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Research Report

Sustained delivery of dbcAMP by poly(propylene carbonate) micron fibers promotes axonal regenerative sprouting and functional recovery after spinal cord hemisection

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ABSTRACT

This study describes the use of poly(propylene carbonate) (PPC) electrospun fibers as vehicle for the sustained delivery of dibutyryl cyclic adenosine monophosphate (dbcAMP) to the hemisected spinal cord. The dbcAMP and PPC were uniformly mixed with acetonitrile; then, electrospinning was used to generate micron fibers. The release of dbcAMP was assessed by ELISA *in vitro*. Our results showed that the encapsulation of dbcAMP in the fibers led to stable and prolonged release *in vitro*. The PPC micron fibers containing dbcAMP and the PPC micron fibers without dbcAMP were then implanted into the hemisected thoracic spinal cord, followed by testing of the functional recovery and immunohistochemistry. Compared with the control group, sustained delivery of dbcAMP promoted axonal regenerative sprouting and functional recovery and reduced glial scar formation, and the PPC micron fibers without dbcAMP did not have these effects. Our findings demonstrated the feasibility of using PPC electrospun fibers containing dbcAMP for spinal cord injury. The approach described here also will provide a platform for the potential delivery of other axon-growth-promoting or scar-inhibiting agents.

Abbreviations: PPC, poly(propylene carbonate); dbcAMP, dibutyryl cyclic adenosine monophosphate; SCI, spinal cord injury; CO_2, carbon dioxide; SEM, scanning electron microscopy; GAP-43, growth-associated protein 43; NF-200, neurofilament-200; GFAP, glial fibrillary acidic protein; BBB, Basso, Beattie, and Bresnahan; chABC, chondroitin sulfate ABC; DMEM, Dulbecco's modified Eagle's medium; DAB, 3,3′-diaminobenzidine; ELISA, enzyme-linked immunosorbent assay

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1. Introduction

Numerous therapeutic strategies have been tested for axonal regeneration and functional recovery after spinal cord injury (SCI). These strategies also involve a variety of administration methods. Until now, the administration methods have included direct injection (Qiu et al., 2002), intraventricular injection (Fouad et al., 2009), intrathecal injection, sustained microinjection (Neumann et al., 2002), and local slow release (Rooney et al., 2011). Several of these methods have disadvantages. Injection methods that involve repeated punctures could result in pain and infections. Implanted tubes used for sustained microinjection could cause tissue damage in the cannula placement site (Fouad et al., 2009). Compared with the strategies above, using sustained delivery agents by implanting biodegradable materials could maintain the local concentration at an effective level and decrease the side effects caused by systematic administration. PPC, which is biodegradable, has been synthesized by carbon dioxide (CO_2) and propylene oxide and can degrade into CO_2 and water (Du et al., 2004). In addition, electrospinning is an effective method to make nanofibers or micron fibers, which can be sculpted into different forms such as membranes, tubes, and scaffolds. Previous studies have shown that biodegradable polymer materials made of PPC generated by electrospinning technology possess optimal biocompatibility and biodegradation properties (Mo et al., 2004). However, in clinical practice, most SCIs are contusion, so using a membrane with drugs is superior to using scaffolds.

dbcAMP, a membrane-permeable analog of cAMP, activates protein kinase A (PKA) signaling by cAMP-related pathways (Rydel and Greene, 1988; Qiu et al., 2002) and then regulates diverse functions within the nervous system. These functions include the following: reduction of apoptosis in cultures of spinal motor neurons (Hanson-Jr et al., 1998) and retinal ganglion cells (Meyer-Franke et al., 1995), as well as rat sympathetic and sensory neurons (Rydel and Greene, 1988), guiding the growth cone of axons (Meyer-Franke et al., 1995; Song et al., 1998), promoting the growth of axons (Rydel and Greene, 1988; Cai et al., 2001, 2002; Neumann et al., 2002; Nikulina et al., 2004), improving axonal regeneration in SCI (Neumann et al., 2002; Qiu et al., 2002; Nikulina et al., 2004), attenuating the formation of glial scars (Nikulina et al., 2004), and decreasing the formation of capillaries in the presence of mesenchymal stem cells (MSCs) (Rooney et al., 2011).

Here, we prepared micron fibers of PPC, which were infused with dbcAMP by electrospinning technology, to assess the possibility of the local treatment of spinal cord injuries. This study aimed at establishing a novel method of dbcAMP delivery to the hemisected spinal cord *via* PPC membranes.

2. Results

Fibers were readily generated from PPC emulsion by electrospinning. The fibers had a smooth surface and relatively uniform morphology, with an average diameter of 3 μm (Fig. 1A).

2.1. *Study of dbcAMP release by infused PPC micron fibers*

The weight ratio of dbcAMP to PPC of our micron fibers was 1:9. The micron fibers supported a stable release of dbcAMP over an 8 day period (Fig. 1B) in Dulbecco's modified Eagle's medium (DMEM) at 37 °C and 5% CO_2. The concentrations of dbcAMP in each time point ranged from 6.80 to 127.45 nmol/ml, and the concentration peak appeared after 8 h.

2.2. *Sustained delivery of dbcAMP regulated the expression of growth-associated protein-43*

Growth-associated protein-43 (GAP-43) is a nervous-tissue-specific protein; increasing its expression in neurons promoted axon regeneration (Buffo et al., 1997; Gianola and Rossi, 2004). The numbers of GAP-43-positive neurons in the control group were 3.40 ± 1.14, 10.20 ± 1.34, 7.00 ± 1.58, and 7.00 ± 2.345 at 1, 2, 3, and 4 weeks, respectively, and the maximum appeared in the second week. In addition, the numbers of GAP-43-positive neurons in the PPC group were 4.00 ± 1.23, 11.00 ± 2.00, 7.40 ± 1.32, and 6.60 ± 1.95 at 1, 2, 3, and 4 weeks, respectively, and the maximum also appeared in the second week. Between these two groups, there was no significant difference at any time point (Fig. 2N). In the experimental group, the average numbers of GAP-43-positive neurons were 7.00 ± 1.58, 13.40 ± 1.34, 14.00 ± 1.58, and 20.00 ± 2.12 at 1, 2, 3, and 4 weeks, respectively, and the maximum number appeared in the fourth week. There were significant differences in the experimental group at each time point compared to the control group and the PPC micron fibers without dbcAMP group ($p<0.05$) (Fig. 2N). Neurons in animals that received dbcAMP were prone to regrowth, with higher expression of GAP-43. Compared with the other three groups, no GAP-43-positive neurons were detected in the sham-operated animals (Fig. 2M). Intra-group comparison of the experimental group showed that the number of positive

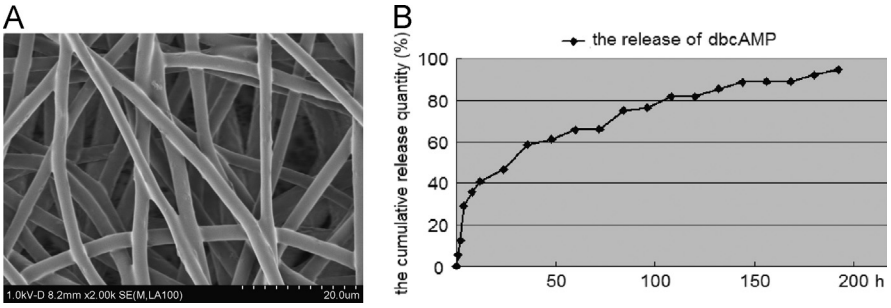

Fig. 1 – (A) Surface and morphology of the micron fibers. (B) Release curve of dbcAMP.