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Mini review

Therapeutic potential of controlled drug delivery systems in neurodegenerative diseases

N. Popovic *, P. Brundin

Neuronal Survival Unit, Department of Experimental Medical Science, BMC A10, 22 184 Lund, Sweden

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Abstract

Several compounds that exhibit a therapeutic effect in experimental models of neurodegenerative diseases have been identified over recent years. Safe and effective drug delivery to the central nervous system is still one of the main obstacles in translating these experimental strategies into clinical therapies. Different approaches have been developed to enable drug delivery in close proximity to the desired site of action. In this review, we describe biodegradable polymeric systems as drug carriers in models of neurodegenerative diseases. Biomaterials described for intracerebral drug delivery are well tolerated by the host tissue and do not exhibit cytotoxic, immunologic, carcinogenic or teratogenic effects even after chronic exposure. Behavioral improvement and normalization of brain morphology have been observed following treatment using such biomaterials in animal models of Parkinson's, Alzheimer's and Huntington's diseases. Application of these devices for neuroactive drugs is still restricted due to the relatively small volume of tissue exposed to active compound. Further development of polymeric drug delivery systems will require that larger volumes of brain tissue are targeted, with a controlled and sustained drug release that is carefully controlled so it does not cause damage to the surrounding tissue.

Keywords: Biomaterials; Biocompatibility; Microspheres; Nanoparticles; Rods; Neurodegenerative diseases

1. Introduction

The last decade has brought considerable advances in our understanding of the mechanisms involved in the development of neurodegenerative disorders. An extended list of therapeutics has also been identified while drug delivery appears to be one of the main obstacles to efficient treatments for neurodegenerative diseases.

Drug accessibility to the central nervous system (CNS) is limited by the blood–brain barrier (BBB), which restricts the selection of applicable compounds depending on their size and endothelial permeability. High dose parental drug administration is often necessary to reach sufficient concentrations of the drug in the brain parenchyma. Many compounds found to be neuroprotective in vitro induce side effects when administered to animals. Peptides and small molecules with lipophilic characteristics satisfy penetrability requirements. However, their limited stability due to rapid systemic degradation before reaching the site of action is a major disadvantage.

Different approaches have been developed to overcome difficulties associated with systemic drug administration. Selective drug biodistribution has been improved by conjugating active compounds with carriers exhibiting a high affinity to the BBB and a capacity to promote active transport of drug across the barrier. To enable drug application in close proximity to the desired site of action, direct drug delivery has been applied by: (a) local infusion using mini-osmotic pumps, (b) implantation of drug-containing biodegradable devices like microparticles, microcapsules, and nanoparticles, or (c) transplantation of non-biodegradable devices that accommodate cells that synthesize and release deficient trophic factors, hormones or neurotransmitters. These systems have resolved some of the problems of drug delivery. Direct drug infusion allows the greatest degree of control of dosage, polymer implants supply the highest local drug concentrations, while implantation of encapsulated drug-releasing cells potentially provides sustained drug delivery. Nevertheless, numerous problems remain to be solved. In this review, we elaborate performance of biodegradable polymeric

* Corresponding author. Tel.: +46 46 222 0524; fax: +46 46 222 0531.
E-mail address: natalija.popovic@med.lu.se (N. Popovic).

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drug delivery systems applied in the treatment of neurodegenerative diseases.

2. Implantable polymeric drug delivery systems

Drug incorporation into an implantable polymeric carrier for controlled release was invented by Folkman and Long (1964) who implanted digoxine-releasing silicone rubber devices into the myocardium of dogs (Benoit et al., 2000). Since then, many natural or synthetic materials have been implanted in different organs and their biocompatibility examined. Polymers have shown to be especially suitable for the preparation of implants. Non-biodegradable polymers such as poly(tetrafluoroethylene) (Teflone) or polyethylene have been extensively used for preparation of vascular and orthopedic implants, respectively. Synthetic poly(α-hydroxy-acids) of the aliphatic polyesters, including poly(lactic acid) (PLA), poly(glycolic acid) (PGA) and their copolymers poly(lactic-*co*-glycolic acid) (PLGA), and polyanhydrides are degradable polymers affected by non-enzymatic hydrolytic processes. The rate of biodegradation varies from less than one month to a few years. Their byproducts are shown to be biocompatible, nontoxic and readily excreted.

3. Can polymeric drug delivery systems be beneficial for neurodegenerative diseases?

Treatment of neurodegenerative disorders is based on the strategies that either prevent cell degeneration and death, stimulate cell proliferation or compensate depletion of neuroacitve substances. Neurotransmitter, hormone and trophic factor-loaded polymer delivery systems have been used in different models of neurodegenerative diseases. In this article we will elaborate the results of polymer implantation in animal models of Parkinson's, Alzheimer's and Huntington's diseases (Table 1).

3.1. Parkinson's disease

The main neuropathological hallmark of Parkinson's disease (PD) is a degeneration of nigro-striatal dopaminergic neurons. The unilateral destruction of the nigro-striatal pathway by stereotaxic injection of toxin 6-hydroxydopamine (6-OHDA) has been widely used to study the hemiparkinsonism in rodents. This animal model displays deficits in several well established tests of motor function. For example, upregulation of postsynaptic dopamine receptors in the denervated striatum induces contralateral rotation behavior if the animal is treated with a dopamine receptor agonist, e.g. apomorphine.

Levodopa (L-DOPA) still remains the backbone of effective drug therapy of PD. Due to limited transport across the BBB it has to be administered at high doses. Generally, L-DOPA is combined with a peripheral decarboxylase inhibitor (e.g. carbidopa-CD or benzeraside) that inhibits peripheral L-DOPA decarboxylation to dopamine. Recently L-DOPA- and CD-loaded microspheres have been produced and their therapeutic efficiency have been tested in rats with 6-OHDA lesions of the substantia nigra. Intrastriatal implants of a mixture of L-DOPA/CD-loaded microspheres were found to reduce apomorphine-induced rotational motor asymmetry in hemiparkinsonian rats. The reduction in asymmetry was detectable as early as 1 week after lesion, but only reached the relatively modest level of 25% at 8 weeks after lesion (Arica et al., 2005).

Implantation of dopamine or norepinephrine-loaded PLGA microspheres into the striatum has been reported to stimulate regrowth of dopaminergic fibers accompanied by a 80% decrease in apomorphine-induced rotational behavior for up to 4 weeks (McRae and Dahlström, 1994; McRae et al., 1994). A major problem with interpreting functional effects based solely on apomorphine-induced rotation is that there are risks for non-specific effects of the treatment. Since apomorphine-induced rotation is dependent upon the integrity of dopamine receptors on striatal neurons, non-specific damage to striatal neurons, e.g. due to the implantation surgery or the implants themselves, can result in a reduction in apomorphine-induced rotation.

Glial cell line-derived neurotrophic factor (GDNF) was considered a highly promising growth factor for PD treatment. Two open-label trials involving continuous GDNF infusion into the putamen of five PD patients in Bristol, UK (Gill et al., 2003; Patel et al., 2005) and ten PD patients in Lexington, Kentucky (Slevin et al., 2005) showed that the growth factor could improve motor symptoms that stayed on also during the 1 month washout period (Slevin et al., 2005). Positron emission tomography (PET) of ^{18}F-DOPA uptake demonstrated a significant increase in putamen dopamine storage indicating a direct effect of GDNF on dopamine function (Gill et al., 2003). However, a double blind placebo controlled study on GDNF infusion into PD patients presented by the biotechnology corporation AMGEN

Table 1
Neuroprotective effect of polymeric drug delivery systems

Implant	Lesion	Implantation Time	Recovery parameters (%)		Recovery period	Reference
			Morphological	Behavioral		
MS-DA or MS-NE	Striatum (PD)	After lesion	Significant	30–50	12 weeks	McRae and Dahlström (1994)
MS-L-DOPA/CD	Substantia nigra	4 weeks after lesion	N.D.	25	8 weeks	Arica et al. (2005)
MS-GDNF	Striatum (PD)	2 weeks after lesion	Significant	75	22 weeks	Jollivet et al. (2004b)
MS-GDNF	Striatum (PD)	Immediately after lesion	26	Significant	6 and 3 weeks, resp.	Gouhier et al. (2002)
MS-NGF	Septum-Hippocampus	Immediately after lesion	30	N.D.	6 weeks	Péan et al. (2000)
Rod-NGF	Fimbria-Fornix	Immediately after lesion	46	N.D.	2 weeks	Hoffman et al. (1990)
MS-Bethanechol	Fimbria-Fornix	2 weeks after lesion	Significant	Significant	40 days	Howard et al. (1989)
MS-NGF	Striatum (HD)	1 week before lesion	40	N.D.	2 weeks	Menei et al. (2000)

DA: dopamine; HD: Huntington's disease; N.D.: not determined; NE: norepinephrine; MS: microspheres; PD: Parkinson's disease; resp.: respectively.

dampened the enthusiasm since the results were negative (Amgen Incorporated, 2004; Pollack, 2005). Essentially there were no beneficial effects in the patients, and in non-human primate safety studies AMGEN reported unwanted side-effects. As a result, all clinical development of GDNF treatment in PD was halted. However, GDNF may still be an interesting candidate for the future. The problems in the AMGEN trials could be related to dose and mode of delivery of the growth factor and therefore polymer-based drug delivery systems could be valuable in this respect.

In several PD models, GDNF has been shown to protect mesencephalic dopaminergic neurons from 6-OHDA induced degeneration if it is administered via pump or repeated injections at the site of toxic insult (Björklund et al., 1997; Kirik et al., 2004). There are also positive results when GDNF has been delivered using biodegradable microparticles. Thus, intrastriatal injection of GDNF-releasing microspheres into the striatum 2 weeks after 6-OHDA lesion induces sprouting of the preserved dopaminergic neurons. Tyrosine hydroxylase (TH, the rate limiting enzyme in dopamine synthesis) expression increased by 26% and the density of striatal dopamine transporter (DAT) went up by 17% in the striatum 6 weeks after implantation of microspheres loaded with 2.5–3.75 μg of GDNF. As a consequence, almost half of the rats displayed a reduction in the number of ipsilateral turns and functional improvement in a test of sensorimotor function, between 8 and 22 post-implantation weeks (Jollivet et al., 2004a,b). When GDNF-releasing microspheres were implanted immediately after 6-OHDA injection, however, significant improvement in amphetamine-induced rotation test was detected only during the first three post-operative weeks (Gouhier et al., 2002). It is known that direct intrastriatal injection of 10 μg GDNF induces a 50% reduction of apomorphine-induced rotations 2 weeks after injection, but this effect does not last until 4 weeks (Aoi et al., 2000). Continuous intraventricular infusion of GDNF at 3 μg/day, from 2 to 6 weeks after lesion has efficiently protected nigral dopaminergic neurons with long lasting improvement in the drug-induced rotation and stepping test while instrastriatal infusion showed only transient beneficial effect on behavior (Kirik et al., 2001). These data indicate that a satisfactory therapeutic effect in hemiparkinsonian rats is achievable only by continuous GDNF delivery and the choice of optimal site may be influenced by disease severity.

3.2. Alzheimer's disease

Alzheimer's disease (AD) is characterized by learning and memory impairments. Plaques and tangles clearly are an important hallmark of AD pathology. The symptoms, however, are also well correlated with the degeneration of basal forebrain cholinergic neurons and consequent cortical and hippocampal cholinergic denervation. The transection of the fimbria-fornix pathway in rodents induces a similar hippocampal cholinergic denervation that results in spatial memory deficits. In this rodent model of AD, intrahippocampal implantation of microspheres loaded with bethanechol, an acetylcholinesterase (AChE)-resistant cholinomimetic, ameliorated memory deficits (Howard et al., 1989). Intraseptal implantation of 3 mg microspheres loaded with 5 μg of NGF, with estimated release of 70 ng/day, immediately after transection of the septohippocampal pathway, promoted the survival of axotomized cholinergic neurons. The percent of AChE-immunopositive neurons was increased from approximately 30% in non-treated-animals to more than 60% in NGF-treated animals (Péan et al., 2000). Intraventricular implantation of 4 mm length poly(ethylene-vinylacetate) (EVA) rods with 3 μg of incorporated NGF for 2 weeks reduced the loss of AChE-positive neurons by 46% in the medial septum and vertical limb of the diagonal band of Broca in rats with unilateral fimbria-fornix lesions (Hoffman et al., 1990). By comparison, continuous infusion of NGF at the low rate of 0.13 μg/day for 2 weeks, starting 3 days before fimbria-fornix transection, prevented 50% of the neurons in the rat medial septum and almost 100% of those in the vertical limb of the diagonal band of Broca from undergoing degeneration and death (Williams et al., 1986).

3.3. Huntington's disease

Huntington's disease (HD) is characterized by striatal, hypothalamic and cortical degeneration resulting in involuntary movements, personality changes, weight loss and dementia. A widely used animal model that mimics some of the pathology and symptoms of HD is based on intrastriatal injection of excitotoxins such as quinolinic acid. In this model, the effect of NGF-microspheres has been tested. Microspheres, weighing 0.8 mg and containing 1.5 μg of NGF, were intrastriatally implanted 1 week before the lesion was induced. The size of the lesion was reduced by 40%, with protection of several types of striatal cells, including cholinergic and GABAergic neurons, up to 3 months after surgery (Menei et al., 2000). In contrast, daily intrastriatal injection of 1 μg NGF for 1 week in the same rat model of HD protects only cholinergic neurons without apparent effect on glutamic acid decarboxylase (GAD)-immunopositive neurons (Venero et al., 1994). Thus, a constant release of NGF from biodegradable microparticles may be more effective than injections of NGF. Particles producing human ciliary neurotrophic factor (hCNTF) poly(acrylonitrile-*co*-vinyl chloride) have been implanted into the lateral ventricle 8–12 days before quinolinic acid injections into the rat striatum. These particles completely protected cholinergic neurons and 90% of GAD-immunopositive neurons and reduced apomorphine-induced rotation behavior (Emerich et al., 1996, 1997a), demonstrating that CNTF can protect against excitotoxic injury.

4. Delivery of neuroactive agents by living cells

The focus of this chapter is the use of polymer systems to achieve delivery of bioactive compounds to the brain in models of neurodegenerative diseases. A slightly different angle on this approach is the use of polymer-encapsulated progenitor or immortalized cells programmed by ex vivo gene transfer to produce growth factors. This technique circumvents the inherent risks of either tumor formation due to overgrowth of the grafted cells or immune rejection of the transplanted cells and ensures

Table 2
Neuroprotective effect of encapsulated drug-releasing cells

Implant	Lesion	Implantation time	Recovery parameters (%)		Recovery period	Reference
			Morphological	Behavioral		
GDNF-BHK cells	MFB	1 week before lesion	38	72	1 week	Tseng et al. (1997)
NGF-SCT-1 cells	Fimbria-Fornix	Immediately after lesion	40	N.D.	3 weeks	Schinstine et al. (1995)
CNTF-BHK cells	Striatum (HD)	12 days before lesion	Significant	Significant	70 days and 4 weeks, resp.	Emerich et al. (1996)

BHK cells: baby hamster-kidney cells, DA: dopamine, MFB: medial forebrain bundle, N.D.: not determined, PC12 cells: pheochromocytoma cells, resp.: respectively, SCT-1 cells: Schwannoma cells

long term drug delivery without necessity of repeated surgical interventions (Table 2).

Implantation of encapsulated dopamine- and L-DOPA-producing rat pheochromacytoma (PC12) cells into the striatum of hemiparkinsonian rats normalized motor asymmetry for up to 6 months (Lindner and Emerich, 1998). This demonstrated that encapsulated cells could be used to replace a neurotransmitter locally in the brain. Encapsulated baby hamster-fibroblast (BHF) or kidney (BHK) cells genetically engineered to produce growth factors have been used in neuroprotection studies in models of PD and HD. Long term secretion of growth factors such as human NGF for over 1 year was achieved by implantation of BHK cells in the lateral ventricles of rodents (Winn et al., 1996). The average daily release was 2.2 ng of NGF. Rats receiving implants of BHK cells releasing 5–10 ng GDNF/day, were implanted unilaterally into the substantia nigra 1 week before transection of the medial forebrain bundle. Some reduction in rotational behavior was observed 1 week after treatment which correlated with a partial protection of the dopaminergic neurons that had been axotomized by the transection procedure (Tseng et al., 1997; Zurn et al., 2001).

Delivery of CNTF to the striatum can protect against excitotoxic damage, as mentioned earlier. Implants of CNTF-releasing BHK cells into quinolinic acid-lesioned primate striatum preserved cholinergic, GABAergic and diaphorase-positive neurons (Emerich et al., 1997b). Intraventricular implantation of encapsulated BHK cells releasing hCNTF induced complete, partial or no neuroprotection depending of the amount of hCNTF released (Emerich, 2004). Development of this technique has recently led to trials with intraventricular implantation of hCNTF-producing BHK encapsulated cells in patients affected by HD. After a follow-up of 2 years, the estimated amount of released CNTF was 0.15–0.5 μg/day. The effect, if any, on neurologic symptoms has not yet been reported in detail (Bloch et al., 2004).

Implantation of encapsulated cells is advantageous when trying to achieve long term localized delivery of neuro-active compounds into the brain. It circumvents problems of drug denaturation or repeated refilling associated with other therapeutic delivery techniques. However, the estimated diffusion distance of 0.2 mm (Lindner and Emerich, 1998) to 1 mm (Kordower et al., 1996) does not appear to be sufficient when transferring the technology to many diseases that affect the human brain. Moreover, it is a major practical problem that cells within the capsule die due to, e.g., failing supply of nutrients as the pores in the capsule clog up with extracellular proteins.

5. Performances of polymer drug delivery systems

Intracerebral implantation of polymeric drug delivery systems to treat neurodegenerative disorders is faced with several practical problems related to the design of the drug delivery system (Table 3). They fall within at least four different areas: (a) loading capacity and controlled and sustained release of active agent, (b) drug instability and particle degradation, (c) drug penetrability, and (d) particle biocompatibility and safety of the implantation procedure.

5.1. Loading capacity and releasing profile

The ability of polymer systems to release the drug over prolonged periods of time is crucial. The solubility of the protein determines loading capacity and high solubility allows a more uniform distribution of protein within the particles. This, in turn, minimizes the risk of unwanted burst release. High immediate drug release from polymers can be caused by a non-uniform distribution of the drug within the matrix and an enhanced adhesion to the surface of the particle making the drug more accessible once the particle is hydrated by the surrounding medium.

Protein release kinetics from microspheres depends on the used encapsulation technique although it is difficult to make generalized estimation. In one of the particular analyses it was found, for example, that microspheres prepared by spontaneous emulsification technique (SE) release less than 10% of the total amount of loaded protein over the first 5 days. After approximately 8 weeks, over 75% of the total protein is released. In contrast to that, microspheres produced by double emulsion method (DE) release almost 20% of the total protein loaded within the first 2 h of incubation. This is followed by slow release of the remaining 70% during the period of 8 weeks (Fu et al., 2003). During the first 24 h, the in vitro delivery rate of GDNF- and NGF-microspheres has reached 17% or 28%, respectively of the total amount. During the following 5 weeks, 50% of the total encapsulated NGF is released (Menei et al., 2000). Therefore, the first 10-fold decrease of NGF release occurred during the first 10

Table 3
Properties of microspheres drug delivery system

Advantages	Disadvantages
Extended period of drug release	High initial drug release
Biocompatibility	Particle degradation
Reduced risk of side effects	Limited drug penetration

days after implantation while second the 10-fold decrease takes place during the ensuing 6 post-implantation weeks (Krewson and Saltzman, 1996). For GDNF particles, around 28% of the growth factor is released by 7 weeks (Jollivet et al., 2004a).

Drug loading in nanospheres is lower and polymer degradation occurs more rapidly than in microspheres. Initial high burst can occur even in nanospheres that are prepared by the SE method, due to the large surface to volume ratio. The kinetics of NGF release from EVA rods also showed the burst phase during the first 24 h with 12% of loaded protein being released (Hoffman et al., 1990). During the following 2 weeks, NGF was released at a rate of 4–9% per day, leading to a total release of 72% (Hoffman et al., 1990). This corresponds to an average NGF release of 100–275 ng/day. Delivery of 100 ng/day NGF has been found to be sufficient for therapeutic effects in rodent models of AD (Tuszynski et al., 1991; Naumann et al., 1994). Even as little as 5–15 ng/day of NGF, released from polymer-encapsulated genetically modified cells, supported a 75–90% increase in cell survival in the fimbria-fornix transection model of AD (Martinez-Serrano et al., 1995). In a comparative study, 5 mm long GDNF-releasing polymer rods initially displayed similar amounts of released protein as genetically modified encapsulated cells and a gene therapy strategy using lentiviral vectors (Bensadoun et al., 2002). In the long term, the encapsulated cells and lentiviral vector strategy were more effective at releasing GDNF than the polymer rods (Bensadoun et al., 2002).

5.2. Drug instability and particle degradation

Denaturation of the therapeutic protein can occur during one or more of several steps during production of the microparticles. Firstly, during the emulsification step or aggregation following the initial rehydration of the particles. Secondly, the active protein can undergo denaturation during adsorption to the polymer or, thirdly, during acidification induced by products generated during particle degradation. The rate of degradation of the microparticle is an important factor to consider in the formulation of the drug delivery system, since an overly rapid degradation of a polymer particle is associated with uncontrolled high release of the active compound. Degradation rates depends on the lactic acid/glycolic acid ratio and the influence of surrounding implantation area (Spenlehauer et al., 1989; Winn et al., 1996).

5.3. Drug penetration

Active compounds released from implanted polymers distribute into the surrounding brain parenchyma with a concentration decreasing exponentially with distance away from the implant. Drug penetrability can be enhanced by a higher rate of diffusion and a reduced overall rate of elimination. Drugs are usually eliminated by binding to tissue components, cellular internalization and metabolism. The majority of drugs delivered to brain tissue by polymeric systems diffuse 2–3 mm (Krewson et al., 1995; Mahoney and Saltzman, 1999; Saltzman et al., 1999). Conjugation of active compounds with a substance that is eliminated slowly from the brain enhances the penetration of the therapeutic agent. The elimination rate of dextran-NGF conjugates, for example, is approximately seven times lower than of unmodified NGF (Krewson et al., 1996).

5.4. Polymer biocompatibility and safety of implantation procedure

Polymeric systems that undergo a slow degradation can conceivably present a strong stimulus for the immune system. Degradation products can interact with the surrounding tissue, bind to tissue carriers and become antigenic (Saini et al., 1997; Fournier et al., 2003). However, polymers are widely considered to be non-mutagenic, non-cytogenic and non-teratogenic and safe for administration if properly sterilized. Particularly the CNS tolerates implantation of slow-degradable polymers well (Rosen et al., 1983; Leong et al., 1986; Brem et al., 1983; Tamargo et al., 1989; Fournier et al., 2003).

In contrast to other organs, the brain is devoid of a fibroblast capsule formation that would represent a diffusion barrier. Histological analyses have revealed astro- and microglial reactions around NGF-loaded and unloaded microspheres 2–6 weeks after implantation (Péan et al., 2000; Fournier et al., 2003). Infiltration of T-lymphocytes was not observed at any time point. The number and size of depots can influence host tissue response. Activation of microgila and phagocytosis was detectable after implantation of 7 μm microspheres, but not around those that were 30 μm in size (Nicholas et al., 2002). The severity and length of inflammatory reaction also depends of the polymer composition and its rate of degradation. Hydrophobic polymers – due to their slow degradation rate – induce slight inflammatory reactions while hydrophilic formulations tend to cause a greater inflammatory responses. Polymers like poly-[pyromellitylimidoalanine-*co*-1,6-bis(carboxy)hexane] produce minimal inflammation even after implantation in subcutaneous tissue (Ibim et al., 1998).

6. Conclusions

Improvement of both morphological and functional parameters has been detected after implantation of biodegradable microparticles in animal models of PD, AD and HD. Limited drug diffusion restricts the size of brain volume being treated and is a disadvantage in therapy of diseases where the neuropathologic alterations are widespread. Further development of polymeric systems for drug delivery in neurodegenerative disorders is needed. The focus should be on controlled, sustained and self-regulated drug delivery using materials that cause minimal damage to the surrounding brain parenchyma.

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