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Research article

Neuroprotective effect of 4-(Phenylsulfanyl)butan-2-one on optic nerve crush model in rats

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ABSTRACT

This study is to investigate the effect of coral-related compound, 4-(phenylsulfanyl)butan-2-one (4-PSB-2) on optic nerves (ON) and retinal ganglion cells (RGC) in a rat model subjected to ON crush. The ONs of adult male Wistar rat (150–180 g) were crushed by a standardized method. The control eyes received a sham operation. 4-PSB-2 (5 mg/kg in 0.2 mL phosphate-buffered saline) or phosphate-buffered saline (PBS control) was immediately administered after ON crush once by subcutaneous injection. Rats were euthanized at 2 weeks after the crush injury. RGC density was counted by retrograde labeling with FluoroGold (FG) application to the superior colliculus, and visual function was assessed by flash visual evoked potentials (FVEP). TUNEL assay, immunoblotting analysis of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX2) in the retinas, and immunohistochemistry of ED1 in the ON were evaluated. Two weeks after the insult, the RGC densities in the central and mid-peripheral retinas in ON-crushed, 4-PSB-2-treated rats were significantly higher than that of the corresponding ON-crushed, PBS-treated rats FVEP measurements showed a significantly better preserved latency of the P1 wave in the ON-crushed, 4-PSB-2-treated rats than the ON-crushed, PBS treated rats. TUNEL assays showed fewer TUNEL positive cells in the ON-crushed, 4-PSB-2-treated rats. The number of ED1 positive cells was reduced at the lesion site of the optic nerve in the ON-crushed, 4-PSB-2-treated group. Furthermore, administration of 4-PSB-2 significantly attenuated ON crush insult-stimulated iNOS and COX2 expression in the retinas. These results demonstrated that 4-PSB-2 protects RGCs and helps preserve the visual function in the rat model of optic nerve crush. 4-PSB-2 may work by being anti-apoptotic and by attenuation of the inflammatory responses involving less ED1 positive cells infiltration in ON as well as suppression of iNOS/COX-2 signaling pathway in the retinas to rescue RGCs after ON crush injury.

1. Introduction

Traumatic optic neuropathy (TON) occurs in 0.5–5% of patients presenting with close head trauma and is often devastating cause of permanent visual loss (Steinsapir, 1999). Damage to the optic nerve (ON) causes immediate shearing of and induces a vicious cycle of swelling and ischemia lead to RGCs and axon injury. In the majority of cases, the first critical event is RGC axonal damage, possibly mediated by glial dysfunction, following the apoptotic cell signaling, retrograde axonal degeneration and Wallerian degeneration. RGC death after axonal injury involves apoptosis (Berkelaar et al., 1994; Levin and Louhab, 1996; Quigley, 1995). After ON transection, RGC begin dying by apoptosis by first 3–7 days and the remainder (50–90%) have disappeared from the retina taking weeks to sometimes months (Allcutt et al., 1984; Berkelaar et al., 1994; Peinado-Ramon et al., 1996; Villegas-Perez et al., 1993). Axonal injury also induces a burst of superoxide within the RGC soma, following induction of downstream oxidative events and cytotoxic cytokines and results in apoptosis (Kanamori et al., 2010;

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Lieven et al., 2006). Therefore, therapies that stimulate both neuronal viability and axon growth may prove beneficial after ON lesion (Levin, 2007).

The optic nerve crush model is an effective model for studying pathophysiology of RGCs death in axon injury and to evaluate the neuroprotective ability of several strategies for acute optic neuropathies. ON crush induces a retrograde degeneration of the RGCs after the injury. Prior to that, the myelin sheath of the axons degenerates, the ED-1 positive phagocytes (including macrophage and microglia) infiltrate (Morishita et al., 2014; Ohlsson et al., 2004a; Tonari et al., 2012) and remove the myelin debris. Macrophage and microglia accumulation at the site of the insult contributes to glial scar formation in the ON, which is an obstacle for regeneration (Ohlsson et al., 2004a, 2004b; Stoll et al., 1989). These observations have implicated important roles of inflammatory processes in the ON crush injury. Inhibition of glial activation by both nitric oxide inhibitors and anti-inflammatory cytokines has been used to rescue RGC from apoptosis after axon injury (Koeberle and Ball, 1999; Koeberle et al., 2004; Wilhelmsson et al., 2004).

In recent years, numerous marine invertebrates based compounds have been reported to show extensive anti-inflammatory activities, stimulation of neurogenesis, and modulation of receptors or voltage gated channels in central nervous system (CNS) (Grosso et al., 2014; Mayer et al., 2013). Previous studies of bioactive marine natural products have led to isolation of several compounds with neuroprotective (Chen et al., 2012; Tseng et al., 2009; Wen et al., 2010) and anti-inflammatory activities (Chao et al., 2008; Chen et al., 2014; Lee et al., 2013; Lin et al., 2013) from soft corals. Austrasulfone, a bioactive substance isolated from the Formosan soft coral Cladiella australis, exhibits potent neuroprotective effects against the 6-OHDA-induced neurotoxicity in neuroblastoma SH-SY5Y cells (Wen et al., 2010). Dihydroaustrasulfone alcohol, the synthetic precursor of austrasulfone, not only exhibited anti-inflammatory activity in vitro, but also showed potent therapeutic potential in the treatment of inflammatory-related diseases (Wen et al., 2010). In order to easily pass through the cell membrane, the polar hydroxyl group (−OH) of hydroxylated sulfone was replaced with benzene ring in a straightforward synthesis to yield the compound, 4-(Phenylsulfanyl)butane-2-one (4-PSB-2). 4-PSB-2 also show anti-inflammatory effect by inhibiting LPS-stimulated inducible nitric oxide synthase (iNOS) expression in murine macrophage RAW 264.7 cells (Wen et al., 2014). To the best of our knowledge, 4-PSB-2 has not been evaluated in neuroprotection or anti-inflammatory effect on retinal neurons. The purpose of this study was to examine the effect of 4-PSB-2 on neurodegeneration of ON and RGCs after ON crush in rats.

2. Materials and methods

Animals Forty five adult male Wistar rats weighing 150–180 g (7–8 weeks old) were used in this study (Table 1). Rats were obtained from the breeding colony at BioLASCO Co., Taiwan. Animal care and experimental procedures were conducted in accordance with the Association for Research in Vision and Ophthalmology (ARVO) statement for the use of animals in ophthalmic and vision research. The Institutional Animal Care and Use Committee (IACUC) at Tzu Chi University approved all animal experiments. All manipulations were performed with animals under general anesthesia induced by an intramuscular injection of a mixture of ketamine (40 mg/kg body weight (BW)) and xylazine (4 mg/kg BW; Sigma, St Louis, Mo., USA). In addition, topical 0.5% Alcaine eye drops (Alcon, Puurs, Belgium) were used. The rats had free access to food and water. They were maintained in cages in an environmentally controlled room that was held at a temperature of 23 ± 1 °C, a humidity of 55 ± 5% and had a 12-h light–dark cycle (light period: 7 AM to 7 PM).

Table 1
Summary of the rats used and the study design.

Method	Group	Number of rats
Flash VEP,TUNEL,IHC	Sham	6
	Crush + PBS	6
	Crush + 4-PBS-2	6
FG retrograde labeling	Sham	6
	Crush + PBS	6
	Crush + 4-PBS-2	6
Immunoblotting analysis	Sham	3
	Crush + PBS	3
	Crush + 4-PBS-2	3

2.1. Preparation of 4-(phenylsulfanyl)butan-2-one (4-PSB-2)

The compound was partly provided by Professor Jyh-Horng Sheu, the Department of Marine Biotechnology and Resources of National Sun Yat-sen University, and synthesized by the Research Center of National Research Program for Biopharmaceuticals (Taipei, Taiwan) (Wen et al., 2014). Briefly, the reaction of bezenethiol (2.0 g, 98%, 17.8 mmole) and triethylamine (0.25 mL, 1.78 mmole) were added in a round bottom flask containing 10 mL of acetone. Followed by stirring at 0 °C, a solution of methyl vinyl ketone (1.38 mL, 90%, 17.8 mmol) in 4 mL of acetone was slowly added into the mixture. The temperature of the mixture was raised to room temperature and the reaction was continued for 16 h. The solvent free product was subject to silica gel column chromatography, eluting with n-hexane/ethyl acetate (25:1), to afford 4-(phenylsulfanyl)butan-2-one (2.80 g, yield 87%). The reaction was shown in Fig. 1.

2.2. Optic nerve crush injury experiment

An ON crush injury was induced as described in our previous report (Tsai et al., 2008). Briefly, after general anesthesia and topical Alcaine eye drop application, the ON was exposed and isolated. Care was taken to avoid damaging the small vessels around the ON. A vascular clip (60 g micro-vascular clip, World Precision Instruments, FL, USA) was then applied to the ON 2 mm posterior to the globe for 30 s. After the surgery, Tobradex eye ointment (Alcon, Puurs, Belgium) was administered. The rats were kept on electric heating pads at 37 °C for recovery. The left eyes received a sham operation that entailed ON exposure without the crush. 4-PSB-2 (5 mg/kg in 0.2 mL phosphate-buffered saline) or phosphate-buffered saline (PBS control) was immediately administered after ON crush once by subcutaneous injection.

2.3. Flash visual-evoked potentials (FVEPs)

For the functional evaluation of the ON, FVEPs were recorded 2 weeks after ON crush in 18 experimental rats. An isolated silver plate electrode was placed extradurally through a 2-mm diameter craniotomy over the visual cortex using the stereotactic coordinates (bregma −8 mm, lateral 3 mm) and a modified method described by Ohlsson et al. (Ohlsson et al., 2004a) We used a visual electrodiagnostic system (UTAS-E3000, LKC Technologies, Gaithersburg, MD, USA) to measure FVEPs (Huang et al., 2011; Tsai et al., 2008). After 10 min of light adaptation, we performed photopic FVEP, based on the report showing no significant differences of latency between photopic and scotopic VEP in Wistar rats (Heiduschka and Schraermeyer, 2008). The settings were background illumination off, a flash intensity of Ganzfeld 0 db, single flash with flash rate on