—Original—

Comparison of the Protective Effects of Prostaglandin Analogues in the Ischemia and Reperfusion Model of Rabbit Eyes

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Abstract: This study was planned to investigate the neuroprotective potentials of three commercially available prostaglandin analogues (PGA), in the ischemia and reperfusion model (I/R). Thirty New Zealand rabbits were divided into 5 groups and except for the control group (non-ischemic, non-treated), 0.9% NaCl, bimatoprost, latanoprost, or travoprost were applied to both eyes of animals of the respective groups for 1 week. At the end of treatment, ischemia was induced in both eyes of the 4 treatment groups by anterior chamber irrigation of the animals for 60 min. Following 24 h reperfusion, the animals were sacrificed. Enucleated eyes and retinal tissues were investigated by light microscopy, electron microscopy, immunohistochemistry for retinal histopathology, intracellular and apoptotic cells and by retinal morphometry. Vitreous samples were biochemically investigated for probable role of reactive oxygen species, by measuring xanthine oxidase (XO) activity. Analysis of morphometric measurements and vitreous XO activity revealed significant differences between the PGA-treated groups and the NaCl-treated group ($P<0.05$). Similarly, apoptotic cell counts in different retinal layers showed that PGA-treated groups had fewer apoptotic cells in all retinal layers than the NaCl-treated ischemic group ($P<0.05$). PGA may have high protective potential for different retinal layers and cells. Biochemical analysis of vitreous showed that all PGAs decreased vitreous XO activity significantly compared to the NaCl-treated group ($P<0.05$). However we could not find any statistically significant differences among the analogues. PGAs may reduce the injury induced by I/R, through the inhibition of XO activity, and it seems that their effects are elicited through numerous pathways.

Key words: bimatoprost, ischemia and reperfusion, latanoprost, travoprost, xanthine oxidase

Introduction

Retinal ischemia contributes to visual impairment and blindness in diseases such as acute closed angle glau-

coma [36] and diabetic retinopathy [6]. Hypoxia leads disruption of the cellular energy metabolism and initiates several harmful cascades [1]. The generation of reactive oxygen species (ROS), degradation of the antioxidant

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system, induction of cytokine production via transcriptional factors, and extracellular accumulation of glutamate that is excitotoxic to neuronal elements are the main alterations secondary to ischemia [1, 16, 18, 19, 34, 38, 40, 41]. Reperfusion after initial ischemia paradoxically maintains the destruction process, perhaps due to increased levels of extracellular neurotransmitters, ROS, and waste products damaging previously unharmed cells when being reoxidized [1, 5, 28]. In this process, it is considered that xanthine oxidase (XO) is a major source of ROS [22, 39].

Glaucoma is one of the major causes of irreversible blindness on all over the world. Vision loss in glaucoma is attributed to retinal ganglion cell loss, and raised intraocular pressure (IOP) is the major modifiable risk factor [15]. Although the clinical picture of glaucoma is well described, the exact mechanism leading to this specific type of damage is not yet clear [11]. One of the most frequently used models for the investigation of molecular mechanisms and potential therapeutic strategies for retinal ischemia has been an experimental model of acute elevation of IOP followed by reperfusion [43].

IOP-lowering treatment, either by use of topically applied drugs or by surgery, is the only treatment modality for glaucoma. Among various topical agents, prostaglandin analogues (PGA) are the newest class of glaucoma medications that have been introduced into the anti-glaucoma market. These medications have powerful ocular hypotensive effects through facilitated uveoscleral outflow. There are currently three internationally approved analogues (bimatoprost, latanoprost, and travoprost) for the treatment of patients with elevated intraocular pressure. In addition to the IOP lowering effect, a recent paper documented the retinal cell protective potential against ischemic conditions, *in vivo* and *in vitro* for latanoprost [10]. Clinically apoptosis has been shown to occur in retinas with glaucoma [27].

The aim of this study was to compare the anti-apoptotic potential of three prostaglandin analogues (bimatoprost, latanoprost, and travoprost) in the ischemia and reperfusion (I/R) model. We also aimed to investigate the potential of these agents against ROS production through the measurement of xanthine oxidase (XO) activity in vitreous.

Materials and Methods

Thirty adult male New Zealand rabbits, weighing 900 to 1,100 g, were used in compliance with the ARVO Statement for the use of Animals in Ophthalmic and Vision Research. The animals were allowed free access to standard food diet and water in the animal laboratory at Inonu University. The animal handling and experimental protocol were reviewed and approved by the ethical committee for animal care and use of Inonu University. The rabbits were quarantined for 8 days at 22–24°C with a 12-h light / 12-h dark cycle before beginning the experimental process for adaptation.

Experimental model of ischemia and reperfusion

The animals were randomly divided into 5 groups. No medication was applied to the animals of the first group (n=6) and this group is hereafter described as the control group. In the other 4 groups (n=6), animals were treated with 0.9% NaCl, bimatoprost (Lumigan, Abdi Ibrahim Ilac Inc., Turkey), latanoprost (Xalatan, Pfizer Turkey Inc.), or travoprost (Travatan, Alcon Turkey Inc.) 1×1 drop/day for one week. At the end of one week, ischemia was created as described previously [9, 30, 33]. In short, animals were anesthetized with ketamine (50 mg/kg) and xylazine (5 mg/kg) and topical anesthetic was dropped on to the cornea of the animals. A steel 30-gauge infusion needle connected to a saline reservoir, elevated to 1.70 m, was inserted into the anterior chamber of both eyes of the animals. Except for the control group, the reservoir was opened for 60 min and the increase of intraocular pressure was monitored by indirect ophthalmoscopy by observing the cessation of blood flow at the optic disk. At the end of 60 min, the cannulating needle was removed and intraocular pressure normalised by allowing reperfusion for 24 h. At the end of the reperfusion period, the animals were sacrificed with an overdose of xylazin (10 mg/kg) and ketamin (150 mg/kg) and their eyeballs were removed for analysis.

Preparation of tissue samples

Both eyes of the animals were enucleated, and the left eyes were dissected quickly under a stereo microscope. The eyeball was cut posteriorly to the limbus, and there-

after the anterior segment was discarded. Immediately, the vitreous was obtained for biochemical analysis, and the retina was carefully dissected from the sclera/choroid/pigment epithelium.

Xanthine oxidase (XO) activity assay and protein level determination

XO activity was determined spectrophotometrically as described by Bergmeyer *et al.* [4]. To 50 mM phosphate buffer, pH 7.5, containing 1 mM xanthine, aliquots of homogenate were added and changes in optical density were recorded at 290 nm. One unit of XO was defined as 1 μ mol of xanthine converted to uric acid in 1 min at pH 7.5 and 25°C. The specific activity of XO was expressed in milli-units (mU) per mg protein. Protein levels of the samples were measured spectrophotometrically according to the method of Lowry *et al.* [21].

Light microscopy and immunohistochemical evaluation

For light microscopic and immunohistochemical evaluation, right ocular globes were fixed in 10% neutral buffered formaldehyde and embedded in paraffin wax. Sections from paraffin blocks were cut at 5 μ m, mounted on slides. Sections were stained with haematoxylin and eosin (HE) and then stained immunohistochemically using a TUNEL System (DeadEnd Colorimetric TUNEL System, Promega Corporation, Madison, USA) kit. The sections were observed, analysed and photographed using a Leica DFC280 light microscope and a Leica Q Win Image Analysis System (Leica Micros Imaging Solutions Ltd., Cambridge, UK).

Electron microscopic examination

For electron microscopic examination, samples of retina were fixed in 2.5% glutaraldehyde buffered with 0.2 M $\text{NaH}_2\text{PO}_4 + \text{NaHPO}_4$ (pH=7.4) and postfixed in 1% OsO_4 . After dehydration in acetone, they were embedded in Araldite CY 212. Ultrathin sections were stained with uranyl acetate and lead citrate and examined by a Zeiss Libra 120 transmission electron microscope.

Immunohistochemical examination

Five- μ m thick sections were mounted on poly-L-lysine coated slides, subsequently deparaffinized by

immersion in xylene for 5 min at room temperature, then rehydrated in a graded alcohol series. A Colorimetric Apoptosis Detection System was used according to the manufacturer's instructions. Briefly, sections were treated with 20 μ g/ml proteinase K for 10 min, washed in PBS and refixed in 10% buffered formaldehyde in PBS and treated with equilibration buffer for 10 min at room temperature. Subsequently, add the rTdT (Terminal deoxynucleotidyl transferase) reaction mix was added to the sections on the slides. The slides were incubated at 37°C for 60 min inside a humidified chamber. After washing, 0.3% hydrogen peroxidase was used to block endogenous peroxidase. Streptavidin HRP (1:500 in PBS) was added for 30 min at room temperature. DNA strand breaks were visualized by using hydrogen peroxidase and chromogen 3,3'-diaminobenzidine tetrahydrochloride. Sections were counterstained with haematoxylin. Negative controls were performed by omitting TdT enzyme.

Morphometric analysis

The thickness of retinal layers was measured on 10 different sections under the light microscope with $\times 40$ objective lens. The TUNEL-positive cells at the ganglion cell layer (GCL), the inner nuclear layer (INL) and the outer nuclear layer (ONL) were counted on 10 different sections under the light microscope with $\times 40$ objective lens.

Statistical analysis

Statistical analysis was performed with SPSS for Windows version 12.0 program (SPSS Inc., Chicago, IL). All data are reported as means $\pm$ standard error of mean (SEM). For statistical analysis of retinal thickness measurements among groups the Mann-Whitney U was test used. For statistical analysis of XO measurements, variables in groups were determined by the Shapiro Wilk test. The variables did not show normal distribution ($P > 0.05$). So, the Kruskal Wallis test and the Mann-Whitney U test with Bonferroni correction were used for comparisons of variables among and between the studied groups. A value of $P < 0.05$ was considered statistically significant.

Table 1. Mean $\pm$ SEM thickness of retinal layers in microns (μ) among study groups

Group	GCL	INL	ONL	PRL	Total
Control	20.8 $\pm$ 0.6	20.2 $\pm$ 0.8	33.5 $\pm$ 1.3	30.3 $\pm$ 0.94	125.3 $\pm$ 1.6
I/R + NaCl	18.8 $\pm$ 0.2 ^{a)}	24.0 $\pm$ 0.7 ^{a)}	27.4 $\pm$ 0.5 ^{a)}	24.8 $\pm$ 1.14 ^{a)}	117.7 $\pm$ 1.6 ^{a)}
I/R + Bimatoprost	19.6 $\pm$ 0.4	22.4 $\pm$ 0.3	33.0 $\pm$ 0.4 ^{b)}	32.7 $\pm$ 0.8 ^{b)}	128.8 $\pm$ 1.6 ^{b)}
I/R + Latanoprost	20.3 $\pm$ 1.0	22.2 $\pm$ 0.9	34.6 $\pm$ 1.1 ^{b)}	33.0 $\pm$ 1.1 ^{b)}	129.7 $\pm$ 3.6 ^{b)}
I/R + Travoprost	20.0 $\pm$ 1.2	22.2 $\pm$ 0.8	33.6 $\pm$ 1.5 ^{b)}	33.3 $\pm$ 0.8 ^{b)}	128.3 $\pm$ 1.6 ^{b)}

GCL: Ganglion cell layer, INL: Inner nuclear layer, ONL: Outer nuclear layer, PRL: Photoreceptor layer, I/R: Ischemia and reperfusion. ^{a)}: Statistically significant difference compared to control group $P < 0.05$. ^{b)}: Statistically significant difference compared to I/R + NaCl group $P < 0.05$.

Results

Light microscopy

As shown in Table 1, morphometric analysis showed that treatment with PGA prevented atrophy of the retinal layers particularly ONL and photoreceptor layer (PRL) compared to the NaCl-treated group. Under a light microscope, sections from the control group were normal in histological appearance (Fig. 1). However, in the NaCl-treated group, a decrease in the number of cells in INL and ONL, loss in GCL, edema in INL (Table 1), nuclear condensation in ONL, and degeneration at the PRL were detected (Fig. 2). In the bimatoprost-treated group, the histological picture of the retina was very similar to that of the control group, except for mild edema in PRL (Fig. 3). In the latanoprost treatment group, minimal edema in ONL was noticed (Fig. 4). Finally, in the travoprost treatment group, there was minimal edema in INL and PRL (Fig. 5).

Immunohistochemical analysis

No apoptotic cell in any of the retinal layers was observed in the control group. However, following I/R, TUNEL-positive cells appeared as dark brown cells, as shown in Figs. 2, 3, 4, and 5. In the control group, we did not detect any TUNEL-positive cells in any layer. On the other hand, in the I/R group treated with NaCl, TUNEL-positive cells increased throughout INL and GCL, and were much more intense and numerous in ONL (Fig. 2). In the bimatoprost and travoprost treatment groups, there were a few TUNEL-positive cells scattered throughout all the retinal layers (Figs. 3 and 5). In the latanoprost treatment group, TUNEL-positive cells were scattered in all retinal layers, but were more prominent

Fig. 1. [Control retina] Up Left; Normal histological appearance in retina (HE; $\times 40$), Up Right; no TUNEL-positive cell were found (TUNEL; $\times 40$), Bottom Left; electron micrograph of INL cells normal ultrastructural appearance ($\times 8,000$), Bottom Right; normal ultrastructural appearance in PRL cells ($\times 10,000$).

in GCL than in the other treatment groups (Fig. 4). As shown in Table 2, treatment with PGA yielded statistically significantly lower apoptotic cell counts than the NaCl-treated group in GCL, INL, and ONL ($P < 0.05$). Also, there were significantly better results in the travoprost treatment group compared to the other prostaglandin analogues, particularly in INL and ONL.

Fig. 2. [0.9% NaCl treated group] Up Left; degeneration in PRL and nuclear condensation in GCL, INL, ONL, and edema in INL (HE; $\times 40$), Up Right; TUNEL-positive cells appear dark brown in GCL, INL, and ONL (TUNEL; $\times 40$), Bottom Left; highly intercellular space distention and lightly nuclear chromatin condensation in INL ($\times 6,656$), Bottom Right; extracellular edema and mitochondrial degeneration in PRL ($\times 10,000$).

Fig. 3. [Bimatoprost treated group] Up Left; edematous transparent areas in PRL (HE; $\times 40$), Up Right; a few TUNEL-positive cells in GCL, INL, and ONL (TUNEL; $\times 40$), Bottom Left; Bimatoprost; mild intercellular space distention and lightly nuclear chromatin condensation in INL ($\times 8,000$), Bottom Right; normal ultrastructural appearance in PRL cells ($\times 6,300$).

Transmission electron microscopy

In the control group, electron microscopic features of all cells from all layers of the retina were normal in appearance (Fig. 1). In the NaCl-treated group, in INL, nuclear condensation, and dilatation of intercellular spaces were obvious (Fig. 2). In PRL, there was extracellular edema and mitochondrial degeneration (Fig. 2). In all of the treatment groups, in INL there was nuclear condensation and intercellular space distention, but these alterations were mild compared to the NaCl-treated ischemia group (Figs. 3, 4, and 5). In PRL, the ultrastructure of cells were normal in all prostaglandin analogue-treated groups as shown in Figs. 3, 4, and 5.

Vitreous XO measurements

The activity of XO was significantly different among the study groups ($P < 0.05$). Dual comparisons between groups show that there was a statistically significant difference between the NaCl-treated group and the others ($P < 0.05$) (Fig. 6). On the other hand, the measured XO

activity in the prostaglandin-treated groups was higher than the control group, but the difference was not statistically significant ($P > 0.05$). Similarly, we did not detect any statistically significant differences among groups treated with prostaglandin analogues ($P > 0.05$).

Discussion

As in most tissues, there are highly complex mechanisms for the regulation of retinal circulation. The regulation system is under the control of many different local and systemic factors. Since the retinal tissue lacks vascular innervation, retinal arterial tone is largely regulated by local factors [17]. Previous studies have shown that ROS produced during ischemia activate cyclo-oxygenase and released prostaglandins mediate the vasodilator action of free radicals in the eye [17].

Interruption of blood supply results in ischemic injury which rapidly damages metabolically active tissues. Paradoxically, restoration of blood flow to the ischemic tissue initiates a cascade of events that may lead to ad-

Fig. 4. [Latanoprost treated group] Up Left; Normal histological appearance in retina (HE; $\times 40$), Up Right; TUNEL-positive cells at INL, ONL, and more prominent in GCL (TUNEL; $\times 40$), Bottom Left; mild intercellular space distention and lightly nuclear chromatin condensation in INL ($\times 6,300$), Bottom Right; normal ultrastructural appearance cells and few degenerative cells in PRL ($\times 6,300$).

Fig. 5. [Travoprost treated group] Up Left; minimal edema in ONL and mild edematous transparent areas in PRL (HE; $\times 40$), Up Right; TUNEL-positive cells were rarely noticed in INL and ONL (TUNEL; $\times 40$), Bottom Left; lightly intercellular space distention and nuclear condensation in INL cells ($\times 8,000$), Bottom Right; normal ultrastructural appearance cell segments in PRL ($\times 6,300$).

Fig. 6. Vitreous xanthine oxidase activity assay among groups. I/R: Ischemia and reperfusion, †: statistically significant compared to I/R + NaCl group ($P < 0.05$).

ditional cell injury which is known as reperfusion injury [22]. A growing body of evidence indicates that the tissue injury observed after reperfusion is due to the reintroduction of oxygen rather than a delayed manifestation of injury caused by ischemia [31, 35].

Up to now, various experimental designs have been

described for the induction of ischemia-reperfusion damage in the retina, as vessel occlusion by clamping [1, 8, 9, 13, 24, 28, 30, 32, 33]. In the present study, we used the method of raising intraocular pressure above the arterial opening pressure. This method enables easy reversible occlusion with visually controlled blood flow.

Table 2. Mean $\pm$ SEM of apoptotic cell counts in different layers of retina among groups

Group	GCL	INL	ONL
Control	0.0 $\pm$ 0	0.0 $\pm$ 0	0.0 $\pm$ 0
Ischemia + NaCl	4.5 $\pm$ 0.6 ^{a)}	44.8 $\pm$ 4.6 ^{a)}	194.57 $\pm$ 18.4 ^{a)}
Ischemia + Bimatoprost	1.4 $\pm$ 0.4 ^{a, b)}	27.9 $\pm$ 2.4 ^{a, b, c)}	33.42 $\pm$ 3.5 ^{a, b, c, d)}
Ischemia + Latanoprost	1.8 $\pm$ 0.3 ^{a, b)}	24.4 $\pm$ 2.6 ^{a, b, c)}	25.19 $\pm$ 2.2 ^{a, b, c)}
Ischemia + Travoprost	1.2 $\pm$ 0.4 ^{a, b)}	15.9 $\pm$ 1.5 ^{a, b)}	13.69 $\pm$ 2.3 ^{a, b)}

GCL: Ganglion cell layer, INL: Inner nuclear layer, ONL: Outer nuclear layer. ^{a)}: Statistically significant compared to control group $P < 0.05$. ^{b)}: Statistically significant compared to I/R and NaCl treatment group $P < 0.05$. ^{c)}: Statistically significant compared to I/R and travoprost treatment group $P < 0.05$. ^{d)}: Statistically significant compared to I/R and latanoprost treatment group $P < 0.05$.

Also elucidation of surgical operation is another advantage of this method. We applied 60 min of ischemia, which should have been sufficient to have induced substantial ischemic damage.

It has been suggested that both apoptosis and necrosis are involved in I/R-induced neuronal damage [9]. During I/R, apoptosis plays a major role because oxygen-derived reactive species (ROS) are known to trigger apoptosis [37, 38]. Several studies have shown that application of exogenous free radical scavengers [3, 9] and topically applied medications including PGA may reduce I/R-induced damage to the retina [2, 20, 26, 29].

Nakanishi *et al.* showed that latanoprost rescued cells from apoptosis, secondary to serum deprivation, by inhibiting caspase-3, probably mediated by the mitogen-activated protein kinase pathway coupled to FP receptors in a dose-dependent manner [26]. In our study, we detected a significant alteration in the NaCl treated group compared to the control group. These alterations were decrements in thickness of all layers except the INL layer, and the total retinal thickness was significantly thinner in the NaCl-treated group than in the control group. Meanwhile, treatment with PGA provided a defence mechanism which was statistically significant in ONL and PRL, as shown in Table 1. On the other hand, unoprostone isopropyl, is also a PGF2 α -related ocular hypotensive, and Mukuno *et al.* showed it has an anti-apoptotic effect which works through the Akt or/and protein kinase G pathway, unlike latanoprost [25]. Probably, PGF2 α -related compounds have diverse intracellular signaling pathways specific for each derivative.

Guenoun *et al.* compared PGA, *in vitro*, with regard

to their protective effects against benzalkonium chloride (BAC) toxicity on conjunctiva-derived epithelial cells [14]. They found that latanoprost and travoprost have the potential to protect on human conjunctiva-derived epithelial cells from BAC toxicity. This property could not be documented for bimatoprost because of its low toxicity, making a protective effect impossible to reveal. The protective effect of PGA has been explained as inhibition of the proapoptotic effects of BAC through the oxidative stress pathways. In our study, in the investigated sections, we also counted the apoptotic cell counts in GCL, INL, and ONL, and we detected statistically significant differences among and between the groups as shown in Table 2 ($P < 0.05$). We also noticed that in the travoprost treatment group the apoptotic cell count was significantly lower than in the other analogue treatment groups ($P < 0.05$). Generally, the order of groups from minimum to maximum apoptotic cell counts was: travoprost < latanoprost < bimatoprost < NaCl.

Glutamate has neurotoxic potential and it appears to be related to excessive calcium influx through ionotropic receptor stimulation and the subsequent activation of enzymes such as XO, nitric oxide (NO) synthase and phospholipase A2. These different enzymes, respectively, produce superoxide anions, NO and arachidonic acid (AA) that are neurotoxic in various experimental models [7, 23, 42]. However, Drago *et al.* clearly documented that latanoprost may protect retinal cells against glutamate toxicity at particular concentrations, probably through the blockade of glutamate receptors. They speculated that the *in vivo* neuroprotective activity of PGA may be the result of multiple actions on different

biological targets [10]. In our study, probably inhibition of these pathways with PGA yielded significantly lower levels of XO in the vitreous. As a result of this reduction, the retinas of the animals were exposed to relatively low levels of ROS, and we observed a relatively low number of apoptotic cells in the retinal layers. However, we also noticed that some of the medications we used were more effective on some retinal layers, as shown in Tables 1 and 2. Even though this can be accepted as a coincidence, a relatively high affinity of a particular PGA to certain retinal cells and layers is another possibility. Moreover, as mentioned by Drago *et al.*, the *in vivo* neuroprotective potential of these agents could be the result of multiple actions on different biological targets and their variable potency on different targets could be related to their chemical compositions [10].

The presence of XO in the endothelium of retinal vessels and in the photoreceptor layers of human and bovine retina has recently been documented [12]. Inhibitors of XO have been successfully used to reduce ischemia-induced changes in model systems [32]. In our study, we detected significantly lower levels of XO activity in the PGA treatment groups. Although, we looked for differences between treatment groups, we could not find any statistically significant differences ($P>0.05$) (Fig. 6). However, the effect of the three PGAs on vitreal and retinal anti-oxidant enzymes could be the subject of another experimental study.

Although there is substantial evidence that many oxidative pathways contribute to damage resulting from I/R, it seems unlikely that any one pathway is sufficiently critical to define the outcome. The present results confirm that topically applied PGA acts as a neuroprotective agent against retinal I/R. This conclusion is based on the finding that the treatment of animals for one week with PGA reduced the histological alterations which occur after ischemia. Moreover, the rationale for the protection achieved by topical PGA is supported by the observation that I/R induced increase of vitreal XO activity was counteracted by these medications.

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References

1. Agardh, C.D., Gustavsson, C., Hagert, P., Nilsson, M., and Agardh, E. 2006. Expression of antioxidant enzymes in rat retinal ischemia followed by reperfusion. *Metabolism* 55: 892–898.
2. Aktaş, Z., Gürelik, G., Akyürek, N., Onol, M., and Hasanreisoglu, B. 2007. Neuroprotective effect of topically applied brimonidine tartrate 0.2% in endothelin-1-induced optic nerve ischaemia model. *Clin. Experiment. Ophthalmol.* 35: 527–534.
3. Baudouin, C., Pisella, P.J., Ettaiche, M., Goldschild, M., Becquet, F., Gastaud, P., and Droy-Lefaix, M.T. 1999. Effects of EGB761 and superoxide dismutase in an experimental model of retinopathy generated by intravitreal production of superoxide anion radical. *Graefes Arch. Clin. Exp. Ophthalmol.* 237: 58–66.
4. Bergmeyer, H.U., Gawehen, K., and Grassl, M. 1974. Enzymatic assay of xanthine oxidase. pp. 521–522. In: *Methods of Enzymatic Analysis*, 2nd edition, Vol. I (Bergmeyer, H.U. ed.), Academic Press, New York.
5. Bonne, C., Muller, A., and Villain, M. 1998. Free radicals in retinal ischemia. *Gen. Pharmacol.* 30: 275–280.
6. Bresnick, G.H., Engerman, R., Davis, M.D., de Venecia, G., and Myers, F.L. 1976. Patterns of ischemia in diabetic retinopathy. *Trans. Sect. Ophthalmol. Am. Acad. Ophthalmol. Otolaryngol.* 81: 694–709.
7. Chi, D.W. 1988. Calcium-mediated neurotoxicity: relationship to specific channel types and role in ischemic damage. *Trends Neurosci.* 11: 465–467.
8. Daugeliene, L., Niwa, M., Hara, A., Matsuno, H., Yamamoto, T., Kitazawa, Y., and Uematsu, T. 2000. Transient ischemic injury in the rat retina caused by thrombotic occlusion/thrombolytic reperfusion. *Invest. Ophthalmol. Vis. Sci.* 41: 2743–2747.
9. Dilsiz, N., Sahaboglu, A., Yildiz, M.Z., and Reichenbach, A. 2006. Protective effects of various antioxidants during ischemia-reperfusion in the rat retina. *Graefes Arch. Clin. Exp. Ophthalmol.* 244: 627–633.
10. Drago, F., Valzelli, S., Emmi, I., Marino, A., Scalia, C.C., and Marino, V. 2001. Latanoprost exerts neuroprotective activity in vitro and in vivo. *Exp. Eye Res.* 72: 479–486.
11. Flammer, J. and Orgül, S. 1998. Optic nerve blood-flow abnormalities in glaucoma. *Prog. Retin. Eye Res.* 17: 267–289.
12. Fox, N.E. and van Kuijk, F.J. 1998. Immunohistochemical localization of xanthine oxidase in human retina. *Free Radic. Biol. Med.* 24: 900–905.
13. Gelbach, P.L. and Purple, R.L. 1994. A paired comparison of two models of experimental retinal ischemia. *Curr. Eye Res.* 13: 597–602.
14. Guenoun, J.M., Baudouin, C., Rat, P., Pauly, A., Warnet, J.M., and Brignole-Baudouin, F. 2005. In vitro comparison of cytoprotective and antioxidative effects of latanoprost,

- travoprost, and bimatoprost on conjunctiva-derived epithelial cells. *Invest. Ophthalmol. Vis. Sci.* 46: 4594–4599.
15. Guo, L., Moss, S.E., Alexander, R.A., Ali, R.R., Fitzke, F.W., and Cordeiro, M.F. 2005. Retinal ganglion cell apoptosis in glaucoma is related to intraocular pressure and IOP induced effects on extracellular matrix. *Invest. Ophthalmol. Vis. Sci.* 46: 175–182.
 16. Hangai, M., Yoshimura, N., and Honda, Y. 1996. Increased cytokine gene expression in rat retina following transient ischemia. *Ophthalmic Res.* 28: 248–254.
 17. Hardy, P., Beauchamp, M., Sennlaub, F., Gobeil, F. Jr., Tremblay, L., Mwaikambo, B., Lachapelle, P., and Chemtob, S. 2005. New insights into the retinal circulation: inflammatory lipid mediators in ischemic retinopathy. *Prostaglandins Leukot. Essent. Fatty Acids* 72: 301–325.
 18. Jo, N., Wu, G.S., and Rao, N.A. 2003. Upregulation of chemokine expression in the retinal vasculature in ischemia-reperfusion injury. *Invest. Ophthalmol. Vis. Sci.* 44: 4054–4060.
 19. Kuriyama, H., Waki, M., Nakagawa, M., and Tsuda, M. 2001. Involvement of oxygen free radicals in experimental retinal ischemia and the selective vulnerability of retinal damage. *Ophthalmic Res.* 33: 196–202.
 20. Lafuente, M.P., Villegas-Pérez, M.P., Mayor, S., Aguilera, M.E., Miralles de Imperial, J., and Vidal-Sanz, M. 2002. Neuroprotective effects of brimonidine against transient ischemia-induced retinal ganglion cell death: a dose response in vivo study. *Exp. Eye Res.* 74: 181–189.
 21. Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J. 1951. Protein measurement with the folin phenol reagent. *J. Biol. Chem.* 193: 265–275.
 22. Mallick, I.H., Yang, W., Winslet, M.C., and Seifalian, A.M. 2004. Ischemia-reperfusion injury of the intestine and protective strategies against injury. *Dig. Dis. Sci.* 49: 1359–1377.
 23. Meldrum, B. and Garthwaite, J. 1990. Excitatory aminoacid neurotoxicity and neurodegenerative disease. *Trends Pharmacol. Sci.* 11: 379–383.
 24. Mosinger, J.L. and Olney, J.W. 1989. Photothrombosis-induced ischemic neuronal degeneration in rat retina. *Exp. Neurol.* 105: 110–113.
 25. Mukuno, H., Nakamura, M., Kanamori, A., Nagai, A., Negi, A., and Seigel, G. 2004. Unoprostone isopropyl rescues retinal progenitor cells from apoptosis in vitro. *Curr. Eye Res.* 29: 457–464.
 26. Nakanishi, Y., Nakamura, M., Mukuno, H., Kanamori, A., Seigel, G.M., and Negi, A. 2006. Latanoprost rescues retinal neuro-glial cells from apoptosis by inhibiting caspase-3, which is mediated by p44/p42 mitogen-activated protein kinase. *Exp. Eye Res.* 83: 1108–1117.
 27. Okisaka, S., Murakami, A., Mizukawa, A., and Ito, J. 1997. Apoptosis in retinal ganglion cell decrease in human glaucomatous eyes. *Jpn. J. Ophthalmol.* 41: 84–88.
 28. Osborne, N.N., Casson, R.J., Wood, J.P., Chidlow, G., Graham, M., and Melena, J. 2004. Retinal ischemia: mechanisms of damage and potential therapeutic strategies. *Prog. Retin. Eye Res.* 23: 91–147.
 29. Osborne, N.N., DeSantis, L., Bae, J.H., Ugarte, M., Wood, J.P., Nash, M.S., and Chidlow, G. 1999. Topically applied betaxolol attenuates NMDA-induced toxicity to ganglion cells and the effects of ischemia to the retina. *Exp. Eye Res.* 69: 331–342.
 30. Oz, O., Gürelik, G., Akyürek, N., Cinel, L., and Hondur, A. 2005. A short duration transient ischemia induces apoptosis in retinal layers: an experimental study in rabbits. *Eur. J. Ophthalmol.* 15: 233–238.
 31. Parks, D.A. and Granger, D.N. 1986. Contributions of ischemia and reperfusion to mucosal lesion formation. *Am. J. Physiol.* 250: 749–753.
 32. Rosenbaum, D.M., Rosenbaum, P.S., Singh, M., Gupta, G., Gupta, H., Li, B., and Roth, S. 2001. Functional and morphologic comparison of two methods to produce transient retinal ischemia in the rat. *J. Neuroophthalmol.* 21: 62–68.
 33. Sakamoto, K., Yonoki, Y., Kuwagata, M., Saito, M., Nakahara, T., and Ishii, K. 2004. Histological protection against ischemia-reperfusion injury by early ischemic preconditioning in rat retina. *Brain Res.* 1015: 154–160.
 34. Sanchez, R.N., Chan, C.K., Garg, S., Kwong, J.M., Wong, M.J., Sadun, A.A., and Lam, T.T. 2003. Interleukin-6 in retinal ischemia reperfusion injury in rats. *Invest. Ophthalmol. Vis. Sci.* 44: 4006–4011.
 35. Sasaki, M. and Joh, T. 2007. Oxidative stress and ischemia-reperfusion injury in gastrointestinal tract and antioxidant, protective agents. *J. Clin. Biochem. Nutr.* 40: 1–12.
 36. Schwartz, B., Takamoto, T., and Nagin, P. 1991. Photogrammetry and Image Analysis of Optic Disc, Retinal Nerve Fiber Layer and Retinal Circulation in Glaucoma, Saunders, Philadelphia.
 37. Sugawara, T. and Chan, P.H. 2003. Reactive oxygen radicals and pathogenesis of neuronal death after cerebral ischemia. *Antioxid. Redox Signal.* 5: 597–607.
 38. Szabo, M.E., Droy-Lefaix, M.T., Doly, M., Carré, C., and Braquet, P. 1991. Ischemia and reperfusion induced histologic changes in the rat retina. Demonstration of a free radical-mediated mechanism. *Invest. Ophthalmol. Vis. Sci.* 32: 1471–1478.
 39. Warner, D., Sheng, H., and Batinic-Haberle, I. 2004. Oxidants, antioxidants and the ischemic brain. *J. Exp. Biol.* 207: 3221–3231.
 40. Vorwerk, C.K., Lipton, S.A., Zurakowski, D., Hyman, B.T., Sabel, B.A., and Dreyer, E.B. 1996. Chronic low-dose glutamate is toxic to retinal ganglion cells. Toxicity blocked by memantine. *Invest. Ophthalmol. Vis. Sci.* 37: 1618–1624.
 41. Yoneda, S., Tanihara, H., Kido, N., Honda, Y., Goto, W., Hara, H., and Miyawaki, N. 2001. Interleukin-1 β mediates ischemic injury in the rat retina. *Exp. Eye Res.* 73: 661–667.
 42. Zeevalk, G.D. and Nicklas, W.J. 1990. Chemically induced hypoglycemia and anoxia: relationship to glutamate receptor mediated toxicity in retina. *Pharmacol. Exp. Therapeut.* 1253: 1285–1292.
 43. Zheng, L., Gong, B., Hatala, D.A., and Kern, T.S. 2007. Retinal ischemia and reperfusion causes capillary degeneration: similarities to diabetes. *Invest. Ophthalmol. Vis. Sci.* 48: 361–367.