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A drug delivery hydrogel system based on activin B for Parkinson's disease

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ABSTRACT

Parkinson's disease (PD) is one of the most common neurodegenerative diseases. Activins are members of the superfamily of transforming growth factors and have many potential neuroprotective effects. Herein, at the first place, we verified activin B's neuroprotective role in a PD model, and revealed that activin B's fast release has limited function in the PD therapy. To this end, we developed a multi-functional crosslinker based thermosensitive injectable hydrogels to deliver activin B, and stereotactically injected the activin B-loaded hydrogel into the striatum of a mouse model of PD. The histological evaluation showed that activin B can be detected even 5 weeks post-surgery in PD mice implanted with activin B-loaded hydrogels, and activin B-loaded hydrogels can significantly increase the density of tyrosine hydroxylase positive (TH^+) nerve fibers and reduce inflammatory responses. The behavioral evaluation demonstrated that activin B-loaded hydrogels significantly improved the performance of the mice in the PD model. Meanwhile, we found that hydrogels can slightly induce the activation of microglia cells and astrocytes, while cannot induce apoptosis in the striatum. Overall, our data demonstrated that the developed activin B-loaded hydrogels provide sustained release of activin B for over 5 weeks and contribute to substantial cellular protection and behavioral improvement, suggesting their potential as a therapeutic strategy for PD.

1. Introduction

Parkinson's disease (PD), the second most common neurodegenerative disease, is a chronic progressive disorder characterized by cellular loss of nigrostriatal dopaminergic neurons that project to the striatum. Loss of dopamine neurons in the substantia nigra (SN) results in both motor dysfunction and nonmotor symptoms [1,2]. Recently, several studies have been focused on the therapeutic effects of cytokines on diseases in the central nervous system (CNS) [3–8]. Activins are members of the superfamily of transforming growth factors [9,10]. It has been demonstrated that activin A has neuroprotective effects in such neurological diseases as spinal injury, cerebral ischemia, and acute encephalitis [11–14], also activin A can increase rapidly to achieve its neuroprotective and anti-inflammatory functions [14]. Although activin B affects the same receptors and activates a series of downstream signaling pathways as activin A, the effects of activin B in neurodegenerative diseases, such as in PD animal model are not reported yet. Kupershmidt et al. firstly found that activin B can protect neuronal

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cell line SY5Y from being damaged [15]. We also demonstrated that activin B can make a neuroprotective role in both 1-methyl-4-phenylpyridinium ion (MPP^+)-induced SY5Y cell model and the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced mouse models of PD by improving histological and behavioral parameters (Supplementary Fig. 3A, B, D–H). However, the short half-life of activin B, which has been directly injected, necessitates a biocompatible vehicle to deliver activin B with a prolonged release (Supplementary Fig. 3D).

The delivery systems for neuro-related diseases include polymer formulations, mini-pumps, and genetically modified cells [16–20]. Among all, hydrogels performed both similar physical and chemical structures to extra-cellular matrices and have therefore obtained ideal treatment effects in various disease models such as cerebral ischemia, spinal injury, Huntington's disease (HD), and Alzheimer's disease (AD) [21–23]. The applicability of a biohybrid hydrogel platform consisting of chemically cross-linked heparin and star-PEG for neuronal cell replacement strategies in Parkinson's or Huntington's disease treatment has been reported [24]; also, it is found that hydrogel delivery provides significant trophic support to damaged primary afferents and is a promising treatment modality for nerve root compression-mediated pain [25]. Hydrogels are mainly composed of crosslinked hydrophilic macromolecules with water-swollen properties [26]. They can be directly injected into the body and sol-gel transition occurs when specific chemical functional groups are introduced to them [16]. Bioactive components or therapeutic agents can be introduced into hydrogels to mimic extracellular matrix for tissue engineering applications [27]. As is so often pointed out, hydrogels incorporated with these bioactive agents is an universal appeal as they contribute to better cell adhesion, migration, and axon growth [19]. For examples, poly (ethylene glycol) hydrogels combined with the ciliary neurotrophic factor (CNTF) were found to stimulate neurite outgrowth and promote CNTF's sustained release in the body [28]. Acrylated polymerization of acrylated poly(lactic acid)-b-poly(ethylene glycol)-b-(poly lactic acid)PLA-b-PEG-b-PLA hydrogels combined with neurotrophin-3 (NT-3) significantly improved the behavioral results, and increased axon growth in a spinal cord injury model [29].

Thermosensitive hydrogels have been widely used in drug delivery applications. Each application has its specific requirements for hydrogel properties and drug release profile. Hydrogel properties such as thermal properties, chemical properties, injectability, biocompatibility and biodegradability determine the drug/hydrogel interactions. Their properties can be manipulated to meet the desired drug release kinetics when designing a drug delivery system using thermosensitive hydrogels. Several studies have reported the controlled release applications of hydrogels to extend the duration of the drug exposure; it was proven through both in vitro and in vivo tests, to have better efficiency compared to using of the pure molecule [30,31].

Poly(N-isopropylacrylamide) (PNIPAM), a typical and widely studied thermosensitive polymer, exhibits a reversible volume change as the surrounding temperature varies across a temperature that is called the lower critical solution temperature (LCST) [32,33].

It has been reported that PNIPAM based hydrogel holds promise for helping to cure CNS disorders. Omolli et al. found that PNIPAM-co-poly (ethylene glycol) (PNIPAM-PEG) introduced less invasive, injectable scaffold platform for repairing the spinal cord injury (SCI). They found that the release of neurotrophic factors was sustained for up to four weeks. It is also shown that their hydrogel is compatible with bone marrow stromal cells, improving their survival and attachment for up to one month [34]. So as to enhance the biocompatibility, PNIPAM hydrogels has been modified with some other polymers like Collagen [35], hyaluronic acid [36,37].

In our recent work, a novel multifunctional poly (amidoamine) (PAA) was synthesized as a crosslinker for NIPAM to form biocompatible, biodegradable and injectable hydrogels which precipitate cell adhesion and proliferation [38]. This novel multifunctional crosslinker together with NIPAM have introduced promising applications in tissue engineering [39,40].

In this study, an injectable thermosensitive hydrogel is developed based on our developed multifunctional crosslinker and NIPAM to release activin B in a prolonged way. It is hypothesized that activin B-loaded injectable hydrogels have neuroprotective effects on the (MPP^+) and (MPTP)-induced PD cells and mouse model. To this end, we first stereotactically injected activin B-loaded hydrogels into the striatum of MPTP-induced mice. Histological analyses were performed to detect the release of activin B, the TH^+ neuron fiber density, F4/80 for microgial activation, GFAP immunostaining for astrocytic reaction and TUNEL analysis for apoptosis. Behavioral tests were carried out by open field test and rotarod test. The results of the present study may reveal a promising PD treatment.

2. Materials and methods

2.1. Hydrogel and hydrogel/activin B composites preparation

The mutifunctional crosslinker was synthesized as previous reported with modifications [41]. Briefly, the guanidine like agmatine sulfate salt (Sigma Chemical Co., St. Louis, MO, USA), and disulfide contained N,N′-Cystaminebisacrylamide (CBA) (Sigma Chemical Co., St. Louis, MO, USA) were utilized to synthesize the multifunctional poly (amido-amine) crosslinker (PAA) by Michael addition. The molar ratio between CBA (1.8 mmol) and agmatine sulfate (AS) (1 mmol) was 1.8:1 and PAAs are terminated with vinyl bonds. CBA and AS were mixed with methanol/water solvent (50% volume each) to make a final mass concentration of 116 mg/ml using the vortex. 1 mmol of $LiOH \cdot H_2O$ powder was added to solution to tune pH around 6.5. After 72 h reaction at 55 °C in oil batch, the solvent was rotary-evaporated, and the hydrogel was frozen under −20 °C overnight, then put in a −20 °C freeze dryer (CHRIST, Epsilon 2–4 LSC, Germany) for 12 h. Forty-four milligrams (mg) of NIPAM and 1.6 mg of PAA crosslinker were added in 900 μL sterile double distilled water by sonication. Then 9 μL of 100 mg/mL APS (ammonium persulfate, Sigma) was added followed by adding 1 μL N,N,N′,N′-tetramethylethane-1,2-diamine (TEMED, Sigma Chemical Co., St. Louis, MO, USA) and reacted on ice batch for 30 min.

The hydrogel/activin B composites were prepared by mixing the isometric hydrogel with 50 ng/μl activin B on ice. The activin B was added into the hydrogel solution. After thorough mixing, the hydrogel/activin B mixture was stored at 4 °C.

2.2. Characteristics of the physical properties of the hydrogels

2.2.1. Characteristics of the internal structure of the hydrogels

After the hydrogels were prepared, they were frozen for sectioning and gold coating. The morphology of the hydrogels was observed using a scanning electron microscopy (SEM, Hitachi H-7500, Japan).

2.2.2. Measurement of swelling behaviors

The hydrogels were prepared in the shape of a column, with a diameter of 10 mm and a height of 2 mm. After being lyophilized in a freeze dryer, the dry weights of the hydrogels were measured (W_0). Next, the hydrogels were immersed into a phosphate buffered saline (PBS) (0.01 M, pH 7.4) at 37 °C for 24 h and then weighed (W_t) (So as to simulate body conditions phosphate buffered saline (PBS) was selected as the swelling medium and in vitro degradation