PRIMARY GLAUCOMA AS NEURODEGENERATIVE PATHOLOGY: NOVEL OPTIONS OF TREATMENT

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Glaucoma is a chronic disease that causes vision loss through the degeneration of retinal ganglion cell (RGC) neurons and their axons in the optic nerve. It is a leading cause of irreversible blindness estimated to affect 79.6 million people worldwide by 2020 [1]. RGCs and the optic nerve, integral parts of the CNS, have demonstrated similar mechanisms of cell death in glaucoma to those of Alzheimer's disease (AD), marking glaucoma as a neurodegenerative disease [2].

The intraocular pressure (IOP) is one of the main risk factor blamed for the pathology. The increased IOP in glaucoma may cause oxidative stress in retinal neurons. Although retinal circulation has an autoregulatory system to maintain a constant oxygen supply to the retina, it might be impaired under increased ocular pressure. A diminish in the retinal blood flow may lead to a kind of ischemic injury to retina, characterized by the formation of toxic reactive oxygen species (ROS) [3].

The ROS formation is a key process in many pathologic conditions. The relationship of glaucomatous optic excavation and retinal oxidation is still under investigation. Growing evidence supports that retinal ganglion cells in glaucomatous eyes are under oxidative stress that is associated with pathogenic mechanisms leading to glaucomatous neurodegeneration. Increased ocular pressure triggers molecular oxidation in the retina [4]. The reasons of increase in ROS in glaucoma were suggested to be due to different mechanisms. The retina is especially susceptible to oxidative stress because of its high consumption of oxygen, its high proportion of polyunsaturated fatty acids, and its exposure to light.

The antioxidant enzymes act as free radical scavengers to cope with oxidation. Therefore, the antioxidative therapy might be of help in glaucomatous neuron degeneration.

Lowering IOP generally slows progression in glaucoma, but does not necessarily stop degeneration and there is a significant proportion of glaucoma patients who despite adequate IOP control continue to lose vision. In glaucoma, therefore, 'neuroprotection' is restricted to mean therapy directed specifically at neurons in the whole visual pathway which is independent of its effects on IOP.

Intervention in the apoptotic cascades may provide therapeutic targets for drugs that could delay or ameliorate RGC loss in these chronic retinal neurodegenerative conditions. As the topical ocular hypotensive treatment is the main option in glaucoma nowadays, the direct neuroprotective effect of hypotensive eye drops is very important.

Evidence is mounting that topical ocular hypotensive exert direct neuroprotective effects in RGCs, both in vitro and in vivo.

The protective effect of beta-blocking medication on the visual fields of glaucoma patients has been studied, but with conflicting results [5]. In vivo experiments using topical and systemic application of β-blockers demonstrated the neuroprotective properties of betaxolol [6]. According to the Low Pressure Glaucoma Treatment Study2 (LoGTS), the α2 agonist brimonidine may have a beneficial effect on visual function independent of IOP lowering [7]. Recent study demonstrated that topical brimonidine decreased MDA and increased GPx and catalase; hence it may act as an antioxidant [8].

Our recent research of antioxidant activity of carboanhydrase inhibitors for topical glaucoma treatment revealed that fixed that they had the advantages over other eye drops, using for glaucoma, due to their high antioxidant activity [9].

Prostaglandin (PG) analogues have been a first line drug for glaucoma treatment, because maximum intraocular pressure reduction is derived by once daily application without any systemic side effect in all kinds of glaucoma types. As well as the other ocular hypotensive drugs, PG analogues have been expected to have an additional effect to protect neurons independent of IOP reduction. Latanoprost showed neuroprotective effect on glutamate-induced

retinal ganglion cell (RGC) death in vitro, and ischemia or axotomy-induced optic neuropathy mimicking glaucoma in animal models [10].

Nowadays fixed combinations are becoming more and more popular in the treatment of glaucoma as well, they are not inferior by their hypotensive activity comparing to PG analogues. However, there are no data in the literature about the antioxidant properties either of PG analogues, or of fixed combinations.

Purpose: to compare the antioxidant activity (AOA) of the most widely used prostaglandins analogs and fixed combinations for topical glaucoma treatment.

Methods: the model of oxidation-induced haemolysis was used to study AOA of travoprost, bimatoprost and latanoprost and fixed combinations (dorzolamide/timolol (Cosopt), dorzolamide/timolol (Dorzopt Plus), brinzolamid/timolol, latanoprost/timolol, brimonidin/timolol, travoprost/timolol and bimatoprost/timolol).

Antioxidant activity (AOA) was measured by a method based on the induced erythrocytes hemolysis. When adding solutions of tretbutyl (TB) and clotrimazole (CT) to a suspension of red blood cells washed from the plasma, their hemolysis is observed. Compounds with antioxidant activity (AO) inhibit hemolysis caused by free radicals (TB radicals). Clotrimazole (CT) has the ability to amplify the intensity of hemolysis. According to the degree of inhibition of free hemolysis it is possible to judge about the AO activity of the test compound. The degree of hemolysis was determined by the change in hemoglobin concentration in the incubation medium. This method in detail has been described by us previously [9].

In this paper we investigated the antioxidant activity of three PG analogues: latanoprost (latanoprost 0.005%, Pfizer), travoprost (Travoprost 0.004%, Alkon) and bimatoprost (Bimatoprost ophthalmic solution 0.03%, Allergan), and the main fixed combinations such as dorzolamid/timolol (Cosopt: Dorzolamide hydrochloride 2% + Timolol maleate 0,5%, MSD Pharmaceuticals), dorzolamid/timolol (Dorzopt Plus: Dorzolamide hydrochloride 2% + Timolol maleate 0,5%, S.C. Rompharm Company S.R.L.), brinzolamid/timolol (Brinzolamid 1% + Timolol maleate 0,5%, Alcon), latanoprost/timolol (Latanoprost 0,005% + Timolol maleate 0,5%, Pfizer), travoprost/timolol (Travoprost 0.004% + Timolol maleate 0,5%, Alkon), brimonidin/timolol (0,2% Brimonidin tartrato + Timolol maleate 0,5%, Allergan) and bimatoprost-timolol (Bimatoprost 0,03%+ Timolol maleate 0,5%, Allergan).

Results have revealed the highest AOA in travoprost (17%). However, it decreased upon adding larger quantities of the drug into the model system. In contrast to this, bimatoprost showed an increase of its AOA in higher concentration. Its AOA peaked at 38% in 120 mcl of bimatoprost. AOA in latanoprost was the lowest. Upon reaching 120 mcl, latanoprost exhibited oxidative properties. The data is represented in Fig.1.

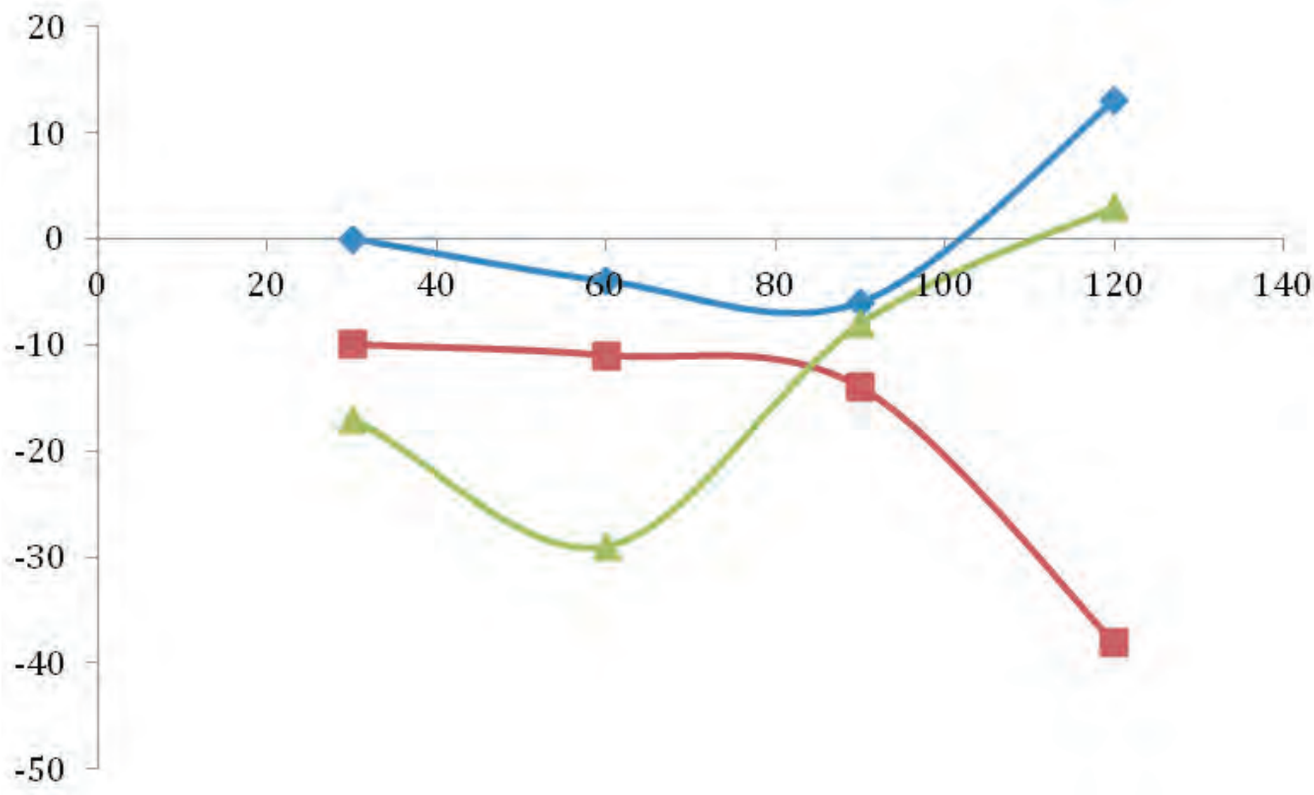

Fig.1. Comparative characteristics of the antioxidant activity of prostaglandins analogues for topical hypotensive treatment of glaucoma

Note: The abscissa shows the volume (mcl) of the PG analogues solution added to the model system (erythrocytes + solution of tretbutyl and clotrimazole). The vertical axis shows the percentage of inhibition of erythrocytes hemolysis when adding the drug into the model system containing the solution of tretbutyl and clotrimazole.

The blue colour shows the curve indicating the antioxidant activity of latanoprost, the red - bimatoprost, and the green - travoprost. It is noticeable that at the minimum of the investigated drug's volume the highest AOA was observed in travoprost; at the maximum volume – in bimatoprost. In volume up to 100 mcl of latanoprost and up to 110 mcl of travoprost these drugs lead to the increased erythrocyte hemolysis.

Figure 2 shows the comparative characteristics of AOA fixed combinations in the study of different volumes of drugs added to the model system. The highest antioxidant activity was recorded in the combination of dorzolamide/timolol (Cosopt). It exceeded the AOA of other fixed combinations when analyzing all the investigated volumes. It was increasing with the increase of the amount of the drug added to the model system (from 30 mcl to 60 mcl to 90 mcl), and accounted for 40 %, 52 % and 75%, respectively. It is noteworthy that the AOA of Cosopt twice exceeded the AOA of its analog - Dorzop Plus (Figure 2).

According to its antioxidant characteristics another drug is approaching to those fixed combinations, also containing a carbonic anhydrase inhibitor (brinzolamide/timolol), the AOA of which was 16%, 42% and 54%, respectively, when the volume of the test drug was 30, 60 and 90 mcl. Among the fixed combinations with prostaglandins, latanoprost/timolol took the lead regarding its antioxidant properties (Fig. 1).

Discussion

In this study we compared the main medications for glaucoma treatment for their novel properties, concerning their antioxidant activity. The results showed that among PG analogues the highest antioxidant activity at the minimum doses of the studied drugs was characteristic for travoprost. This activity increased and reached the highest value at a dose of 60 mcl of travoprost, and further increasing the added amount of the drug decreased its AOA. A different pattern was observed with respect to latanoprost. This PG analogue showed the lowest antioxidant activity and behaved as an oxidant, increasing hemolysis, when was tested with 120 mcl of the drug Among all the tested PG analogues only bimatoprost showed direct dose-dependent antioxidant activity: AOA of the drug increased with its volume under the study.

Takano N., et al. have recently demonstrated protective effects of bimatoprost on RGS in vitro, using the model of oxidative stress. Interestingly, this effect was dose-dependent: the greater the concentration of bimatoprost was added to the model system, which reproduced the reaction of free radical oxidation, the greater the surviving retinal ganglion cells were observed [11]. These results are consistent with those that we found in the model system in vitro, where oxidative stress led to hemolysis, and this hemolysis was inhibited by bimatoprost in dose-dependent manner. It has been postulated that PG analogues may have a potential to exert

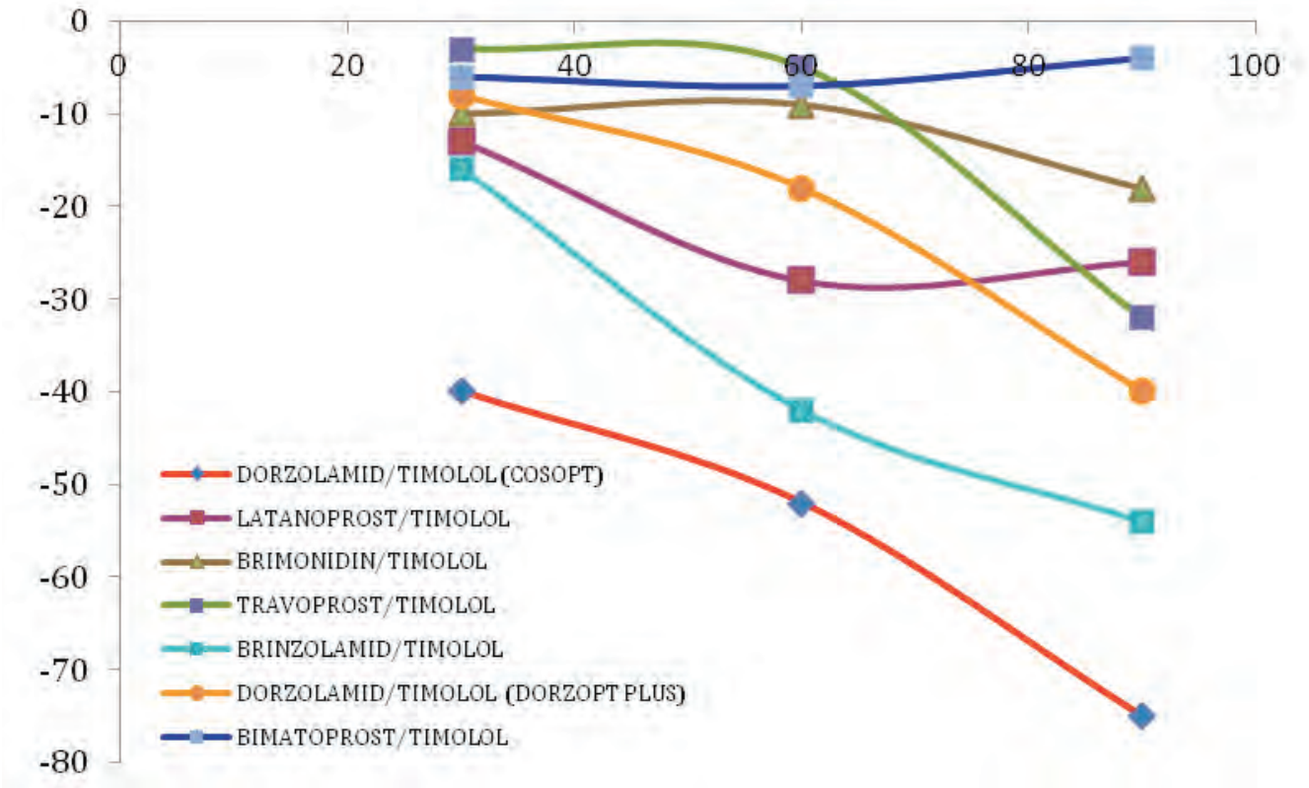

Fig.2. Comparative characteristics of the antioxidant activity of fixed combinations for topical hypotensive glaucoma treatment

Note: The abscissa shows the volume (mcl) of the antiglaucoma fixed combination added to the model system (erythrocytes + solution of tretbutyl and clotrimazole). The vertical axis shows the percentage of inhibition of erythrocytes hemolysis when adding the drug into the model system containing the solution of tretbutyl and clotrimazole.

neuroprotective effect by local administration. In some of the recent studies PG analogues indicated IOP independent neuroprotective effect on glutamate- and hypoxia-induced RGC death at clinically available intracameral concentration [12].

Recent studies by N.Osborne have shown an antioxidant effect of latanoprost: it was found that the drug promoted the development of the endogenous antioxidant glutathione [13].

The neuroprotective effect of PG analogues may be derived from the other mechanisms unrelated to the FP receptor stimulation. Many papers suggest that prostanoid EP2 is neuroprotective [14, 15]. Additionally, cyclooxygenase 2 (Cox2), one of the key enzymes to produce intrinsic PG by FP receptor stimulation, was reportedly neuroprotective [16].

In the course of the present study, for the first time PG analogues were compared in terms of their antioxidant activity, which refers to the neuroprotective properties of drugs.

Antioxidant protection plays a key role in oxidative stress conditions. Probably, with different neurodegenerative diseases, the activity of antioxidant defense molecules, that normally counteract the harmful effects of ROS, reduced. For example, the antioxidant enzymes superoxide dismutase (SOD), catalase, glutathione peroxidase (GSHPx) - and glutathione reductase (GSHRd) have reduced activity in the affected areas of the brain in Alzheimer's disease and in the retina in glaucoma. For the first time we showed a decrease of the antioxidant protection of aqueous humor in glaucoma in 1996[17].

Application in the present study a model system with erythrocytes, hemolysis of which was caused by an oxidative stress, let us with a certain probability judge about the antioxidant activity of the tested PG analogs and fixed combinations. The results showed that antiglaucoma fixed combinations exhibited the highest antioxidant activity, containing carbonic anhydrase inhibitors (ICA). At the same time, the highest antioxidant activity was found in combination of dorzopt/timolol (drug Cosopt). This coincided with the data of the high antioxidant activity of dorzolamide obtained by us earlier [9].

Recent studies have shown that dorzolamide and timolol were able to protect mitochondrial DNA trabecular endothelial cells from oxidative stress [18], and with the combination of these drugs the antioxidant effect was amplified, which prevented trabecular from the damaging effect of hydrogen peroxide [19]. It should be emphasized that the explicit antioxidant properties of dorzolamide were particularly evident in relation to intact mitochondria trabecular endothelium, when the trabecular tissue was at least partially functioning. This substantiates the early administration of the dorzolamid, which may be the most effective in the initial stage of glaucoma. Clinical observations by V.Zanon-Moreno et al. about the high antioxidant activity of dorzolamide confirm this conclusion [20]. Attention should be paid to the recent findings about the ability of a fixed combination of dorzolamide with timolol to enhance the perfusion of the optic disk and retina [21].

Conclusion. Antioxidant activity of hypotensive drugs for glaucoma treatment is their important new features that are associated with the direct neuroprotective effect. The most commonly used drugs at the present day are PG analogues and fixed combinations. They demonstrate a different antioxidant activity. Dorzolamide/ timolol fixed combination has potential advantages over all other studied drugs, including fixed combinations and PG analogues, due to its highest antioxidant activity. This data should be taken into consideration as a novel option in glaucoma treatment.

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