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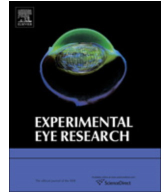

Neuroprotective effects of topical CB1 agonist WIN 55212-2 on retinal ganglion cells after acute rise in intraocular pressure induced ischemia in rat

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ABSTRACT

Neuroprotection in retinal experimental work consists primarily of preventing retinal ganglion cell (RGC) loss after exposure to a hostile event. We have studied the neuroprotective effect on RGCs in an ischemia-reperfusion model by activation of the cannabinoid receptor CB1 using topical application of WIN 55212-2. Intraocular pressure (IOP) was increased by continuous infusion of phosphate buffer saline (PBS) into the anterior chamber of the eye. Mean intraocular pressure was increased up to 88.5 ± 0.29 mm Hg (control normal IOP 15.1 ± 0.25 mm Hg), for 35 min. Animals were distributed in 3 groups. Left eyes underwent acute rise in intraocular pressure. First group was treated with topical Tocrisolve™ 100 in both eyes. Second group was treated with 1% solution of CB1 agonist WIN 55212-2 in both eyes. Third group was treated with WIN 55212-2 1% and CB1 antagonist AM 251 1% solutions in both eyes. Subsequently, RGCs were immunolabeled with Brn3a and automated quantification of retinal mosaics of RGCs were performed. The ischemic damage led to a mean loss in RGC density of 12.33%. After topic administration of WIN 55212-2, mean loss of RGCs was of 2.45%. Co-treatment with CB1 antagonist AM 251 abolished almost completely the neuroprotective effect of WIN 55212-2. Topic 1% WIN 55212-2 showed a neuroprotective effect on RGC degeneration after ischemia-reperfusion without pre-activation of CB1 receptors.

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Neuroprotection consists of preventing the death of damaged neurons and the degeneration of those cells that had undergone the hostile environment created by an initial insult. By definition, in ocular neuroprotection, the target neurons should be located in the visual pathway, including retinal ganglion cells (RGCs) (Wheeler et al., 2001). Ocular neuroprotection has been investigated for various diseases affecting RGCs and the optic nerve, such as Leber's optic neuropathy (Johns and Colby, 2002), traumatic/compressive optic neuropathy (Ben Simon et al., 2006), toxic–metabolic optic neuropathies (Pinar-Sueiro et al., 2010), ischemic (Wilhelm et al., 2006), inflammatory (Bessero and Clarke, 2010), demyelinising neuropathies (Croxford et al., 2008), diabetic retinopathy (Verma, 1993), and glaucoma (Nucci et al., 2007). All these neuropathies have several common degeneration pathways such as glial activation, oxidative stress, and excitotoxicity (Yuan and Neufeld, 2000; Seki and Lipton, 2008; Hare and Wheeler, 2009). Except for glaucoma, no treatment has been proven to be sufficiently effective as to establish a gold standard. For these reasons, efforts are consistently being directed toward the development of new drugs to prevent RGC degeneration from diverse optic neuropathies.

Cannabinoid receptors (CB) were classified as G protein coupled receptors with high affinity for the agonist tetrahydrocannabinol. Two types of CB receptors have been described, CB1 and CB2 (Howlett, 2002) and both have been found in retina (Cabral et al., 2008).

WIN 55212-2 is a synthetic aminoalkylindole that mainly binds to CB1 cannabinoid receptor (Chien et al., 2003). Cannabinoid agonists were first investigated as a possible treatment for glaucoma

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due to their efficacy to decrease intraocular pressure (Hepler and Frank, 1971; Porcella et al., 2001). Progress in the role of cannabinoids in providing neuroprotection in traumatic, ischemic, inflammatory and neurotoxic damage to neurons has been made (Van der Stelt and Di Marzo, 2005; de Lago and Fernández-Ruíz, 2007; Mechoulam and Shohami, 2007). Other studies have shown that CB1 agonists (THC and cannabidiol) protect RGCs from glutamate-induced excitotoxicity (El Remessy et al., 2003; Opere et al., 2006), and secondary degeneration in an experimental model of glaucoma in rat (Crandall et al., 2007; Nucci et al., 2007). Several cannabinoid agonists have been shown to act as non-competitive NMDA antagonist (Feigenbaum et al., 1989) thereby providing neuroprotection for RGCs (Yoles et al., 1996). However the direct neuroprotective effect on RGCs of a topic CB1 agonist (without preconditioning) has not been described in an ischemia-reperfusion animal model. The aim of the present study was to investigate the neuroprotective effect on RGCs of topical application of 1% solution of CB1 agonist WIN 55212-2.

27 female Sprague–Dawley rats, weighing 250–300 g were used. Animals were housed with ad libitum access to food and water, in a room with a 12:12 h light: dark cycles at 21 °C. All experimentation adhered to the ARVO Statement for the Use of Animal in Ophthalmic and Vision Research.

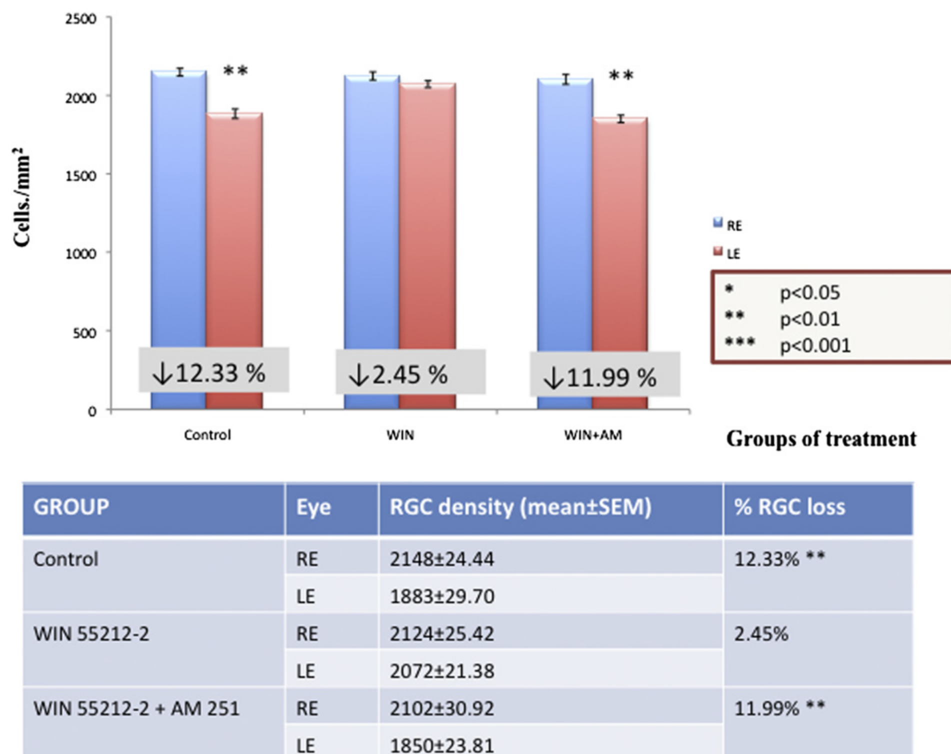
The rats were placed under deep anesthesia with intramuscular injection of ketamine and xylazine (66.7 mg/kg and 6.7 mg/kg body weight, respectively). Ischemia was induced by blocking off blood supply to the retina from the retinal artery by increasing IOP. Increased pressure was achieved by infusion of 100 μl of phosphate buffer saline (PBS) into the anterior chamber for 35 min through a 32-gauge needle. The needle was attached to Tygon tubing (Hamilton) linked to a syringe pump (Panlab, Kd Scientific) with a flow rate of 2.9 μl/min. Retinal ischemia was confirmed by observing bleaching of the retina. IOP was monitored every 5 min and the absence of retinal perfusion was maintained. Increased IOP was maintained for 35 min and then infusion was removed allowing the eye to reperfuse. Contralateral right eyes were treated by inserting a 32-gauge needle into the anterior chamber of the eye through the cornea without the infusion and it served as control. Eyes undergoing continuous infusion reached increased intraocular pressure up to 88.5 ± 0.29 mm Hg ($n = 11$) with respect to their control right eyes (mean basal IOP of 15.1 ± 0.25 mm Hg).

Eye drops were applied in both eyes once daily at 9:00 h a.m. 48 h after the ischemia, rats were sacrificed by decapitation and eyes were enucleated for the posterior retinal fixation, immunolabeling and RGC quantification.

WIN 55212-2 mesylate salt (Cat. No. 1038, Tocris, Madrid, Spain) and AM-251 (Cat. No. 1117, Tocris, Madrid, Spain) were dissolved in Tocrisolve™ 100 (Cat. No. 1684, Tocris, Madrid, Spain) solution. Final solutions consisted of 1% WIN 55212-2; and 1% AM 251, and were kept in borosilicate glass tubes (Sigma–Aldrich, Madrid, Spain).

Animals were distributed in 3 groups. Left eyes underwent acute rise in intraocular pressure for 35 min. First group ($n = 11$) was treated with topical Tocrisolve™ 100 (20 μl) in both eyes. Second group ($n = 8$) was treated with 1% WIN 55212-2 solution (20 μl) in both eyes. Third group ($n = 8$) was treated with 1% WIN 55212-2 and 1% AM 251 solution (20 μl) in both eyes. Both eyes in each animal were subjected to the same treatment, for providing the best pair group for statistics and avoiding complications due to crossover of drug into the contralateral eye.

Topical treatments of Tocrisolve™ 100, 1% WIN 55212 and 1% AM 251 were applied immediately following ischemia/reperfusion. Treatments were repeated once daily, at the same time of the day (9 a.m.) for the following 48 h. IOP was measured using electronic indentation tonometry (TonoLab, Icare®LAB; Icare, Finland). Increased IOP was monitored every 5 min under deep anesthesia during ischemia induction, and, afterward, it was measured in awake animals every 24 h, at 8:00 h a.m. daily to avoid natural IOP



GROUP	Eye	RGC density (mean±SEM)	% RGC loss
Control	RE	2148±24.44	12.33% **
	LE	1883±29.70	
WIN 55212-2	RE	2124±25.42	2.45%
	LE	2072±21.38	
WIN 55212-2 + AM 251	RE	2102±30.92	11.99% **
	LE	1850±23.81	

Fig. 1. Retinal ganglion cell densities in different treatment groups. Abbreviations: RE: Right eye; LE: Left eye; WIN 55212-2: Group treated with topic 1% WIN 55212-2; WIN 55212-2 + AM 251: Group treated simultaneously with 1% WIN 55212-2 and 1% AM 251; Control: Control group, treated with Tocrisolve™ as placebo; SEM: Standard error of the mean.

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