Available online at www.sciencedirect.com
ScienceDirect
Surgical Neurology 69 (2008) 568 – 577
SURGICAL NEUROLOGY
www.surgicalneurology-online.com

Spine

Benefit of chondroitinase ABC on sensory axon regeneration in a laceration model of spinal cord injury in the rat

Lisa B.E. Shields, MD, Yi Ping Zhang, MD, Darlene A. Burke, MA, Rebecca Gray, BA, Christopher B. Shields, MD*

Kentucky Spinal Cord Injury Research Center, University of Louisville School of Medicine, Louisville, KY 40202, USA
Department of Neurological Surgery, University of Louisville School of Medicine, Louisville, KY 40202, USA

Received 6 July 2007; accepted 4 February 2008

Abstract

Background: Chondroitin sulfate proteoglycans are up-regulated in the spinal cord after SCI, creating a molecular barrier inhibitory to axon growth. Chondroitinase ABC degrades CSPGs in vitro and in vivo.

Methods: We studied whether IT ChABC promotes axonal regeneration in a laceration model of SCI. Three groups of Sprague-Dawley rats were used: control and rats treated with low-dose and high-dose IT ChABC. Chondroitin sulfate proteoglycan breakdown products were measured by 2-B-6 expression, and intact CSPGs by CS-56 expression. Sensory axonal regeneration was traced after CTB injection into the median, ulnar, and sciatic nerves.

Results: CS-56 expression was down-regulated and 2-B-6 expression was increased in the groups treated with IT ChABC but not in the control. Laminin and GFAP immunoreactivity was unaltered in the ChABC groups. The number of axons growing into the scar was 3.1 times greater ($P < .01$) in the high-dose ChABC group and 2.1 times greater ($P < .01$) in the low-dose group compared with the controls. The length of axonal growth after high- and low-dose ChABC was 9.9 ($P < .01$) and 8.3 ($P < .01$) times greater, respectively, than in the control group. Axons extended across the lesion gap and into the distal spinal cord stump in 2 of 8 (low dose) and in 3 of 9 (high dose) rats compared with none in the control group.

Conclusions: Intrathecal ChABC administration caused a slight decrease in CSPGs in the scar after a laceration SCI with a minimal increase in sensory axonal regeneration into and across the laceration gap.

© 2008 Elsevier Inc. All rights reserved.

Keywords: Chondroitinase; Spinal cord injury; Spinal cord laceration; Spinal cord regeneration; Axonal regeneration; Glial scar; Chondroitin sulfate proteoglycan; Extracellular matrix; Vibraknife; Sensory axon

Abbreviations: ANOVA, analysis of variance; BDNF, brain-derived neurotrophic factor; ChABC, chondroitinase ABC; COBRE NIH, Centers of Biomedical Research Excellence National Institutes of Health; CNS, central nervous system; CSPGs, chondroitin sulfate proteoglycans; CTB, cholera toxin B; DRG, dorsal root ganglia; GAG, glycosaminoglycan; GFAP, glial fibrillary acidic protein; HBSS, Hanks balanced salt solution; HSD, honestly significant difference; IACUC, Institutional Animal Care and Use Committee; IM, intramuscular; IP, intraperitoneal; IR, immunoreactivity; IT, intrathecal; MAG, myelin-associated glycoprotein; NT3, neurotrophic 3; OD, outer diameter; OMgp, oligodendrocyte myelin glycoprotein; PBS, phosphate-buffered solution; PBST, phosphate buffered saline Tween-20; PN, peripheral nerve; SAS, subarachnoid space; SCI, spinal cord injury; Sema, semaphorins.

* Corresponding author. Department of Neurological Surgery, University of Louisville, Louisville, KY 40202, USA, Tel.: +502 629 5510; fax: +502 629 5512.

E-mail address: cbshields1@gmail.com (C.B. Shields).

0090-3019/$ – see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.surneu.2008.02.009

1. Introduction

Spinal cord axons are incapable of regeneration after SCI in adult mammals; thus, axon regeneration is 1 strategy currently being studied to treat SCI. Failure of axon regeneration is attributable in part to the intrinsic incompetence of mature neurons to grow and to the unfavorable molecular and physical environment encountered by axon growth cones. Growth cones of severed axons grow in response to inhibitory and permissive signals in the nervous system, respectively [28,61]. After injury, the spinal cord scar consists of a mixture of growth-promoting molecules such as laminin [10,39], cell adhesion molecules [57], and growth factors in combination with growth-inhibitory molecules. Unfortunately, inhibitory molecules dominate, resulting in a net inhibition of axon growth.

Several inhibitory molecules have been identified including myelin-derived Nogo [18,19,21,52], OMgp [34,58], MAG [13,38,41,56], and membrane-bound Sema [20,26]. The family of CSPGs serves as another major inhibitory molecule. After the atraumatic microtransplantation of DRG neurons into the adult rat CNS [11], CSPGs significantly inhibit their neurite outgrowth to an even greater extent than components of CNS white matter. Abortive attempts of neurite spouting from transplanted DRG neurons correlate with increased proteoglycan levels within the extracellular matrix at the transplant interface [14]. Sensory neuron implantation into adult CNS white matter also leads to inhibition of axon regeneration at CSPG-rich sites [2]. Members of the CSPG family inhibit neurite outgrowth in vitro [15,48] and undergo altered expression after spinal cord and brain injuries in vivo [16,36,37,44]. Certain CSPGs, specifically, NG2, neurocan, brevican, and versican, are up-regulated in the glial scar after SCI [9,12,28,31,32]. Chondroitin sulfate proteoglycan up-regulation adjacent to the CNS injury inhibits axon regeneration, axon sprouting, and growth [45,50,62].

Chondroitin sulfate proteoglycan receptors have not been identified on growth cones [47], and therefore, ChABC degradation of several CSPG components may provide a strategy for axon regeneration. Such treatment has promoted neurite outgrowth in vitro [39,40] as well as CNS axonal regeneration in vivo [42], including improved locomotor function and proprioception in rats [5].

Experimental SCI in rats is complicated by the formation of posttraumatic spinal cord cysts that create a physical barrier to axonal regeneration [63]. Posttraumatic cysts occur after intramedullary hemorrhage, ischemia, and cell death. Our model of SCI was created by a precisely controlled laceration using the Vibraknife, which contributed to the reduction in size of the posttraumatic cyst [64]. In this study, we have evaluated the effect of IT administration of ChABC on the digestion of CSPG and sensory axon regeneration.

The study of a laceration SCI is important because this type of injury represents a major problem clinically. Twenty-five percent of all SCI are caused by lacerations (knife, gunshot wound, sharp bone fragments); thus, our model is clinically significant [6]. We have created a dorsal spinal cord hemisection, and therefore, only sensory axon regeneration will be evaluated.

2. Materials and methods

Surgical procedures and animal care adhered to the guidelines outlined by the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, National Research Council, 1996) and the University of Louisville IACUC.

2.1. Surgical model

Twenty-two adult female Sprague-Dawley rats (200-225 g) were anesthetized with sodium pentobarbital (50 mg/kg, IP) and treated with prophylactic gentamicin (0.03 mg/kg, IM). A C3-5 laminectomy was performed, the dura opened, and the C3 vertebra immobilized by stainless steel fixation clamps attached to the Vibraknife base. A C3 dorsal hemisection using the Vibraknife was made with a 3.6-mm blade, which covered the width of the spinal cord. The laceration was created by elevating the rat spinal cord to the vibrating blade. This produced a laceration 1.5 mm deep [51,64], which extended across the central canal, effectively transecting all fibers in the dorsal funiculus. Lubrication at the blade-spinal cord interface was maintained using sterile HBSS.

A polyethylene (PE-10) catheter (Becton Dickinson, Sparks, MD) was placed through a dural opening at C4-5 (Fig. 1A) and threaded rostrally in the SAS to reach the C3 spinal cord hemisection (Fig. 1B). The PE-10 tubing was heated and tapered to obtain a 100-μm OD at its tip. The entire catheter (80 mm long) contained a volume of 3 μL. The tubing was heparinized before placement into the SAS to prevent occlusion by blood. The extradural end of the tube was coiled in the submuscular area and stabilized with three 6-0 sutures (Ethicon, Somerville, NJ) tied to the overlying muscle and passed transcutaneously through a separate skin incision on the back of the animal. Dural closure at C3 was performed using a 10-0 monofilament nylon suture (Ethicon). The dural flap was curved to prevent the dural closure at C3 from being superimposed over the spinal cord laceration gap (Fig. 1C). The dural repair at C3 and the dural exit of the PE-10 tubing at C4-5 were sealed with fibrin glue (Baxter, Glendale, CA). The tip of the tubing was visualized transdurally at the laceration site. Muscle and skin were subsequently closed. The exposed end of the PE-10 tubing was occluded by a stainless steel plug and opened only for injections into the IT space.

2.2. Treatment

Rats were anesthetized with 2.5% halothane gas administered by a custom-made rat anesthesia mask. On the day of surgery and alternate days for a total of 5 injections (days 0,

Download English Version:

https://daneshyari.com/en/article/3092954

Download Persian Version:

https://daneshyari.com/article/3092954

Daneshyari.com