

Toxic effects of antiglaucoma drugs on superficial tissues of the eye

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SUMMARY

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Glaucoma is a chronic disease which, if untreated, can lead to blindness. Although its pathogenesis still remains unclear the only management of glaucoma consists in lowering of the elevated intraocular pressure, the main risk factor of the disease has a rational basis. In this paper a current opinion on the toxic effects of the application of ophthalmic drops on the surface components of the eye (conjunctiva, cornea, and the corneal stroma) is presented. Apart from the active components conservatives present in the eyedrops are of prime importance in the induction of cytotoxicity. Effects of BAK and the newly developed conservatives, such as SofZia or SOC, on the cornea are discussed. Although the topical application of antiglaucoma drugs can have severe side effects its usefulness in the treatment of glaucoma is undisputed. A once-a-day application of the preservative-free, BAK-free, long-acting eyedrops should be recommended in almost all of the glaucoma cases.

Key words: topical antiglaucoma therapy, toxic effects, apoptosis, inflammation

Glaucoma is a neuropathy of the optic nerve. It is characterized by the atrophy of the optic disc and the resulting defects in the visual field [1]. It is a slowly progressing disease and, if left untreated, can lead to the total loss of vision [2]. As one of the main causes of blindness worldwide glaucoma constitutes a significant social problem. Unfortunately, pathogenesis of this disorder is still not completely understood and, currently, the only possible treatment is aimed at inhibiting or slowing down its progression. The present day management of glaucoma relies on elimination of its main risk factor which is a high intraocular pressure (IOP) [2,3]. This could be achieved either by a topical use of antiglaucoma

STRESZCZENIE

Toksyczny wpływ leków przeciwjaskrowych na powierzchowne tkanki oka

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Jaskra jest przewlekłą chorobą, która nieleczona prowadzi do utraty wzroku. Chociaż jej patogenezę jest nadal niewyjaśniona, jedynym sposobem leczenia tego schorzenia jest obniżanie podwyższonego ciśnienia wewnątrzgałkowego – głównego czynnika ryzyka dla choroby. Obecna praca przedstawia aktualne poglądy na toksyczne efekty podawania kropli okulistycznych na tkanki powierzchni oka (spojówka, rogówka). Oprócz aktywnych składników kropli dodawane do preparatów konserwanty mają największe znaczenie w indukcji cytotoksyczności. Wpływ BAK oraz nowo opracowanych konserwantów, takich jak SofZia oraz SOC, na rogówkę i spojówkę został również przedyskutowany. Chociaż miejscowe podawanie przeciwjaskrowych leków może prowadzić do powstania poważnych objawów ubocznych, ich użyteczność w leczeniu jaskry nie podlega dyskusji. Godnym polecenia u większości pacjentów z jaskrą jest stosowanie kropli raz dziennie, kropli bez BAK, czy innych konserwantów, jak również kropli długodziałających.

Słowa kluczowe: miejscowe leczenie, działania toksyczne, apoptoza, zapalenie

drugs or by a laser and/or incision surgery [4]. The topically applied antiglaucoma drugs have to cross the cornea and conjunctiva to reach their sites of action. This is why epithelial cells of the eye's front are the first tissues to be exposed [5]. A medical intervention associated with a potential adverse outcome is defined by the WHO as "any noxious, unintended, and undesired effect of a drug, which occurs at doses used in humans for prophylaxis, diagnosis, or therapy" [6]. This outcome can be allergic or toxic. Because glaucoma is a chronic disease the topical treatment usually has to be continued for a long time and adverse effects thereof may cause serious problems.

Nowadays, the most commonly used medications in the topical antiglaucoma therapy include prostaglandin derivatives and prostamides (latanoprost, travoprost, bimatoprost, tafluprost, unoproston), β -blockers (timolol, betaxolol, carteolol, metipranolol, pindolol), adrenergic agonists (non-selective dipivefrin, epinephrine and selective- brimonidine, apraclonidine, clonidine), carbonic anhydrase inhibitors (dorzolamid and brinzolamid), and parasympathomimetics (pilocarpine, carbachol) [7]. In order for the IOP-lowering drugs to be active they must penetrate the superficial tissues of the eye (i.e., conjunctiva, sclera, and cornea) to reach their therapeutic targets [2]. Adverse (toxic) reactions of the topical antiglaucoma medication may range from minimal to very severe. Inflammation is the first sign of a toxic bolus to the conjunctiva. This inflammation can be papillary, with generalized injection, or follicular, caused by proliferation of lymphocytes and plasma cells. The mucopurulent discharge may be present. A cytotoxic effect of a drug causes damage to and death of the conjunctival and corneal epithelial cells. In the cornea punctate epithelial erosions are observed which may later become confluent and deeper. The cornea may become opaque and neovascularised. These reactions are nearly always accompanied by acute or chronic inflammatory responses leading to keratinization and scarring of the conjunctiva with the formation of symblepharons called the drug-induced pseudophthalmos [8].

The strength of toxicity and the effect of antiglaucoma drugs on a given tissue may vary. *Cheong* et al. using human corneal and retinal epithelial cell lines investigated the in vitro cytotoxicity elicited by eight different β -blockers. These authors observed that the cytotoxic effect of these compounds occurred very rapidly, but large differences in the effects on the same cell line were noted between different, although similar in structure, β -blockers. In order to find any tissue-specific differences in susceptibility, cytotoxicity of these compounds was also tested on human skin fibroblasts and keratinocytes. It appeared that each drug was equally cytotoxic for cells from different tissues [5]. *Stewart* et al. didn't find any differences between the punctate corneal epitheliopathy induced by different prostaglandin derivatives [9]. The next part of the cornea to be crossed by drugs applied to the surface of the eye is the corneal stroma with the not very abundant keratocytes which play a crucial role in the production of the collagen fibrils and other matrix substances during embryogenesis and healing of the corneal wounds. Hence, the undisturbed proliferation and function of these cells is critical for sustaining normal corneal physiology. *Wu* et al. reported that antiglaucoma drugs such as dif-

ferent β -blockers, dipivefrin, and pilocarpine inhibited proliferation of cultured human keratocytes [10]. This could explain the impediment in regeneration of and abnormalities in the corneal stroma after a prolonged antiglaucoma therapy. Also, activation of certain stromal enzymes may contribute to alterations in the corneal physiology. It has been established that prostaglandin analogs decrease the central corneal thickness (CCT) [11], probably through activation of the matrix metalloproteinases (MMPs) and reduction of the synthesis of different collagen types. Changes in the normal corneal architecture are probably responsible for this phenomenon [12, 13]. The endothelial layer is the last corneal part to be crossed by drugs penetrating to the anterior chamber of the eye. It plays a crucial role in maintaining transparency of the cornea, by transferring the aqueous humour entering the stroma back to the anterior chamber. Disturbances in either the function or the number of endothelial cells may make the cornea opaque and edematous. In the study of *Inoue* et al. topical application of 1% dorzolamide increased CCT, but did not affect the morphology of the corneal endothelium [14]. Dorzolamide inhibits carbonic anhydrase, an enzyme present in both the ciliary body and the corneal endothelium that leads to elevation of CCT by reducing the endothelial pumping function and increasing thereby the corneal stroma water content [14]. However, loss of the corneal endothelial cells was observed during prolonged topical administrations of epinephrine, timolol, betaxolol, and dorzolamid [15, 16]. *Wu* et al. demonstrated that betaxolol, brimonidine, dorzolamide, dipivefrin, latanoprost, and unoprostone induced cytotoxicity in bovine corneal endothelial cultured cells killed bovine corneal endothelial cells in culture, as assessed by the lactate dehydrogenase (LDH)-release assay [17]. All antiglaucoma drugs may affect the corneal endothelial cell function through alteration of the Ca^{2+} mobility [18]. Corneal swelling in the absence of extracellular calcium is probably due to the induction of morphologic changes in the endothelium, such as rounding of the cells, loss of their apical junctions, and increased membrane permeability for calcium [18, 19].

Chronic exposure of the eye to the antiglaucoma drugs leads to increase in the numbers of macrophages, lymphocytes, and fibroblasts in the conjunctiva and the Tenon's capsule as well as to decrease in the number of the conjunctival goblet cells [20-22]. In the tear samples obtained from patients undergoing a chronic glaucoma therapy the levels of the proinflammatory cytokines (IL-1, IL-6, IL-12, and TNF) are significantly increased in the conjunctiva. Such an upregulated cytokine production by the conjunctival cells occurs in response to toxic stimuli and

reflects the inflammatory activation of these cells [23]. Unfortunately, such inflammatory changes create a potential risk of a potential glaucoma filtration surgery failure [24-26]. Biopsies taken from patients treated with the topical antiglaucoma drugs showed enhanced apoptosis among the conjunctival epithelial cells, as measured by the TUNEL method, compared with the specimens taken from healthy subjects [27]. Chronic topical treatment with the IOP-lowering medications induces a marked squamous metaplasia which can also be tracked down by investigating the conjunctival impression cytology specimens [28].

With respect to the toxic effects of topical drugs on the ocular surface, evaluation of the contribution of each component of the ophthalmic drops (active substance, vehicle, or preservatives) is important. The role of preservatives in the ophthalmic solutions is to suppress microbial growth and prevent decomposition of the active drug in the container [29]. Adverse effects of the preservative-containing topical antiglaucoma medications on the ocular surface is well documented [30]. Benzalkonium chloride (BAK), a cationic detergent used at concentrations ranging from 0.004 to 0.02%, is the most common preservative of the eye drops [2, 29]. BAK has a high affinity to membrane proteins and, by changing the ionic resistance of the cell membranes, acts as a detergent. It also causes protein denaturation and disruption of the cytoplasmic membranes [29]. When exposed to BAK the corneal epithelium, the main barrier of intraocular penetration, is impaired and the corneal permeability increases. Toxicity of BAK for the corneal and conjunctival epithelium is probably related to its pro-oxidative activity [31]. It may also induce immuno-allergic reactions [2, 32]. Its biological half-time in the corneal and conjunctival epithelial cells is relatively long. Moreover, BAK can accumulate and for a long time persist in the ocular tissues. Its cytotoxic effects are dose-dependent [2, 33, 34]. This cytotoxicity is responsible for its pro-apoptotic and necrotic effects [35]. *De Saint et al.* [34] demonstrated that after addition of the 0.01% to 0.05% solution of BAK to a human conjunctival cell line all the cells died within 24 hours showing characteristic features of apoptosis, such as chromatin condensation, DNA fragmentation, reduction in the cell volume, and expression of the apoptotic marker Apo 2.7. Concentrations of BAK less than 0.005% induced cell death in a dose-dependent manner [34, 36]. There are a lot of other studies showing that the long-term use of the BAK-preserved ophthalmic solutions induces indirect and direct toxic effects to the ocular surface, including the reduced cellular viability, infiltration of the conjunctival stroma, and overexpression of the inflammation- or apoptosis-related molecules such as HLA-DR, ICAM-1, Fas (CD45), and Apo2.7

[35, 37-41]. Similar results were revealed by exploration of the impression cytology specimens obtained from patients with glaucoma. Moreover, harmful effects of the above described phenomena on the morphologic and functional statuses of the conjunctiva were confirmed by the decreased expression of MUC5AC on the conjunctival cells present in the specimens [41]. This observations explains the mucin deficiency and the development of dry eyes in patients undergoing a prolonged antiglaucoma therapy. *Guenoun et al.* assessed expression on the conjunctival epithelium of the inflammation- and immune-related markers, such as the intercellular adhesion molecule-1 (ICAM-1; CD54), the platelet-endothelial cell adhesion molecule-1 (PECAM-1; CD31), and HLA-DR, after the contact of these cells with latanoprost, travoprost, bimatoprost, and BAK solutions. These authors noted that the cytotoxic effects of prostaglandins were proportional to the concentrations of BAK in the solutions. The bimatoprost eyedrops containing the lowest concentration of BAK were less toxic than latanoprost and travoprost [43]. Interestingly, the preserved latanoprost and travoprost exhibited less pronounced pro-apoptotic effects than their respective preservative formulations [42]. Similarly, the latanoprost eyedrops induced in vivo fewer HLA-DRs than the preserved, topically applied β -blockers, despite the higher BAK content of the former drug and its lower toxicity compared to the same concentration of BAK. These results suggest that prostaglandins exert a protective effect against the cytotoxicity of BAK [41-43]. The BAK-preserved antiglaucoma drugs may also induce apoptosis of the trabecular meshwork cells and affect either the outflow of the aqueous humour or the site of action of the antiglaucoma drugs [44, 45].

To minimize these side-effects of the long-term therapy with the IOP-lowering drugs solutions containing less or no BAK or with alternative preservatives have been introduced into the topical glaucoma therapy [2]. Compared with the preserved latanoprost, travoprost, and bimatoprost the preservative-free solution of tafluprost (PF tafluprost) showed reduced toxicity and very low pro-apoptotic or pro-oxidative activities in the human conjunctival epithelial cell lines *in vitro* [46]. *Liang et al.* assessed the *in vivo* reactions of the conjunctiva and cornea to PF tafluprost, the commercially available latanoprost, and the 0.02% benzalkonium chloride. As demonstrated by the *in vivo* confocal microscopy, partial desquamation of epithelial cells, irregular cell shapes, anisocytosis, loss of cell borders, abnormal reflectivity patterns, cell swelling, and inflammatory infiltrations were present in the cornea of the rabbit eyes treated with latanoprost and the BAK-solution. In contrast, the

corneal epithelium of rabbits treated with PF tafluprost had a regular polygonal mosaic appearance, the brightly reflective nuclei, and no obvious signs of desquamation or swelling. Only after the administration of BAK a slight inflammatory infiltration in the anterior corneal stroma could be detected. Apparent inflammatory infiltrations in the peripheral cornea and the limbus area were noted after the exposure to latanoprost or BAK, but not after the exposure to PF tafluprost. Likewise, after the application of BAK or latanoprost the conjunctival stroma vessels were infiltrated by inflammatory cells. In contrast, after the application of PF tafluprost no signs of inflammation were found in the tested blood vessels which looked virtually normal. As demonstrated by the conjunctival impression cytology a significant inflammatory infiltration and abundant inflammatory cell patches were present after application of latanoprost and BAK, respectively, to the rabbit eyes. Moreover, more CD45+ cells were present in specimens obtained from the eyes exposed to latanoprost and BAK than in those obtained from the eyes treated with PF tafluprost. Similarly, expression of TNF was highest in the cells originating from the eyes exposed to BAK or latanoprost. Also, few as opposed to numerous, TUNEL+ cells were observed after the application of PF tafluprost and latanoprost as opposed to the administration of BAK, respectively. Interestingly, latanoprost appeared to be less toxic than the 0.02% solution of BAK alone [47]. This latter observation supports the view that analogues of prostaglandins may protect against the BAK-induced injury [41-43].

One of the novel preservatives introduced to topical medications is Purite, a stabilized oxychloro complex (SOC) consisting of 99.5% chlorate, 5% chlorite, and trace amounts of chlorine dioxide. SOC has been shown to possess antimicrobial properties including the fungicidal, viricidal, and bactericidal activities. This compound oxidizes unsaturated lipids and glutathione in the cell [29]. In the *in vivo* studies brimonidine Purite produced significantly less pronounced corneal damage (loss of microvilli, cell membrane wrinkling, increase in the number of epithelial holes, cell peeling, loss of hexagonal shape, retraction of cell borders) and conjunctival inflammatory response (lymphocytic infiltration) than drugs containing BAK [29]. Other new preservation formulas include polquaternium-1 (PQ-1, Polyquad), an antimicrobial quaternary ammonium compound, and the SofZia preservative system [2]. SofZia (travoprost Z, Travatan Z) is a proprietary buffer system made of carefully selected combinations of ions and buffers at a specific concentration providing preservative efficacy [2]. The preservative SofZia is an oxidizing compound containing borate, zinc, and sorbitol [48]. No cytotoxicity was observed in cells exposed *in vitro* to travoprost Z

containing no BAK. The BAK-containing latanoprost and travoprost as well as BAK alone exerted significant cytotoxic (apoptotic) effects on the human conjunctival cells [37]. Similarly, in the *in vivo* studies changes in the cornea and conjunctiva after application of the preserved latanoprost were significantly more pronounced than the respective changes after the application of travoprost Z. There was a significant inflammatory infiltration and fibroblast proliferation in the conjunctiva of the eyes treated with latanoprost. The corneas from these eyes exhibited decreased numbers of the surface microvilli and disruption of the architecture [48]. In yet another study, corneas from the eyes exposed to latanoprost showed increased epithelial permeability and loss of tight junctions, as compared to the corneas exposed to travoprost Z [49].

In conclusion, the present review indicates that, because of serious side-effects of the topical ophthalmic medication, chronic topical treatment of the glaucoma patients may be very difficult. The applied drugs may severely injure superficial tissues of the patients' eyes and may exacerbate any preexisting ocular diseases, such as the dry eye syndrome. A rational decision about the treatment is very important and choices between the one day-dosing drugs, combined formulations, preservative-free, or less toxic medicines should always be considered.

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