification and formulation of new drug delivery systems, drugs and imaging biomarkers.

Another mostly and simply used imaging technique is the autoradiography. Autoradiography with Beta imager is an imaging technique providing a fast, accurate and efficient detection of many diseases and gives the opportunity to work on small sections and slides. However, to perform research on sections obtained from specific slices of desired organs or tissues in small animals, some rapid and practical imaging modalities can be performed such as autoradiography in frozen brain sections after incubating with the specific radioligands (162). In this research, autoradiography was used to evaluate and monitor the therapeutic efficacy of nanosized, PEGylated, neutral, Pramipexole encapsulated liposomes and niosomes in brain sections of 6-OHDA lesioned rats after sacification at 21 dpl.

2.7.2.1. Autoradiography

Autoradiography is the process to obtain the autoradiograph which is an image on an X-ray film or nuclear emulsion produced by the pattern of decay emissions (e.g., β-particles or α-rays) from a distribution of a radioactive substance.
It is also available as a digital image (digital autoradiography), with the use of scintillation gas detectors (163) or rare earth phosphorous imaging systems. Autoradiography was first used in 1867 accidentally after observing a blackening on emulsions of silver chloride and iodide by uranium salts. These studies were contributed to the discovery of radioactivity. Although, the discovery of autoradiography was very early, its use as a biological technique was begun after World War II after the development of photographic emulsions and silver halide comprising stripping films (164-166). Lacassagne and collaborators developed the first autoradiographic method as film emulsion to localise radioactive Polonium in biological specimens in 1924 (167). Film has some special characteristics that make it suitable for autoradiography. The sensitivity of the film and the background noise are controlled by the proportion of the crystals per unit area. The number of crystals should not exceed 10 crystals per 10,000 square micron, higher counts of crystals may cause low signals and effect the evaluation of experimental data (168,169). These grains are ionised by beta-particles emitted from the tissue sample and cause image formation. The extremely small size of these grains causes an excellent intrinsic spatial resolution (169). Figure 2.14. designates the schematic presentation of autoradiography from a radioactive ligand incubated tissue section (170).

![Figure 2.14](image)

**Figure 2.14.** The schematic representation of autoradiography from a radioactive ligand incubated tissue section (170).
Typically, biomolecules are labeled with $^{32}$P or $^{35}$S and detected by overnight film exposure. Other from these radionuclides, $^{125}$I and $^{3}$H can also be used for the detection in autoradiography. These radioisotopes emit beta-particles which are fast electrons. Table 2.2. gives the amount of commonly used isotopes which can be detected by overnight autoradiography (171,172).

**Table 2.2.** Detection limits of some isotopes (171).

<table>
<thead>
<tr>
<th>Isotope</th>
<th>CPM Necessary for Detection</th>
<th>Energy Per Emission (MEV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{3}$H</td>
<td>$&gt;10^7$</td>
<td>0.0055</td>
</tr>
<tr>
<td>$^{14}$C</td>
<td>2000</td>
<td>0.050</td>
</tr>
<tr>
<td>$^{35}$S</td>
<td>1000</td>
<td>0.167</td>
</tr>
<tr>
<td>$^{32}$P</td>
<td>1000</td>
<td>0.70</td>
</tr>
<tr>
<td>$^{125}$I</td>
<td>10</td>
<td>(gamma)</td>
</tr>
</tbody>
</table>

The film or emulsion is apposed to the labeled tissue section to obtain the autoradiograph or autoradiogram. It was indicated by the auto-prefix that the radioactive substance is within the sample in which the sample is X-rayed using an external source. In the case of micro-autoradiography, the localization of silver grains on the interiors or exteriors of cells or organelles can be detected microscopically (172,173).

Autoradiography has many applications in the laboratory including measuring the pathways of many different biomolecules. These applications are generally based on the identification, localisation and quantification of neurotransmitter receptors in brain tissue sections (169). For instance, autoradiography can be used to analyze the length and number of DNA fragments after they are separated by gel electrophoresis and to determine tissue or cell localization of a radioactive substance, either introduced into a metabolic pathway, bound to a receptor (174,175) or enzyme, or hybridized to a nucleic acid (172,176). Autoradiography is a method used to map the distribution of radiolabeled biomolecules, tracers, deposited in thin tissue specimens (169) and the location of radiolabeled ligands to visualize and quantify receptors in tissue. Autoradiography can also be used to determine hormonal uptake and indicate
receptor location in the field of endocrinology (172). It is used to trace neurons by axonal transport of radioactively labeled amino acids, certain sugars or transmitter substances. It measures DNA production (e.g., $^3$H-thymidine). Autoradiography produces a permanent record of the positions and relative intensities of radiolabeled bands in a gel or blot.

Autoradiography has 2 types. One is in vivo autoradiography and the other one is in vitro autoradiography. Receptors are labeled in intact living tissue by systemic administration of the radioligand for in vivo autoradiography. Slide-mounted tissue sections are incubated with radioligand so that receptors are labelled under very controlled conditions which is called in vitro autoradiography (170). In this research, in vitro autoradiography was used to observe the therapeutic efficacy of antiparkinsonian effect of formulations by the decrease in the loss of DAT binding (%) at the ipsilateral (lesioned) side in striatum of brain sections. DAT specific ligands are generally labeled with $^3$H or $^{125}$I.

Autoradiography can also be used to define the mechanism of drugs, ligands and their interaction with specific receptors after radiolabeling with a suitable radioisotope. Thus when the ligand under study binds with a specific receptor, the location of this receptor binding is identified by detection of the radioactivity emitted by the radioisotope (169).

The principle of autoradiography with Beta-imager was given as a scheme in Figure 2.15.
Autoradiography has some disadvantages. Binding to everything can cause some misinterpretations in the results. This process needs no biochemical or physiological criteria for assessing the binding specificity. The presence of a high-affinity radiolabeled receptor does not necessarily imply that the receptor has physiological significance and ligands are not always very specific.

Although autoradiography has some disadvantages, it also has many advantages such as; being a highly specific tool to pharmacologically characterize receptors in tissue, providing location of receptor or protein in tissue, enabling characterization of receptors in different tissues between different animals or brain regions and being a technically easy process (170). Autoradiography is a less costly and practical method to detect the small animals in preclinical research field.
3. MATERIALS

3.1. Chemicals

1,2-Dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) Avanti Polar Lipids, USA
1,2-Distearyl-sn-glycero-3-phosphoethanolamine-N-[Metoxy (Polyethylene glycol)-2000] (Ammonium salt) (PEG2000-DSPE) Avanti Polar Lipids, USA
2,4,5-Trihydroxyphenethylamine hydrochloride, Sigma-Aldrich, USA
2,5-Dihydroxytyramine hydrochloride, 2-(2,4,5-Trihydroxyphenyl)ethylamine hydrochloride (6-OHDA)

2-Methylbutane (Isopentane) Sigma-Aldrich, USA
Absolute ethanol Riedel-de-Haen, Germany
Ascorbic acid Sigma-Aldrich, USA
Ascorbic acid (Vit C) Atabay, Turkey
Bone Wax Ethicon, France
Carboglace (Dry Ice) Carboglace
Chloroform (%98) Riedel-de-Haen, Germany
Cholesterol (5-Cholesten-3β-ol) Sigma Chemicals, USA
Cocaine Cooper, France
D-Amphetamine-d₃ sulfate salt Sigma-Aldrich, USA
Diethyl ether anhydride Merck, Germany
Diethylene Triamine Penta Acetic Acid (DTPA) Sigma-Aldrich, USA
D-saccharose Sigma-Aldrich, USA
Eye Protectan (Ocry-gel®) TVM, France
Hydrochloric acid (%36-38) J.T.Baker, Netherlands
Hydrogen bromide (solution in acetic acid (%32)) Merck, Germany
Instant freezing spray for anatomical pieces Cryoral, RAL Diagnostics, France.
Isoflurane (Aerrane®) Baxter, Dublin
N-(3-iodopro-2E-enyl)-2beta-carbomethoxy-3beta-(4’-methylphenyl) nortropane (PE2I)  
Synthesized by Team 3, Molecular Imaging and Brain, U930, Tours Univ., France.

Pargyline hydrochloride  
Sigma-Aldrich, USA

Perchloric acid (%70)  
Riedel-de Haen, Germany

Potassium chloride (KCl)  
Sigma-Aldrich, USA

Potassium phosphate monobasic (KH$_2$PO$_4$)  
Sigma-Aldrich, USA

Pramipexole dihydrochloride monohydrate  
Abdi Ibrahim, Turkey

Rhodamine-PE (0,5% mmole)  
Avanti Polar Lipids, USA

Sodium chloride (NaCl)  
Sigma-Aldrich, USA

Sodium phosphate dibasic anhydrous (Na$_2$HPO$_4$)  
Sigma-Aldrich, USA

Stearylamine (SA)  
Sigma Chemicals, USA

SURII (Alcool cetylique polyglycerole)  
L’Oreal, France

TRIS [(Tris(hydroxymethyl)-aminomethan]  
Meck, Germany

Attachment factor (4Z0-210)  
Cellsystems, Germany

Fetal bovine serum (5%)  
Sigma Chemicals, USA

Penicilin/streptomycin (1%) solution  
Sigma Chemicals, USA

ECGS (%1)  
Sigma Chemicals, USA

Asyrocyte growth factor (1%)  
Sigma Chemicals, USA

Rhodamine-PE (0,5% mmole)  
Avanti Polar Lipids, USA
### 3.2. Equipments

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<th>Equipment</th>
<th>Supplier</th>
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<tr>
<td>Aspiration Unit</td>
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</tr>
<tr>
<td>Asyroyocyte growth factor (1%)</td>
<td>ScienCell, USA</td>
</tr>
<tr>
<td>Attachment Factor</td>
<td>CSC Certified, Cell-Ststems.</td>
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<td>Automatic Shaker</td>
<td>Lab-line, India</td>
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<td>Balance</td>
<td>Mettler Toledo AB104-S, Swiss</td>
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<td>β-imager</td>
<td>β-imager TM 2000, Biospace Lab, Paris, France</td>
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<tr>
<td>Cryotome</td>
<td>Leica, CM 3050 S, France</td>
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<td>Dialysis Cellulose Membrane (13000 MW cut off)</td>
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<td>Lipex, Biomembranes, Canada</td>
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<td>Fethal Bovine Serum (5%)</td>
<td>ScienCell, USA</td>
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<td>Micromotor High-Speed Drill</td>
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<td>Pore Dialysis Membrane</td>
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<td>Rotometer</td>
<td>Imetronic Behaviour Instruments, Bordeaux,</td>
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<td>Sonicator</td>
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<td>Temperature Controller</td>
<td>CMA/150, Fredom, USA</td>
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<td>Transwell cell culture inserts</td>
<td>Sigma-Aldrich, Germany</td>
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<td>UV Spectrophotometer</td>
<td>Agilent 8453, USA</td>
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<tr>
<td>Vacuumed Incubator</td>
<td>Shel Lab (SL), Spain</td>
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<td>Veterinary Anesthesia Equipment</td>
<td>Minerve, France</td>
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<td>Water Bath</td>
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<td>Wistar rats</td>
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<tr>
<td>Zeta Sizer</td>
<td>Malvern Instruments, Nano-ZS, United Kingdom</td>
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4. METHODS AND RESULTS

One of the most recently observed neurodegenerative disease among aged-related diseases is PD. It is degeneration of dopamine producing cells in substantia nigra. Currently there is a deficiency in early diagnosis and therapy of this disease due to the limited brain penetration of potential drug treatments. Brain is protected by tight, protective barriers composed of tight endothelial cells and tight junctions such as BBB limiting penetration of drugs and molecules. Although there is a variety of approaches for BBB penetration, the utilization of drug delivery systems is one of the most frequently investigated one.

Pramipexole is one of these effective dopamine agonists with high relative in vitro specificity and full intrinsic activity at D2 subfamily of dopamine receptors and with a higher binding affinity to D3 receptor subtypes. Recently, nanosized drug delivery systems have been investigating for BBB penetration.

Liposomes and niosomes are one of the most commonly investigated drug delivery systems used for delivering both drugs and diagnostic agents to the targeted area. Both liposomes and niosomes have proper characteristics such as being non-toxic, biocompatible and biodegradable. Passive targeting of liposomes and niosomes can be performed with the help of formation of a steric hindrance created by PEGylation and particle size reduction.

Although a variety of studies were performed about therapeutic efficacy of Pramipexole (6,7,19-22) and use of some delivery systems for the therapy of PD (23,24), the formulation of Pramipexole encapsulated liposomes and niosomes have not been studied before as an alternative to oral Pramipexole dosage form.

The aim of our study was to formulate novel, effective, nanosized, PEGylated, pramipexole encapsulated, neutral and positively charged liposomes and niosomes for effective therapy of PD. It was also aimed to perform characterization and in vitro release kinetics of formulations and to evaluate BBB penetration by BBB cell co-culture model. In vivo effectiveness of nanosized, neutral, pramipexole-encapsulated liposomes and niosomes was aimed to evaluate in 6-OHDA lesioned rats by rotometer test and autoradiography.
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Manuscript Type: Article

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DEVELOPMENT OF NANOSIZED, PRAMIPEXOLE-ENCAPSULATED LIPOSOMES AND NIOSOMES FOR THE TREATMENT OF PARKINSON’S DISEASE

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Keywords: Nanosized pramipexole liposomes, nanosized pramipexole niosomes, brain delivery, BBB penetration, Parkinson’s Disease therapy.
ABSTRACT

Parkinson’s disease (PD) is characterized by the degeneration of the dopamine-producing cells in the substantia nigra. Early diagnosis and therapy is essential at the molecular level before initiation of symptomatic changes. Blood-brain barrier (BBB) penetration still remains a major challenge. Increased brain penetration and targeting can be achieved by formulating nanosized drug delivery systems using liposomes and niosomes. Other studies have been performed using pramipexole, but our study is novel in evaluating the penetration and antiparkinsonian effect of nanosized, PEGylated pramipexole-encapsulated liposomes and niosomes. Nanosized, polyethylene glycol (PEG)ylated, neutral and positively charged pramipexole-encapsulated liposomes and niosomes were formulated, characterized, and the release kinetics evaluated. In vitro penetration of all formulations was evaluated using the BBB cell co-culture model. In vivo effectiveness of neutral, pramipexole-encapsulated liposomes and niosomes was evaluated in 6-hydroxydopamine
(6-OHDA) lesioned rats by rotometer testing and autoradiography. All formulations exhibited approximately 10% encapsulation efficiency and around 100 nm particle sizes and fitted first-order release kinetics. All formulations were permeable in vitro as determined by fluorescent images and fluorospectroscopy. Therefore, nanosized, neutral pramipexole-encapsulated niosomes showed better effects at a dosage approximately 9 times less than that administered using conventional pramipexole tablets for human in routine treatment. Nanosized polyethene glycolylated pramipexole liposomes and niosomes were blood brain barrier permeable. Nanosized pramipexole-encapsulated neutral niosomes showed potential therapeutic effects in a Parkinson’s disease animal model depending on the nanosize and non-ionic surfactant properties of the niosomes. Further experiments are currently being performed to improve the therapeutic effects.

INTRODUCTION

Parkinson’s disease (PD) is generally diagnosed in patients over the age of 50. Around 5% of cases occur in younger people (less than 20 years old) and are referred to as juvenile PD. While the disease incidence is 0.2–0.3% among the community, it is 1% among people who are over the age of 55.

Levodopa administration remains the gold standard of treatment for PD, but motor complications such as dyskinesia and wearing off phenomenon may occur following its long-term administration. Dopamine agonists can be used to reduce the duration of immobile periods and dependence on levodopa for improving motor impairments. Pramipexole is one of these dopamine agonists, a nonergot dopamine agonist with high relative in vitro specificity and full intrinsic activity at the D2 subfamily of dopamine receptors, and can also be used for monotherapy. It can be used alone for the treatment of non-serious PD. The occurrence of side effects is very rare. The probability of orthostatic hypotension is much lower (depending on specificity to D3 receptors) when compared with that of other dopamine agonists. There is an immediate release formulation of pramipexole which was approved by the Food and Drug Administration (FDA) in 1997 for individual use
or in use combination with Levodopa for idiopathic PD. The FDA also approved the extended release formulation of pramipexole (MirapexER®) for early phase PD in 2010.4

The brain is protected by several barriers such as the blood-brain barrier (BBB) with the blood-cerebrospinal fluid (CSF) interface and the CSF-blood interface.5, 6 Although these tight and rigid barriers protect the brain from harmful substance and chemicals, they also prevent penetration of drugs that could have therapeutic effects in a variety of brain disorders such as PD. There are different approaches to BBB delivery of molecules. The use of a drug delivery systems is one of the approaches currently being researched.

Nanotechnology deals with materials with dimensions of nanometer scale length (1-100 nm), and thus can be used for a broad range of applications in both biochemical and medical field with production of various types of nano materials and nano devices.7, 8 Controlling of physical and chemical structure of nanomaterials and nanosized delivery systems is very essential for their behavior in vivo which needs a multidisciplinary working of related fields.9 Nanomaterials have many applications for medical and biological purposes. Especially nanosized drug delivery systems are very popular systems in a variety of different diseases depending on their proper structures for targeting diseased site and ability to properly modify. Simvastatin encapsulated Monomethoxy poly(ethylene glycol)-poly(lactic acid) (MPEG-PLA) nanoparticles were observed to improve the anabolic bone effects of Simvastatin in rabbit cranium.10 Folic acid-γ cyclodextrin-C60 was synthesized as a carrier system for tumor-targeted drug delivery. It was observed that Folic acid-γ cyclodextrin-C60 increased the intracellular uptake and cytotoxicity of carboplatin.11 Functional targeting daunorubicin liposomes were developed by modifying the liposomes with distearoylphosphatidylethanolamine polyethylene glycol-polyethylenimine (DSPE-PEG2000PEI600 and a lipid-glucose derivative (DSPE-PEG2000-GLU). The functional targeting daunorubicin liposomes were found successful to transfer across BBB and exhibited efficacy in killing glioma and glioma stem cells in brain of glioma-bearing mice.12 Indomethacin encapsulated lipid-core nanocapsules were prepared and found BBB permeable and effective in glioblastoma model in C57Bl/6 mice after oral and i.v.
administration. Transferrin (Tf)-targeted self-assembling nanoparticles (Tf-PLCaPZ NPs) were formulated to use zoledronic acid in the treatment of glioblastoma. Zoledronic acid encapsulated nanoparticles found effective in inhibiting cell growth in LN229 cells and in mice xenografted with U373MG when compared with free zoledronic acid.

The use of nanosized drug delivery systems has advantages including biocompatibility, biodegradability, non-toxicity, targetability, and increasing the blood circulation time. Liposomes and niosomes are the most commonly investigated drug delivery systems used for releasing both drugs and diagnostic agents to a target area. Liposomes are formed by self-sustainable bilayered structures comprising phospholipids. Niosomes are very similar to liposomes but are non-ionic surfactant vesicles. They can also carry a variety of drugs with different physicochemical properties such as hydrophilic, lipophilic, and amphoteric molecules via entrapment inside the hydrophilic core or anchoring on the lipid bilayer. Liposomes and niosomes can be passively targeted with the help of nanosized formulations and the formation of steric hindrance created by altering surface charge, surface properties, particle size, proper phospholipid packing, and the extent of steric hindrance. Brain targeting can be obtained by increasing blood circulation time rather than targeting reticuloendothelial system (RES) organs like the liver and spleen. Sterically stabilized long circulating liposomes and niosomes can penetrate the brain. Tyrosinase-encapsulated liposomes administered by stereotaxic injection resulted in a significant increase in dopamine levels in the rat brain. In another study, dopamine-encapsulated liposomes were stereotactically implanted into the corpus striatum of PD model rat brains. A sustained release of dopamine was obtained for 40 days. A rise in extracellular dopamine levels and partial behavioral recovery were achieved with the use of dopamine liposomes when compared with control liposomes, and the combination of both liposomal formulations resulted in a synergistic effect. It was also observed that dopamine was efficiently delivered to the brain by passive targeting and protected from brain degradation by incorporation into liposomes when compared with plain dopamine, L-dopa preparations, and a marketed formulation of L-dopa containing carbidopa (Syndopa®).
Although numerous studies were performed about the therapeutic efficacy of pramipexole\(^1, 2, 24-30\) and the use of some delivery systems for PD treatment\(^20, 23\), the antiparkinsonian effect and brain penetration of pramipexole using liposome and niosome formulations have not yet been explored.

Accurate and specific diagnosis with molecular imaging at the cellular and molecular level via different modalities is useful for providing early and effective PD therapy. Imaging of the dopamine transporter (DAT) can be performed by positron emission tomography (PET) and single-emission photon tomography (SPECT) imaging using radiolabeled cocaine derivatives such as \(^{18}\text{F}\)-fluorinated-N-3-fluoropropyl-2-\(\beta\)-carboxymethoxy-3-\(\beta\)-(4-iodophenyl)nortropane (\([^{18}\text{F}]\text{FP-CIT}\)) and \(^{99}\text{Tc}\)-TRODAT-1, which are commercially available.\(^31\) Autoradiography can also be used for \textit{in vitro} and \textit{ex vivo} imaging of animal models using target-specific ligands such as \(\beta\)-CIT\(^32\) and PE2I\(^33\) after radiolabeling with a proper radionuclide. N-(3-iodopro-2E-enyl)-2\(\beta\)-carbomethoxy-3\(\beta\)-(4'-methylphenyl) nortropane (PE2I) is a cocaine derivative and a very potent radiopharmaceutical for imaging DAT using SPECT, PET, and autoradiography techniques after radiolabeling.\(^33\) 2\(\beta\)-carbomethoxy-3\(\beta\)-(4'-iodophenyl) tropane (\(\beta\)-CIT)\(^32\) has a greater affinity for DAT compared to that of cocaine (Ki = 1.6 vs. 221 nM). The structure of \(\beta\)-CIT was modulated for obtaining a more DAT-selective ligand. The pharmacological properties of PE2I are ideal in regards to having a good affinity for DAT (4 nM) and being one of the most selective DAT ligands. \textit{Ex vivo} autoradiography performed in rats has shown that high levels of \([^{125}\text{I}]\text{PE2I}\) accumulate in the striatum and also in the substantia nigra and ventral tegmental areas.\(^33\) Other DAT radioligands have also been developed such as \([^{18}\text{F}]\text{-FE-PE2I}\), \([^{18}\text{F}]\text{FECT}\), and \([^{18}\text{F}]\text{LBT-999}\).\(^36, 37\)

Although numerous studies were performed about the therapeutic efficacy of pramipexole\(^1, 2, 24-30\) and the use of some delivery systems for PD treatment\(^20, 23\), the antiparkinsonian effect and brain penetration of pramipexole using liposomes and niosomes have not yet been explored as an alternative to oral Pramipexole dosage form using in neurology clinics for PD treatment. The objective of this study was to formulate novel,
nаносизированный антипаркинсонийный препарат (прамипексол диидроклорид гидрат) включает нейтральные и положительно заряженные PEGylated "stealth" липосомы и низомы для эффективной терапии PD. Низомы могут служить хорошей альтернативной системой для проникновения в BBB в зависимости от их нон-ионной поверхностной активной природы. Характеризация и исследование кинетики релизиса формулаций были проведены. BBB проникновение липосом и низомов было оценено в модели BBB Cell Co-Culture с использованием интенсивности флуоресценции и микрофлуоресцентных изображений. Инвивидуальная терапевтическая эффективность наносизированных, прамипексола включенных липосомальных и низомальных формулаций была мониторирована и сравнивала с помощью дополнительных методов, таких как вращательное поведение и ауторадиография в эксперименте на крысах с PD, полученных под влиянием частичного 6-гидроксидопамина (6-OHDA) билатерального штамбиального повреждения. Ожидается, что в 6-OHDA поврежденных крысах будет получен потенциальный результат, который также может привести к дальнейшим исследованиям с большим количеством животных, что может привести к созданию коммерческих препаратов также для пациентов с PD в клиниках в будущем для эффективной терапии с уменьшенными побочными эффектами и уменьшенной частотой введения, что является очень значимым для пациента по соблюдению терапии.

**METHODS**

**Materials**

Дипалмиотилфосфатидилхолин (DPPC) (Phospholipon GmbH, Cologne, Germany) как фосфолипиды, алкохолят цетилпигментной полиэтиленгликолевой (polyglyceryl-3 cetyl ether; SUR II, Loreal, France) как нонионный поверхностно активный компонент, холестерол (Chol; Sigma-Aldrich, St. Louis, Missouri, USA) как билярный конденсатор и стеариламин (Sigma Chemical Co, USA) как положительный ионный индуктор были использованы в формулациях. 1,2-Дистеарил-сп-глицерол-3-фосфатэтаноламин-N-[метокси(полиэтиленовый гликоль)-2000] (аммонийная соль) (PEG2000-DSPE; Avanti Polar Lipids, Inc., Alabaster, Alabama, USA) использовался для PEGylation. Прамипексол диидроклорид гидрат (Abdi Ibrahim İlaç Sanayi, Turkey) использовался как нерготный дофаминергический агент и как антипаркинсонийный препарат. Прамипексол диидроклорид гидрат использовалась вместо прамипексола из-за высокой водной растворимости (более 20%). Слово "прамипексол" используется вместо "прамипексол диидроклорид гидрат" в течение всего текста. Все остальные реагенты и компоненты буферного раствора использовались в качестве аналитического-класса подготовки.
Formulation of Nanosized, PEGylated, Pramipexole-Encapsulated Liposomes

Long-circulating, pramipexole-encapsulated, PEGylated, neutral and positively charged liposomes [DPPC:Chol:PEG2000-PE (200 µmol.ml\(^{-1}\)) (10:5:1 %, molar ratio) and DPPC:Chol:PEG2000-PE:SA (10:4:1:1 %, molar ratio)] were prepared according to the film method.\(^{30}\) Briefly, lipid mixtures were dissolved in chloroform. After removing chloroform at reduced pressure in a vacuum, the lipid film was then hydrated with pramipexole (5 mg ml\(^{-1}\)) comprising Tris (20 mM, pH 7.4) buffer at 70°C for 30 min.

Multilamellar vesicles (MLV) were obtained and then extruded at 70°C with an extrusion unit (Avestin Inc., Germany). Liposomal dispersions were extruded two times by passing through a polycarbonate filter with gradually decreasing pore sizes of 0.6 \(\mu\)m, 0.4 \(\mu\)m, and 0.2 \(\mu\)m (Nucleopore® Track-Etch Membrane; Whatman Inc., New Jersey, USA). Dialysis was performed against Tris (20 mM, pH 7.4) buffer through a dialysis cellulose membrane (13000 MW cut off) (Sigma-Aldrich Chemie GmbH, Munich, Germany) for 12 h with stirring to remove unencapsulated pramipexole.

Formulation of Nanosized, PEGylated, Pramipexole-Encapsulated Niosomes

Long-circulating, pramipexole-encapsulated, PEGylated, neutral and positively charged niosomes [SURII:Chol:PEG2000-PE (200 µmol.ml\(^{-1}\)) (10:5:1 %, molar ratio) and SURII:Chol:PEG2000-PE:SA (10:4:1:1 %, molar ratio)] were prepared according to the film method.\(^{38}\) The only differences were the temperature (40°C) of the hydration and the extrusion procedures.

The codes of pramipexole-encapsulated liposomes and niosome dispersions are listed in Table 1.
Table 1. Codes of pramipexole encapsulated, neutral and positively charged liposome and niosome dispersions.

<table>
<thead>
<tr>
<th>Formulations</th>
<th>Codes</th>
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<tbody>
<tr>
<td>Pramipexole encapsulated DPPC:Chol:PEG2000-PE</td>
<td>PPX-DPPC</td>
</tr>
<tr>
<td>Pramipexole encapsulated SURII:Chol:PEG2000-PE</td>
<td>PPX-SURII</td>
</tr>
</tbody>
</table>

Characterization of Pramipexole-Encapsulated Liposome and Niosome Formulations

The mean particle size, zeta potential, and encapsulation efficiency of nanosized PPX-DPPC and PPX-DPPC:SA liposome and PPX-SURII and PPX-SURII:SA niosome formulations were evaluated. Additionally, liposomal phospholipid quantity was determined for both PPX-DPPC and PPX-DPPC:SA liposomes. In addition, the release of pramipexole from encapsulated liposome and niosome dispersions was assessed in vitro.

Mean Particle Size and Zeta Potential of Liposomes and Niosomes

Mean particle size and zeta potential of PPX-DPPC and PPX-DPPC:SA liposomes and PPX-SURII and PPX-SURII:SA niosomes were measured using the dynamic light scattering method with the Nano-ZS (Malvern Instruments, Malvern, UK) at 25°C.

Encapsulation Efficiency (%) of Liposomes and Niosomes

After removing unencapsulated pramipexole by dialysis, liposome and niosome vesicles were lysed by ethanol. The encapsulated pramipexole amount was determined spectrophotometrically at a wavelength of 263 nm. Encapsulation efficiency (%) was calculated with the help of standard line and line equations obtained previously. The percentage of entrapped drug was calculated by applying the following equation:

\[
\% \text{ Entrapment} = \frac{(D_E \times 100)}{(D_i)},
\]

(1)
where $DE$ is the amount of entrapped drug and $DI$ is the initial amount of drug.

**Liposomal Phospholipid Amount**

Phospholipid contents of PPX-DPPC and PPX-DPPC:SA liposome formulations were determined by the modified Rouser method, relying on the determination of phosphorus content after perchloric acid destruction at 797 nm spectrophotometrically.

**In Vitro Release Kinetics of Pramipexole from Liposome and Niosome Formulations**

*In vitro* drug release studies of pramipexole-encapsulated, neutral and positively charged liposomes and niosomes were performed using the dialysis method with a dialysis membrane (MWCO: 3.5 kDa) at 37 ± 1°C with 100 rpm against a release medium of Tris buffer (20 mM, pH=7.4). Briefly, 1 ml of pramipexole-encapsulated liposome and niosome dispersions in dialysis tubing were immersed in 10 ml of Tris buffer (20 mM, pH=7.4), used as release medium, and agitated in a shaker at a rate of 100 oscillations per minute at 37°C. Then, 1 ml samples were taken at specific time intervals (15 min and every half hour up to 7 h) and replaced with the same volume of fresh Tris buffer (20 mM, pH=7.4). The amount of pramipexole in the release medium was measured spectrophotometrically at a wavelength of 262 nm. The release kinetics of PPX-DPPC and PPX-DPPC:SA liposomes and PPX-SURII and PPX-SURII:SA niosomes were calculated using absorbance values with the *In Vitro-In Vivo Kinetics, Version 1.0.40* program.

**In Vitro BBB Cell Co-Culture Studies**

*In vitro* BBB penetration of fluorescence-labeled, nanosized, PEGylated, pramipexole-encapsulated, neutral and positively charged liposomal and niosomal dispersions were investigated using a BBB cell co-culture model in Transwell® plates with astrocytes in the bottom and endothelial cells in the top part.

To obtain a polarized BBB-symbolizing co-culture model, 50,000 cells cm$^{-2}$ were seeded on attachment factor (4Z0-210, Cellsystems)-coated 6-well plates with 0.4 μm pores.
in semi-transparent membrane indentations (0.3 cm² indentation⁻¹). Endothelial cell layers divided this system into apical (blood side) and basolateral (brain side) sides. This setup provides a model of both sides of the BBB. Human brain microvascular endothelial cell membranes were sustained with fetal bovine serum (5%), penicillin/streptomycin (1%) solution, and endothelial cell growth supplement (ECGS) (1%). In order to formulate a co-culture model, astrocytes were seeded on 6-well plates (1.9 cm² well⁻¹) at 50,000 cells cm⁻². Human astrocyte medium consisted of fetal bovine serum (5%), penicillin/streptomycin (1%) solution, and astrocyte growth factor (1%). All cells were cultured in a humidified incubator at 37°C with CO₂ (5%). Medium was changed every 5–7 days until the cells were properly attached.

To prepare the fluorescence-labeled liposome and niosome dispersions, Rhodamine-PE (0.5% mmol) was added to the lipid bilayer during the preparation procedure. After the preparation procedure, Rhodamine-labeled formulations were dialyzed against Tris (20 mM, pH 7.4) buffer through a dialysis cellulose membrane (13000 MW cut off) (Sigma-Aldrich Chemie GmbH, Munich, Germany) for 12 h with stirring to remove unlabeled free Rhodamine-PE. These neutral and positively charged liposomes and niosomes were added to the endothelial cells (1.5 ml) (n=6 for each group). Formulations were incubated for 2 h with human astrocyte and human brain microvascular endothelial cells at 37°C with CO₂ (5%). At 0 and 120 min after incubation, 100 µl samples were collected from the bottom of each Transwell® plates. Fluorescent microscope (Inverted microscope Olympus, U.K.) images were taken (40 times extension) and relative fluorescence intensity was measured using fluorospectroscopy (Spectrofluorometry (RF-5301PC), Shimadzu, Japan) at 0, 30, 60, 90, 120, 150, and 180 min. To observe fluorescence intensity of penetration, excitation and emission spectrums were scanned. Excitation was determined as 560 nm and emission was determined as 583 nm.
In Vivo Small Animal Studies

According to the characterization and release studies, PPX-DPPC liposomes and PPX-SURII niosomes were selected as optimum formulations, and they were investigated for their therapeutic efficacy in a PD animal model.

All procedures were conducted in accordance with the requirements of the European Community Council for the care of laboratory animals 86/609/EEC and after approval of the regional ethical committee (No: 00434.02). Experiments were carried out on 26 adult male Wistar rats each weighing 250–300 g at the beginning of the experiments. Animals were housed in groups of two per cage in a temperature and humidity controlled environment (temperature 22ºC ± 1ºC; relative humidity 40% ± 7%), with a 12 h light/dark cycle and standard feeding conditions (ad libitum).

In Vivo Parkinson Model by 6-Hydroxydopamine (6-OHDA) Lesion

All rats were administered 6-OHDA in order to generate a Parkinson’s model. Lesions were made as previously described.43 Twenty minutes before surgery, animals were injected intraperitoneally with pargyline (50 mg.kg⁻¹). Rats were then anesthetized with isoflurane (5% for induction) and placed on a stereotaxic apparatus. They were maintained under isoflurane (3%) throughout surgery. To protect the eyes and prevent drying, a protector gel (Ocry-gel) was applied. The skull was exposed and small holes were made with a dental drill. Lesions were made using unilateral intrastriatal injection of 6-OHDA hydrochloride (1 mg.ml⁻¹). A total of 10 µg of 6-OHDA was administered in two points of the right striatum (1 mg.ml⁻¹ in 0.01% ascorbic acid, pH 4.5, i.e., 5 µg in 5 µl for each point) with a Hamilton syringe (gauge 25) at a flow rate of 1 µl min⁻¹ for 5 min. The coordinates from bregma were AP = +0.5 mm, L = -2.5 mm, P = -5 mm, and AP = -0.5 mm, L = -4 mm, P = -5 mm, according to the atlas of Paxinos and Watson (1986).44 The syringe was left in place for 4 min after injection and then removed slowly to optimize toxin diffusion. After surgery, the rats were given buprenorphine (0.05 mg/kg subcutaneously) for post-operative pain and were allowed to recover from surgery for 7 days before being subjected to the induced-rotation test.
Study Design

All animals were tested for their rotational behavior in response to D-amphetamine sulfate injection (3 mg.kg\(^{-1}\), i.p.) both for determination of a successful lesion and therapeutic efficacy of pramipexole-encapsulated, nanosized liposomal and niosomal drug delivery systems at 7, 14, and 21 days post lesion (dpl). After amphetamine injection, the rats were placed in automated rotometer bowls, and 15 min later full body turns were monitored by a computer for 90 min. The number of turns the animal made on itself clockwise (ipsilateral rotations) (turns\(^{-}\)) and anticlockwise (contralateral rotations) (turns\(^{+}\)) were automatically recorded. General activity was calculated by (turns\(^{-}\) - turns\(^{+}\)) and some behaviors such as grooming, rearing, or absence of behavior were not included in this count. The results are expressed as the mean number of ipsilateral turns/min ± SEM.

Only those rats showing on average more than 8 ipsilateral turns per min at 7 dpl were selected for further experiments as it was assumed that they represented a good PD model.\(^{43, 45, 46}\) A total of 24 rats were selected as fitting this description. A schematic representation of the experimental protocol is depicted in Figure 1.

**Figure 1.** Schematic representation of experimental protocol.
The rats were divided into four groups in which PPX-DPPC liposomes, PPX-SURII niosomes, pramipexole solution, or Tris buffer (control group) (0.2 ml of all formulations per one injection) were applied by i.p administration 3 times a week for 21 days. All formulations contained the same amount of pramipexole (0.5 mg/ml⁻¹) to compare the therapeutic efficacy. Therapeutic efficacy was evaluated by rotational behavior at 14 and 21 dpl for each group and with autoradiography of lesioned and intact striatum of rats after sacrifice.

**Monitoring of Therapeutic Efficacy by Rotameter and Autoradiography Studies**

All formulations containing the same amount of pramipexole (0.5 mg.ml⁻¹) were compared for their therapeutic efficacy. Therapeutic efficacy was evaluated by rotational behavior after 14 and 21 dpl for each group with autoradiography of both lesioned and intact striatum.

**Rotameter Study**

All animals were tested for their rotational behavior in response to D-amphetamine sulfate injection (3 mg.kg⁻¹, i.p.) both for determination of a successful lesion and therapeutic efficacy of pramipexole-encapsulated, nanosized liposomal and niosomal drug delivery systems at 7, 14, and 21 dpl.

After amphetamine injection, the rats were placed in automated rotometer bowls, and 15 min later full body turns were monitored by a computer for 90 min. The number of turns the animal made on itself clockwise (ipsilateral rotations) (turns⁻) and anticlockwise (contralateral rotations) (turns⁺) were automatically recorded. General activity was calculated by (turns⁻ - turns⁺) and possible behaviors such as grooming, rearing, or absence of behavior were not included in this count. The results are expressed as the mean number of ipsilateral turns/min ± SEM.

The increase in the therapeutic antiparkinsonian efficacy of the formulations could be observed by the decrease in the mean number of ipsilateral turns/min.
**Autoradiography Study**

The DAT density was measured by quantitative autoradiography after sacrificing of rats at 22 dpl using $^{125}$IPE2I, as previously described. After sacrifice, the brains were removed and frozen in isopentane cooled to -35°C. Coronal sections (16 µm thickness) were then cut on a cryostat microtome and thaw-mounted on Super-FrostPlus® slides. Sections were maintained at -80°C until use. For binding experiments, sections were allowed to equilibrate at 25°C for 3 h. They were then incubated with 100 pM $^{125}$IPE2I in 100 µl of a pH 7.4 phosphate buffer (10.14 mM NaH$_2$PO$_4$, 137 mM NaCl, 2.7 mM KCl, 1.76 mM KH$_2$PO$_4$) at room temperature for 90 min. Nonspecific binding was assessed on adjacent sections in the presence of 100 µM cocaine. Slides were then washed twice for 20 min in ice-cooled phosphate buffer (4°C), rinsed for 1 sec in distilled water, and dried at room temperature. Dried slides were made conductive by the application of metal electric tape on the free side and then they were placed in the gas chamber of the β-imager. Data from brain sections were collected for at least two hours. The regions of interest (left and right striatum) were selected manually. Using the β-vision software, the level of bound radioactivity was directly determined by counting the number of β-particles emitted from the delineated areas. The radioligand signals in the regions of interest (ROIs) were measured on at least eight sections for each rat and are expressed as cpm.mm$^{-2}$. Specific binding was determined by subtracting nonspecific binding from total binding (SB = TB - NSB). The results are expressed as the percentage of binding on the ipsilateral compared to contralateral side. The increase in the therapeutic antiparkinsonian efficacy of formulations can be observed from the decrease in the loss of binding [(Non-lesioned part - lesioned part)/Non-lesioned part] (%) at the ipsilateral side.

**Statistical Analysis**

All values are expressed as the mean ± SEM. If the number of data points was less than 30, nonparametric test methods were used for data evaluation. Depending on the group number, the Mann-Whitney U test was used for comparison of two groups and the Kruskal Wallis was used for comparison of three or more groups. To compare the rotational behavior
between groups and at different dpl, one way analysis of variance (ANOVA) and Mann-Whitney U tests were performed. To analyze the autoradiography data, the Mann-Whitney U test was used. All tests were two-sided. The significance level was set at p < 0.05.

RESULTS

Characterization of Nanosized, Pramipexole-Encapsulated Liposomes and Niosomes

Long-circulating, PPX-DPPC and PPX-DPPC:SA liposomes and PPX-SURII and PPX-SURII:SA niosomes were prepared and characterized for the treatment of PD. Particle size and size distribution, zeta potential, encapsulation efficiency (EE%) of liposomes, and niosome dispersions are listed in Table 2. Phospholipid amounts of PPX-DPPC and PPX-DPPC:SA liposome dispersions were evaluated and the data are listed in Table 3.

Table 2. Mean particle size, zeta potential and encapsulation efficiency of neutral and positively charged pramipexole encapsulated liposomes and niosomes (Data represented as mean ± SD (n=6)).

<table>
<thead>
<tr>
<th>FORMULATION</th>
<th>MEAN PARTICLE SIZE (nm) and PDI</th>
<th>ZETA POTENTIAL (mV)</th>
<th>EE (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPX-DPPC</td>
<td>122 ± 0,13 (0,12*)</td>
<td>-10,60 ± 0,12</td>
<td>9,42 ± 0,01</td>
</tr>
<tr>
<td>PPX-DPPC:SA</td>
<td>115 ± 0,21 (0,14*)</td>
<td>-9,71 ± 0,09</td>
<td>8,94 ± 0,02</td>
</tr>
<tr>
<td>PPX-SURII</td>
<td>103 ± 0,37 (0,13*)</td>
<td>-13,80 ± 0,21</td>
<td>10,51 ± 0,01</td>
</tr>
<tr>
<td>PPX-SURII:SA</td>
<td>112 ± 0,19 (0,17*)</td>
<td>-11,70 ± 0,35</td>
<td>9,84 ± 0,02</td>
</tr>
</tbody>
</table>

Data presented as mean ± SD (n=6).
Table 3. Phospholipid amount and phospholipid efficiency of neutral and positively charged nanosized, pramipexole encapsulated liposomes.

<table>
<thead>
<tr>
<th>FORMULATIONS</th>
<th>PHOSPHOLIPID AMOUNT (µmol lipid.mL⁻¹)</th>
<th>PHOSPHOLIPID EFFICIENCY (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPX-DPPC</td>
<td>90,4 ± 2,1</td>
<td>91,6 ± 2,2</td>
</tr>
<tr>
<td>PPX-DPPC:SA</td>
<td>86,6 ± 3,2</td>
<td>87,7 ± 3,3</td>
</tr>
</tbody>
</table>

Data presented as mean ± SD (n=6).

There were no significant differences observed between particle size and zeta potential of formulations. No statistically significant differences were observed within the neutral and positively charged liposomes and niosomes (p>0.05).

In Vitro Release Kinetics of Pramipexole from Liposome and Niosome Formulations

In vitro release kinetics of neutral and positively charged, nanosized liposomes and niosomes were evaluated by the dialysis method.⁴⁸

For a better comparison, cumulative release (%) and in(cumulative release) versus time graphs are shown in Figure 2 and Figure 3 for both neutral and positively charged, nanosized, pramipexole-encapsulated, PEGylated liposomes and niosomes.
Figure 2. In vitro release of pramipexole from neutral and positively charged liposomal and niosomal formulations in Tris (20 mM, pH 7.4) buffer [Cumulative Release(%)/Time(h) curve] (n=6).

Figure 3. In vitro release of pramipexole from neutral and positively charged liposomal and niosomal formulations in Tris (20 mM, pH 7.4) buffer [ln(Cumulative Release)/Time(h) curve] (n=6).

As shown in Figures 2 and 3, both neutral and positively charged liposomes and niosomes were fitted to first-order release kinetics. First-order release kinetics can be used to describe the drug dissolution in pharmaceutical dosage forms containing water-soluble
drugs in porous matrices. First-order release kinetics apply when a constant portion of drug concentration is available at a time. Therefore, the process depends on the initial drug concentration and the rate of the process is directly proportional to the drug concentration. A number of studies were performed designating first order release kinetics to liposomal formulations, supporting our data related to the first order release of pramipexole from liposomal and niosomal formulations.

**In Vitro BBB Cell Co-Culture Studies**

*In vitro* BBB penetration of pramipexole-encapsulated neutral and positively charged liposomal and niosomal dispersions was investigated in a BBB cell co-culture model using Transwell® plates with astrocytes at the bottom and endothelial cells at the top. Fluorescence microscopy images were taken at 0 and 2 h after the application of PPX-DPPC, PPX-DPPC:SA, PPX-SURII, and PPX-SURII:SA formulations on the Transwell® plates (Fig. 4).

**Figure 4.** Bright light images and fluorescence microscopy images showing the penetration of BBB Cell Co-Culture model of neutral and positively charged liposomes and niosomes at different time points (Magnification: 40x).

In order to observe the significance of penetration through the BBB cell co-culture model, relative fluorescence intensity was also measured after sampling at the bottom part of the model to evaluate BBB penetration of pramipexole-encapsulated neutral and positively charged liposomes and niosomes.
charged liposomal and niosomal dispersions by spectrofluorometry at excitation: 560 nm and emission: 583 nm wavelengths at 0, 30, 60, 90, 120, 150, and 180 min (Fig. 5).

**Figure 5.** Relative fluorescence intensities showing penetration of BBB Cell Co-Culture model of neutral and positively charged liposomes and niosomes at different time intervals (n=6).

All formulations showed an increase in relative fluorescence intensity at 120 min when compared with 30, 60, and 90 min (p<0.05). No significant increase was observed in relative fluorescence intensity from 120 to 180 min (p>0.05).

**Monitoring of Therapeutic Efficacy**

**Rotometer Test**

The rotational behavior was evaluated in 6-OHDA-lesioned rats intraperitoneally administered PPX-DPPC liposomes, PPX-SURII niosomes, pramipexole solution, or Tris buffer (control group) at 7, 14, and 21 dpl (Figure 6).
Figure 6. The mean values of number of ipsilateral turns in min. at 7, 14 and 21dpl after i.p. administration of nanosized, PPX-DPPC liposomes, PPX-SURII niosomes, pramipexole solution and control (n=6).

Regarding the delay after lesion, no significant difference in the number of ipsilateral turns was observed between 7, 14, and 21 dpl in the control and PPX groups. However, a slight increase was observed in the PPX-DPPC group at 21 dpl (p<0.0421 vs. control) and in the PPX-SURII group at 14 and 21 dpl (p<0.0362 and 0.0459, respectively). At 7 and 14 dpl, the number of turns was similar in the 4 groups. However, at 21 dpl a slight increase was observed in the PPX-SURII compared to the control group (p<0.0294).

Autoradiography Studies

DAT [125I]PE2I autoradiography was performed in both ipsilateral and intact striatum in order to evaluate the efficacy of PPX-DPPC liposome and PPX-SURII niosome formulations on the integrity of dopamine neurons. Illustrations of the autoradiograms are shown in Figure 7.
Figure 7. Autoradiograms of brain striatum of 6-OHDA lesioned rats i.p. administered (a) nanosized, PPX-DPPC liposomes, (b) nanosized, PPX-SURII niosomes, (c) pramipexole solution and (d) Tris buffer (20 mM, pH:7.4) (while upper parts representing ipsilateral (lesioned part) of the striatum, bottom parts represents controlateral parts of the striatum of the brain (control)).

It was observed from the autoradiograms of striatal cross-sections that while loss in the accumulation of $[^{125}\text{I}]\text{PE2I}$ in the lesioned vs. intact striatum of rats administered PPX-SURII niosomes or pramipexole solution were significantly lower, this was due to less dopamine neurodegeneration after 21 days of therapy. Enhanced therapeutic effect and less dopaminergic cell loss were observed in the rats i.p. administered PPX-SURII niosomes after
21 days of therapy. However, as shown in the autoradiograms of striatal cross-sections of rats administered PPX-DPPC liposomes and the control group, loss in the accumulation of \[^{125}\text{I}]\text{PE2I}\) in the lesioned vs. intact striatum was higher compared with the group treated with PPX-SURII niosomes and pramipexole solution.

The percent loss in the accumulation of \[^{125}\text{I}]\text{PE2I}\) in the lesioned vs. intact striatum was calculated and results are shown in Figure 8.

![Figure 8](image)

**Figure 8.** The percent loss in the radioactivity accumulation in the lesioned part of 6-OHDA partial lesioned PD model rats after i.p. administration of nanosized, PPX-DPPC liposomes, PPX-SURII niosomes, pramipexole solution and Tris buffer (control) (n=4).

The percent loss of \[^{125}\text{I}]\text{PE2I}\) binding was similar in the control and PPX-DPPC groups. In contrast, this loss was significantly reduced in both the PPX and PPX-SURII groups.

**DISCUSSION**

Nanosized, long-circulating PPPX-DPPC and PPX-DPPC:SA liposomes and PPX-SURII and PPX-SURII:SA niosomes were formulated for PD therapy. Because of the high
water solubility (more than 20%) of pramipexole dihydrochloride monohydrate,\textsuperscript{58} it was encapsulated in liposomal and niosomal formulations as the salt form of pramipexole for PD therapy. The characterization of neutral and positively charged liposomes and niosomes was performed.

Less liposomal phospholipid loss was observed in PPX-DPPC liposomes when compared with PPX-DPPC:SA liposomes, but this difference was not statistically significant (p>0.05).

All formulations were defined as nanosized (around 100 nm). This is very significant for long-circulation and passive targeted by PEGylation to obtain “stealth” formulations that are essential for BBB penetration.\textsuperscript{59, 60} Niosomes have a slightly smaller particle size when compared with liposomes. This may be related to their non-ionic surfactant characteristics, and vesicle size may easily be reduced by extrusion because they have more elasticity than phospholipids. Positively charged liposomes and niosomes had slightly increased particle sizes when compared with neutral ones.

Zeta potential designates the magnitude of the electrostatic repulsion/attraction between particles or vesicles of formulations. It is one of the basic parameters affecting stability and shelf-life of formulations. It provides detailed information about dispersion, aggregation, or flocculation and can be applied to improve the formulation of dispersions, emulsions, and suspensions (Malvern Instruments-Zeta Potential). The surface charge of the vesicles also plays an important role in the in vivo performance of liposomes and niosomes.\textsuperscript{61} A high absolute value of the zeta potential indicates stability of colloidal distributions due to a greater electric charge on the surface of the nanoparticles, preventing aggregation via strong repellent forces among the particles or vesicles.\textsuperscript{62, 63} As the zeta potential increases, the charged particles repel one another and they become more stable against aggregation.\textsuperscript{64} Nanoparticles with zeta potentials greater than +25 mV or less than -25 mV typically have high degrees of stability. Dispersions with very low zeta potential values will eventually aggregate as a result of Van Der Waals inter-particle attractions.\textsuperscript{65} The zeta potential of niosomes was slightly higher when compared with liposomes. This may be
due to their non-ionic surfactant characteristics. Neutral liposomes and niosomes showed slightly higher zeta potentials when compared with positively charged ones. The results revealed that the zeta values of the niosome vesicles seem to be more negative when compared with liposome vesicles.

Pramipexole encapsulation efficiency of niosomes seemed to be slightly higher than that of liposomes. Although the hydrophilic solution encapsulated within the liposome vesicles was very limited (5–10%), all formulations exhibited about 10% encapsulation efficiency, which is very high for hydrophilic drugs. This may be a result of the higher encapsulation ability of niosomes containing non-ionic surfactant vesicles. Both neutral liposomes and niosomes had slightly higher encapsulation efficiencies when compared with positively charged ones.

In vitro release kinetics of liposomes and niosomes are meaningful for predicting an efficient therapeutic effect. The release of active ingredients is faster from drug delivery systems composed of liquid-crystalline type phospholipids, depending on the distance within the polar head groups of liquid crystalline-type phospholipids, and is also faster than in gel state phospholipids. Differently charged liposome formulations containing DPPC as the phospholipid showed slightly better in vitro pramipexole release rates. The release from liposomes may be more effective than niosome formulations. However, this difference was not statistically significant. The release rate of positively charged liposomes and niosomes was less than that of neutral ones initially. However, this difference was not statistically significant. Our results were comparable with previously published results. It was observed that diffusion from the liquid phase leads to formation of first-order release kinetics from liposomes. Some deviations may be observed in obtaining a linear line while drawing the log cumulative release versus time plots for liposomal and niosomal delivery systems depending on the phospholipid structure and hydrophilic polymer (PEG) on the liposomal vesicles. This is related to the release of active substances from the phospholipid structure and hydrophilic polymer at the outer shell.
The penetration of fluorescence-labeled, pramipexole-encapsulated neutral and positively charged liposomal and niosomal dispersions was evaluated in a BBB cell co-culture model by inspection of fluorescent microscope images and measuring relative fluorescence intensity obtained by fluorospectroscopy.

The increase in the fluorescence intensity in fluorescent images was related to the penetration. Fluorescence intensity was increased in all formulations at 120 min (p<0.05); neutral liposomal and niosomal formulations exhibited higher fluorescence intensity when compared with positively charged ones.

No significant increase was observed in relative fluorescence intensity from 120 to 180 min. The difference between relative intensities of liposomal and niosomal formulations was not significant. According to both fluorescent microscope images and relative fluorescence intensity obtained by fluorospectroscopy, it can be concluded that all formulations penetrated the BBB cell co-culture with a maximum observed at 120 min.

Based on the in vitro results, the optimum neutral, pramipexole-encapsulated liposome and niosome formulations were applied to animals to monitor therapeutic efficacy. Smaller particle size, proper zeta potential, larger encapsulation efficiency, and better release kinetics were obtained with these neutral formulations when compared with positively charged ones.

The rotational behavioral test can be used for observing the effect of new drugs on dopaminergic activity. Briefly, after a unilateral lesion of the nigrostriatal dopamine receptor (DA) system with the neurotoxin 6-OHDA and following amphetamine injection, animals circle towards the side with the 6-OHDA lesion (ipsiversive). The increase in therapeutic antiparkinsonian efficacy of formulations can be observed by the decrease in the mean number of ipsilateral turns/min.

The number of ipsilateral turns after i.p. administration of nanosized PPX-DPPC liposomes, PPX-SURII niosomes, pramipexole solution, and Tris buffer (as control) were similar at 7, 14, and 21 dpl. The results indicated that no reduction was observed in the
control group or treated groups, regardless of the treatment. In addition, an increased number of ipsilateral turns per minute was observed after i.p. administration of nanosized PPX-DPPC liposomes and PPX-SURII niosomes at 21 dpl (p<0.05). Although this unexpected observation may indicate aggravation of the lesion after treatment with PPX-DPPC liposomes and PPX-SURII niosomes, this is not possible because of the phospholipid structure of the liposomes and nonionic surfactant structure of the niosomes.

In order to evaluate the therapeutic efficacy of formulations in greater detail, autoradiography studies were performed. Autoradiography is a quantitative and image-based test, and it is more reliable than rotometer testing based on the ability to visualize and quantify the location of radioactivity obtained from DAT. The increase in the antiparkinsonian effect of formulations can be observed from the decrease in the loss of binding [(Non-lesioned part - lesioned part)/Non-lesioned part] (%) at the ipsilateral side. The therapeutic effect of both PPX-SURII and pramipexole solution was found to be similar in the autoradiograms. The increase in the loss of binding [(Non-lesioned part - lesioned part)/Non-lesioned part] (%) was found to be higher after therapy with PPX-DPPC liposomes when compared with PPX-SURII niosomes (p<0.05). The percent loss of binding was decreased in both PPX-SURII niosomes and pramipexole solution when compared with neutral PPX-DPPC liposomes (p<0.05). Both PPX-SURII niosomes and pramipexole solution had beneficial PD therapeutic effects (p<0.05). The autoradiography study results indicated that treatment with pramipexole-encapsulated niosomes had a positive therapeutic effect.

Better efficacy of neutral, pramipexole-encapsulated, nanosized niosomes likely depends on their non-ionic surfactant properties and nanosize, which enhance the BBB penetration properties. Permeability and penetration of niosomes through the BBB is enhanced by the opening of tight and rigid junctions of endothelial cells. Neutral niosomes had a beneficial therapeutic effect that was similar or even better when compared with pramipexole solution in autoradiography studies, even at dosages less than those administered in neurology clinics. The administration protocol of pramipexole tablets in clinics is three times of 0.125 mg doses per day during the first week before being increased
to a maximum of 4.5 mg day\(^{-1}\). The pramipexole dose given with pramipexole-encapsulated, nanosized, PEGylated liposomes and niosomes was 0.043 mg day\(^{-1}\). This dose administered to rats is approximately 9 times less than the conventional minimal dose of pramipexole tablets for PD patients in neurology clinics. Nanosized, PEGylated, neutral pramipexole-encapsulated niosomes were found to be beneficial for PD therapy, BBB permeable, and effective at a dosage 9 times less than that administered in humans in neurology clinics.

**CONCLUSION**

The characterization of both neutral and positively charged, nanosized, pramipexole-encapsulated, PEGylated liposome and niosome formulations was performed. It was observed that with i.p. administration of nanosized, PEGylated, pramipexole-encapsulated, neutral niosomes, similar or even better therapeutic effects were achieved in 6-OHDA-lesioned rats even at a dosage approximately 9 times less than that administered with conventional pramipexole tablets in neurology clinics for Parkinson’s Disease patients. This effect is likely due to the non-ionic surfactant properties of niosomes and the nanosize, which can enhance the BBB penetration properties. This significant reduction in pramipexole dose could result in a significant decrease in possible side effects and prevent drug resistance and adverse effects in PD patients. Therefore, these findings could lead to the development of more specific, targeted, nanosized drug delivery systems for both diagnosis and/or therapy of PD in the future.

**ACKNOWLEDGEMENTS**

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DECLARATION OF INTEREST

The authors report no conflicts of interest.

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5. DISCUSSION

BBB penetration of nanosized, pramipexole encapsulated, PEGylated, liposomal and niosomal formulations was evaluated by both fluorescent microscope images and relative fluorescence intensity obtained by fluorospectroscopy and it can be concluded that all formulations penetrate BBB Cell Co-Culture and maximum penetration was observed at 120 min. Pramipexole encapsulated neutral liposomes and niosomes were selected as optimum formulations due to smaller particle size, higher absolute value of zeta potential, larger encapsulation efficiency and better release kinetics were observed with neutral formulations when compared with positively charged ones. When liposomes and niosomes were compared depending on the charge, neutral ones were chosen as optimum formulations to carry out the in vivo studies. Additionally, it would also be better to continue in vivo studies with these neutral formulations depending on higher BBB penetration chance with the neutral ones when compared with charged particles.

Therapy effect of neutral, pramipexole encapsulated liposomes and niosomes were evaluated in 6-OHDA lesioned rats by rotameter test and autoradiography studies. Generally, a stable turn number was observed at 7, 14 and 21 dpl in control and PPX groups and a tendency to increase between 7 and 14 dpl in PPX-DPPC and PPX-SURII and then stabilization was observed between 14 and 21 days for rotameter studies. A tendency of increase was observed at 14 and 21 dpl for both liposomes and niosomes vs control and PPX solution. The therapy effect of neutral, Pramipexole encapsulated DPPC:Chol:PEG2000-PE liposome and SURII:Chol:PEG2000-PE niosome was observed very similar with Pramipexole solution by number of ipsilateral turns in min. This insignificance and similarity in rotameter results may be due to the nature of rotameter which is probably less adapted for a partial lesion. When compared with autoradiography, rotameter test is less reliable and can be effected from different conditions such as small failures in sliding of amphetamine injection site and in performing 6-OHDA lesioned rats and light existence at experimental medium. Of course, the result of rotometer test is not very accurate by itself and should be supported with autoradiography studies which will be very precise to decide.
The increase in the therapeutic efficacy of antiparkinsonian effect of formulations can be observed from the decrease in the loss of binding [(Non-lesioned part-lesioned part)/Non-lesioned part] (%) at the ipsilateral side. The percent loss of DAT binding [(Non-lesioned part-lesioned part)/Non-lesioned part] (%) was found lesser with both Pramipexole encapsulated SURI:Chol:PEG2000-PE niosomes and Pramipexole solution than neutral DPPC:Chol:PEG2000-PE liposomes and Tris buffer. Niosomes gave slightly better results than Pramipexole solution.

With i.p. administration of nanosized, PEGylated, pramipexole encapsulated, neutral niosomes similar but slightly better therapeutic effect was achieved with pramipexole solution at same doses in 6-OHDA lesioned rats. If this dose is compared with conventional pramipexole tablets used for PD patients in Neurology clinics, a beneficial therapeutic effect was achieved at approximately 9 times lesser doses. Because the administration protocol of pramipexole tablets in clinics is three times of 0,125 mg.day$^{-1}$ during the first week then then it can be increased till maximum 4,5 mg.day$^{-1}$ (178,179). The pramipexole dose which was given with pramipexole encapsulated, nanosized, PEGylated liposomes and niosomes is 0,043 mg.day$^{-1}$ (Briefly the encapsulated pramipexole dose of liposomes and niosomes are similar and about 0,5 mg.mL$^{-1}$. The pramipexole dose in pramipexole solution is the same as 0,5 mg.mL$^{-1}$. We administered rats 3 days a week about 0,2 mL of formulations each so the administered volume to a single rat is 0,6 mL.week$^{-1}$. The pramipexole dose administered to a single rat is 0,3 mg.week$^{-1}$ which corresponds to a dose of 0,043 mg. day$^{-1}$) in our study. This dose is approximately 9 times lesser dose given with minimum dosed conventional pramipexole tablets. Nanosized, PEGylated, neutral, pramipexole encapsulated niosomes found BBB permeable, potential and effective in PD therapy. It may be very beneficial to try nanosized, pramipexole encapsulated, neutral liposomes in a large number of 6-OHDA lesioned animals to evaluate antiparkinsonian effect better in the future.
6. CONCLUSION

CNS disorders are one of the first ordered disease to endorse their research in the diagnosis and therapy with several framework projects in Europe and all around the World. The huge gap in the issue of efficient CNS drug delivery and the success of PD therapy needed to be researched and investigated. For the therapy of PD, there is still a long way to go through. As being the first study in the literature, liposomal and niosomal formulations of Pramipexole have never been studied before.

The characterization of both neutral and positively charged, nanosized, Pramipexole encapsulated, PEGylated liposome and niosome formulations was found proper. Pramipexole encapsulated all formulations (DPPC:Chol:PEG2000-PE, DPPC:Chol:PEG2000-PE:SA, SURII:Chol:PEG2000-PE, SURII:Chol:PEG2000-PE:SA) exhibited nanosize and proper zeta potential. All formulations designated about 100nm particle size and a large encapsulation efficiency for Pramipexole (about 10%). Both, neutral and positively charged liposomes showed proper phospholipid content showing the quality of liposome formulations. Additionally, all formulations fitted to first-order release kinetics. BBB penetration of neutral liposome and niosome formulations was found better than positively charged ones at in vitro BBB Cell Co-Culture studies. However, this difference was found statistically insignificant.

Although the therapeutic efficacy of PPX-DPPC and PPX-SURII was found insignificant in rotameter test, nanosized, neutral PPX-SURII niosome designated similar even better effect than pramipexole solution in autoradiography studies in 6-OHDA lesioned rats. This pramipexole dose is approximately 9 times lesser doses applied with conventional pramipexole tablets for humans in Neurology clinics. Therefore, a beneficial therapeutic effect was achieved at significantly lesser doses with nanosized, neutral niosomes. It is most probably depends on the non-ionic surfactant properties of nisomes which can enhance BBB penetration properties. This beneficial effect may probably help reduction in possible side effects and prevent observation of drug resistance and adverse effects at PD patients. It may be very beneficial to try nanosized, pramipexole encapsulated, neutral liposomes in a large
number of 6-OHDA lesioned animals to evaluate antiparkinsonian effect better in the future. These results can also enlighten the further human studies for therapy of PD in the future. Future studies may lead in developing commercial preparations for neurology clinics for effective therapy of PD with decreased side effects and frequency of administration which is very significant for patient’s compliance to the therapy.
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SUPPLEMENTS AND PUBLICATIONS

Supplement 1.

CV

She was borned at Ankara in 1984. She was graduated from Hacettepe University in 2006 and she was won a scholarship from Erasmus Student Exchange Programme to resume her undergraduate programme at Ljubljana University Faculty of Pharmacy in Ljubljana/Slovenia for 5 months in 2005. She was completed her master thesis in 2009 at Hacettepe University Faculty of Pharmacy Department of Radiopharmacy. She began her doctoral thesis at the same year and she was won a scholarship from Campus France in 2011 to proceed her cotutelle doctoral thesis in a collaboration between Hacettepe University and François Rabelais de Tours University. She has been working as a research assistant at Hacettepe University Faculty of Pharmacy Department of Radiopharmacy since 2006 and she is the treasurer of Association of Radiopharmacy since 2007.
Supplement 2.

Publications Performed within the Concept of PhD Thesis

- **Articles**


- **Oral Presentations**


- **Poster Presentations**
  - Silindir. M, Erdoğan, S, Özer A Y. Characterization of Nanosized Theragnostic Liposomes for the Diagnosis and Therapy of Parkinson’s Disease, 16th International Pharmaceutical Technology Symposium (IPTS-
16), Proceedings Book, Lara The Marmara Hotel, Antalya-Turkey, September 10-12 2012. (pp. 121-123).


- **Silindir Gunay M**, Ozer AY, Erdogan S, Baysal I, Guilloteau D, Chalon S. In Vitro Studies on BBB Penetration of Pramipexole Encapsulated Theranostic Liposomes for the Therapy of Parkinson’s Disease. 18th European Symposium on Radiopharmacy and Radiopharmaceuticals, 07-10. April 2016, pp. 79, Salzburg/Austria.

- **Project**
  - Preparation of Theragnostic Immunoliposomes for the Diagnosis and Therapy of Parkinson’s Disease, TUBITAK, Project No: 112S244, December 2012-2013 (As Scholarship Student) (budget: 29.600 TL).

- **Scholarship**
  - French Embassy Coutotelle Doctorate Thesis Scholarship supported by Campus France which was supervised by François Rabelais Université de Tours, Faculty of Medicine, Equipe 3, Molecular Imaging and Brain, Inserm U930 and Hacettepe University, Faculty of Pharmacy, Department of Radiopharmacy (2011-2015).
Supplement 3.

Articles

CURR NEUROPHARMACOL. 2015 Dec 30. [Epub ahead of print]

DRUG DELIVERY SYSTEMS FOR IMAGING AND THERAPY OF PARKINSON'S DISEASE.

Gunay MS¹, Yekta Cizer A², Chatin S³.

Author information

Abstract

Although a variety of therapy approaches are available for Parkinson's disease (PD) which is one of the most commonly seen neurodegenerative disease, limited blood brain barrier penetration and delivery of drugs to the target brain tissue and side effect observations depending on long term administration of anti-parkinsonian drugs are still major challenges that should be surpassed for effective PD therapy. Similar to other diseases, the use of drug delivery systems like liposomes, niosomes, micelles, nanoparticles, nano capsules, gold nanoparticles, microspheres, microcapsules, nanobubbles, micro bubbles and dendrimers are investigating for diagnosis and therapy of PD. It is significant to transport and deliver sufficient amounts of drugs or radio contrast agents to brain to provide better efficacy or imaging within the desired period of time without exposing to an undesired metabolism or enzymatic degradation both for the therapy and imaging of diseases. PD is one of the widely seen neurodegenerative disease formed in elderly ages, especially. Current treatments for PD focus on motor symptoms but they generally do not deal with modifying the course of disease. Beyond pharmaceutical drugs, the identification of abnormal proteins such as α-synuclein, parkin or leucine-rich repeat serine/threonine protein kinase 2 could represent promising alternative targets for molecular imaging and therapy of PD. The improvement of drug delivery systems could have a high potential to enhance PD therapy effectiveness and reduce its side effects. This review focuses on formulation, development and advantages of nano sized drug delivery systems which can penetrate to central nervous system for the therapy and/or diagnosis of PD and highlights the future nano technological approaches.

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The benefits of pramipexole selection in the treatment of Parkinson's disease

Mine Silindir · A. Yekta Ozer

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Abstract Levodopa administration as a gold standard in Parkinson's disease (PD) treatment is very valuable; however, long-term administration may cause some motor complications such as abnormal unintended movements and shortening response to each dose (wearing off phenomenon). Dopamine agonists were developed to reduce duration of immobile off periods and dependence in levodopa for improving motor impairments (Ch Juan et al., Cochrane Libr 1:1–23, 2000). Pramipexole is one of these nonergotic dopamine agonists with high relative in vitro specificity and full intrinsic activity at D2 subfamily of dopamine receptors, with a higher binding affinity to D3 than to D4 or D2 receptor subtypes (Fieri et al., Clin Neuropharmacol 21:141–151, 1998). It can be advantageously administered as monotherapy or adjunctive therapy to levodopa to decrease side effects and increase effectiveness in both early and advanced PD treatment.

Keywords Pramipexole · Parkinson's disease

Introduction

Due to increase in lifetime, an increase is observed in the incidence of geriatric diseases, particularly in research in this field across the world. One of the most recently observed neurodegenerative diseases is PD diagnosed over age 50.

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Generally; however, it can also be seen in young people about 5 % among all patients and is called juvenile PD. While disease incidence is 0.2-0.3 % in the community, it is 1 % among people over the age of 55. PD took its name from an English doctor James Parkinson in 1817, who published about the disease first in A Essay on Shaking Palsy. Its motor symptoms took its source from degeneration or death of dopamine-generating cells in substantia nigra of midbrain [3]. While movement-related symptoms are observed in earlier PD, cognitive, behavioral problems are generally observed in progressive levels of disease. In some cases of subsequent advanced stages, dementia may be occurred [4]. Antiparkinsonian drugs comprise dopaminergic and antimuscarinic agents. While dopaminergic are used for potential actions of dopamine, antimuscarinics are used for reducing excessive central cholinergic effects. Antimuscarinics are grouped as tertiary amines. Dopaminergics are grouped as levodopa, peripheral dopa-decarboxylase inhibitors, aminocytes, and amantadine and amantadine, ergot derivatives, various other nonergotic dopamine agonists including pramipexole, specific monoamine oxidase type B inhibitors, and catecho-β-methyltransferase inhibitors [5]. Pramipexole is developed as either adjuvantive therapy to levodopa or monotherapy in PD treatment.

Phytochemical properties

Pramipexole dihydrochloride monohydrate (5)-2-amino-4,5,6,7-tetrahydro-6-propylamino-benzotriazole dihydrochloride) is a white to off-white crystalline powder (302.27 g mol⁻¹) and stable under ordinary conditions. Its solubility is more than 20 % in water, about 8 % in methanol, 0.5 % in ethanol and practically insoluble in dichloromethane (6). Due to high solubility and high permeability
Liposomes and their applications in molecular imaging

Mine Silindik, Sura Erdoğan, A. Yekta Özer, and Serge Malet

Abstract

Molecular imaging is a relatively new discipline with a crucial role in diagnosing and treating various diseases through characterization and quantification of biological processes at cellular and sub-cellular levels of living organisms. These molecular targeted systems can be conjugated with reporter agents or molecules to obtain specific molecular probes for the purpose of diagnosis of diseases more accurately by different imaging modalities. Nowadays, an interesting new approach to molecular imaging is the use of highly organized drug delivery systems such as liposomes having convenient properties such as low hazardous biocompatibility and specificity and they can specifically be targeted to desired disease tissues by connecting them with specific targeting ligands and probes. The targeted liposomes as molecular probes in molecular imaging have been evaluated in this review. Therefore, the essential point is detection of molecular target of the disease which, is present from normal conditions such as increase or decrease of a receptor, transporter, hormone, enzyme on identification of a novel target. Transport of the diagnostic probe specifically to targeted cellular, sub-cellular or even intracellular entities can be performed by molecular imaging probes. This may lead to production of targeted therapies for imaging and/or therapy of diseases at earlier stages.

Keywords: Molecular imaging, molecular targeted imaging, targeted liposomes, targeted delivery systems, liposomes for imaging

Introduction

Considerable progress has been achieved in recent years of molecular imaging due to formation and development of new modalities, molecular biology, and anatomy (Khan and Wang, 2010). Molecular imaging is defined as the measurement, characterization, and visualization of biological processes at cellular and molecular level in human and other living systems (Silindik, 2009; Chang and Chiu, 2008). Imaging of multiple cellular processes is performed in the same time through gene expressions or protein-protein interactions, signaling of cell targeting, drug and gene therapy optimization, determination of the progression of molecular pathogenic diseases, imaging of drug effect at molecular and cellular level, supplying rapid, reproducible and three-dimensional computerized images with quantitative results of therapeutic effects of gene products on animals or patients, therapy tuning are some significant application fields of molecular imaging (Blasberg, 2001; Masoud and Gambhir, 2003; Saha, 2004). Many branches like molecular biology, cellular biology and imaging technology are related with molecular imaging (Masoud and Gambhir, 2003). Table 1 represents significant properties of some imaging modalities and molecular probes (Masoud and Gambhir, 2003). The basic principle of molecular imaging depends on obtaining significantly high signal intensity by the use of minimal amount of molecular probes. Studies on the development of special reagents, ligands, protocols and devices for molecular imaging have been carried out over the past two decades. Better biocompatible probes/ligands for selecting appropriate cellular and sub-cellular targets for imaging were developed. The delivery of these probes by overcoming biological barriers and image amplification for detection of trace amount of target concentrations such as picomolar-range (pM–nM) and development of imaging systems to a level of higher spatial/temporal...
Supplement 4.

Oral Presentations
Radiopharmaceuticals are radioactive drugs composed of a radionuclide and a pharmaceutical for the diagnosis and/or therapy of many diseases comprising neurodegenerative diseases. While about 97% of the radiopharmaceuticals are used for the diagnostic purposes, the rest can be used for therapeutic purposes of the diseases (1). Although they are used in very minimal amounts, they should be sterile and pyrogen free, and they have to be undergone all the quality control measurements for drugs and also some special tests for the radioactive part (1). A variety of different applications were performed for the diagnosis of PD at Nuclear Medicine clinics with the use of specific radiopharmaceuticals. The precise differential diagnosis can be performed with the assessment of the accumulation of pathologic proteins and dopaminergic system besides brain perfusion and glucose metabolism (2).

Parkinson’s disease (PD) is one of the widely seen neurodegenerative disease formed in elderly ages. Although PD is diagnosed over the age of 50 generally, it is not the disease only seen in geriatrics and can be seen as 5% in young people that is called as Juvenile PD. While about 10 million people suffer from PD worldwide, about 100-130 thousand people were diagnosed with PD in Turkey. It is expected that this number will be duplicated in 2030 in Turkey in which about 10 thousand people is diagnosed with PD every year (3, 4). Current treatments for PD generally focuses on curbing the motor symptoms however these treatments generally do not deal with modifying the course of disease. Although different diagnosis and therapy approaches are available for PD, there are still major challenges that should be surpassed for effective PD therapy such as limited blood brain barrier (BBB) penetration, effective drug delivery to the target brain tissue and enhanced side effect observation depending on long term administration of antiparkinsonian drugs. It is significant to transport and deliver sufficient amount of drugs or radiopharmaceuticals to the brain for obtaining the efficacy or imaging in the desired period of time without exposing to an undesired metabolism or enzymatic degradation both for the therapy and imaging of diseases. Similar to other diseases, the use of nanosized drug delivery systems like liposomes, niosomes, micelles, nanoparticles, nanocapsules, gold nanoparticles, microspheres, microcapsules, nanobubbles, microbubbles and dendrimers are investigating for diagnosis and therapy of PD for highlighting the future nanotechnological approaches.

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Biyomedikal Mühendisliği

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5biyosemp.biyomedtekarge.org

25 Mart 2016 CUMA
16:30 - 11:15 Açılış
> Doç. Dr. Betül Taşdelen
(Namık Kemal Üniversitesi, Biyomedikal Mühendisliği Bölüm Başkanı)
> Yrd. Doç. Dr. Hale Pınar Zangelidilelı
(Namık Kemal Üniversitesi, Biyomedikal Mühendisliği Bölümü, Sempozyum Başkanı)
16:45 - 11:15
> Prof. Dr. Musa Halak Ayşeb
(Yıldız Teknik Üniversitesi, Biyomedikal Mühendisliği Bölüm Başkanı)
> Doç. Dr. Kamuran A. Kadıoğlu
(Yıldız Teknik Üniversitesi, Biyomedikal Mühendisliği Bölüm Başkanı)
Biyomedikal Mühendisliği Öğrenci Sempozyumu
24-25 Mart 2016
11:15 - 11:45 Sermi Bilgiler
(Istanbul 2. Hastanesi, Biyomedikal Mühendisliği) İlahie Trubuzcu ve Medical Teknoloji Setleri Giriş
11:45 - 12:30 Osman Pınar Küçükövereli
(Kardiyoloji A.Ş., Şirket Otlaç ve Proje Yöneticisi)
Kardiyoloji Teknoloji ve Ar-Ge Çalışmaları
12:30 - 14:30 ARA
14:00 - 14:30 Ufuk Karanfı
(Istanbul Çelik Nano Kamu Hastaneleri Birliği Genel Sekreteri) Beyaz Uzmana, Tıbbi Hizmetler Bennetteli Koordinatörü
Kamu Hastaneleri Birliği Kamu Mühendislik Hizmetleri ve Biyomedikal Mühendislik Kurgusu
14:30 - 16:00 Korem SÜNDER
(Büyük Sağlık, Hayvancılık ve Orman Uygulama Merkezi Kürsüsü)
Dijital Dünyada Büyük Kullanım
15:00 - 15:30 Kursat AKTAŞ
(BTECH Innovation A.Ş., Genel Müdürü)
3 Boyutlu Teknoloji: Sağlık Sektöründe Kullanım
15:30 - 16:00 Uzm. Ecz. Mine Silindir Güngör
(Nihatpasa Üniversitesi)
Reddettiklerinizi ve Yeni Gelişimleri
16:00 - 16:15 "Multimodal Işlevselliği" (Medical Imaging Academy Kürsüsü)
Medical Gürültülere
16:15 - 17:00 GÜZÜNLER BULUŞUYOR
> Aydın Ashoav
(Edek Tek Biyoloji, Biyomedikal Mühendisliği)
> Canan Altun
(Türkçe ve İslam Bilimleri, Biyomedikal Mühendisliği)
> Geren Yoğuz
(Medikal Teknik Elektronik A.Ş., Biyomedikal Mühendislik)
> Yaşar Birgit
(Türkçe ve İslam Bilimleri, Biyomedikal Mühendisliği)
17:00 Kapanış

YER: EXPOMED Töyap Fuar ve Kongre Merkezi
Karadeniz Salonu
Radyofarmasötikler ve Yeni Gelişmeler

Mine Silindir Güney, A. Yekta Özer

Hacettepe Üniversitesi, Eczacılık Fakültesi, Radyofarmasi ABD., 06100, Sihhiye, Ankara, Türkiye.


Nükleer tip ve Radyoloji alanında kullanılan görüntüleme cihazlarından dahi gelişmeleri bağlı olarak, hedefe spesifik olarak gidebilene ve hastalığın olduğu bölgeyi etrafındaki normal dokuya dayanmış ve özgünlediği radyofarmasötikleri intiyaç duymaktadır. Bu amaçla pek çok hastalığa spesifik olarak hedeflenerek modifiye edilmiş multifonksiyonel nanopartiküler tayfesi sistemlerin hazırlanması ile teşhis ve veya tedavi sağlanabilmektedir. Dünyada olduğu gibi ülkemizde de son yıllarda kanser, nörodegeneratif hastalıklar, kartiyoloji ve pek çok alanlarda yüzey modifiyasyonu, polimer kinyası ve hemofylinde ile hedefe spesifik radyofarmasötik ve terapostenkler üzerinde uzun vaad eden çalışmalar yürütülmektedir.

Anıktar kelimeler: Radyofarmasötikler, radyofarmasi, hedefe spesifik multifonksiyonel nanopartiküler sistemler.

References:

Supplement 5.

Poster Presentations
Characterization of Nanosized Theragnostic Liposomes for the Diagnosis and Therapy of Parkinson’s Disease
M. Silindir, S. Erdoğan, A.Y. Özer
Hacettepe University, Faculty of Pharmacy, Department of Radiopharmacology, Ankara, Turkey.

Introduction
One of the most recently observed neurodegenerative disease among geriatric diseases is the Parkinson’s Disease (PD) which is diagnosed generally over age 50 generally in Turkey and the world. It can also be seen in 5% of young people. PD is hard to diagnose. By using the imaging modalities such as Positron Emission Tomography (PET) and Single Photon Emission Computed Tomography (SPECT), the decline in the accumulation of the radiotracer in substantia nigra of the brain can be detected. Therefore, some molecular targets such as Dopamine (DA) and Dopaminergic Transporter (DAT) should be specifically chosen. Drug therapy is one of the mostly used methods for the therapy of PD. One of the most crucial points in drugs that are used for the therapy is the limited amount of the drug penetrated into brain. However, any increase in the drug concentration also increases the risk of side effect observation as proportionally. Therefore, it is needed to increase the drug concentration by not causing any rise in the side effects. Nanosized drug delivery systems such as liposomes have many advantages in brain delivery for the purpose of either therapy or diagnosis which can be targeted passively or actively. The studies performed in recent years generally depend on the development of drug delivery systems for the purpose of both the diagnosis and therapy which are called theragnostics. Diagnosis can be managed at the same time the therapy and the effectiveness of the therapy can also be evaluated.

In this research, it is aimed to develop PolyEthylene Glycol (PEG) coated, nanosized, radiolabeled with 111In, for molecular imaging with SPECT and antiparkinson drug pramipexole dihydrochloride monohydrate (pramipexole) encapsulated liposomes for the diagnosis and therapy of PD. Following the preparation of liposomal dispersions, the characterization studies were also performed.

Keywords:
Brain Targeting, Molecular Imaging, Parkinson’s Disease, Theragnostic Liposomes, Pramipexole.

Materials and Methods
Dipalmitoylphosphatidylcholine (DPPC) (Avanti Polar Lipids Inc.) is used as phospholipid, cholesterol (Chol, Sigma) is used as bilayer condenser and DSPE-PEG$_3$ (Lipoid) for PEG-coating. Pramipexole (Abdi Ibrahim) is used as antiparkinson agent.

Synthesis of DTPA-PE to prepare DTPA-PE for radiolabeling of liposomes, 4 mL of chloroform was added on 0.2 mmol Egg PC and stirred. 30 μL triethylamine was added on the mixture. 1 mmol DTPA (Diethylene triamine penta acetic acid) anhydride was dissolved in 20 mL of DMSO (dimethyl sulfoxide) in another place and this mixture was added dropwise on the other mixture in the existence of argon gas and incubated for 3 hours. Precipitation was obtained after adding distilled water on the mixture and filtered and lyophilized (1, 2). The synthesis of DTPA-PE was observed by IR spectra with pressed potassium bromide tablets at 4000-40 cm$^{-1}$ bands. TLC determination was also performed for the formation of the complex by observing the spot at UV lamp with 254 nm.

Preparation of Theragnostic Liposomes
Pramipexole encapsulated DPPC:Chol:PE$_{3}$:DTPA-PE (10:5:1:0.16 mole ratios %) and positive charged DPPC:Chol:PE$_{3}$:DTPA-PE (10:5:1:0.16 mole ratios %) liposomes were prepared according to the film method (3). Briefly, phospholipid and other ingredients were dissolved in chloroform and afterwards chloroform was evaporated in a rotovapor under vacuum system. The film layer was hydrated with Tris buffer (20 mM pH 7.4) containing pramipexole (5 mg/mL) for 30 min at 70°C. Liposomes were
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Emerging Challenges for Global Delivery

203. Biodegradable Nanoparticles for Intracellular Hydrogen Peroxide Generation. Presenter: Ho-Jung Mun, Department of Biometrics, School of Medicine, Taipei Medical University, Taipei, Taiwan. Authors: F. R, Y. Su, S. Yu, D. Tong, Y. Hu, A. Chao. VIEW ABSTRACT

204. Bladder cancer targeting nanocarrier. Presenter: Toshihiko Umeda, University of u.S.A. Authors: T. Umeda. VIEW ABSTRACT


207. Comparison of the Acoustic Release of Doxorubicin from Targeted and Non-targeted Polymeric Micelles. Presenter: Ghulam Husain, American University of Sharjah, UAE. Authors: A. A. Husain, L. Khedr, R. H. Pitt, J. A. Husain. VIEW ABSTRACT


216. Dual-targeting polyelectrolyte-based nanoparticles for the treatment of glioma in mice. Presenter: Sheng Fu, Department of Pharmacology, School of Pharmacy, Fudan University, Shanghai, China. Authors: J. Peng, G. Chang, T. Lu. VIEW ABSTRACT

217. Dynamic contrast enhanced computed tomography imaging of perfusion predicts the heterogeneous spatial distribution of opossums. Presenter: Shawn Magness, University of Eastern, Canada. Authors: E. Storrs, M. Fuller, A. Allen, B. A. Minor. VIEW ABSTRACT

218. Effect of Polymers on API Precipitation Determined by Polarized Light Microscopy. Presenter: Annette Mobrand, Department of Pharmacy, University of Copenhagen, Denmark. Authors: C. Jensen, J. X. Xu, T. Perd, J. Kostianov, B. Strehlau, A. Mikkelsen. VIEW ABSTRACT


222. Fluorescence Real-time Imaging of Cell Dynamics in Tissue of a Living Mouse Using Tissue Flaying Section Device. Presenter: Masahiko Fujii, Graduate School of
International Conference on Clinical PET-CT and Molecular Imaging (IPET 2015): PET-CT in the Era of Multimodality Imaging and Image-Guided Therapy

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- World Association of Radiopharmaceutical and Molecular Therapy (WARMTH)
- World Federation of Nuclear Medicine and Biology (WFNMIB)
- World Molecular Imaging Society (WMIS)
Comparative Evaluation of Pramipexole Encapsulated Theranostic Liposomes and Niosomes for Parkinson’s Disease

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Background: Parkinson’s disease (PD) is defined as a degenerative disorder of CNS. It is a chronic and progressive movement disorder. Its motor symptoms result from the death of dopamine generating cells in the substantia nigra of midbrain. PD is assumed as one of the most recently observed neurodegenerative disease among geriatric diseases diagnosed generally over age 50 all around the world. However, it can also be seen in 5% of young people. PD is hard to diagnose. By using the imaging modalities such as positron emission tomography (PET) and single photon emission computed tomography (SPECT), the decline in the accumulation of the radiotracer in substantia nigra can be detected.

For PD treatment, drug therapy is one of the mostly used methods. Limited amount of drug penetrating into the brain is one of the most crucial points. It is needed to increase the drug concentration by not causing any rise in side effects. Passively or actively targeted nano-sized drug delivery systems such as liposomes and niosomes have many advantages in brain delivery for the purpose of either therapy or diagnosis. Recent studies performed around this issue generally depend on development of new drug delivery systems in which diagnosis can be managed together with therapy, which is called theranostics, and therapy effectiveness can also be evaluated.

Methodology: In this research, liposomes and niosomes were formulated for the diagnosis and therapy of PD through passive brain targeting: they were coated in polyethylene glycol (PEG), nanosized, neutral or positively charged, 99mTc labeled for SPECT imaging, encapsulated with pramipexole dihydrochloride monohydrate (pramipexole). Following preparation of liposomal and niosomal dispersions, their characterization and release kinetics were evaluated and compared.

Results: In both formulations nanosized particles and proper zeta potentials and about 90% pramipexole encapsulation efficiency was observed.

Conclusion: Promising characterization and release profiles for both diagnosis and therapy of PD were obtained with these theranostic systems. To obtain better results it is needed to perform further in vivo animal studies and our studies are continuing.

(The authors would like to thank for generous gifts of Abdı Brahim İlaç for Pramipexole, to Lipoid for PEG-PE. This study was supported by the grant of TUBITAK (Project No: 112S244)).
PP21
In Vitro Studies on BBB Penetration of Pramipexole Encapsulated Theranostic Liposomes for the Therapy of Parkinson’s Disease
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1Department of Radiopharmacy, Faculty of Pharmacy, Hacettepe University, 06100, Ankara, Turkey.
2UMR INSERM U930, Université François Rabelais de Tours, Tours, France.
3Department of Biochemistry, Faculty of Pharmacy, Hacettepe university, 06100, Ankara, Turkey.

Introduction: Brain penetration and targeting is hard due to complex structure with different barriers such as blood–brain barrier (BBB) with blood–cerebrospinal fluid (CSF) interface and CSF–blood interface (1, 2). Although these tight and rigid barriers protect brain, they also prevent penetration of molecules, drugs and radiopharmaceuticals for diagnosis and therapy of many neurodegenerative diseases. Parkinson’s disease (PD) is assumed to be one of the most frequently observed neurodegenerative disease among geriatric disorders. PD comprises motor symptoms resulting from the death of dopamine generating cells in the substantia nigra. By using the imaging modalities such as single photon emission computed tomography (SPECT), the decline in the accumulation of specific radiotracer in the striatum can be detected. For PD treatment, limited brain penetration of drug is a major concern. Passively or actively targeted, nanosized drug delivery systems such as liposomes have different interests for either therapy or diagnosis. Recent studies generally depend on the development of new delivery systems, theranostics, in which diagnosis can be managed together with therapy by evaluating therapeutic effect. Materials and Methods: Theranostic liposomes were formulated by polyethylene glycol (PEG) coated, nanosized, either neutral or positively charged, 99mTc labeled for SPECT imaging and pramipexole encapsulated for PD therapy. Their characterization and in vitro release kinetics were evaluated. In vitro penetration of both formulations was evaluated in a BBB cell co-culture model. Results: Both neutral and positively charged liposomes showed proper characterization with about 10% encapsulation efficiency and around 100 nm particle sizes. All formulations fitted to the first-order release kinetics (3). Both formulations were found BBB permeable in cell culture studies with fluorescent images and fluorospectroscopy. Conclusions: Promising characterization and release profiles were obtained with theranostic liposomes for both diagnosis and therapy of PD. Both neutral and positively charged formulations found BBB permeable in vitro. In vivo animal studies are continuing to obtain more accurate data. (The authors thank to Abdi ibrahim ilg for Pramipexole. This study was supported by the grant of TUBITAK, Project No: 115S244).

References:
THE PREPARATION OF THERAGNOSTIC IMMUNOLIPOSOMES/IMMUNONIOSOMES FOR THE DIAGNOSIS AND THERAPY OF PARKINSON’S DISEASE

Résumé

Parkinson’s Disease (PD) is degeneration of dopamine producing cells in substantia nigra. Blood-brain barrier (BBB) is a strong obstacle in PD therapy. More penetration and accumulation in the target tissue can be obtained by preventing RES uptake via “stealth effect”. Liposomes and niosomes are the promising systems for being biodegradable, bioavailable, non-toxic and targetable. Although CNS disorders are the first to endorse at their research in the diagnosis and therapy with several framework projects in Europe and over the world, there is still a huge gap in CNS drug delivery and the success of PD therapy. Although different studies have performed with pramipexole, evaluation of penetration and antiparkinsonian effect of pramipexole encapsulated liposomes and niosomes has never been studied before.

Among this thesis, nanosized, polyethylene glycol (PEG) coated, neutral and positively charged, pramipexole encapsulated liposomes and niosomes were formulated, characterized and release kinetics of the systems were evaluated. In vitro penetration of all formulations was evaluated in BBB cell co-culture model. Therapeutic efficacy of neutral, pramipexole encapsulated liposomes and niosomes were evaluated in 6-hydroxydopamine (6-OHDA) lesioned rats by rotometer test and autoradiography.

All formulations have approximately 10% encapsulation efficiency, around 100 nm particle sizes and fitted to first-order release kinetics. All formulations were found BBB permeable at in vitro cell culture studies. Nanosized, neutral niosomes designated similar but slightly better effect than pramipexole solution in autoradiography studies in 6-OHDA lesioned rats. This pramipexole dose is approximately 9 times lesser doses applied with conventional pramipexole tablets for humans in Neurology clinics. Nanosized, pramipexole encapsulated, neutral niosomes showed potential PD therapeutic effect in PD animal model depending on non-ionic surfactant properties of niosomes.

Key Words: Pramipexole Liposomes, Pramipexole Niosomes, Brain Targeting, Parkinson’s Disease Therapy, Dopamine Transporter Autoradiography.