Biomedicine & Pharmacotherapy 86 (2017) 354–362

Available online at
ScienceDirect
www.sciencedirect.com

Elsevier Masson France
EM|consulte
www.em-consulte.com/en

Original article

The combination of db-cAMP and ChABC with poly(propylene carbonate) microfibers promote axonal regenerative sprouting and functional recovery after spinal cord hemisection injury

Tongliang Xia[a,1], Bin Huang[a,1], Shilei Ni[a], Lei Gao[b], Jiangang Wang[a], Jian Wang[a,c], Anjing Chen[a], Shaowei Zhu[d], Benlin Wang[a], Gang Li[a], Shugan Zhu[a], Xingang Li[a,*]

[a] Department of Neurosurgery, Qilu Hospital of Shandong University and Brain Science Research Institute, Shandong University, 107# Wenhua Xi Road, Jinan, 250012, PR China
[b] Jiyang County People's Hospital of Shandong Provence, 9# Xinyuan Street, Jinyang County, Jinan, 251400, PR China
[c] Department of Biomedicine, University of Bergen, Jonas Lies Vei 91, 5009 Bergen, Norway
[d] Department of Neurology, Qilu Hospital of Shandong University, 107# Wenhua Xi Road, Jinan, 250012, PR China

ARTICLE INFO

Article history:
Received 25 June 2016
Received in revised form 8 December 2016
Accepted 8 December 2016

Keywords:
SCI
Electrospinning
ChABC
db-cAMP
PPC

ABSTRACT

This study describes the use of poly(propylene carbonate) (PPC) electrospun microfibres impregnated with a combination of dibutyryl cyclic adenosine monophosphate (db-cAMP) and chondroitinase ABC (ChABC) in the treatment of right-side hemisected spinal cord injury (SCI). Release of db-cAMP and/or ChABC from the microfibres was assessed in vitro using high-performance liquid chromatography (HPLC). Drug-impregnated microfibres were implanted into the hemisected thoracic spinal cord of rats, and treatment was evaluated using functional recovery examinations and immunohistochemistry. Our results demonstrated that the microfibres containing db-cAMP and/or ChABC displayed a stable and prolonged release of each agent. Sustained delivery of db-cAMP and/or ChABC was found to promote axonal regenerative sprouting, functional recovery, and reduced glial scar formation when compared to untreated control animals. The combination of both db-cAMP and ChABC was determined to be more effective than using either drug alone in the treatment of SCI. These findings demonstrate the feasibility of using PPC electrospun microfibres for multi-drug combination therapy in SCI.

1. Introduction

Spinal cord injuries (SCI) are global medical concerns, primarily due to the inability of this tissue to regenerate. This lack of regenerative ability is thought to result from several factors, including immune responses, glial scar formation, and the lack of sufficient neurotrophic support after SCI [1–5].

Several experimental drugs have recently been reported to promote recovery of neural function and neural axon regeneration after SCI [6–10]. However, despite much effort, methods to selectively deliver these drugs to the SCI site in a timely manner are currently lacking, due in large part to the presence of the blood brain barrier. To date, the methods of administration that have shown the most promise include direct injection [9], intraventricular injection [11], intrathecal injection, sustaining microinjection [12], and local slowly releasing [13]. However, each of these methods has associated shortcomings. For example, irrespective of injection site/method, repeated punctures are required, which are often painful and increase the risk of secondary infections. Further, the use of implanted tubes to sustain microinjections can cause tissue damage in the site of cannula placement [11].

We have previously determined that biodegradable polymer materials made of poly (propylene carbonate) (PPC) using electrospinning technology possessed the optimal combination of biocompatibility and biodegradation ability to deliver drugs to a SCI site [14]. Since PPC is synthesized from carbon dioxide (CO_2) and propylene

Abbreviations: SCI, spinal cord injuries; CS, Chitosan; PPC, poly(propylene carbonate); db, cAMP dibutyryl cyclic adenosine monophosphate; ChABC, chondroitinase ABC; PVP, polyvinylpyrrolidone; GFAP, glial fibrillary acidic protein; NF-200, GAP-43 growth-associated protein-43; CSPGs, chondroitin sulfate proteoglycans.

* Corresponding author.
E-mail address: lixg@sdu.edu.cn (X. Li).

[1] These authors have contributed equally to the work.

http://dx.doi.org/10.1016/j.biopha.2016.12.045
0753-3322/

oxide, its biodegradation products (CO_2 and water) are non-toxic. In addition, electrospinning was found to be an ideal method to make nano- or microfibres from this material, since they can be easily sculpted into membranes, tubes, and/or scaffolds [15–18]. Meanwhile, recent reports have shown that dibutyryl cyclic adenosine monophosphate (db-cAMP) and chondroitinase ABC (ChABC) exhibit properties that promote axonal regeneration. Specifically, db-cAMP was found to inhibit neuronal apoptosis, exhibit beneficial effects on axons [19–22], attenuate the reactive gliosis and formation of the glial scar [23], and reduce capillary formation [13]. ChABC decomposes the glycosaminoglycan (GAG) side chain components of chondroitin sulfate proteoglycans (CSPGs), which are known to be obstacles for axonal regeneration [4,5,24–27].

Since the two agents promote axonal regeneration with different functioned ways, and due to the aforementioned PPC-based delivery system is well suited for SCI therapeutic applications, we thus evaluated PPC-based microfibres containing both db-cAMP and ChABC to determine whether this combination products exhibits synergistic axonal sprouting and/or functional recovery effects compared to their individual use and whether the system is suitable for multidrug. In this context, we found that the combination of the two agents was superior to the single use and they are allowed to be sustained local delivery of multidrug in the SCI.

2. Experimental procedures

2.1. Materials and anti-bodies

Chitosan (CS), polyvinylpyrrolidone (PVP), and haematoxylin were purchased from Aladdin Reagents (Shanghai, China). Acetic acid, tripolyphosphate, dichloromethane, and paraformaldehyde were obtained from Beijing Chemical Reagent (Beijing, China). ChABC was purchased from Sigma-Aldrich Company (Santa Clara, USA), and db-cAMP and PPC were purchased from Inner Mongolia Mengxi High-Tech Group Co. (Erdos City, China). Hydrogen peroxide, 3,3′-diaminobenzidine, 0,0-dimethyl-0-2,2-dichloroethylene phosphate, goat serum, and phosphate-buffered saline (PBS) were purchased from ZSGB-BIO (China). Anti-GAP-43 primary antibody, anti-neurofilament-200 (NF-200) primary antibody, anti-glial fibrillary acidic protein (GFAP) primary antibody, anti-chondroitin sulfate (CS-56) primary antibody, 4–6-diaminidino-2-phenylindole, anti-rabbit IgG conjugated with fluorescein isothiocyanate, and anti-mouse IgG conjugated with Texas red were all purchased from Abcam (England).

2.2. Preparation of CS microspheres with ChABC

CS was weighed and poured into a 30 × 50 mm bottle that was filled with 1% w/w aqueous acetic acid solution. The mixture was then stirred for 6 h until the powder had been completely dissolved in the solution. The CS quality percentage was 0.5%. PVP was dissolved in deionized water to create a 1% PVP solution. ChABC (0.056% w/w) was then added to the PVP solution, and equal volumes of CS and PVP solutions (with or without ChABC) were mixed and stirred for 0.5 h. The mixed solution was added to the tripolyphosphate 5% w/w solution, and the resulting mixture was stirred for 3 h. The final mixture was centrifuged at 2000 × *g* for 30 min, and the resulting precipitate was then washed twice with deionized water and freeze-dried to yield CS microspheres, either with or without ChABC.

2.3. Fabrication of microfibres containing ChABC/db-cAMP

PPC powder and CS microspheres/db-cAMP were weighed and poured into a 30 × 50 mm bottle. Dichloromethane was added to the bottle, and the mixture was stirred for 6 h until the powder completely dissolved in the solution. The electrospinning process was then performed as previously described [14]. The distance and voltage between the nozzle and a grounded collection target was 12 cm, and 10 kV, respectively. For the fibres containing a single drug, the weight ratio of ChABC or db-cAMP was 1%. For the combination fibers, the weight ratios of ChABC and db-cAMP were each 1%.

2.4. Characterization of fibers

Fibers were examined for morphology and size using scanning electron microscopy (Hitachi SU70, Japan). Samples were sputtered with platinum at a current of 10 mA for 20 s under argon gas, and then viewed on the scanning electron microscope at acceleration voltages of 1.0 Kv.

2.5. Determination of in vitro ChABC/db-cAMP release from fibers

To quantify the in vitro drug release, PBS (5 mL) was added to 15 mL centrifuge tubes each containing a fiber (10 mg, n = 3 for each treatment group) that were fixed in a bath incubator at 37 °C. Aliquots of PBS were removed at the following time points: 0.5, 1, 2, 4, 8, 12, 24, 48, 60, 72, 84, 96, 108, 120, 132, 144, 156, 168, 180, 192, 204, 216, 228, 240, and 252 h and stored at −20 °C until analysed. Chondroitin sulfate A was added to the residual PBS, and ChABC activity was measured using a microplate reader after 10 min at O. D232. The tubes were replenished with fresh PBS at each time point, and were kept on ice during these changings.

2.6. Spinal cord right-side hemitransection surgery and postoperative care

All experiments were approved by the Shandong University Animal Care Committee and conducted in accordance with criteria established by the Chinese Council on Animal Care. In total, 120 adult female Wistar rats (200–230 g) were used in these experiments. The rats were assigned to four groups: a combination group (that received fibres containing ChABC and db-cAMP), a ChABC group (that received fibres containing ChABC only), a db-cAMP group (that received fibres containing db-cAMP only) and a control group (that did not receive microfibres). Each rat was anesthetized with an intraperitoneal injection of 3 mL/kg chloral hydrate (10%). A 2-cm midline incision was made to expose the T7–T9 spinous processes and corresponding vertebral laminae. A laminectomy was then performed, and after identifying the posterior median

Table 1
Beam-walking scores [27].

1. The rat is unable to place the affected hindpaw on the horizontal surface of the beam.
2. The rat places the affected hindpaw on the horizontal surface of the beam and maintains balance for at least 5 sec.
3. The rat traverses the beam while dragging the affected hindpaw.
4. The rat traverses the beam and at least once places the affected hindpaw on the horizontal surface of the beam to aid its step.
5. The rat crosses the beam and places the affected hindpaw on the horizontal surface to aid more than one, but less than half its steps.
6. The rat crosses the beam and places the affected hindpaw on the horizontal surface to aid more than half its steps, but has more than two footslips.
7. The rat crosses the beam with no more than two footslips