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ORIGINAL ARTICLE

Protective effects of cerebrolysin in a rat model of optic nerve crush

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Abstract To investigate the effects of cerebrolysin (Cbl) on optic nerves (ON) and retinal ganglion cells (RGC) in a rat model of ON crush. Rats received intravitreal injection of Cbl ($n = 20$), intra-ON injection of Cbl ($n = 20$), intraperitoneal injection (IPI) of Cbl ($n = 20$), or phosphate buffered saline (PBS; $n = 20$) every day for 2 weeks after ON crush injury. At 3 weeks post-trauma, RGC density was counted by retrograde labeling with FluoroGold and visual function was assessed by flash visual-evoked potentials. Activities of microglia after insults were quantified by immunohistochemical analysis of the presence of ED1 in the optic nerve. At 3 weeks postcrush, the densities of RGCs in the Cbl-IVI group ($1125 \pm 166/mm^2$) and in the Cbl-IPI treatment group ($1328 \pm 119/mm^2$) were significantly higher than those in the PBS group ($641 \pm 214/mm^2$). The flash visual-evoked potential measurements showed that latency of the P1 wave was significantly shorter in the Cbl-IVI- and Cbl-IPI-treated groups (105 ± 4 ms and 118 ± 26 ms, respectively) than in the PBS-treated group (170 ± 20 ms). However, only Cbl IPI treatment resulted in a significant decrease in the number of ED1-positive cells at the lesion sites of the ON (5 ± 2 cells/vs. 30 ± 4 cells/high-power field in control eyes). Treatment with intra-ON injection of Cbl was harmful to the optic nerve in the crush model. Systemic administration of Cbl had neuroprotective effects on RGC survival and visual function in the optic nerve crush model.
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Introduction

Optic nerve (ON) injury leads to anterograde and retrograde degeneration, consequentially producing a scar at the site of injury and loss of retinal ganglion cells (RGCs). Although pulse steroid therapy is frequently used to treat acute traumatic optic neuropathy [1], there are no convincing data to indicate any effective treatment for traumatic optic neuropathy. In our previous studies we found that systemic human granulocyte colony-stimulating factor, but not corticosteroid treatment, had a neuroprotective effect in a rat model of optic nerve crush [2,3].

Neurotrophic factors that are upregulated by endogenous opioids are not sufficient to enable the rescue of damaged neurons [4]. Various neurotrophic factors are present in the central nervous system after injury, including brain-derived neurotrophic factor, nerve growth factor, ciliary neurotrophic factor, and glial cell line-derived neurotrophic factor [5]. Cerebrolysin (Cbl), the only drug available for clinical use that contains active fragments of some important neurotrophic factors [6,7], has been found effective in a number of clinical trials for the treatment of vascular dementia, stroke, and other neurodegenerative diseases [8—12]. Studies of the effectiveness of Cbl as a neuroregeneration treatment have been conducted in different injury models including traumatic brain injury, spinal cord injury, ischemic stroke, hyperthermia, drugs of abuse, and neuropathic pain [6,7,12—17]. In this pilot study that aimed to extend this research, we investigated whether Cbl has neuroprotective effects in an animal model of optic nerve crush.

Materials and methods

Cbl administration

Cbl; 215.2 mg/mL (EVER Neuro Pharma GmbH, Unterach, Austria) was used as the target drug in this crush model.

Animals

Adult male Wistar rats weighing 150—180 g (age 7—8 weeks) were obtained from the breeding colony of BioLASCO Co., Yu-Lan, Taiwan. The Institutional Animal Care and Use Committee at the Tzu Chi Medical Center, Hualien, Taiwan approved all animal experiments. All manipulations were performed with standard procedures as described in our prior paper [18].

Study design

A total of 80 rats underwent ON crush procedures in the right eyes and sham operations in the left eyes. Rats then received one intravitreal injection (IVI) of Cbl 2 μL (0.43 mg; $n = 20$), one intra-ON injection (IONI) of Cbl 2 μL (0.43 mg; $n = 20$), intraperitoneal injection (IPI) of Cbl (5 mL/kg; $n = 20$), or phosphate buffered saline (PBS; $n = 20$) every day for 2 weeks. Three weeks after surgery, RGC density was measured by retrograde labeling with FluoroGold (Fluorochrome LLC, Denver, CO, USA), and visual function was assessed by flash visual-evoked potentials (fVEP). Microglia activity after insult was quantified by immunohistochemical analysis of ED1 expression in the optic nerve.

Optic nerve crush injury experiments

ON crush injuries were induced as described in our previous reports [2,18]. Briefly, a standardized vascular clip (60-g microvascular clip; World Precision Instruments, Sarasota, FL, USA) was then applied to the ON at a distance of 2 mm posterior to the globe for 30 seconds. The left eyes received a sham operation that entailed optic nerve exposure without the crush procedure.

Retrograde labeling of RGCs with FluoroGold and morphometry of the RGCs

The detailed procedures have been described in our previous reports [2,3,18]. We performed retrograde labeling of the RGCs 1 week prior to when the rats were euthanized. In brief, the rats were anesthetized and placed in a stereotactic apparatus (Stoelting, Wood Dale, IL, USA). The 1.5 μL of 5% FluoroGold was injected into the superior colliculus on each side through a Hamilton syringe. One week after the labeling, the eyeballs were harvested after the animals had been euthanized. The retinas, examined with a 400× epi-fluorescence microscope (Axioskop; Carl Zeiss Meditech Inc., Thornwood, NY, USA), were examined for RGCs at a distance of 1 mm from the center to provide the central RGC densities. We counted at least five randomly chosen areas of 62,500 μm^2 each in the central regions of each retina, and their averages were taken as the mean density of RGCs per retina ($n > 6$ in each group).

fVEP

An isolated silver plate electrode was placed extradurally through a 2-mm diameter craniotomy over the visual cortex using stereotactic coordinates (bregma −8 mm, lateral 3 mm) [18,19]. We used a visual electrodiagnostic system (UTAS-E3000, LKC Technologies, Gaithersburg, MD, USA) to measure the fVEP [18]. When the wave was nonrecordable, the latency of P1 was set at 200 ms for comparison.

Immunohistochemistry (IHC) of ED-1 (CD68) in the ONs

The experiment was performed as described previously [3,20]. In short, the primary antibody (1:50; AbD Serotec, Oxford, UK) was applied and incubated overnight at 4°C. The secondary antibody conjugated with fluorescein isothiocyanate (FITC, 1:100; Jackson ImmunoResearch Laboratories, West Grove, PA, USA) was applied at room temperature for 1 hour. Counterstaining was performed using 4′,6-diamidino-2-phenylindole (DAPI, 1:1000; Sigma, St Louis, MO, USA). For comparison, the ED1-positive cells

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