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ScienceDirect
Behavioural Brain Research 184 (2007) 11–18
BEHAVIOURAL BRAIN RESEARCH
www.elsevier.com/locate/bbr

Research report

Unilateral implantation of dopamine-loaded biodegradable hydrogel in the striatum attenuates motor abnormalities in the 6-hydroxydopamine model of hemi-parkinsonism

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Received 8 February 2007; received in revised form 20 June 2007; accepted 26 June 2007
Available online 5 July 2007

Abstract

Dopaminergic functional recovery following controlled release of dopamine from biodegradable polymer matrices implanted in the lesioned striatum was investigated in a hemiparkinsonian animal model. Significant dopamine depletion in the striatum ipsilateral to the side of infusion was observed in animals unilaterally infused with 6-hydroxydopamine (6-OHDA) in the substatia nigra. These animals displayed apomorphine-induced contralateral rotational behavior, when examined on the 16th day. Implantation of a controlled release delivery system (hydrogel obtained by mixing dextran dialdehyde cross-linked with gelatin) containing dopamine in the denervated striatum on the 1st day or the 18th day significantly abolished the apomorphine-induced contralateral rotational behavior in these animals. The recovery was visible for about 17 days, thereafter the behavioral bias reappeared. The present results indicate that dopamine released from the polymer matrices alleviates behavioral bias in experimental parkinsonism, implying use of such technologies as an alternative method for the treatment of Parkinson's disease. This approach is useful in reducing the oral dose of drugs that are with severe systemic effects, and that develop tolerance.

Keywords: Parkinson's disease; 6-Hydroxydopamine; Dextran dialdehyde cross-linked gelatin hydrogel; Controlled release; Dopamine; Rotational behavior; Striatal implantation; Apomorphine

1. Introduction

Parkinson's disease (PD) is characterized by a progressive degeneration of dopaminergic neurons in the substantia nigra pars compacta (SNpc) region of the brain, leading to massive loss of dopamine (DA) in the nuclei of caudate and putamen (NCP). The major symptoms of the disease consist of a group of motor abnormalities such as tremor, rigidity, akinesia and postural instability. Current treatment provides for symptomatic relief, but none of the existing drugs are without side effects over prolonged use. The precursor of dopamine, L-DOPA is the first-line drug of choice for the treatment of PD. Prolonged use of L-DOPA develops tolerance, severe side effects and "on–off" effects [16,20]. One of the ways to combat this problem is to develop strategies for reducing the dosage and to make the drug available at the target site.

Intracranial implantation technique has been tested for DA replacement in the striatum as a therapeutic procedure in neurodegenerative diseases. Delivery of drugs specifically to the damaged regions would probably be the most efficient method for alleviating the symptoms of the disease. However the most important criterion for such implantation procedure is the non-toxic and biodegradable nature of the carrier polymers. DA or dopamine producing cells or neurotrophic factor-loaded biopolymer implants in the brain have been shown to reverse the neurological deficits manifested following DA loss in hemi-parkinsonian rat model [4,13,19,26,36]. 6-Hydroxydopamine (6-OHDA) that is toxic to both the central and peripheral

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doi:10.1016/j.bbr.2007.06.025

catecholaminergic neurons [25] has been used in several of these studies. Systemic administration of 6-OHDA is ineffective for CNS injury, since it does not cross the blood brain barrier, while stereotaxic injection of 6-OHDA into the SNpc, NCP and medial forebrain bundle (MFB) could induce degeneration of nigrostriatal DA-ergic pathway and result in a specific pattern of rotational behavior upon challenge with DA agonists [26,30,34]. This classical model of PD has been in use for new drug discovery programs for a long time, especially since the hemiparkinsonian rats exhibited reproducible behavioral asymmetry to the administration of apomorphine, a DA agonist, resulting from post-synaptic DA receptor super-sensitivity [28,30,34].

In vitro and *in vivo* biocompatibility of dextran dialdehyde cross-linked gelatin hydrogel is shown by Draye et al. [10,11]. Immediately thereafter dextran dialdehyde cross-linked gelatin microspheres has been used for controlled release studies of the anti-tumor drug TAPP-Br *in vitro* [8]. Encapsulatation of hepatocytes and controlled delivery of primaquine, an anti-malarial, has been demonstrated employing an injectable drug delivery vehicle, alginate dialdehyde cross-linked with gelatin [2]. Although dextran dialdehyde cross-linked gelatin has never been tried in the central nervous system, several other hydrogel matrices are investigated for sustained release of drugs [3,17] and as scaffolds [18,32] in the brain and the spinal cord. Intracranial implantation of sequestrated cells in hollow fiber made of a permselective polymer membrane (which allow the inward diffusion of nutrients and oxygen and the outward secretion of the trophic factors) has been used to correct behavioral abnormalities in PD animals [26].

In the present study we have used a self-cross-linking dextran dialdehyde cross-linked gelatin as an injectable drug delivery vehicle. We studied the effects of controlled release of DA in the striatum from polymer matrices, on functional recovery in a 6-OHDA-rat hemiparkinsonian model. In this model the dopamine releaser amphetamine and DA receptor agonist apomorphine cause stereotypic ipsilateral and contralateral circling behaviors, respectively indicative of profound striatal DA depletion, and development of post-synaptic DA receptor super-sensitivity. We tested the hypotheses that: (i) early implantation of DA embedded polymer can block the development of stereotypic circling behaviors resulting from unilateral striatal DA loss or development DA receptor super-sensitivity, and (ii) striatal implantation of the DA embedded polymers after the development of striatal DA loss would significantly attenuate the severity of the stereotypic rotations. The former investigation would provide information on the prophylactic nature, and latter study would suggest the therapeutic potential of DA embedded hydrogel in PD.

2. Experimental procedure

2.1. Animals

Experiments were carried out on inbred adult male Sprague–Dawley rats weighing 300–350 g obtained from the institute animal facility. Animals were housed at 22 ± 1 °C, 60 ± 5% humidity, with proper illumination (12 h light-dark cycle), four animals per cage, and with free access to food and water. All the experimental protocols met the National Guidelines on the 'Care and Use of Animals in Scientific Research' (INSA, 2000) and were approved by the Animal Ethics Committee of Indian Institute of Chemical Biology, Kolkata. Adequate measures were also taken to minimize pain and discomfort of the animals.

2.2. Chemicals

The following fine chemicals were procured from Sigma Chemicals, St. Louis, USA: 6-OHDA, dopamine hydrochloride, heptane sulfonic acid, amphetamine, apomorphine, ascorbic acid, ethylenediaminetetracaetic acid disodium salt (EDTA), cresyl violet, tris (hydroxymethyl) aminomethane (Tris), paraformaldehyde and 3,3-diaminobenzidine. Chloral hydrate was purchased from Fluka, Switzerland. The primary anti-rabbit tyrosine hydroxylase, the secondary anti-rabbit IgG-conjugated horseradish peroxidase antibody and normal goat serum (NGS) were procured from Chemicon Inc. Temecula, CA, USA. Dextran dialdehyde and gelatin were prepared in one of our laboratories (SCT, Thiruvananthapuram). All the other chemicals and reagents used were of analytical grade and procured locally. Quartz distilled water was filtered and deionized (TKA, Niederelbert, Germany) for use in HPLC-electrochemistry.

2.3. Unilateral intranigral infusion of 6-OHDA

For the stereotaxic surgery, rats were anaesthetized by chloral hydrate (450 mg/kg i.p.). Animals were then positioned in a stereotaxic frame (Stoelting Co., USA). The flat skull position was achieved when the incisor bar was lowered to 3.5 mm below the horizontal zero. A sagittal incision was made in the scalp with sterile blade, the skin and inferior tissue layers covering the skull were retracted and a small hole was drilled at the following coordinates: lateral (L) 0.20; antero-posterior (AP) −0.53, dorso-ventral (DV) −0.75 from the bregma point. The stereotaxic co-ordinates were calculated for the dopaminergic neuronal cell body region, SNpc following the "Rat Brain Atlas" [23]. Vehicle (0.2% ascorbic acid) or 6-OHDA (8 μg in 1 μl in 0.2% ascorbic acid) was infused unilaterally through a 26-gauge stainless steel cannula into the SNpc region employing a Syringe Pump and 'Worker Bee' Controller (BAS, West Lafayette, USA), through a Liquid Switch (BAS, West Lafayette, USA). Animals were allowed to recover completely before being returned to housing.

2.4. Experimental design

- Experiment # 1. A group of animals were assessed for the stereotypic rotational behavior following unilateral 6-OHDA infusion (8 μg in 1 μl in 0.2% ascorbic acid) into SNpc. The schematic diagram (Fig. 1A) depicts the time schedule of analyzes made after the stereotaxic surgery in the animals. In short, the animals were treated with amphetamine (5 mg/kg, i.p.) on the 14th day following surgery. The stereotypic rotations exhibited by the animals were counted for every 10 min till the rotations persisted. Likewise, on the 16th day these animals were injected with apomorphine (1 mg/kg, s.c.) and the resulting rotations were counted every 10 min while it lasted. In both the studies the ipsilateral and contralateral rotations were recorded separately.
- Experiment # 2. Another group of animals was infused with 6-OHDA (8 μg in 1 μl in 0.2% ascorbic acid) into the SNpc as described above. These animals were divided into two groups and were used for investigating early implantation effects, if any. Half of the animals were implanted with DA embedded hydrogel and the other half with hydrogel alone into the ipsilateral striatum. Injectable biodegradable hydrogel loaded with DA (50 μg/μl) in about 10 μl was stereotaxically implanted in the ipsilateral striatum, following the co-ordinates: AP = 0.02, DV = −0.45 and L = 0.26 mm from the Bregma, immediately after the intranigral infusion of 6-OHDA. The schematic representation of the time schedules for stereotaxic surgery, hydrogel implantation, amphetamine and apomorphine-induced behavioral studies are depicted in Fig. 1B. In short, on the 14th and 16th days, respectively, amphetamine and apomorphine were injected in these rats and the stereotypic circling behaviors were studied.
- Experiment # 3. A third group of animals received intranigrally 6-OHDA (8 μg in 1 μl in 0.2% ascorbic acid) as explained above. These animals were divided into two groups and were used in the studies for therapeutic effects of DA-hydrogel implantation in animals where the disease has already set in. Animals showing more than 350 contralateral rotations during the initial