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Genomic and post-genomic effects of anti-glaucoma drugs preservatives in trabecular meshwork

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ABSTRACT

Oxidative stress plays an important role in glaucoma. Some preservatives of anti-glaucoma drugs, commonly used in glaucoma therapy, can prevent or induce oxidative stress in the trabecular meshwork. The aim of this study is to evaluate cellular and molecular damage induced in trabecular meshwork by preservatives contained in anti-glaucoma drugs. Cell viability (MTT test), DNA fragmentation (Comet test), oxidative DNA damage (8-oxo-dG), and gene expression (cDNA microarray) have been evaluated in trabecular meshwork specimens and in human trabecular meshwork cells treated with benzalkonium chloride, polyQuad, purite, and sofzia-like mixture. Moreover, antimicrobial effectiveness and safety of preservative contents in drugs was tested. In *ex vivo* experiments, benzalkonium chloride and polyQuad induced high level of DNA damage in trabecular meshwork specimens, while the effect of purite and sofzia were more attenuated. The level of DNA fragmentation induced by benzalkonium chloride was 2.4-fold higher in subjects older than 50 years than in younger subjects. Benzalkonium chloride, and polyQuad significantly increased oxidative DNA damage as compared to sham-treated specimens. Gene expression was altered by benzalkonium chloride, polyQuad, and purite but not by sofzia. In *in vitro* experiments, benzalkonium chloride and polyQuad dramatically decreased trabecular meshwork cell viability, increased DNA fragmentation, and altered gene expression. A lesser effect was also exerted by purite and sofzia. Genes targeted by these alterations included Fas and effector caspase-3. The efficacy of the preservatives in inhibiting bacterial growth increased the adverse effects in trabecular meshwork in terms of DNA damage and alteration of gene expression. Presented data indicates the delicate balance between efficacy and safety of drug preservatives as not yet optimized.

1. Introduction

Our previous study demonstrated that oxidative damage occurs at a high level in the trabecular meshwork (TM) of glaucomatous patients [1] and is related to the extent of intra-ocular pressure alteration and visual field damage [2]. Oxidative damage targeting TM in glaucoma is due to the high sensitivity of this tissue to oxidative stress as compared to the other components of the ocular anterior chamber [3]. Evidence has been provided that aqueous humor protein composition undergoes dramatic alterations during glaucoma course mainly oriented toward decrease of antioxidant defenses accompanied by increasing levels of proteins resulting from the damage of TM cells and their intracellular components [4]. Mitochondria damage has a pivotal role as a source of endogenous oxidative stress in the TM of glaucomatous patients [5,6]. These findings underline the importance of oxidative damage as occurring in the TM for glaucoma pathogenesis. Accordingly, it is of interest to evaluate whether or not anti-glaucoma drugs can affect the oxidative damage occurring in the ocular anterior chamber. It has been reported that treatment of mammalian cells with common drug preservatives may result in the occurrence of severe oxidative DNA damage and toxicity [7]. A drug commonly used in glaucoma therapy, *i.e.* timolol, displays antioxidant effects protecting endothelial cells from oxidative stress [8]. This

Abbreviations: TM, trabecular meshwork; HTM, human trabecular meshwork; BKA, Benzalkonium chloride; PLYQ, polyquad; SFZ, sofzia like mixture; PRT, purite; 8-oxo-dG, 8-hydroxy-2′-deoxyguanosine.

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mechanism was confirmed in trabecular meshwork cells and demonstrated for other anti-glaucomatous drugs, *i.e.* dorzolamide. These experiments, performed in TM specimens collected *ex vivo* from human subjects, provided evidence that carbonic anhydrase inhibitors, such as dorzolamide, maximize their anti-oxidant effect whenever TM still bear preserved mitochondria function, while the protective effect decreases in parallel with the mitochondrial dysfunction. Accordingly, it has been suggested that dorzolamide should be used as a first-line drug in glaucoma therapy in patients still having a relatively preserved TM [5].

This scientific background indicates that some drugs, commonly used in glaucoma therapy, can protect or induce oxidative stress in the TM and represent an important issue to be further explored. Indeed, although experimental data are available for timolol and dorzolamide, some important issues remains to be clarified. In particular, the contribution of drug preservative to the oxidative stress should be analyzed. Moreover, some ocular drug preservatives added for antiseptic purposes could be able to induce detrimental effects on living cells, also including oxidative damage.

The present study is addressed to answer the question whether the most commonly used preservatives for anti-glaucoma drugs (*i.e.*, Benzalkonium chloride, Sofzia, PolyQuad, and Purite) cause oxidative stress. Although previous studies demonstrated that benzalkonium chloride and polyQuad have deleterious effects on trabecular meshwork [9], the molecular mechanisms involved have not been investigated. Accordingly, our study was aimed at evaluating molecular mechanisms contributing to TM cell alterations induced by drug preservatives analyzing transcriptome. These endpoints have been tested both *in vitro* in human trabecular meshwork (TM) cell lines and *ex vivo* in TM biopsies collected from corneal donors.

The following analyses have been performed: *in vitro* treatment of HTM cells with drug preservatives; cell viability by MTT test in HTM cells; DNA damage (8-oxo-dG; Comet test), gene expression analysis by microarray in both HTM cells and TM biopsy. Moreover, to evaluate antimicrobial effectiveness of preservative contents in drugs, the comparative antibacterial potency was tested. Indeed the lack of adverse effects on HTM cells could be due to the safety of an effective preservative or to the lack of biological activity of an un-effective anti-bacterial preservative.

Accordingly, the main goal of the present study is the comparative evaluation of the effect of preservatives commonly used in anti-glaucoma drugs on oxidative stress and cell homeostasis in TM. Because oxidative stress plays an important pathogenic role in glaucoma, it is relevant to identify the most suitable and effective drugs to be used in the different stages of glaucoma course and to explore this issue on a comparative basis among different preservatives.

2. Materials and methods

2.1. Challenge and cell viability of HTM cells treated with drug preservatives

Human TM cells (ScienCell Research Laboratories, CA, USA) were cultured in fibroblast medium in poly-L-lysine coated vials, supplemented with 10% fetal bovine serum, 2 mM L-glutamine, penicillin (100 IU/ml) and incubated at 37 °C in a humidified atmosphere of 5% CO_2. When the monolayer was at 80% confluence, cells were detached by trypsin/EDTA treatment, and the cell suspension was seeded in 96-well culture plates (3×10^3 cells/well). After 24 h the cells were treated with preservatives for 15 min as following: (A) untreated HTM cells (Sham); (B) HTM + BKA (Benzalkonium chloride) at concentration range of 0.04–0.00001%; (C) HTM + PLYQ (Poliquad) at concentration range of 0.01–0.02%; (D) HTM + SFZ (Sofzia) (zinc chloride 0.0025%, boric acid 1%, sorbitol 0.25%,

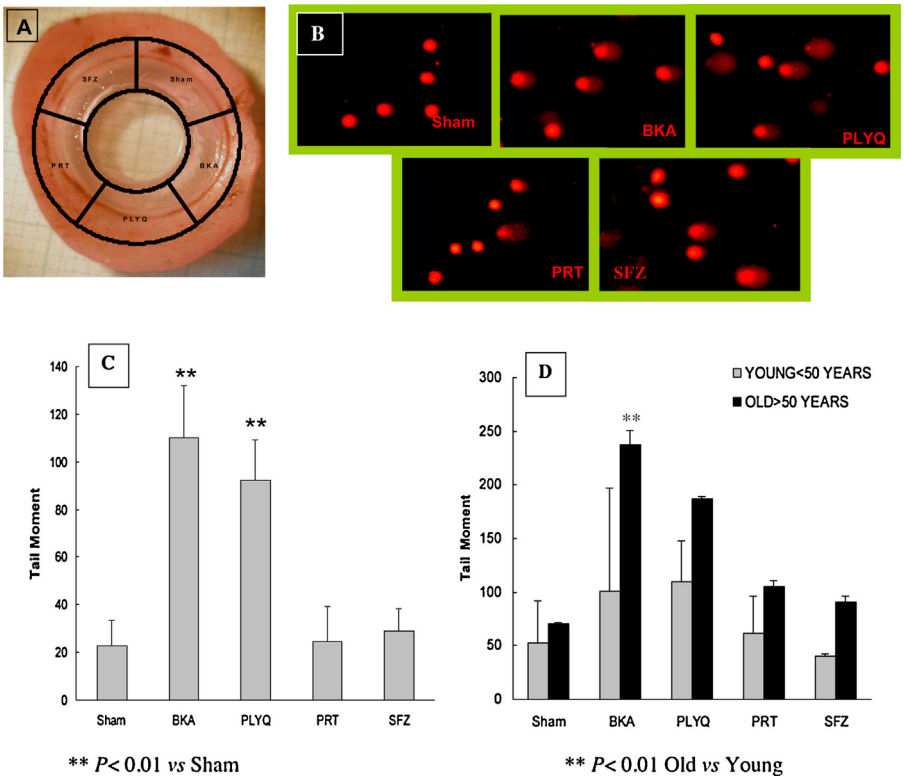

Fig. 1. DNA fragmentation in HTM specimens collected from corneal donors. (A) Specimens divided in five pieces each treated with different drug preservatives. (B) Comet images from TM treated specimens with different drug preservatives. (C) Quantitative data for all specimens (mean ± SD). (D) Data showing different sensitivity of old and young subjects.