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Functional and structural evaluation of lamotrigine treatment in rat models of acute and chronic ocular hypertension

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ABSTRACT

Voltage gated sodium channels (Na_v), are proposed mediators of neuronal damage in ischemic and excitotoxicity disease models. We evaluated the neuroprotective effects of lamotrigine, a Na_v blocker, in the acute and chronic rat ocular hypertension models. Additionally, expression of the main Na_v subtypes in the optic nerve (ON) was assessed to test whether their upregulation plays a role in the pathogenesis of ocular hypertension induced optic neuropathy. Unilateral intraocular pressure (IOP) elevation was induced for 60 min (80 mmHg) and 14–21 days (670–859 mmHg*day) in the acute and chronic models, respectively. Lamotrigine was administered at dosages of 10 mg/kg twice daily and 12.5 mg/kg once daily in the acute ($n = 9$) and chronic ($n = 11$) trials, respectively. Treatment began 2 days prior to IOP elevation until sacrifice. Outer and inner retinal function was evaluated with dark- and light-adapted flash electroretinography and pattern electroretinography, respectively, 6 and 14 days post acute IOP elevation and 13, 28 and 48 days post chronic IOP elevation. Retinal ganglion cell and axon densities and inflammatory reaction were evaluated through Fluorogold, Bielschowsky's silver impregnation and ED1 labeling respectively. Immunohistochemistry for $Na_v1.1$, 1.2 and 1.6 was performed in ONs of untreated rats 7 and 15 days post IOP elevation in the acute model and after 7, 28 and 50 days in the chronic model. In the acute model, no differences were found in the a-wave amplitudes between lamotrigine-treated and vehicle-treated rats. B-wave amplitudes decreased by 40–66% in both treatment groups 6 days post IOP elevation, with no significant difference between groups ($p = 0.38$). However, a partial recovery of b-wave amplitudes was found in lamotrigine-treated rats between day 6 and day 14 post procedure ($p < 0.05$). No differences were found in any other parameter tested in this model. Similarly, lamotrigine treatment did not result in any beneficial effect in structural parameters of the chronic model. Functional evaluation of this model was inconclusive due to super-normal values in the hypertensive eyes. Up-regulation of $Na_v1.1$ and 1.2 expression was found in both models, beginning by day 7; an increase of the former continued in a time-dependent manner in the chronic model. $Na_v1.6$ labeling was inconclusive. In conclusion we found lamotrigine treatment to be mostly ineffective in both acute and chronic ocular hypertension models.

1. Introduction

Glaucoma is a neurodegenerative disease of the inner retina and optic nerve (ON), which leads to progressive visual field loss and possible blindness due to apoptosis and degeneration of retinal ganglion cells (RGC) and their axons.

Although mainstream glaucoma therapy is focused on lowering the elevated intraocular pressure (IOP), much research is devoted to developing drugs that would provide direct protection to the neural

Abbreviations: Na_v, voltage gated sodium channel; LTG, lamotrigine; IOP, intraocular pressure; RGC, retinal ganglion cells; ON, optic nerve; ERG, electroretinogram; FERG, full field ERG; PERG, pattern ERG; IHC, immunohistochemistry.

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tissue. Numerous substances were suggested as candidates for neuroprotective therapy, based on inhibiting various mechanisms of RGC degeneration and apoptosis, or promoting their survival.

Voltage gated sodium channels (Na_v), which normally function in the initiation and propagation of action potentials, are proposed to be mediators of neuronal damage by facilitating excessive depolarization, intracellular sodium overload and presynaptic release of glutamate (Bano et al., 2005; Nikolaeva et al., 2005; Stys, 2005; Tekkök et al., 2007). The presence and function of Na_v in the ON and retina was localized to RGC soma and axons (Boiko et al., 2003), subsets of amacrine cells, horizontal cells (Mojumder et al., 2007) and 2 types of cone bipolars (Cui and Pan, 2008). Na_v are also present in Müller cells where they function in volume regulation (Linnertz et al., 2011). $Na_v1.6$, 1.2 and 1.1 are the main subtypes found in the ON and retina.

Lamotrigine (LTG) is a Na_v channel blocker approved for the treatment of epilepsy, acting by blocking transient, fast inactivating sodium currents (Spadoni et al., 2002) and decreasing glutamate release (Shuaib et al., 1995). LTG was shown to protect excitable neurons in several models (Bechtold et al., 2006; Crumrine et al., 1997). LTG decreased glutamate-induced excitotoxicity in chick retina *ex vivo* (Pisani et al., 2001) and protected rabbit retina from apoptotic effects of intravitreal silicone oil injection (Guizzo et al., 2008) *in vivo*. Guizzo et al. (2005) found LTG to partially protect the rat inner retina from ischemia/reperfusion damage at the histopathological level (vacuolization and cell body densities), though the study did not differentiate RGCs from displaced amacrines.

A previous study on the neuroprotective effects of LTG in a chronic ocular hypertension rat model failed to find a beneficial structural effect (Marina et al., 2012). However, due to the great promise shown by the drug in other ischemic and excitotoxic models, we aimed to further this investigation by studying LTG in two ocular hypertension models, using more extensive methodology. Our goal was therefore to study both functional and structural effects of LTG treatment in the acute and chronic ocular hypertension induced optic neuropathy rat models. Furthermore, based on observed changes in Na_v channel expression in ON axons, macrophages and microglia of experimental allergic encephalomyelitis (EAE) model mouse (Craner et al., 2003, 2005) we evaluated similar changes in two rat ocular hypertension induced optic neuropathy models. We hypothesized that upregulation of Na_v channels in hypertensive eyes would further increase the efficacy of LTG treatment.

2. Methods

We used two rat ocular hypertension models to evaluate:

1. LTG efficacy as a neuroprotective agent by studying electroretinography (ERG), RGC counts, axon density, and microglia/macrophage involvement.
2. Changes in ON $Na_v1.1$, 1.2 and 1.6 expression using histopathology and immunohistochemistry (IHC).

2.1. Animals

10–12 weeks old Lewis/SsNHsd and Wistar/HsdBrlHan (Harlan Laboratories Inc, Israel) male rats were used for the acute and chronic IOP elevation procedures, respectively. The number of rats that were used in each procedure is summarized in Table 1. Rats were reared in 12 h light cycle and controlled temperature. All experimental procedures were in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research, and the guidelines of the Institutional Animal Care and Use Committee. An intraperitoneal (IP) injection of ketamine and xylazine were used for anesthesia in all procedures.

Table 1
Number of rats in each procedure. LTG = lamotrigine. Number of days indicate day of sacrifice (post IOP elevation).

	IOP elevation		ERG	Fluorogold labeling	Optic nerve structural analysis
LTG efficacy (Study I)	Acute	LTG	8	8	9
		vehicle	8	8	8
	Chronic	LTG	11	11	11
		vehicle	8	10	10
Changes in Na_v expression (Study II)	Acute	7 days			4
		15 days	5	5	9
	Chronic	7 days			13
		28 days			14
		50 days			6

2.2. Acute IOP elevation

The anterior chamber of a randomly chosen eye (the contralateral eye served as normotensive control) was cannulated with 30-gauge needle connected to a 0.9% saline reservoir hanging 150 cm above the eye. IOP elevation lasted 60 min and was assessed with a rebound tonometer before, 3 and 57 min after induction of IOP elevation and by observing blanching of the limbal plexus. Visualization of reperfusion to the limbal plexus within 30 s following cannula removal served as an indication of return to physiological IOP.

2.3. Chronic IOP elevation

Limbal plexus laser photocoagulation was used to block venous drainage from the left eye of anesthetized rats as described previously (Levkovitch-Verbin et al., 2002b). In the 1st session, 37–63 cautery pulses of 400 mW were applied. A 2nd photocoagulation session (18–44 cautery pulses, 250–300 mW) was performed 7 days later, except for 4 cases where only one laser session was performed due to marked IOP elevation and blanched appearance of the limbal plexus. IOP was measured with a Tonopen prior to photocoagulation, immediately after the procedure and then on days 1, 7, 14, 21, 27, 33 and 48 post IOP elevation.

2.4. LTG neuroprotection study

2.4.1. Experiment design of LTG neuroprotection trial

The sequence of procedures is depicted in Fig. 1. LTG and vehicle (VEH) administration began 2 days before induction of IOP elevation and continued thereafter, resulting in both a preventive and therapeutic administration, respectively.

2.4.2. LTG administration

VEH was 0.5–0.75% methylcellulose in PBS. LTG was suspended in VEH yielding 3.5% and 1.3% suspensions for acute and chronic

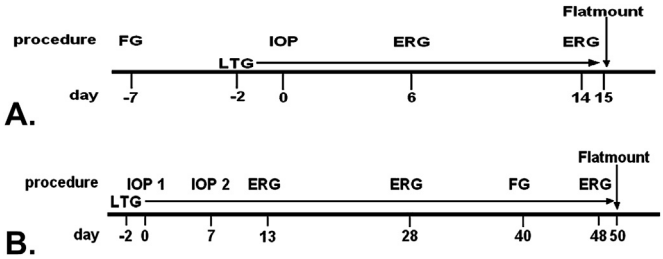

Fig. 1. Timetable of LTG procedures. A) Acute IOP elevation model. B) Chronic IOP elevation model. ERG = electroretinogram; FG = Fluorogold injection; IOP = intraocular pressure elevation procedure; LTG = beginning of daily lamotrigine administration.