

Histopathologic examination of the retina following intra-vitreous propranolol administration in rabbits

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Abstract Propranolol is a nonselective, beta-adrenergic receptor blocker with anti-angiogenic and anti-inflammatory effects that could be used in the treatment and control of many vascular disorder-related retinopathies. In this pilot study, the effects and complications of an intra-vitreous injection of propranolol HCl on the rabbit retina were investigated by clinical and histopathological means as the first step to determination the viability of intra-vitreous propranolol administration. Group 1 ($n=8$) had 15 μg of propranolol HCL given, via intra-vitreous injection, into the right eye. Group 2 ($n=8$) had a matching volume of balanced saline solution also via intra-vitreous injection into the right eye. After 4 weeks, all rabbits were killed humanely and the right eyes from both groups enucleated for histopathological study. Clinical observations and histopathological findings suggest no difference between propranolol and control groups ($P \geq 0.05$). As a result of handling, in some cases, the eyes had light inflammation, hyperaemia and oedema with acute degeneration changes. This study shows that 15 μg of propranolol does not cause any significant abnormalities and is not a toxic dose for intra-vitreous injection in rabbits.

Keywords Propranolol · Histopathology · Retina · Rabbits

Introduction

Propranolol, a nonselective, beta-adrenergic receptor blocking agent, has been used in the treatment of many cardiovascular diseases, such as arterial hypertension, coronary artery disease and heart failure (Degoute 2007; Priviero et al. 2007; Igarashi et al. 2006) and is also used in the treatment of non-cardiovascular disease like severe migraine attacks, some tumours and inflammatory disease (Hoffmann and Reuter 2007; Benish et al. 2008; Kato et al. 2009; Tang et al. 2008). Anti-vascularization and vasoconstriction effects of propranolol were incidentally observed on eyelid haemangioma growth in children treated for heart problems (Léauté-Labrèze et al. 2008). Decreased serum levels of vascular endothelial growth factor (VEGF) following treatment with propranolol in haemangioma have also been observed (Zhao et al. 2011). Beta-adrenergic receptor (AR) blockade with propranolol can lead to downregulation of pro-angiogenic factors like VEGF, reduction of retinal haemorrhage and tufts and interfere with retinal neovascularization in retinopathy of prematurity (ROP) (Filippi et al. 2010). Propranolol also decreased angiogenic factors in leukemic T and monocyte cells on in vitro study (Hajighasemi and Hajighasemi 2009). Due to these properties, propranolol could be an alternative to previous treatment such as cryotherapy, laser therapy and intra-vitreous injections of anti-VEGF drugs (like bevacizumab) to treat neovascularization of retinopathies (Dobson et al. 2006; Kivlin et al. 1996; Early Treatment For Retinopathy Of Prematurity Cooperative Group 2003). Propranolol, compared with previous treatments, is more accessible, cheaper, well tolerated and with fewer side effects (Robinson et al. 2001; Penn et al. 2008).

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Fig. 1 Retina for controlled eyes, detachment because of autolysis; GFAP staining ($\times 10$)

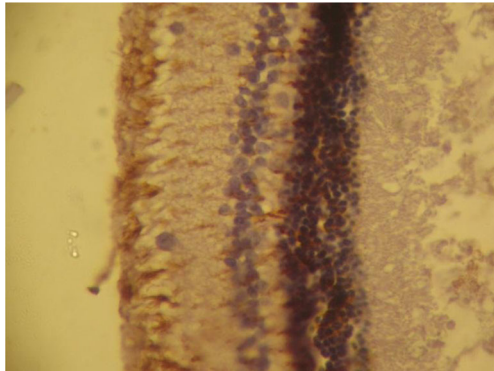
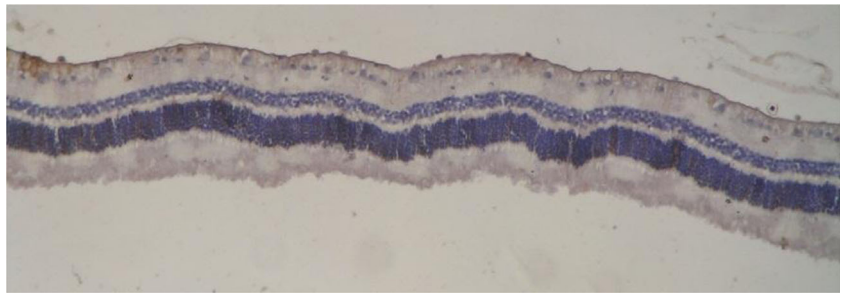


Fig. 2 Retina for controlled group; ganglion and glial cells (Müller cells) had a mild reaction; GFAP staining ($\times 40$)

In this study, we have attempted to investigate the intraocular effects of propranolol following intra-vitreous injection of propranolol HCL; this investigation could be the first step to find the optimum intra-vitreous dosage of propranolol, and we investigated the safety of this route of propranolol administration by ocular examination and histopathology.

Method and materials

Sixteen female New Zealand white rabbits of similar same age and weight were obtained from the Pasteur Institute of Iran.

The rabbits were allocated in to two groups and individually housed, with equal feeding and access to water. Group 1 had 15 μg of propranolol HCL administered intra-vitreally into the right eye (propranolol group) ($n=8$) and group 2 (control group) ($n=8$) received a volume of 15 μl BSS by the same approach. Before intra-vitreous injections, all rabbits were anaesthetized using ketamine hydrochloride (25 mg/kg) and xylazine hydrochloride (2 mg/kg) intramuscularly. All of the intra-vitreous injections were performed in the right eye, using a sterile syringe, with 31-gauge needle (Hamilton syringe), approximately 4 to 5 mm posterior to the limbus in the pars plana area according to the standard model.

Ocular examinations were done before and after injection, and at days 7, 14, 21 and 28. Clinical observation included fundus and slitlamp examinations. After 4 weeks, all animals were anaesthetized and killed humanely by intracardiac injection of 50 % magnesium sulfate. The right eyes of both groups were enucleated and fixed with 10 % neutral buffered formalin solution for shipment to the laboratory of the eye bank of Iran. The eyes were processed for paraffin embedding, sectioned and stained with haematoxylin and eosin (H&E) and glial fibrillary acidic protein (GFAP) separately. All histopathological findings were scored 0 to 5 for statistical evaluation, 0 indicating no change and 5 severe change. Changes within and between two groups were assessed by statistical analysis (SPSS).

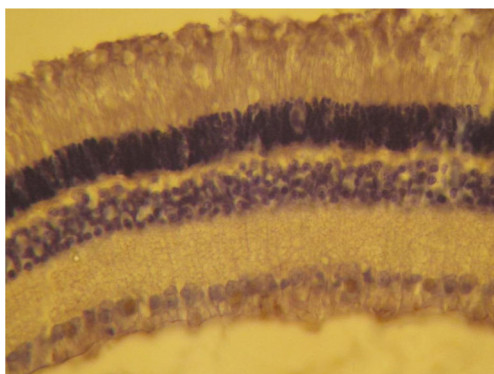


Fig. 3 Retina of control group without any severe abnormalities ($\times 40$)

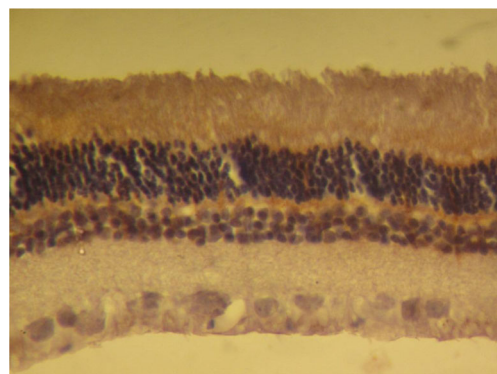


Fig. 4 Mild reactive in the propranolol group, determine in outer plexiform layer and photoreceptor layer. GFAP staining ($\times 40$)

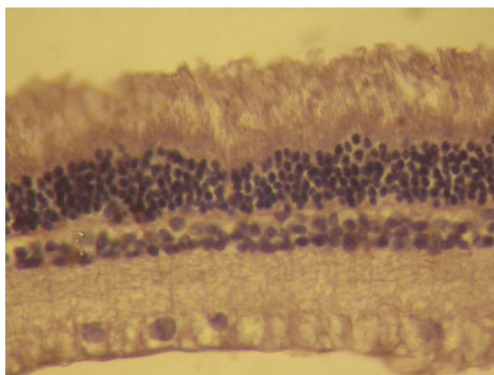


Fig. 5 Normal retina in the propranolol group; GFAP staining (×40)

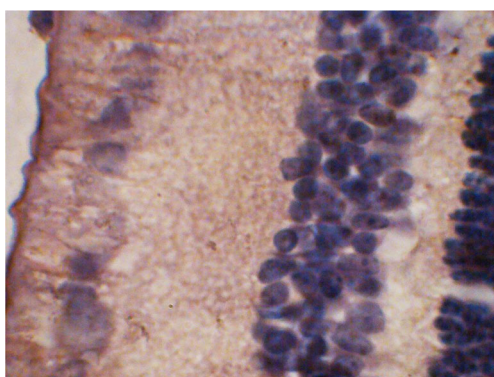


Fig. 6 Retina of control group, ganglion cells had a mild reaction; GFAP staining (×100)

Results

In clinical observations, no flare or cells were seen in the anterior chamber or corneal limbus. Mild local changes in the lens (trace cataracts) were seen in three cases from the control group and two cases from the treated group. Fundus examination revealed no detachment or opacity of the retina. Retinal examination for inflammation or any vitreal haemorrhage was negative. Some cases had mild inflammation, hyperaemia and oedema with no severe degeneration changes for 2 to 3 days after intra-vitreal injection.

Histological findings on H&E staining revealed no inflammation or necrosis in the retinal layers or choroid layer (Fig. 1). There was no intra-vitreal haemorrhage present in any of the eyes examined, and retinal pigment epithelium (RPE) integrity and sensory retinal integrity were preserved in all eyes.

GFAP staining showed mild reaction in the ganglion cells of the retina of three control eyes (Fig. 1) and one treated eye (Fig. 2). Also, the same reaction was seen in other retinal cells like Müller cells and the photoreceptor layer in some sections of one control eye and one propranolol group eye. All of these histopathological findings are mild, and in both groups considered statistically not significant ($P>0/05$) (Figs. 3, 4, 5, 6 and 7).

Discussion

Anti-vascularization and vasoconstriction effects of beta-AR blockers and their effects such as downregulation of pro-angiogenic factors in retinopathies with neovascularization,

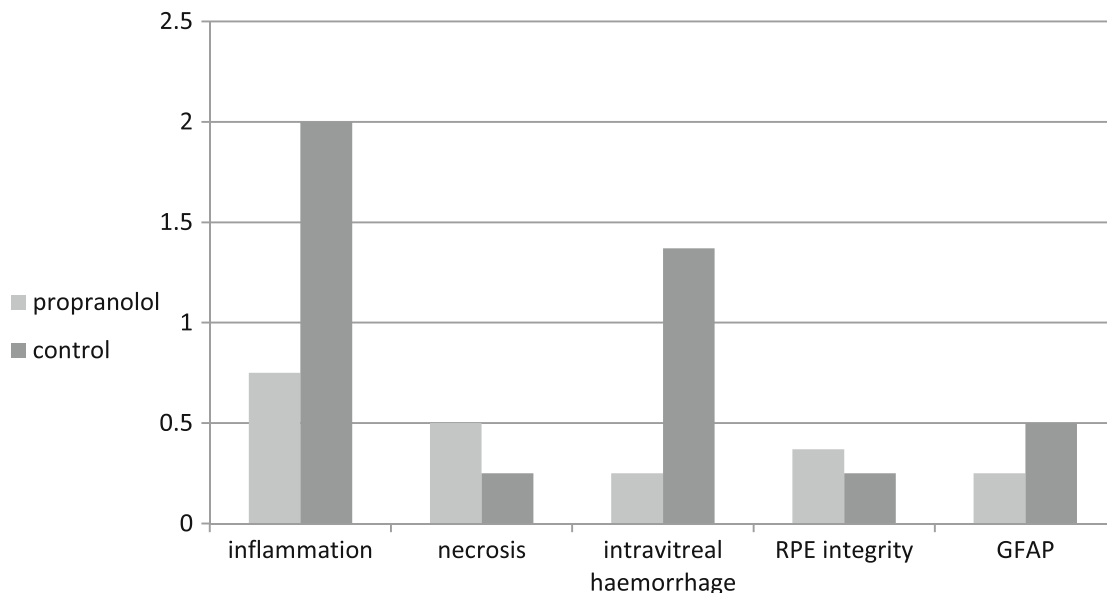


Fig. 7 All of histopathologic findings converted to quantitative data from number 0 to 5 for statistically analysis evaluation. Number 0 shows nothing change and 5 reveals severe change. As observed, all of the changes in both groups were mild and were not significant in statistical analysis ($P>0.05$)

such as ROP and diabetic retinopathy, inspired us to do this investigation (Hajighasemi and Hajighasemi 2009; Filippi et al. 2010). Although some studies indicated propranolol was not effective against the development of retinopathy in mouse model of oxygen-induced retinopathy (OIR) in three routes of delivery (oral gavage, IP injection and SC injection) (Jing et al. 2012), most investigations showed propranolol was effective in angiogenic abnormalities of the retina such as ROP (Filippi et al. 2010; Early Treatment For Retinopathy Of Prematurity Cooperative Group 2003; Ristori et al. 2011; Martini et al. 2011). The objective of this investigation was to check intra-vitreous injection of propranolol as a new route for propranolol administration that could be more effective on the inner layers of the retina without the systemic side effects of this drug that include bronchospasm, heart failure, bradycardia, hypotension and heart block (Frishman 1988).

These results have shown that propranolol, in this dose, has no complications with respect to the retina and other intraocular tissues. The cataract seen in some eyes of both groups was a side effect of intra-vitreous injection—the large size of lens in a rabbit's eye can be damaged through intra-vitreous injections.

Toxic complications of propranolol will occur at serum levels above 2000 ng/kg, and we chose 15,000 ng/kg in this investigation for the intra-vitreous injection. According to the results, this dose of propranolol hydrochloride was not toxic for this route of administration, and further study should be done to check higher doses.

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