Advances in Neurotherapeutic Delivery Technologies

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Abstract

Despite major advances in intracranial surgery and delivery of drugs to the brain, treatment of neurological diseases remains one of the great medical challenges of our days. The complexity of the organ makes surgical procedures complicated, and conventional systemic delivery of drugs to the brain is hampered by low drug selectivity and low drug partitioning over the blood-brain barrier. Due to the high social and economic impacts related to diseases of the central nervous system, development of new improved treatments of brain related disorders is of significant value, both for the patient and for the society. Nanomedicine is a rapidly growing field in the development of novel therapies for treatments of brain pathologies. The scientific progress in nanotechnology has resulted in several new innovative nano-assemblies, with promising medical potentials. Therapeutic benefits related to the use of nanovectors includes, reduced chemical and enzymatic degradation of drugs, increased uptake over biological barriers, improved selectivity by surface modification using targeting ligands, and reduced toxic side effects in non-target tissue. This review discusses various applications of polymeric nanoparticles as nanovectors in treatment of neuronal diseases, specifically illustrated for Alzheimer’s and Parkinson’s diseases and Glioblastoma.

Keywords: Alzheimer’s Disease; Engineered Polymeric Nanoparticles; Glioblastoma; Nanoencapsulation; Nanomedicine; Nanotechnology; Parkinson’s Disease

Introduction

The term “nanomedicine” was introduced about 10 years ago as a consequence of an increasing association between physico-chemistry of nano-assemblies and pharmaceutical and medical sciences [1]. Since then, a robust proof of concept has been established regarding the efficacy of Nanoparticles (NPs) for treatments of various pathologies. Examples of nano-assemblies which has been developed and used in pharmaceutical application, are polymeric nanostructures (polymeric NPs, micelles, dendrimers, carbon nanotubes and nanogels) and lipid NPs (liposomes, solid lipid NPs and lipid nanocapsules) [2-8]. However,
Few nanomedicine candidates have so far reached the gap between pre-clinical and clinical trials, in particular for Central Nervous System (CNS) pathologies. Phase 1 trials with CTP-11 (a topoisomerase I inhibitor) loaded liposomes in patients with recurrent high-grade tumors, at the University of California, is the only clinical trial in progress today using nanomedicine for the treatment of CNS related disorders. Nevertheless, there are several ongoing clinical trials in progress concerning neuropathologies without the use of nanomedicines, i.e., 1457 for Alzheimer’s disease, 1349 for Parkinson’s disease and 932 for glioblastoma (www.clinicaltrials.org). Concerning CNS pathologies, a successful treatment depends largely on early disease detection. Nevertheless, early detection is complicated and CNS pathologies are often detected at late stages in the disease evolution, after the appearance of clinical symptoms. Diagnostic tool-based nanovectors are currently developed to improve the CNS disorder detection (sensibility and specificity), and this task will be beneficial for CNS disorder treatments [9].

The main limitation for CNS disorder therapies after systemic administration is the poor transport of the active agents across the Blood-Brain Barrier (BBB). The barrier surrounds the brain tissue allowing only a very limited uptake of most biological active compounds [10,11]. Moreover, the presence of active efflux mechanisms, such as P-glycoprotein and multidrug resistance-related proteins, at the BBB restrict the penetration of several active agents [12]. Improved selectivity by grafting of targeting ligands (peptide or antibodies) is an important advantage of nano-scale drug delivery systems to increase uptake over biological barriers, such as the BBB [13]. Additional benefits of nanovectors over conventional dosage forms includes, e.g. protection of drugs from metabolic degradation, sustained release than can be adjusted to last from days to months, increased efficacy, decrease multi-drug resistance, reduction in dosage, a decrease in systemic toxicity, and an improved patient compliance [14]. Among all nanostructures, polymeric NPs are, together with liposomes, the most studied for treatment of neurological diseases. In this chapter we will give a general overview of recent advances in the delivery neuro-therapeutic drugs for the main CNS pathologies, Alzheimer Disease (AD), Parkinson Disease (PD) and Glioblastoma Multiforme (GBM), using polymeric NPs, through in vivo preclinical studies.

**Polymeric Nanoparticles for Alzheimer’s Disease Therapy**

AD is one of the most recurrent pathologies of the central nervous system, affecting approximately 35 million people worldwide [15]. Extracellular amyloid-β (Aβ) plaques and intracellular hyperphosphorylated tau neurofibrillar tangles are protein aggregates, always found in the brain of AD patients. These two neurotoxic aggregates produce cognitive dysfunctions (memory, language, orientation, functional abilities, etc.) due to reduced acetylcholine levels, leading to a loss in the cholinergic transmission of the cortical neurons of the brain. Additional negative effects of the protein aggregate toxicity are increased oxidative stress and elevated metal ion concentrations resulting in a degeneration of cholinergic neurons in cortex, hippocampus, basal forebrain and ventral striatum. Available treatments are symptomatic and comprise delivery of acetylcholinesterase inhibitors. The treatment provides some relief in the symptoms for the patient, but no real decline in the disease evolution [16]. Delivery of drugs in nano-scale colloidal carriers, including polymeric NPs, is a promising approach to meet the challenges we are facing in the treatment of AD. Recent in vivo studies have highlighted new strategies to suppress AD symptoms and if possible overturn the degenerative processes. These include acetylcholinesterase inhibitor delivery (to increase acetylcholine concentrations in the brain), neuron protection and stimulation, antioxidant delivery and Aβ aggregate targeting and inhibition.

**Acetylcholinesterase inhibitor strategies**

Acetylcholinesterase inhibitors, e.g. Rivastigmine Tartrate (RT), tacrine, donepezil, huperzine A are drugs used to reduce the enzymatic degradation of low level of acetylcholine in the brain of AD patients in order to improve connection between neurons.
Encapsulation of RT in polymeric NPs is a promising approach to improve the therapeutic effect of the native drug. For example, Pagar et al., synthesized L-lactide-depsipeptide polymeric NPs (140nm), with 64% RT loading, using a single emulsion-solvent evaporation technique [17]. Their study showed that amnesic rats receiving intravenous injections of RT loaded NPs regained memory faster compared to amnesic rats given intravenous injections of RT in solution. Pharmacokinetic parameters (total concentration, mean residence time and clearance), and biodistribution profiles (RT drug concentration in brain) supported the improved efficiency of RT in NPs.

In another study, Fazil et al., developed 185nm RT-loaded chitosan NPs (85% encapsulation efficacy) using an ionic gelation method. The NP formulation significantly improved RT uptake by the brain following intranasal delivery, compared to the free drug in solution [18]. NPs induced two to three times higher RT concentration in the brain (966 ± 21ng/mL at t\textsubscript{max} = 60 min), compared to intravenous (387 ± 30ng/mL, t\textsubscript{max} = 30 min) and intranasal (509 ± 22ng/mL, t\textsubscript{max} = 60 min) administrations of RT in solution.

Improved efficiency of RT in NPs was also demonstrated by Wilson et al., who prepared polysorbate 80 coated Polybutylcyanoacrylate (PBCA) NPs (40nm) with 20% RT loading using an emulsion-diffusion technique [19]. RT uptake by the brain was significantly higher in rats given intravenous administration of polysorbate 80 coated NPs, compared to groups receiving free drug or RT in non-coated NPs. The results demonstrate the influence of polysorbate 80 for the absorption of NPs over the BBB.

NP encapsulation of other acetylcholinesterase inhibitors has also been reported. For example, Wilson et al., investigated the encapsulation of tacrine (15% loading) in polysorbate 80 coated PBCA NPs (35nm) [20]. Intravenous administration of NPs, demonstrated a significant increase in tacrine concentration in the brain, 1h post injection, with polysorbate 80 coated NPs compared to non-coated NPs or the free drug. In addition, the coating reduced the tacrine accumulations in liver, spleen and lungs, compared to non-coated NPs. Tacrine has also been encapsulated in 40nm large Chitosan NPs (approximately 13% loading), with or without polysorbate 80 coating, prepared by a spontaneous emulsification method [21]. Polysorbate 80 coating altered the biodistribution profiles of NPs, with a longer NP systemic circulation time, and a sustained tacrine release. Md et al., encapsulated Donezepil in poly(lactic-co-glycolic acid) PLGA NPs (90nm) (emulsification-diffusion technique) [22]. Using gamma scintigraphy, they demonstrated that the uptake of donezepil in the brain after intravenous administration was higher when NPs were used than for free donezepil. Zhang et al., investigated the synergy of oral administration of borneol and intravenous injection of huperzine A loaded PLGA NPs (85nm), coated with aprotinin [23]. NP uptake in brain capillary endothelial cells was increased after the co-administration of borneol (oral route) and NPs (intravenous route). Moreover, using this therapeutic scheme, the pharmacological effect of huperzine A was increased, reducing the memory impairment of AD rats.

Neuron protection and stimulation strategies

These strategies consist on the delivery of neuroprotective active compounds or growth factors to the brain to decrease the neuronal degeneration due to various stresses involved in AD and PD.

Herrán et al., prepared 200nm Vascular Endothelial Growth Factor (VEGF) loaded PLGA NPs with 45% encapsulation efficiency [24]. Local administration (craniotomy) of VEGF loaded NPs in amyloid precursor protein/presenilin-1 (APP/Ps1) mice, improved the behavioral deficits (T-maze test), decreased Aβ deposit and reduced neuronal loss.

Improved uptake of neuroprotective peptides in polymeric NPs following intravenous administration has also been demonstrated. PLGA NPs (100 nm) encapsulating a NAPVSIPQ Neuroprotective Peptide (NAP), a fragment of an Activity-Dependant Neuroprotective Protein (ADNP), were developed by Liu et al., [25]. A B6 peptide was attached to the NAP-loaded
NPs to improve BBB crossing. *In vivo* administration of B6-modified NAP-loaded NPs increased NAP concentration in the brain as compared to the non-modified NAP-loaded NPs (Figure 1). Furthermore, when administered to AD mouse models, amelioration in learning impairments, decrease in cholinergic disruption and decrease in hippocampal neurons loss were observed with B6-modified NPs, even at low NAP doses.

![Figure 1: (A) Distribution and retention of B6-NPs or NPs in the nude mice following intravenous administration in tail vein. (B) Distribution of B6-NPs or NPs in brain and main organs 1h after administration. (Reprinted with permission from [25]. Copyright 2014 American Chemical Society).](image)

Similarly, Li et al., developed TGNKYKALPHNG peptide (TGN)-grafted PLGA NPs (150nm) to increase uptake of NAP peptide over the BBB [26]. Using Morris water maze experiment, they showed that TGN-grafted NAP-loaded NPs improved spatial learning compared to non-grafted NAP-loaded NPs or NAP solution. Moreover, after hippocampus and cortex recoveries, no morphological disorder and no Aβ plaque were detected in mice treated with TGN-grafted NPs.

Mittal et al., investigated oral administration of estradiol in 130nm tween 80 coated PLGA NPs prepared using an emulsification-diffusion method [27]. NPs were tested in an Ovariectomized (OVX) rat model of AD, again demonstrating the importance of NP surface coating for delivery drugs over the BBB, i.e. the concentration of estradiol was significantly higher in the brain of rats treated with tween 80-coated NPs than in groups treated with non-coated NPs.

**Antioxidant delivery strategies**

Production of reactive oxygen species is induced by the deposition of amyloid-β (Aβ) plaques reacting with activated microglia, leading to severe neuroinflammation in AD patients, which can be reduced using antioxidant drugs, e.g. curcumin and Cyclophosphamide (CYC).

Ray el al., developed a curcumin NPs (registered trademark NanoCurc™) by free radical polymerization of N-isopropylacrylamide, vinylpyrrolidone and acrylic acid [28]. Significant curcumin levels in the brain was observed after intraperitoneal NanoCurc™ injection twice daily in athymic mice, with a decrease in $H_2O_2$ levels, and caspase 3 and 7 activities in the brain.
Another way to reduce cerebrovascular inflammation is to use CYC as reported by Agyare et al., [29]. NPs (140nm) based on Magnevist (MRI contrast agent) and chitosan were prepared using a gelation method and conjugated with putrescine modified F(ab')2 fragments of anti-amyloid antibodies. *In vivo* imaging after intravenous administration showed the ability to target cerebrovascular amyloid in mice. In addition, *ex vivo* experiments showed that the NPs reduced the production of pro-inflammatory cytokines more efficiently than CYC alone.

**Amyloid-β plaques inhibition and targeting strategies**

The disruption of neurotoxin Amyloid-β (Aβ) plaques is one of the strategies to decrease the cognitive impairments in AD patients, Cheng et al., developed curcumin NPs using flash nanoprecipitation of curcumin, with Poly(Ethylene Glycol)-Poly(Lactic Acid) (PEG-PLA) block copolymer and polyvinylpyrrolidone, followed by freeze drying with β-cyclodextrin (<80nm) [30]. Oral delivery of curcumin NPs showed a better bioavailability, with higher curcumin plasma concentrations, than free curcumin. Curcumin NPs also stimulated a significant improvement in cue memory and working memory (tendencies) compared to placebo or free curcumin, in an AD Tg2576 mice model. Moreover, mice treated with curcumin NPs exhibited significantly lower Aβ aggregates than controls, confirming the *in vivo* association of the drug and Aβ protein aggregation blocking.

Neelov et al., developed polyllysine dendrimers of the third and fifth generation and observed the interaction with Aβ aggregation *in silico* [31]. *In vitro* experiments confirmed that the dendrimers could form complex with Aβ protein, inhibiting the aggregation process, and that this association protected human neurablastoma cells (SH-SYSY cell line) against Aβ-induced cytotoxicity. After intraventricular administration, fluorescent-labeled dendrimers were detected in cortex and hippocampus, without toxicity to neuronal and glial cells.

In another strategy developed by Songjiang et al., Aβ subfragments were encapsulated in 15nm chitosan NPs (78% encapsulation efficiency) using an emulsification method [32]. Systemic administration in mice demonstrated an improved uptake over the BBB with NPs, i.e. 81% uptake efficiency for encapsulated peptide, compared to 21% for non-encapsulated peptide. Furthermore, peptide-loaded NPs presented an interesting immunogenicity character, with production of anti-Aβ antibody. This method could potentially be used as vaccine to inhibit Aβ aggregation during AD evolution.

Zhang et al., developed dual-functional PLA NPs (about 100nm diameter), conjugated with TGN and QSH peptides to improve uptake over the BBB and target Aβ aggregates, respectively [33]. After intravenous administration, these NPs targeted the Aβ plaques in the brains of AD model mice (induced after stereotaxic administration of Aβ into the hippocampus).

**Polymeric Nanoparticles for Parkinson’s Disease Therapy**

Despite considerable research on Parkinson’s Disease (PD), the cause of this pathology is still unknown. PD is characterized by progressive degeneration of the nigrostriatal dopaminergic cells, which leads to reduced dopamine production. Some genetic and environmental factors involved in PD pathogenesis have been identified but the diagnosis is essentially based on clinical symptoms, e.g. hypokinesia, bradykinesia, rest tremor and cogwheel rigidity [34]. As for AD, current PD treatments are symptomatic and aim to increase brain Dopamine (DA) levels with DA replacement strategies. Presently there are no treatments which can slow down or stop the progression of the disease [35]. Levodopa (L-Dopa), a dopamine precursor which can cross the BBB and DA agonists (e.g. bromocriptine, pergolide, cabergoline, lisuride, ropinirole and pramipexole) are currently used, but these only improves PD symptoms in the early stages of the disease. Furthermore, long term administration of these drugs has been shown to produce severe side effects [36].
The development of nanoscale drug delivery systems, e.g. NPs, could constitute a new promising approach to treat PD. In vivo studies have reported various strategies, e.g. dopamine replacement, neuron protection and stimulation, antioxidant delivery, to suppress PD symptoms and ideally reverse the degenerative process. In addition, in vitro studies have highlighted α-synuclein fibrillation inhibition as promising strategy for PD treatment.

**Dopamine replacement strategy**

DA replacement is used to increase the DA concentration in the brain (using DA or DA agonist-loaded NPs) in order to balance nigrostriatal dopaminergic cell degeneration.

Pillay et al., prepared DA-loaded polymeric NPs [37]. Using an emulsification-diffusion technique, an alginate scaffold embedding stable DA-loaded cellulose acetate phthalate NPs (200nm) was obtained with 63% drug entrapment efficiency. The scaffold was implanted in the parenchyma of the frontal lobe of a rat model and brain DA concentration was increased between 24h and 30 days, compared to free DA administration. Furthermore, only a very small amount of DA was found in plasma thus reducing the numerous side effects of conventional PD therapy.

A less invasive PD treatment, by intravenous administration, was developed by Trapani et al., using DA-loaded chitosan NPs prepared by the ionic gelation method [38,39]. DA was absorbed on positively charged 110nm NPs with an association efficiency of 81%. DA-loaded chitosan NPs induced a dose and a time dependent increase of DA levels in the rats’ striatum (Figure 2), which confirmed DA-loaded NP uptake over the BBB, probably through an adsorptive-mediated transcytosis.

Ropinirole is a DA agonist (specific D2-receptors), administrated using oral route despite a high hepatic first pass metabolism and non-optimal bioavailability (~50%). It stimulates the post-synaptic DA receptors, and it is used as first line treatment to improve motor symptoms and delay the L-Dopa medication [34]. Surface modified polymer-lipid hybrid NPs were prepared using an emulsification-solvent diffusion technique for intranasal delivery of ropinirole hydrochloride [40]. In vivo pharmacodynamic studies compared therapeutic efficacy of nasal versus oral administration, using a mouse model with chlorpromazine-induced Parkinson’s disease-like signs. The therapeutic efficiency was similar regardless
of the treatment applied, i.e. nasal administration with 3mg/kg body weight or oral administration with 10mg/kg body weight of ropinirole hydrochloride. The study shows that nasal administration of drug-loaded NPs could help in decreasing dose and frequency of the ropinirole administration, with no signs of severe damage on nasal mucosa.

**Neuron protection and stimulation strategy**

Urocortin is a corticotropin releasing factor known to promote long-term restoration of nigrostriatal function. Urocortin loaded PEG-PLGA NPs (120nm) were prepared using a double emulsion and solvent evaporation method (75.5% encapsulation efficiency) [41]. Odorranalectin was conjugated to NPs to improve nose-to-brain drug delivery. It is known that lectins can be grafted onto NPs to recognize sugars and thus more efficiently bind to the glycosylated nasal mucosa. In vivo imaging analysis revealed that NPs were absorbed directly over the nasal mucosa epithelium to brain, and not via the systemic pathway (via blood circulation over the BBB). The therapeutic efficacy of the formulation was tested in hemiparkinsonian rats induced by 6-Hydroxydopamine (6-OHDA). In vivo results showed that conjugated odorranalectin increased brain delivery of urocortin-loaded NPs and that the neuroprotective effects of urocortin were enhanced with intranasal delivery.

Another strategy using urocortin-loaded PEG-PLGA NPs was proposed by Hu et al., [42]. In their study NPs were conjugated with lactoferrin (cationic iron-binding glycoprotein) which is a promising targeting molecule for improving brain delivery, as the lactoferrin-receptor can transport NPs across the BBB. PEG-PLGA NPs with active lactoferrin on the surface were prepared by a double emulsion and solvent evaporation method. Intravenous injection of lactoferrin-modified urocortin-loaded NPs successfully delivered urocortin to the brain and attenuated the striatum lesion caused by 6-OHDA in rats.

Huang et al., developed lactoferrin-modified NPs encapsulating a human gene encoding a glial cell line-derived neurotrophic factor (hGDNF), as a non-viral gene vector [43-45]. The 200nm NPs included a Polyamidoamine (PAMAM) dendrimer with terminal PEGylation, ensuring a good DNA protection. hGDNF is known to induce and produce more dopaminergic neurons. The neuroprotective effects were examined in two rat models of PD: a rotenone-induced chronic model and a 6-OHDA-induced unilateral lesion model. Multiple intravenous injections showed powerful neuroprotective effects in both models, including locomotive activity improvement, dopaminergic neuron loss reduction and improvement of monoamine neurotransmitter levels. Multi-dose could achieve higher and longer expression of therapeutic proteins than a single-dose injection.

Gene therapy was also tested using a plasmid DNA encoding for hGDNF, compacted into DNA NPs (10nm) with 10 kDa PEG-substituted lysine 30-mers (CK(30)PEG10k), and given by intracranial administration [46]. The strategy was to propose a gene therapy which could improve neural cell graft success. Indeed, the majority of the grafted cells dies (necrotic and apoptotic death) soon after the grafting procedure, because the adult brain does not contain enough growth factors for the survival of grafted neurons. The DNA compacted NPs were injected in rats impaired with 6-OHDA and these rats received fetal dopamine neurons grafted one week later. The pretreatment with DNA compacted NPs increased the number of surviving TH+ cells and improved rotational scores and spontaneous forepaw usage. These results suggested that compacted hGDNF-NPs injected into the striatum can result in transfected cells overexpressing hGDNF protein at levels that provide neurotrophic support for grafted embryonic dopamine neurons. DNA compacted NPs were non-immunogenic and non-inflammatory.

Nerve Growth Factor (NGF) regulates growth, maturation and function of neurons. This peptide regulator was studied by Kurakhmaeva et al., who propose a new systemic PD treatment [47]. NGF was adsorbed on PBCA NPs coated with polysorbate-80, and tested in
vivo in a mice model (C57B1/6) with parkinsonian syndrome induced by intraperitoneal injection of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. A single intraperitoneal injection of NGF-loaded PBCA NPs coated with polysorbate-80 decreased rigidity and increased the locomotor activity in mice. Direct measurement of NGF concentrations in the murine brain showed an efficient NGF transport across the BBB.

**Antioxidant delivery strategy**

Similar to AD patients, PD patients show an increased production of reactive oxygen and reactive nitrogen species, which are neurotoxic and contribute to symptom appearance. Catalase is a redox enzyme known to inactivate reactive oxygen species and to reduce inflammation at the site of action. It is known that macrophages can cross the BBB to reach inflammation sites. Thus one treatment strategy that has been investigated is to use blood-borne macrophages as Trojan horses to carry the therapeutic enzyme catalase, loaded in NPs [48,49]. NPs were obtained by coupling the catalase enzyme to a cationic block copolymer, polyethyleneimine-poly(ethylene glycol). Macrophages were then loaded with catalase-NPs and intravenously injected into a PD mouse model (intoxication with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) (Figure 3). The neuroinflammation was reduced and nigrostriatal degeneration was attenuated following treatment with catalase-NP loaded macrophages. In addition, catalase loaded into NPs was protected from degradation by macrophage cytoplasm enzymes.

**Figure 3:** Biodistribution of NP loaded macrophages in MPTP-intoxicated mice by IVIS. MPTP-intoxicated Balb/c mice (15mg/kg) were i.v. injected with A: Alexa Fluor 680-labeled NPs loaded to macrophages (5×10⁶ cells/mouse); B: Alexa Fluor 680-labeled macrophages loaded with non-labeled NPs, and C: Alexa Fluor 680-labeled NPs administered alone. Representative images from n=4 mice per group (dorsal planes) taken at various time points, demonstrating that both components of the formulation, NPs (A) and macrophages (B), were detected in the brain area in MPTP-intoxicated animals. Noteworthy, macrophages and NPs in cell carriers were remained in systemic circulation at later time points compared with NPs administered alone (C). (Adapted from [48] with permission from PMC).

**α-synuclein fibrillation inhibition as promising strategy**

The α-synuclein fibrillation in the central nervous system is responsible for formation of Lewy bodies, which are characteristic of PD. The inhibition of fibril formation and the
destruction of already formed aggregates are potential therapeutic strategies for PD, which have been investigated in vitro.

For example, Rekas et al., proved that PAMAM dendrimers (generation G3 to G5) inhibit fibrillation of α-synuclein and promote the breaking down of pre-existing fibrils [50]. This inhibitor effect increased both with generation number and PAMAM concentration. Using amino or carboxyl groups on the surface of PAMAM dendrimers, Milowska et al., showed that only dendrimers with amino modification inhibited fibrillation of α-synuclein, with a structural reorganization between α-synuclein and PAMAM dendrimers (hydroxyl group of tyrosine and cationic amino groups of dendrimers interaction) [51].

Phosphorus-containing dendrimers (generations G3 and G4) were also studied as potential inhibitors of α-synuclein fibrillation [52]. With α-synuclein/dendrimer ratios of 1/0.1 and 1/0.5, phosphorus-containing dendrimers inhibited fibril formation and the best efficiency was obtained using G3 dendrimers. The β-sheet formation was inhibited through the interaction between the cationic groups of phosphorus dendrimers and the basic amino acid N-terminal region of α-synuclein. However, higher concentrations of dendrimers did not show inhibitor capacity, due to dendrimer association in inactive agglomerates.

Viologen-phosphorus dendrimers with surface modifications, i.e. phosphonate groups or PEG groups, have also been reported [53]. These dendrimers inhibited the α-synuclein β-sheet structure formation. Phosphonate-modified dendrimers were more effective than PEG-modified dendrimers, with a total fibrillation inhibition at low concentrations.

These encouraging in vitro studies, needs to be followed up with in vivo trials to confirm the action of dendrimers as α-synuclein fibrillation inhibitors. If favorable, the dendrimers could provide a therapeutic strategy to break lewy bodies in PD, advantageously in combination with other drugs to obtain synergy effects in treatment of PD patients.

**Polymeric Nanoparticles for Glioblastoma Therapy**

Despite significant medical advances in the field of oncology, cancer remains one of the diseases with the highest mortality rates in the word. GBM is among the most aggressive of all human cancers and the most recurrent brain tumor in adults, representing nearly 50% of all primary gliomas [54]. The incidence rate is approximately 5-10 cases in a population of 100,000 according to the World Health Organization [55,56]. GBM was first described by Bailey and Cushing in 1926, and is classified as a grade IV neuroepithelial tumor, characterized by an abnormal growth of glial cells (glioblasts) in the brain [57]. The tumor typically presents diffuse borders and an extensive infiltration of individual tumor cells into surrounding healthy brain tissue, which complicates removal by surgery [58]. Moreover, conventional chemotherapy is hampered by low selectivity and low drug partitioning over the BBB. Currently available therapies have not been very efficient to improve patient conditions. Generally, the treatment includes surgical excision in combination with chemotherapy and radiotherapy. It provides a mean estimate survival time of only 12-16 month, probably due to glioma recurrence from residual tumors [59,60].

The treatment of GBM remains an important challenge and the development of drug-targeting technologies that enables safe and effective access to the brain is urgently required. In the last decades, different approaches have been developed to overcome the drawbacks of the conventional administration of anticancer agents to the brain by using nanovectors. Particularly polymeric NPs are ideal candidates to deliver DNA, RNA, proteins and chemotherapeutic compounds with high specificity [61-64]. The most recent advances using dual-targeting drug delivery strategies with polymeric NPs are discussed in this section.
Peptide-receptor as dual-targeting drug delivery strategy

One strategy to improve the treatment of GBM has been to identify receptors on tumor cells, which can be targeted using nanomedicines [65]. One example is the Low-Density Lipoprotein (LDL) receptor, a cell-surface receptor, expressed by the BBB cells, and also over-expressed by glioblastoma cells. Angiopep-2 (ANG), a peptide of 19 amino acids, which binds specifically to the LDL receptor, has been shown to enhance delivery of chemotherapeutic agents across the BBB in both in vitro and in vivo models [66,67].

Xin et al., explored the idea of formulating ANG modified NPs with dual targeting function. First, the modified NPs would cross the BBB and then target tumor cells [68]. NPs were prepared by coupling ANG with Maleimide-PEG-PCL copolymer to obtain ANG-conjugated PEG-ePCL NPs (ANG-NPs). ANG-NPs (<100nm) were loaded with Paclitaxel (PTX). In comparison with NPs loaded with PTX without targeting ligand (NP-PTX), loading coefficient and encapsulation ratio of ANG-NP-PTX decreased. The in vivo activity of ANG-NPs was evaluated after intravenous injection in intracranial U87 MG tumor bearing nude mice, using fluorescence labeled NPs. The results showed that the accumulation of ANG-NP-PTX was much higher than the accumulation of NP-PTX in the brain of tumor-bearing mice (Figure 4A). The result was confirmed by ex vivo evaluation of excised tissues (heart, liver, spleen, lung, and kidney), which revealed a selective accumulation of NPs in brain tumor (Figure 4).

The differences in the uptake of ANG-conjugated NPs, in comparison with non-conjugated NPs, could be related to a peptide-induced penetration in the presence of LDL receptors.
both in BBB and tumor cells. The same authors investigated the bioavailability of ANG-PEG-NP-PTX using three-dimensional glioma cell culture model [69]. Transcytosis of ANG-PEG-NP-PTX across BBB cells, followed by endocytosis in tumor cells were demonstrated via LDL receptor recognitions (Figure 5), confirming the dual-targeting strategy.

![Figure 5: A schematic representation of Angiopep-2-conjugated polymer nanoparticles as dual targeting drug delivery system for GBM. (Adapted from [69] with permission from Elsevier.)](image)

The anti-tumor efficacy was tested in vivo, in a U87 MG tumor-bearing mouse model. In comparison with the control group treated with saline, tumor inhibition ratios were 20.5%, 36.1% and 65.6% when mice were treated with Taxol, PEG-NP-PTX or ANG-PEG-NP-PTX, respectively. In addition, mice treated with ANG-PEG-NP-PTX exhibited a median survival time of 37 days, which was significantly longer than for mice treated with Taxol or PEG-NP-PTX. Together the results demonstrate the potential of the dual-targeting strategy using ANG-conjugated NPs. Furthermore, no acute toxicity was detected in the hematological system, liver, kidney and brain parenchyma, after intravenous administration of conjugated non-loaded NPs (100mg/kg/day) during a week [68].

**Dual-targeting of both neovascular and glioma cells**

GBM is one of the most vascular of all solid tumors [70], and neovascularization has a considerable impact on the glioma growth. Zhang et al., described an interesting dual-targeting approach by developing NPs to target neovascular cells as well as to deliver PTX to treat the tumor cells [71]. EGFPeEGF1, a fusion protein, was shown to bind specifically the Tissue Factor (TF) [72], expressed on neovascular and tumor cells. PEG-PLA NPs (105nm) were prepared by the emulsion-solvent evaporation method. It was found that surface-modified NPs were taken up by the cells expressing TF, indicating a mechanism of receptor-mediated endocytosis. In comparison with the non-functionalized NPs, an improved in vivo uptake of the functionalized NPs in neovascular and extravascular tumor cells was observed 4h post intravenous administration [71]. Moreover, animals treated with functionalized NPs had a longer median survival time (41 days) than animals treated with non-functionalized NPs (21-27 days), saline (14 days) or Taxol (13 days).

**Aptamer-peptide conjugates as dual-targeting delivery system**

A targeted delivery system able to cross the BBB was developed by Gao et al., [73,74].
Docetaxel-loaded PEG-PCL NPs (170nm) were prepared by the emulsion-solvent evaporation technique and a 12 amino acid peptide TGYKALHPHNG (TGN) and an aptamer (AS1411) were grafted to the surface of the NPs to improve uptake over the BBB and to target tumor cells, respectively. The tumor targeting efficiency of the NPs was evaluated in vitro using mouse brain endothelial cells. NPs grafted with both TGN and AS1411 exhibited a higher brain uptake in comparison with the AS1411-NPs and non-grafted NPs, which suggested a TGN mediated uptake of NPs through BBB. NPs modified with TGN were observed in healthy brain tissue and also in the tumor cells while NPs modified with both TGN and AS1411 were found mainly inside the GBM cells (Figure 6). The results of the uptake of the NPs into the GBM confirmed the dual-targeting effect of the formulation. The improved effect of the dual-targeting strategy was demonstrated in vivo, i.e. tumor-bearing mice treated with AS1411-TGN NPs, had an increased survival time (up to 36 days), compared to mice treated with AS1411-NPs (30 days) or TGN-NPs (31 days).

**Figure 6:** The in vivo imaging of DiR-loaded NPs (NP), AS1411-NPs (AsNP), AS1411-TGN NPs (AsTNP) and TGN NPs (TNP) in the brain glioma bearing nude mice at several time points with ex vivo imaging of the brain at 24 h. (Adapted from [74] with permission from Elsevier).

**Conclusion**

There has been a rapid development of drug loaded nanovectors over the last decade. Nanovectors have been explored for improved therapeutic effects in the treatment of a wide range of pathologies, including neurological disorders like AD, PD and GBM. Numerous preclinical studies reported in the literature, demonstrates that nano-encapsulation can provide significant improvements in the delivery of drugs to the brain. Specifically, surface modifications of NPs using biobarrier penetration enhancers and targeting ligands can be used to enhance drug absorption over the BBB and the selectivity towards a specific target.

Polymeric NPs-based strategies for treatment of PD and/or AD, which have been discussed in the literature, includes
i) DA replacement and acetylcholinesterase inhibitor delivery;
ii) α-synucleid fibrillation and amyloid-β plaques inhibition and targeting;
iii) Neuron protection and stimulation; and
iv) Antioxidant delivery

In GBM, polymeric NP, have been used to develop novel dual-targeting drug delivery systems, including

i) Peptide dual-targeting drug delivery strategy;
ii) Anti-neovascular therapy; and
iii) A cascade targeting delivery system strategy.

Importantly, as reported for GBM, therapies could be improved combining two or more treatment strategies using a single NP formulation.

Another important field in nanomedicine is diagnosis. A majority of brain pathologies is detected too late through the manifestation of clinical disorders. Early detection is a key step in a successful treatment of a disease and an important application of NPs in CNS disorders is the diagnostic imaging. Thus, a future aim should be to develop complementary strategies of diagnostic analysis and therapy to improve the treatment of CNS disorders.

Perhaps the most challenging part today is to bring the promising nanomedicines to the market. Keeping in mind patient benefits, this issue should be considered as a main concern by using safe materials, safe and scalable protocols, to reach successfully toxicological assays and clinical trials.

References


