The Neuroprotective Effects of Lidocaine and Methylprednisolone in a Rat Model of Retinal Ischemia-Reperfusion Injury

Wen-Chih TASI1), Simon M. PETERSEN-JONES2), Pei-Yun HUANG1) and Chung-Tien LIN1)*

1)Department of Comparative Ophthalmology, School of Veterinary Medicine, College of Bio-Resources and Agriculture, National Taiwan University, Taipei, Taiwan
2)Division of Comparative Ophthalmology, Department of Small Animal Clinical Sciences, Michigan State University. D-208 Veterinary Medical Center, East Lansing, MI 48824–1314, U.S.A.

(Received 13 March 2011/Accepted 30 September 2011/Published online in J-STAGE 14 October 2011)

ABSTRACT. Retinal ischemia is a common cause of visual impairment for humans and animals. The neuroprotective effects of lidocaine (LDC) and methylprednisolone (MP) upon retinal ischemic injury were investigated in a rat model. Sprague-Dawley rats were divided into 3 groups, the IR control, LDC and MP. A very high intraocular pressure (HIOP) and retinal ischemia were induced. In LDC group, LDC bolus (1.5 mg/kg) was IV injected 30 min before ischemia and then a constant rate infusion (CRI) with 2 mg/kg/hr was given until 60 min after reperfusion. In MP group, MP bolus (30 mg/kg) was IV administered twice at 2 min before and immediately after ischemia, respectively. The HIOP damage to retina was evaluated by electroretinogram (ERG) and morphometrical histology. The functional analysis of the retina by ERG revealed a 35.2% reduction of a-wave in the IR group, 49.7% reduction in the LDC group but no significant change in the MP group compared to normal controls. An 81.0% reduction of b-wave was observed in the IR group, 80.7% reduction in the LDC group and 17.6% reduction in the MP group. In the morphometrical histology, the retinal inner plexiform layer/outer nuclear layer (IPL/ONL) ratio was reduced to 48.8% in the IR group, 80.1% in the LDC group and 96.2% in MP group. In conclusion, the MP showed significantly good neuroprotective effects on retinal IR injury, and the LDC showed moderate neuroprotective effects demonstrated in retinal structure but not in retinal function.

KEY WORDS: ischemia-reperfusion injury, lidocaine, methylprednisolone, rat, retina.


Glucoma is a common ocular disease which may impair vision and cause intraocular damage [8, 29]. Retinal ischemia is the leading cause of retinal and optic nerve neuropathy, which causing RGCs (retinal ganglion cells) death in various ocular diseases like glaucoma [27, 28, 33]. The death of RGCs in glaucomatous optic neuropathy following ischemia results from neurotoxic glutamate release [19, 32], Ca2+ influx [27], nitrous oxide (NO) increase, free radicals and apoptosis [26]. Thus, neuroprotection has been considered as an important issue in glaucoma treatment and research in recent years [27].

Lidocaine (LDC) is commonly used to treat ischemia-reperfusion (IR) injury in clinical cases over recent years [6]. The major action to reduce IR injury is by preventing ROS (reactive oxygen species) formation and lipid peroxidation [22, 23]. LDC acts as a Na+/Ca2+ channel blocker, superoxide and hydroxyl radical scavenger, inflammatory modulator, and potent inhibitor of granulocyte functions [6]. In previous studies, lidocaine has been used for treatment of IR injury to a number of tissues including brain [20, 21], heart [5, 14], liver [9] and lung [13]. However, its use in IR injury to the retina has not been reported.

Methylprednisolone (MP) is a potent anti-inflammatory drug. Glucocorticoid steroids function as membrane stabilizers and antioxidants and by decreasing the migration of neutrophils and macrophages to inflammatory sites, they decrease hydrolytic enzyme release, lipid peroxidation, and oxygen radical production [34]. Although the effects of methylprednisolone on IR injury have been widely studied [2, 3, 12, 16, 25, 31, 34], the effect retinal IR injury have not been reported.

The aim of this study was to investigate the neuroprotective effect of two drugs, LDC and MP, in a rat high IOP-induced retinal ischemia model by electroretinogram (ERG) and morphometric histopathology.

MATERIALS AND METHODS

Animals and experimental groups: Male Sprague-Dawley rats (National Yang-Ming Medical University Laboratory Animal Center, Taipei, Taiwan) about 8 weeks old, weighing between 200 and 300 g, were used in this study. Light schedule was set 12-hr periods of light and dark (12L: 12D) with free access to food and water. All procedures of experiment were according to the ARVO (Association for Research in Vision and Ophthalmology) statement for the Use of Animals in Ophthalmic and Vision Research. Rats were grouped into IR- no drug (n=5), IR+LDC (n=7) and IR+MP (n=7).

Retinal ischemia-reperfusion injury model: General anes-
thecia was induced and maintained with 1–2% isoflurane (delivered in 100% O2) by mask. Topical 5% proparacaine (Alcaine®, Alcon) and 0.5% tropicamide (Mydriacyl®, Alcon) was applied to the experimental eye, and then the anterior chamber was cannulated with a 30-gauge needle connected to a saline reservoir. High intraocular pressure (HIOP) was achieved by raising the reservoir to apply 130 mmHg pressure for 45 min. This causes severe retinal ischemia.

**Lidocaine and methylprednisolone administration:** LDC (lidocaine HCl, China Chemical Product®, Taiwan) was given as a 1.5 mg/kg IV bolus 30 min before ischemia and then as a constant rate infusion (CRI) at 2 mg/kg/hr rate for 60 min during reperfusion. MP (methylprednisolone sodium succinate, Nang-Kuang Chemical Product®, Taiwan) was administered as a 30 mg/kg IV bolus 2 min before and then immediately after inducing ischemia.

The neuroprotective effect of LDC and MP were evaluated by ERG and morphometrical histopathology analysis at the 3rd day and 7th day, respectively, after ischemia.

**ERG recording:** The rats were dark-adapted for 12 hr and then anesthetized as described above to record the ERG (BPM-100 Control Program System, ERG/VEP v5.50, RetinoGraphics Inc, U.S.A.). This was recorded prior to the study and then again 3 days after inducing HIOP.

Tropicamide 5% for mydriasis and proparacaine 0.5% for topical anesthetics were given before the examination. The recording electrode (contact lens electrode, ERG-jet®, Universo Plastique Inc, Le Crêt-du-Locle, Switzerland) was placed on the surface of the cornea. The reference electrode (platinum subdermal needle electrode F-E2, Grass-Telefactor Division, Astro-Med Inc, West Warwick, RI, U.S.A.) was placed between the lateral canthus and ear and the ground electrode clamped the ear.

The intensities of light stimuli were set from -20dB to +5dB (-20dB, -15dB, -10dB, -5dB, 0dB, +5dB) to record the stimulus response. Each final result was average of 4 times flash stimuli and the interval flash time was 2 sec.

**Morphometrical analysis of retina:** The test rats were sacrificed at the 7 days after ischemia with an overdose of thiopental. The both globes were enucleated and fixed in 4% paraformaldehyde (PBS solution) for 20 min and then transferred to 10% formalin for 2 days. Globes were paraffin embedded and sectioned vertically through the optic nervehead and stained with hematoxylin and eosin (H&E).

The thickness of retinal layers was measured and the number of nuclei per unit length in the outer nuclear layer counted 250 µm and 500 µm from the optic nervehead dorsally and ventrally. Six areas of each retinal specimen were calculated to get the average with area width 100 µm. The fellow eye not subjected to HIOP acted as a control.

**Statistical analysis:** The results of ERG a- and b-wave were presented in reduction percentage which was analyzed by the Student- test to compare the mean values for the control eyes with corresponding values for the HIOP eyes. In the different medical treated groups, the ANOVA also were used to compare the intra-group intensities influence.

**RESULTS**

**Electroretinogram (ERG) analysis:** The ERG waveforms were different among different experimental groups (Fig. 1). The IR- no drug group revealed a reduction in b-wave after IR injury 3 days compared with the untreated eyes. In the IR+LDC group, the amplitude of the b-wave was significantly reduced 3 days after ischemia but slightly higher than the IR-no drug group. The IR+MP group revealed no obvious reduction in b-wave amplitude at the 3rd day after ischemia (Fig. 1).

**ERG a- & b-waves with various light intensities:** The effects of various stimulus intensities on ERG values were analyzed (Fig. 2). MP had significantly better preservation of the b-wave compared with that in the IR and LDC groups. In individual groups, there were differences in ERG amplitude reduction between the different light intensities in the IR group (ANOVA, \( P < 0.01 \)) and LDC groups (Mean \( \text{ANOVA, } P=0.0519 \)) in the MP group. Within the individual groups, there was no significant differences in a-wave reduction between the stimulus intensities in IR (ANOVA, \( P=0.5749 \)) but not in LDC (ANOVA, \( P=0.0317 \)) or with the exception of between the -20 and -15dB) and high intensities (0 and +5dB) as shown in Fig. 2.

The reduction in ERG a-wave was not as marked as the b-wave reduction (Fig. 3). The a-wave in the IR and LDC groups was reduced, but the a-wave was conserved in the MP group. Within the individual groups, there was no significant differences in a-wave reduction between the stimulus intensities in IR (ANOVA, \( P=0.5485 \)) and LDC group (ANOVA, \( P=0.2634 \)) or with the exception of between the -20 with -10dB intensities (\( P=0.0359 \)) in the MP group (ANOVA, \( P=0.2704 \)).

The mean reduction of ERG a- and b-wave amplitudes by different intensities in the IR, LDC and MP group is shown in Fig. 4. Significant reduction of b-wave was found in both IR (Mean \(-81.00 \pm 4.60\%\), \( P<0.01 \)) and LDC groups (Mean
–80.74 ± 5.11%, \( P < 0.01 \)), but not in MP group (Mean –17.55 ± 13.44%, \( P > 0.05 \)). A similar phenomenon was also seen in a-wave amplitudes, with a reduction in both IR (Mean –35.15 ± 22.29%, \( P < 0.01 \)) and LDC group (Mean –49.72 ± 21.28%, \( P < 0.01 \)), but not in the MP group (Mean 2.05 ± 6.67%, \( P > 0.05 \)).

Retinal morphological analysis: Morphological analysis showed that there were changes in thickness in individual retinal layers between the different treatment groups. The thickness of the IPL was significantly decreased in the IR group. The thickness was not reduced in the LDC and MP group.

There was a marked reduction in the inner plexiform layer thickness in the IR group (compared with the untreated eyes, \( P = 1.55 \times 10^{-5} \)), a slight reduction in the LDC group (compared with the untreated eyes, \( P = 0.0016 \)), but no reduction in the MP group (compared with the untreated eyes, \( P > 0.05 \)) (Fig. 5). However, the reduction of IPL thickness was significantly different between IR and LDC group (\( P = 5.58 \times 10^{-5} \)) which indicated that LDC treatment had a neuroprotective effect. A comparison of the cells counts for each group showed that there was significant reduction in the INL cell number but no significant differences in the ONL. The cells count of INL revealed a significant cell loss in the IR (\( P = 0.0049 \)) and LDC group (\( P = 0.0262 \)).

A comparison of the IPL/ONL ratio in each group is shown in the Fig. 6. The results in IPL/ONL ratio was consistent with the result in thickness of IPL revealing a difference between the LDC group and the untreated control eyes, (\( P = 0.0008 \)); and with the IR group, (\( P = 0.0004 \)) and between the MP group and the untreated control eyes (\( P > 0.05 \)). MP treatment significantly prevented the IPL/ONL ratio reduction suggesting that the neuroprotective effect of methylprednisolone was better than that of lidocaine. However neuroprotective effects were seen in both the LDC and MP groups by histology analysis.

DISCUSSION

A rat model of retinal ischemia was utilized to investigate the neuroprotective effect of lidocaine and methylprednisolone to an induced high IOP. The high IOP is intended to mimic glaucomatous optic neuropathy of acute angle closure glaucoma [27]. The duration of complete ischemia was set as 45 min as this time causes both a reduction in ERG amplitudes and morphological retinal changes [27].

The ERG b-wave is more severely affected than the a-wave by retinal ischemic insult [4, 11, 24, 37]. Thinning of inner retinal layer is also a quantifiable marker in pathological findings [7, 10, 17, 18, 24]. An intensity response series was used to fully investigate the effect on retinal function [24]. The detailed ERG functional analysis of the retina elicited by various intensities in this present study revealed an obvious b-wave loss when using low intensities (–20 and –15dB). The “dip” phenomenon was mentioned by Mukaida in 2004, and his conclusion said the b-wave ampli-
LDC and MP were used to investigate their neuroprotective effects against retinal IR injury in this model. Lidocaine is more commonly used to treat IR injury in clinical cases currently [6, 22, 23]. The therapeutic/clinical effects of lidocaine for IR injury and anti-inflammatory are actions as a Na⁺ and Ca²⁺ channel blocker, decreased neuronal excitotoxicity/glutamate release, hydroxyl radical scavenger, inhibition of neutrophil functions, and cytokine release [6]. Lidocaine also showed neuroprotective ability from IR injury in brain, including inhibiting the release of Ca²⁺ and Na⁺ from mitochondria and suppressing glutamate accumulation in neural tissue [35, 36]. The therapeutic dose of LDC according to Lei and colleagues studies in cerebral ischemic neuropathy was applied to protect against retinal ischemic neuropathy [20, 21].
In the present study, LDC demonstrated a neuroprotective effect against retinal ischemia in histological analysis but did not prevent the IR induced changes to the ERG (Fig. 4). The lidocaine could prevent the reduction of retinal thickness in inner plexiform layer and IPL/ONL ratio, but did not have a marked effect to prevent the loss of cells in the inner nuclear layer (Fig. 6).

The reason that lidocaine failed to provide neuroprotective as assessed by the ERG may be due to a low concentration of lidocaine in the target tissue. In this study, the dose of lidocaine chosen was the low-dose used in the Lei et al. study, this is a dose that is used for treating arrhythmia clinically [1, 20, 21]. A high dose (608 µg/kg/min) of lidocaine was used in regional ischemia in rat [5] and prevention of reperfusion lung injury [13]. Higher doses of LDC were used in a pilot study: two extreme high doses of LDC (5 mg/kg bolus IV with CRI 5 mg/kg/hr and 5mg/kg bolus IV with CRI 10 mg/kg/hr) were administered to the rats but still failed to provide therapeutic effect. However, intermittent lidocaine administrations may not maintain a high concentration in serum. The lidocaine was administered just as one bolus and once as a CRI over the retinal ischemia period in this study.

In the LDC group, there was a slight reduction in IPL thickness, INL cell counts and IPL/ONL ratio, but a significant reduction in ERG amplitudes. The partial neuroprotective effect of lidocaine was better demonstrated on morphology but not in ERG, where there was a dramatic decrease particularly of the b-wave. The ERG b-wave results from ion differences due to bipolar cell function. The INL cells count correlated with b-wave amplitudes was a linear-linear fashion but it revealed the regression line reaching “zero” of the b-wave amplitude despite 45% INL cells remaining. So the remaining INL cells after ischemia insult could only generate little b-wave amplitude as Mukaida’s hypothesis [24]. The exact reason why this study showed poor response to LDC in the ERG evaluation was unclear and more studies are needed to explore the mechanism.

Methylprednisolone was effective in preventing the IR-induced reduction of a- and b-wave. This effect was maintained across the range of stimulus intensities used. Morphometrical pathology evaluation showed that methylprednisolone could prevent reduction in IPL thickness and INL cells counts. The same neuroprotective effect in the IPL/ONL ratio by methylprednisolone was also seen. MP showed very good effect to prevent retinal IR damage in the aspects of retinal functions and histology in this study.

MP has been used in the treatment for IR injury. Methylprednisolone treatment has been used in experimental focal cerebral ischemia at a high dose (105 mg/kg)[34], also to reduce hepatic IR injury [16], and has a beneficial effect in severe brain injury [15] and in reducing IR-induced myocardial injury. ERG is a sensitive method to detect the whole retinal physiological function whereas histology will only show cell loss not altered function [24].
dial apoptosis in immature hearts [30].

In summary, this study shows that lidocaine demonstrated moderate neuroprotective effect against retinal ischemia injury reflected in a preservation of retinal structures. It was not shown to have a significant preservative effect on function as assessed by the ERG. In the LDC group, there was only a slight reduction of thickness and number of cells in the inner retina but little preservation of the ERG b-wave. In contrast, methylprednisolone is effective in preventing retinal IR damage as assessed functionally by ERG and structurally by histopathology in this study. The findings of this study may give an insight to clinical treatment for retinal IR injury using lidocaine and methylprednisolone. In the future, higher doses or continuous administration of lidocaine may be used to try and develop a more effective protocol to use for retinal neuroprotection.

ACKNOWLEDGMENT. The authors would like to thank the support of a research grant (98-2313-B-002-027-MY3) by the National Science Council, ROC.

REFERENCES


