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Review article

Ocular tolerance of preservatives and alternatives

Pascal Furrer[a], Joachim M. Mayer[a], Robert Gurny[b,*]

[a]School of Pharmacy, Institute of Medical Chemistry, University of Lausanne, Lausanne, Switzerland
[b]School of Pharmacy, Department of Pharmaceutics and Biopharmaceutics, University of Geneva, Geneva, Switzerland

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Abstract

Eye drops are multiple dosage forms protected against microbial contamination by means of preservatives. However, the ocular tolerance of these chemicals can vary and this may result in adverse toxic or allergic reactions. This overview presents the pharmacopoeial requirements for the preservation of eye drops, the factors affecting ocular tolerance as well as the adverse external ocular effects induced by preservatives. The alternatives to the use of preservatives are also discussed, including the recent progress in eye drops packaging. © 2002 Elsevier Science B.V. All rights reserved.

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1. Introduction

Topical ophthalmic medications sometimes may cause toxic or allergic reactions resulting in iatrogenic ocular disease [1]. Toxic reactions relate to the direct chemical irritation of tissue whereas allergic reactions imply sensitization and induction of ocular inflammatory processes by the patient's immune system [2]. These adverse external ocular effects of ophthalmic therapy are due to the topically applied drug, or the excipients present in the preparation. Preservatives are among the excipients currently used in ophthalmic preparations.

These substances are chemicals that are meant to prevent microbial spoilage of pharmaceutical preparations [3]. The term 'preservative' should be reserved for these substances in order to avoid confusion with the term 'stabilizing agents' that designates chemicals intended to protect against physical–chemical degradation like oxidation. For instance, sodium (meta)bisulfite and ascorbic acid are antioxidants, and hence stabilizing agents.

The addition of preservatives to eye drops, artificial tears, or contact lens solutions aims at destroying microorganisms (bactericidal effect) or at least preventing their growth (bacteriostatic effect) [4,5]. Indeed, this contamination may cause a physicochemical deterioration of the ophthalmic solutions or a risk of (additional) infection for the patient's eye. The infection of the weakened eye can have serious consequences leading even to ocular perforations [6]. In addition to these serious diseases, the deterioration of the contaminated ophthalmic solutions may alter their efficacy, thus vitiating any beneficial effects on the primary disease and compromising the success of the medical treatment [7]. Such a contamination may occur during the preparation of the medications or during their instillation in the patient's eye [8,9]. During manufacture, the main sources of contamination are the raw materials (especially water), the air, the personnel, and the packing material [10]. During their use, the contamination of the dropper tips or even the solutions inside the bottle can result from physical contact with microbe-harboring surfaces like fingers, eyelashes, etc. [11]. This microbial contamination of eye drops is a significant risk factor of several complications like bacterial keratitis.

Historically it was not until the middle of the 1960s when severe eye injuries were detected in Sweden, caused by eye ointments contaminated with *Pseudomonas aeruginosa*, that the use of preservatives in ophthalmic preparations was required by regulatory authorities in Europe and in the USA [10,12].

Ideally, a preservative should provide numerous qualities like broad antimicrobial activity, chemical/thermal stability, compatibility with the container and other compounds present as well as innocuousness towards ocular tissues [13–15]. Unfortunately none of the preservatives has all the required qualities so as to be used universally for any

* Corresponding author. School of Pharmacy, Department of Pharmaceutics and Biopharmaceutics, University of Geneva, 30. quai E.-Ansermet, CH-1211 Geneva 4, Switzerland. Tel.: +41-22-702-61-46; fax: +41-22-702-65-67.

E-mail address: robert.gurny@pharm.unige.ch (R. Gurny).

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ophthalmic preparation. Indeed preservatives that kill or damage growing microbial cells may also be toxic to growing cells of the ocular tissues. When selecting a preservative for an ophthalmic formulation, the following considerations must be taken into account [16]: (a) irritation potential, (b) pH range for maximal antimicrobial activity, (c) compatibility with other ingredients, (d) synergism or antagonism in antimicrobial activity, and (e) processing conditions such as heat or packaging.

This paper reviews ocular cytotoxic or allergic effects caused by preservatives. It focuses on the validity of the use of preservatives in ophthalmic solutions and the risks associated with their use. A discussion of the alternatives to preservatives closes the review.

2. Classification of preservatives

The main ophthalmic preservatives can be classified according to their chemical type. Table 1 gives an overview of these preservatives, their main synonyms and usual concentrations. The antimicrobial spectrum, the incompatibility with other drugs or excipients, the thermostability as well as the optimal pH for the antimicrobial activity and chemical stability are also mentioned in Table 1.

3. Pharmacopoeial requirements for preservation of ophthalmic preparations

Pharmacopoeias prescribe the ophthalmic preparations to be prepared and packaged so that sterility is assured at the time of first use and maintained during use (Table 2). The preservatives are intended to prevent the microbial spoilage of ophthalmic preparations packaged in multidose containers. However, since they may be irritating to the ocular tissues, the use of preservative is not recommended or is even excluded in certain cases: when ophthalmic solutions are used in surgical procedures, when the solutions are prescribed explicitly without preservatives or when the preparations show suitable antimicrobial activity. In these cases, ophthalmic solutions should as far as possible be packaged in unidose containers.

3.1. Pharmacopoeial tests for preservative effect

The tests for the effectiveness of preservatives are intended to show whether the preservative used provides an effective protection of the ophthalmic preparation in its final container against any accidentally introduced contaminant microorganisms. Both United States and European pharmacopoeias prescribe an evaluation of the effectiveness of the preservative against various microbial strains from the four major classes of eye pathogens, namely gram-positive cocci (*Staphylococcus aureus*), gram-negative rods (*P. aeruginosa*), yeasts (*Candida albicans*) and fungi (*Aspergillus niger*).

However, pharmacopoeial requirements do not exclude eye drop contamination by other microorganisms. Indeed, besides *S. aureus*, eye dropper bottles are frequently reported to be contaminated mainly by *Staphylococcus epidermidis* (gram-positive bacteria) and *Pseudomonas* species as well as *Serratia marcescens* (gram-negative bacteria) [23–27]. Gram-negative bacteria are more frequently isolated from contaminated ocular medications than gram-positive microorganisms [28].

4. Ocular tolerance tests for preservatives

Ocular tolerance can be defined as the ability of ocular tissues to bear a given dose of a chemical without showing evidence of intoxication. The assessment of the ocular tolerance of eye drops is not required by pharmacopoeias but by governmental regulatory bodies like the FDA or OECD, which have published guidelines for eye irritation procedures [29,30]. The following methods are used to assess the effects of preservatives on the eye.

4.1. Methods for the appraisal of ocular tolerance

The tests currently used are carried out in three ways: in vivo, in vitro or ex vivo methods [31–34].

In vivo testing is achieved on a living eye of an animal model (generally rabbits and other rodents, or dogs). The main in vivo test is the so-called Draize test, which relies on macroscopic observation and scoring of changes to corneal, iridic and conjunctival tissues consecutive to the instillation of chemicals onto a rabbit eye [35–37]. In order to refine and improve the sensibility of the Draize test, some technical modifications have been proposed like corneal staining with vital dyes, microscopic observations (specular, slit-lamp or confocal microscopes) or accurate techniques like pachymetry and tonometry [38–41]. For obvious ethical reasons, ocular tolerance tests are not carried out on a large scale on the human eye, although the validity of animal testing in predicting eye irritation potency in humans has been criticized [42,43]. However, some clinical trials on healthy volunteers and several medical reports of patients provide useful information on the human ocular tolerance of preservatives. A particular type of in vivo test refers to wound healing studies. These studies imply the follow-up of the corneal regeneration process after mechanical injuries (corneal debridement) or chemical damages (acid/alkaline, *n*-heptanol or iodine-induced corneal erosion). The corneal erosions are generally stained by fluorescein and the eroded area measured planimetrically. These wound healing models enable detection of the influence of preservatives on delaying corneal repair.

Ex vivo testing refers to the fact that the eye is intact and living at the moment of the treatment (wound injury), but has been removed from the animal for the observation – generally made by electron microscopy. This method

Table 1
Overview of current ophthalmic preservatives [3,17–22]

Chemical class	Preservative	Synonym	Usual concentration (%)	Antimicrobial activity[a]	Incompatibility with other chemicals	Optimal pH[b]	Processing conditions, packaging
Quaternary ammoniums	Benzalkonium chloride						
	Alkyldimethylbenzylammonium	0.01–0.02	Y, F, G +, G −	Anionic drugs/ surfactants		Thermostable[c]	
					4–10		
	Cetrimide	Cetrimonium bromide[d]	0.005		Anionic surfactants, phenylmercuric nitrate	7–9	Thermostable
	Cetylpyridinium chloride	–	0.025	G +, F	Soaps, anionic surfactants	–	Thermostable
	Benzododecinium bromide	–	0.012	G +, F	Soaps, anionic surfactants	–	Thermostable
	Benzethonium chloride	–	0.01–0.02	Y, F, G −, G +	Anionic surfactants	4–10	Thermostable
Mercurials	Phenylmercuric nitrate (PMN)/ acetate (PMA)/borate (PMB)		0.002–0.004	G +, G −, F	Halides (precipitate)	7–8	Loss from polyethylene bottles, thermostable, sensitive to light
	Thiomersal	Merthiolate, thiomersalate, thiomerosal	0.001–0.02	F, G +, G −, Y	Boric acid, EDTA, halides, phenylmercuric salts, benzalkonium	7–8[e]	Adsorbed by rubber closures, thermostable, sensitive to light
Alcohols	Chlorobutanol	Chlorbutanol, acetone chloroform	0.5	G +, G −, F	Polysorbate 80, carboxymethylcellulose, plastic vials	<5.5	Not thermostable, volatile (musty odor)
	Benzyl alcohol	Phenylmethanol, phenylcarbinol	0.5	G +, F, Y	Non-ionic surfactants, methylcellulose	<5	Adsorbed by rubber closures, thermostable
	Phenoxyethanol		1	G −, *Pseudomonas*	Non-ionic surfactants, cellulose derivatives	<6	Thermostable
	Phenylethyl alcohol	Phenylethanol, benzylcarbinol	0.5	G −, G +	Oxidizing agents, polysorbates, proteins	<5	Sensitive to light, thermostable, absorbed by polyethylene containers, volatile
Carboxylic acids	Sorbic acid[f]	E200	0.2	F	Bases, oxidizing and reducing agents	4.5	Sensitive to light
Phenols	Methyl/propyl paraben	E218/E216	0.1	Y, G +	Non-ionic surfactants	4–9	Thermostable, poor soluble
Amidines	Chlorhexidine digluconate		0.005–0.01	G +, G −	Anionic soaps, chloride, borate, carbonate, citrate, phosphate, sulfate	5–8	Unstable above 70 °C, hydrolysis to 4-chloroaniline
Miscellaneous	EDTA[f]	Disodium edetate	0.01–0.1	G −, Y, F	–	4–10	Thermostable

[a] Y, yeast; F, fungi; G +, gram-positive bacteria; G −, gram-negative bacteria.
[b] Optimal for the antimicrobial activity and chemical stability.
[c] Stable at 121 °C (autoclaving).
[d] The name cetrimonium bromide (hexadecyltrimethylammonium bromide) was formerly applied to cetrimide (mainly trimethyltetradecylammonium bromide).
[e] Bactericidal at acidic pH and bacteriostatic and fungistatic at alkaline and neutral pH.
[f] Used in combination with other preservatives.

Table 2
Pharmacopoeial requirements for preservation of ophthalmic preparations

Prescriptions	USP 23[a]	EUR 1998[b]
Sterility	Yes	Yes
Preservative	Demanded if multidose containers intended for individual use on intact ocular surface	Demanded if multidose packaging
	Not recommended if intended for use in surgical procedures	Excluded if preparation contains a drug with intrinsic antimicrobial activity or if prescribed without preservative or if intended for use in surgical procedures

[a] United States Pharmacopoeia 23.
[b] European Pharmacopoeia 1998.

gives ultrastructural details of the eye state but sometimes with artifacts due to the sample preparation [44,45].

Finally, in vitro assays are carried out on cell cultures of enucleated eyes or even non-ocular tissues and measure a wide range of endpoints such as cytotoxicity, inflammatory parameters, membrane permeability or metabolism [46,47]. Perfusion techniques on isolated corneas mounted in special diffusion chambers allow electrophysiological monitoring of the corneal permeability. In vitro tests have the advantages of being rapid, reproducible, quantitative, painless, and inexpensive [48,49]. However, there is no mechanistic link between the endpoints evaluated in vitro and the response of the living eye [50].

4.2. Factors affecting the ocular tolerance of preserved ocular medications

In the usual concentrations and at low-frequency dosages, preservatives used selectively in eye medications do not notably damage the eye, as proven by the millions of daily applications all over the world [51]. However, the ocular adverse effects induced by preservatives may increase under certain circumstances [51]: the combination of preservatives, their chemical purity, the concentration of preservatives, the frequency of instillation, the duration of the treatment, the state of the cornea, the wearing of contact lenses and the use of polymers in the formulation of ophthalmic preparations.

4.2.1. Combination of preservatives

Preservatives can be combined in order to broaden the antimicrobial spectrum. For instance, disodium edetate (EDTA), a stabilizing and chelating agent which helps in restricting metal-catalyzed oxidation of various drugs, promotes the action of antimicrobial preservatives [16]. It is often associated at a 0.01–0.1% w/v concentration with benzalkonium (BAK) chloride or other preservatives to enhance the antimicrobial activity against strains of *Pseudomonas* [52].

The association of preservatives can, however, affect the tolerance of eye drops. For example, the addition of EDTA (0.05%) to thiomersal (0.02%) or chlorhexidine (0.025%) induced morphological alterations on the corneal surface as revealed by electron microscopy, whereas thiomersal or chlorhexidine alone and at the same concentration did not cause any changes [51].

4.2.2. Chemical purity of preservatives

The chemical purity of the preservatives that go into ophthalmic preparations can affect their ocular tolerance. Indeed, it was shown that the ocular tolerance of BAK, which is composed of a mixture of different alkylbenzyldimethylammonium chlorides with alkyl chain lengths ranging from C_8 to C_{18}, depends on the relative proportions of the chain lengths, the short chain C_{12} being less toxic than its higher homologues [53].

4.2.3. Concentration of preservatives

The likelihood of preservatives inducing ocular damage is greatly influenced by the used preservative concentration to which ocular tissues are exposed [54]. Increasing the concentration generally results in increased ocular injuries. Fig. 1 shows the effect of increasing the concentration of preservatives on the murine cornea.

Other examples of the influence of the concentration of some preservatives on the ocular adverse effects are compiled in Table 3. The severity of ocular damage

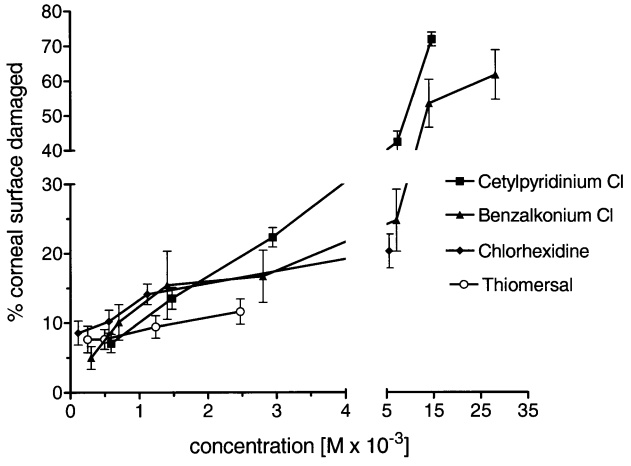

Fig. 1. Influence of instilled concentration of four common preservatives (benzalkonium, cetylpyridinium, thiomersal and chlorhexidine) on murine corneal surface damage. For the four compounds, the tested concentration range goes from the currently used concentration up to at least a ten-fold larger concentration. Mean ± SD ($n = 6$). (From Ref. [15] with permission.)

Table 3
Influence of increasing the concentration of preservatives on ocular adverse effects

Preservative (usual concentration, %)	Method of investigation	Concentration range (regime of application)[a]	Observed ocular damages	Ref.
Chlorhexidine (0.01)	Ex vivo observation on rabbit eye with electron microscope	0.001–0.0025% (1)	No damage detected	[56]
		0.005–0.075% (1)	Loss of microvilli	[57]
		0.01% (1)	Occasional cell ruffling and uplifting	
		0.1%[b]	Wrinkling of plasma membrane	
		0.5% (1)[b]	Exfoliation of total upper cell layer	
Thiomersal (0.01–0.02)	In vivo study on rabbit according to the Draize test	0.005–0.5% (1)	No discernible ocular reactions	[58]
		0.005% (7/5)	No discernible ocular reactions	
		0.005–0.5% (4/21)	Some corneal injection and conjunctivitis	
	In vivo observation on rabbit eye with light and electron microscope	0.1–1% (2/5)	No gross abnormalities	[59]
		2% (2/7)	Mild conjunctival redness, no corneal defect	
Benzalkonium (0.01)	Ex vivo observation on rabbit eye with light and electron microscope	0.1% (2/7)	No gross damage	[59]
		1% (2/7)	Corneal cloudiness, conjunctival injection	
		2% (2/1)	Conjunctival necrosis, corneal ulcers, haziness, massive iritis, endothelial defects	
	Ex vivo observation on rabbit eye with electron microscope	0.001% (1)	No changes	[56]
		0.0025–0.005% (1)	Some cell wrinkling, dissolution of membrane	[60]
		0.0075–0.01% (1)	Cell lifting and peeling	[51]
		0.02% (1)	Superficial cell desquamation	
		0.2% (1)	Marked desquamation affecting up to three cell layers	
Parabens (0.1)	Ex vivo observation on rabbit eye with light and electron microscope	0.04%	Small vacuoles in the endothelium	[57]
		0.16%	Large vacuoles and disruption of the endothelium	[61]
		0.6%	Loss of microvilli	
		3%	Desquamation of the entire upper cell layer	
Cetylpyridinium (0.025)	In vivo observation on rabbit	10–100 μg (1)[c]	No substantive corneal changes	[62]
		0.2–1 g (1)	Severe conjunctival chemosis and hyperemia, stromal edema, corneal neovascularization	
	Ex vivo observation on rabbit with electron microscope	0.7% (13/1)	Few anatomic changes	[63]
		1.9% (13/1)	Loss of microplicae on epithelial cells	

[a] Number of instillations/number of days; (1), single instillation.
[b] Thirty minutes of irrigation.
[c] Applied as a dry powder.

increases with the used concentration of preservatives. More precisely, at concentrations below the effective antimicrobial concentration, no or little damage is detected, whereas at ten-fold increased concentration, more severe lesions appeared.

Imayasu et al. [55] observed superficial punctate fluorescein staining in corneas treated with 0.005% BAK (two drops instilled 15 times at 5 min intervals) and corneal epithelial erosion and conjunctival chemosis on eyes treated with 0.01 and 0.02% BAK. Testing various concentrations of BAK in rabbit and cat eyes, Burstein [56] found the threshold to induce morphological damage to be between 0.001 and 0.01%, i.e. below the concentration of 0.01% commonly used in eye drops.

4.2.4. Frequency of instillation of eye drops

The instillation frequency of preserved eye drops can influence the exposure dose of ocular tissues and hence the extent of possible ocular damage.

Using two instillation regimens of a 0.02% BAK solution, a mild one (two drops instilled every 30 min for 2 h, i.e. eight drops in total) and an exaggerated one (two drops every 3 min for 1 h, i.e. 40 drops in total), Berdy et al. [64] observed a four-fold increase in the corneal damage score with the exaggerated instillation regimen. The mild regimen induced only minimal morphological changes (partial loss of microvilli) whereas the exaggerated one caused severe ultrastructural changes (cell peeling, retraction of cell membrane borders, and total loss of microvilli).

4.2.5. Duration of the treatment

Some patients receive topical ocular medications several times a day, over years or for life, especially in the treatment of chronic ocular diseases such as glaucoma, dry eye or allergy. The prolonged exposure of corneal epithelium to preservatives is susceptible to increasing the irritation potential of the preservatives [65]. In particular, the use of preservative in long-term antiglaucomateous topical medications is debatable [66,67]. BAK has been suspected to exacerbate a dry eye state in glaucoma patients and to be the cause of failure in glaucoma filtering surgery [68,69]. Furthermore, in healthy volunteers, BAK in beta-blocker eye drops has been shown to break the tear film stability [70].

4.2.6. Pathophysiological state of the cornea

Eyes that have undergone surgery may be more sensitive to the deleterious effect of some preservatives that delay the wound healing (see below) or influence the corneal permeability. Ashton et al. [71] stressed that changes in corneal integrity induced by stripping the corneal epithelium or using preservative like 0.01% chlorhexidine may dramatically affect the corneal penetration of some inert excipients in ophthalmic formulations and lead to possible toxicity. The adverse effect of preservatives may be much more relevant in older patients and those suffering from ocular surface disorders [70].

4.2.7. Wearing of contact lenses

Contact lenses can in some cases exacerbate the ocular cytotoxicity of preservatives (this aspect is developed in more detail in Section 5.7).

4.2.8. Use of thickening agents

Viscosity-enhancing agents are used in ophthalmic preparations to prolong the corneal contact time of the drug and increase its bioavailability. However, the corneal contact time of the preservative is also extended and can sometimes give rise to ocular damage. For instance, it has been reported that hydroxyethylcellulose (HEC), a thickening agent largely used in eye drops, prolonged the contact time of BAK with the rabbit cornea resulting in corneal epithelial damage that was not observed with BAK or HEC alone [72]. The use of thickening agents may be balanced in respect to the possible increased adverse effects of preservatives. These effects concern different parts of the eye.

5. Adverse external ocular effects induced by preservatives

Problems caused by preservatives topically applied on the eye are reviewed below according to the affected ocular structure, starting with the outermost ocular part, the conjunctiva, and ending with the crystalline lens. Comments on allergy induced by preservatives are made and problems associated with preserved care solutions for contact lenses are outlined.

5.1. Preservatives and conjunctiva

The conjunctiva is part of the eyelid. It is a delicate membrane that lines the posterior side of the eyelid (palpebral conjunctiva) and covers the anterior part of the eyeball (bulbar conjunctiva) [32]. The conjunctiva contributes to the tear secretion and also has protective functions. This tissue is able to react in a number of ways in response to topical ocular medication [73]. The mechanisms by which the conjunctiva can react include cicatrization (pseudopemphigoid), allergic reactions, papillary or follicular irritative/toxic conjunctivitis (redness, chemosis), deposition or dyschromia and microbial imbalance accompanied by tearing (discharge). Some preservatives can cause adverse effects on the conjunctiva. A single instillation of a 0.02% BAK solution has been reported to lead to a slight conjunctival hyperemia in humans [74]. Thiomersal also caused a conjunctival hyperemia on rabbit and human eye [74,75]. Finally, Hamill et al. [76] showed that a 40 μl drop of 2% chlorhexidine caused bulbar conjunctival hyperemia and eyelids mattering for 60 h after instillation.

5.2. Preservative and tears

The toxicity of preservatives toward the cornea is exerted either directly by modifying the anatomic and physiological integrity of the epithelium or indirectly by altering the tear film. Tightly bounded to the corneal epithelium, the tear film plays an important role for its optical and metabolic functions and for the protection of the eye from dehydration and other external harmful agents [77,78]. The tear film is a three-layered fluid of 7 μm thickness composed of lipid, aqueous and mucin phases [79]. Any discontinuity and instability of this tear film over the cornea may lead to ocular non-wetting tears disorders called dry eye: of these, keratoconjunctivitis sicca and Sjögrens syndrome are the most frequent [80,81]. Tear disorders are assessed by different clinical tests including the break-up time test, the Schirmer test, fluorescein and rose bengal staining and biomicroscopy [82]. Tear substitutes are the mainstay of treatment for all forms of tear deficiency. Besides polymers, artificial tears contain preservatives [83]. The most often used preservatives are BAK, chlorobutanol, thiomersal, chlorhexidine and EDTA [80]. These preservatives can have deleterious effects on the tear film stability as evidenced by several studies. BAK is the most disruptive of the ophthalmic preservatives to the stability of the lipid film [84]. BAK caused almost instantaneous disruption of the tear film by solubilizing the tear lipid layer [85–87]. Thiomersal, which is not surface-active, as well as chlorobutanol and EDTA seem not to influence the tear film stability [74,85,88].

5.3. Preservative and cornea

5.3.1. Cytotoxicity of preservatives

Cytotoxicity refers to the potential harmful effects of chemicals toward cells. These cells can be either microbial or human, the cytotoxicity being mostly non-specific. Preservatives affect microorganisms in several ways, depending on their chemical structure [89]. Alcoholic compounds disorganize the bacterial membrane, increasing its permeability, thus causing the leakage of the cytoplasmic contents. Phenolic chemicals disrupt the cell wall and the cytoplasmic membrane, also causing a cellular lysis. Quaternary ammoniums, due to their surface-active properties, break the cellular membrane and precipitate the cytoplasmic enzymes. Sorbic acid inhibits the oxidation of fumarate, a bacterial enzyme. Chlorhexidine inhibits potassium transmembrane transport. Finally, mercurial preservatives poison microbial enzymes fixing their thiol groups and kill microorganisms by affecting internal cell respiration.

Cytotoxic effects on human cells can be expressed by morphological changes (observed by histochemical and microscopic techniques), by alteration of membrane integrity or cellular metabolism (evidenced by leakage or absorption of various markers), by electrophysiological disruption (followed by microelectrodes) or by inflammation responses (studied by monitoring inflammatory factors). The development of culture techniques for corneal tissues has allowed the assessment of the ocular cytotoxicity of preservatives. Primary explants and sustained cultures can be conveniently monitored and long-term effects can be demonstrated [90]. However, it must be recognized that isolated cells are without the normal overlaying mucin layer and buffering tear film; they are also exposed to agents for a far longer period than would be clinically comparable. That is why the question of the clinical relevance of the cell cytotoxicity must always be kept in mind.

By means of scanning electron microscopy, the typical sequence of morphological cytotoxic events at the cellular level has been described [57]. The loss of microvilli from the periphery of epithelial cells was the first effect, followed by loss of microvilli from the whole surface area. Thereafter, the contacts between neighboring cells disappeared and the first dying cells were seen to be wrinkled and shriveled. Finally, by exfoliation of the superficial cells, further cell layers underwent the same process. Many in vitro studies have investigated the cytotoxicity of preservatives. BAK was shown to induce cellular physiological changes and impair the viability of corneal epithelial or conjunctival derived cells [65,91–94]. Cetrimide also promoted necrosis of human cultured conjunctival cells [95]. When used below the usual concentration for eye drops, thiomersal and sorbic acid also negatively affect the proliferation and survival of rabbit corneal epithelial cells [96].

5.3.2. Adverse effects of preservatives on cornea

The human cornea is a half micron thick, transparent tissue composed of five layers (from anterior to posterior): epithelium, Bowman's membrane, stroma, Descemet's membrane and endothelium. The cornea has two major functions: protection of the intraocular contents and refraction of light [97]. Due to its position, the cornea is the ocular tissue most exposed to external toxicants. Corneal damages can be caused by preservatives contained in topical ocular medications [98]. Some representative examples of the toxic effects induced on the cornea by ophthalmic preservatives are given in Table 4. The resulting ocular damages are listed according to the chemical class of the preservatives. The methods of investigation used to assess the ocular damages as well as the concentration and the regimen of application of the studied preservatives are specified.

A few comments can be made on the basis of the listed ocular damages. Firstly, it appears that preservatives with surfactant properties like BAK dissolve the membrane of corneal epithelial cells. At low concentration, they induce a loss of microvilli from the cell surface and at a higher concentration they cause lifting of cell borders and cellular desquamation [119,120]. Preservatives with surfactant properties also interact with cellular proteins: the head of cationic compounds like BAK interacts with anionic residues on proteins containing glutamate and aspartate [91]. Secondly, other clinical evidence of preservative-induced ocular damages includes allergic reactions (see below) caused by thiomersal and non-specific irritation reactions (edema, eye redness) generated by chlorhexidine and alcoholic preservatives.

The endothelium, the deepest layer of the cornea, consists of a single layer of hexagonal cells that do not regenerate [121]. This tissue is essential for maintaining dehydration of the cornea and hence its optical properties. Therefore, damage to the endothelium is far more serious than damage to the epithelium and is accompanied by marked corneal swelling and results in loss of the corneal transparency. Prolonged use of topical medications containing BAK has been assumed to induce toxic endothelial degeneration in human eyes requiring corneal transplantation [103]. However, at a low instillation regimen (six times a day for 7 days), a 0.005% BAK solution has not been observed to induce pathological changes in rabbit eyes [122]. Yet, the adverse effects of BAK on rabbit endothelium can be magnified if BAK is associated with EDTA or if it is instilled in keratectomized eyes [101]. Parabens have also been described to cause morphologic changes of the rabbit endothelium [61]. Two ultrastructural studies also brought to light the adverse effects on rabbit endothelium of thiomersal that triggered intracellular edema and damage to organelles [110,123]. Finally, in vitro tests on isolated rabbit corneas showed that chlorhexidine perfused over the endothelium can induce corneal swelling as well as BAK and cetylpyridinium [124,125].

Table 4
Compilation of published studies on corneal toxicity induced by preservatives

Type of preservatives	Preservatives (usual concentration, %)	Ocular damages	Concentration used (regime of application)[a]	Method of investigation	Ref.
Quaternary ammoniums	Benzalkonium chloride (0.01)	Decrease of the healing rate after lamellar keratectomy	0.02% (4/12)	In vivo testing on rabbit eye stained with fluorescein	[99]
		Loss of epithelial cells, membrane disruption	0.01% (1)	Ex vivo, ultrastructural study on rabbit eyes with electron microscopy	[7]
		Severe damage of the stromal keratocytes	0.02% (9/2)		[100]
		Slight endothelial edema of previously keratectomized eye	0.02% (9/2)		[101]
		Severe exfoliation of corneal cells, loss of microvilli	0.05% (1)		[57]
		Tear film destabilization in form of a hastened apparition of dry spot	0.0001% (1)	In vivo study on human/rabbit eye examined by slit lamp	[87]
		Three-fold increase of fluorescence intensity compared to untreated eye	0.1% (1)	Ultrastructural study on rabbit eyes ex vivo with electron microscopy and epifluorescence microscopy	[102]
		Up to 5% of exfoliating cells compared to 0.5% in control eye	0.05% (1/2)	Ultrastructural study on rabbit eyes ex vivo with electron microscopy	[11]
		Severe endotheliopathy, corneal edema, endothelial degeneration requiring corneal transplantation	0.004% (NS[b])	Medical report of one patient	[103]
		Loss of microvilli, wrinkling of plasma membrane, exfoliation of upper cell layer	0.05% (30 min irrigation)	Ultrastructural study on rabbit eyes ex vivo with electron microscopy	[57]
		Reduced break up time	0.005% (1/3)	Clinical trial on human volunteers	[70]
		Burning or dry eye sensation	0.01%		[68]
	Cetylpyridinium chloride (0.025)	Severe irritation (large corneal opacity, chemosis and discharge, hemorrhage on the iris)	100 mg[c]	In vivo irritation test on rabbit eyes	[104]
Chelators	EDTA (0.01–0.1)	No delay in healing rate after partial lamellar keratectomy	0.1% (4/8)	In vivo testing on rabbit eye stained with fluorescein	[99]
			0.005% (3/1)		[105]
		Slight stinging	0.34% (1)	In vivo study on human volunteers	[106]
		Severe stinging	1.0% (1)		
Organic mercurials	Thiomersal (0.001–0.02)	Ocular delayed hypersensitivity (corneal infiltrates, redness, lacrimation, foreign body sensation)	0.04% (12/1)	In vivo, recurrence test on human after supposed allergic reaction to eye drops	[107–109]
		Acute hypersensitivity (corneal edema, corneal infiltration and erosion, iritis, occasional corneal neovascularization)	0.1% (1)	In vivo study on rabbits wearing contact lenses soaked with thiomersal	[75]
		No morphological changes	0.01% (1)	Ex vivo ultrastructural study on rabbit eyes with electron microscopy	[7]
		Endothelial edema	0.004% (24/2)		[110]
		Some edematous stromal cells	0.004% (9/2)		[100]
		No interference with corneal epithelial healing rates	0.001% (3/1)	In vivo testing on rabbit eye stained with fluorescein	[105]

Table 4 (*continued*)

Type of preservatives	Preservatives (usual concentration, %)	Ocular damages	Concentration used (regime of application)[a]	Method of investigation	Ref.
		No delay in healing rate after partial lamellar keratectomy	0.004% (4/6)		[99]
Alcohols	Chlorobutanol (0.5)	No corneal reaction	2% (2/7)	In vivo evaluation (light and electron microscope) on rabbits	[59]
		Some membrane disruption, some stromal edema	0.5% (9/2)	Ex vivo ultrastructural study on rabbit with electron microscopy	[100]
		No epithelial ultrastructural changes	0.5% (1)		[7]
		Up to 9% of exfoliating cells vs. up to 0.5% (control eye)	0.5% (1/2)		[111]
		Only occasional intracellular or extracellular edema	0.1% (9/2)	Ex vivo ultrastructural study on rabbit with electron microscopy	[100]
	Phenylethyl alcohol (0.5)	Smarting sensation	0.5% (1)	In vivo irritation study on human volunteers and patients	[74]
		Irritation	0.6% (NS)		[112]
Others	Chlorhexidine (0.01)	Fairly persistent mild irritation and some occasional corneal opacities	0.005%[d]	In vivo study on rabbit eye wearing soft contact lenses	[113]
		Corneal edema, desquamation, delayed healing rate after mechanical injury of the cornea	4% (1)	In vivo and ex vivo study on rabbit, by slit-lamp and electron microscope	[114]
		Eye redness, pain, diminished vision developing to severe corneal damage (opacification)	4% (1)	Medical report of four patients	[115]
		Pain, corneal edema, bullous keratopathy in two patients	4% (1)	Medical report of two patients	[116]
		Intense pain, blepharospasm, punctate corneal ulcerations	NS	Medical report of five patients	[117]
			NS	Medical report of one patient	[118]
		Delayed healing rate after corneal abrasion	2% (1)	In vivo study on rabbit	[76]
	Parabens (0.1)	Smarting sensation	0.02–0.04% (1)	In vivo study on human	[74]

[a] Number of instillations/number of days; (1), single instillation.
[b] NS, not specified.
[c] Applied as a dry powder.
[d] 6 h contact with soft lenses soaked in preserved solution.

5.3.3. Preservatives and corneal drug permeation

Preservatives used in topical drops have been reported to increase corneal permeability of drugs, in particular BAK which possesses surfactant properties and whose bactericidal mode of action is not specific. BAK dissolves membranes not only of microbial cells, but also of epithelial cells [119]. BAK has been shown to cause a dose-related dissolution of the plasma membranes of rabbit corneal epithelial cells as well as loosening of cells by action on cell junctions [57,60,126]. Numerous authors reported an increase of corneal permeability of various tracers (fluorescein, inulin, horseradish peroxidase) and drugs (prednisolone, pilocarpine, beta-blockers) due to BAK [127–137]. For instance, Lee and Lee [138] reported a 17-fold increase of systemic absorption of atenolol formulated with BAK 0.025% compared to a non-preserved atenolol solution. Removal of BAK from a topical timolol formulation was found to be as effective as a preserved formulation in reducing intraocular pressure in glaucomateous patients' eyes, but it also resulted in a reduction of burning sensation or dry eye [139]. Moreover, the undesirable systemic absorption of ophthalmic drugs can lead to serious and even fatal systemic side effects [140,141]. Cetylpyridinium chloride has also been shown to enhance penicillin penetration across the cornea in a similar manner to that found for BAK [142]. EDTA, a calcium chelator that loosens the tight junction between the epithelial cell, also increases the corneal permeability of various drugs [138,143,144]. Chlorhexidine has also been demonstrated to increase rabbit corneal permeability of various labels (fluorescein, sorbitol) [71,127]. Thiomersal has only little effect on the corneal epithelial barrier [136]. Finally, chlorobutanol 0.5% formulated in a polyvinyl alcohol 1.4% hydrogel does not negatively affect the corneal epithelial permeability in dry eye patients contrary to a BAK 0.05% hydrogel [145]. Furthermore, some authors have suggested the use of preservatives as permeation enhancers for drugs like insulin or beta-blockers [146–148].

However, despite increased corneal permeation due to preservatives, it is not possible to sufficiently increase the corneal permeability without causing unacceptable ocular damage. Saettone et al. [149] demonstrated that BAK at the usual concentration of 0.01% did not affect the bioavailability of topicamide in aqueous solutions neither in rabbit nor in human. On the contrary, BAK was shown to depress the bioavailability enhancement of the drug produced by polymeric solutions. The hypothesis was advanced that BAK reduced adhesion of the solution to the corneal surface. In a crossover randomized double blind study on human volunteers, Baudouin and de Lunardo [70] noticed that the intraocular pressure lowering effect of a beta-blocker, carteolol, was not reduced by suppressing BAK from the eye drop. This second example shows that preservatives may not be adequate permeation enhancers clinically exploitable for the ocular route.

5.4. Preservatives and wound healing

Preservatives used in postoperative treatment after surgical operations like keratoplasty can negatively affect the wound healing rate and interfere with corneal reepithelialization [150]. Testing the effect of various preserved formulations on denuded rabbit cornea, Rucker et al. [151] made the following ranking from the least to the greatest healing time inhibition: thiomersal 0.004%+EDTA 0.1% <BAK 0.00004%+EDTA 0.1%+polyvinylalcohol<BAK 0.0002%+methylparaben 0.02%+EDTA 0.1%. BAK has also been shown in other studies to significantly delay corneal epithelium regeneration [99,152,153]. The mechanism by which corneal healing is retarded by BAK involves a breakdown of the anatomical and physiological diffusion barrier, possibly by lysis of the cell membranes, thus hampering cell migration and adhesion over the denuded surface [154]. Chlorhexidine was also claimed to significantly slow the healing rate of rabbit debrided corneas whereas thiomersal had little or no effect on wound healing after mechanical lesion [51,105,155].

5.5. Preservatives and crystalline lens

The crystalline lens, the focusing system of the eye, is a biconvex, multilayered structure located behind the iris. It is composed of a dense capsule and a deeper layer of epithelial cells delimiting a fibrous and lamellar substance [121,156]. The lens may be affected by deposits of mercury contained in certain preservatives. This complication called mercurialentis is characterized by a yellowish brown coloration of the anterior lens capsule occurring in subjects exposed to mercury for a prolonged period [157]. This pathology is found either in occupational disease like thermometer fabrication inducing mercury poisoning or in iatrogenic disease, i.e. long-term use of mercury preserved ocular medications [158–160].

Mercurial preservatives include thiomersal and phenylmercuric salts (acetate, nitrate and borate). Mercury pigmentation of the lens capsule can occur secondary to topical medication with phenylmercury preserved eye drops whereas thiomersal is not likely to induce lens deposits [157]. This may be explained by the chemical and physical differences between both mercurial preservatives. Thiomersal is much more soluble than phenylmercuric salts and is unlikely to precipitate on the lens capsule [161]. Furthermore, mercury is bonded in the molecule of thiomersal by stable covalent bonds, contrary to the partly inorganic bonding of the metal in phenylmercuric salts [161].

5.6. Preservatives and allergy

Contrary to widespread belief, allergic reactions are much less common than toxic reactions, the former accounting for only about 10% of all ocular adverse reactions [162]. However, the potential allergenicity of preservatives in topi-

cal ophthalmic medications, though rare, should not be underestimated as they may cause complications like conjunctival scarring and prolongation of symptoms during treatment [163].

There are three important predisposing factors to allergic reactions: individual susceptibility, prolonged use of medication and preexisting cutaneous disease of the eyelids [2].

Allergic contact reactions occur after sensitization of thymus derived lymphocytes (T cells) in the regional lymph nodes and represent type IV (delayed) hypersensitivity in the Gell and Coombs classification [164]. Topical medications act as haptens (partial antigens) and must combine with tissue protein to form complete antigens that can sensitize lymphocytes and produce inflammation. This sensitization takes weeks to years to develop, but once the patient has been sensitized, allergic reaction can appear in as little as 48 h [1]. The investigation of this kind of reaction is generally carried out by means of patch tests [165].

Evidence of allergic contact reactions includes: follicular conjunctivitis, eczematoid blepharitis, periocular dermatitis preceded by conjunctival hyperemia, discharge and edema [2]. Other symptoms encompass itching and burning sensations of the eye, epiphora, photophobia and foreign body sensation [163].

The main preservatives involved in allergic contact reactions are thiomersal, chlorhexidine, EDTA, benzethonium chloride, BAK, sorbic acid, phenylmercuric nitrate and polyquat [2,108,166–168]. Herbst and Maibach [169] have extensively reviewed reports of contact dermatitis caused by ophthalmic drugs and particularly preservatives. Thiomersal acts as a hapten and it is the most allergenic preservative [109,170]. The incidence of hypersensitivity to thiomersal ranges from 6.5 to 8% among individuals routinely screened for contact allergy in the USA [171]. The prevalence rises even to 10% among soft contact lens wearers [172,173]. BAK tends to substitute thiomersal because of a wider antibacterial spectrum and for environmental reasons (thiomersal contains mercury), but one should be aware of the fact that BAK also has an allergenic potency and can cause severe allergic reactions [167,174]. In this connection, a 1 year study on the frequency of sensitization to common preservatives has concluded that BAK could even be more allergenic in some cases than thiomersal (5.5% of positive reactions vs. 4.2% for thiomersal on a total of 2295 patients) [175]. As in all allergic pathologies, drug reactions resolve slowly after the causative medications are stopped [2].

5.7. Preservatives and contact lenses

Contact lens wearing can lead to various adverse ocular effects [176]. Indeed, a slight corneal abrasion may occur during normal wear, as well as anoxia and accidental trauma during insertion of the lens. These factors predispose the cornea to infection as the lens provides a medium for introducing pathogens into the eye [177]. For this reason, the use of preservatives is sometimes required in some contact lens care solution, as is the case in the Swiss Pharmacopoeia 8. However, other pharmacopoeias like the European and US pharmacopoeias do not specify the addition of preservatives in contact lens care solutions.

Besides other compounds – such as surfactants, enzymes, oxidizing agents, viscolysers, buffering agents and tonicity agents – preservatives are used in various contact lens care solutions. Preservatives are contained in cleaning solutions (aiming at eliminating proteinaceous, lipid, mineral, environmental or cosmetic deposits that accumulate at the surface of the contact lens), in soaking or disinfecting solutions (used to remove microorganisms during the storage of the contact lenses while they are not being worn), in rinsing solutions (used to remove remaining cleaning or soaking solution) and in wetting solutions (used to provide cushioning and lubrication between the lens and the cornea) [178].

These preservatives can negatively affect both the contact lens and the ocular surface. Few studies have investigated undesirable reactions of a preservative on a contact lens. However, a case was reported where sorbic acid, a popular preservative, when allowed to degrade, produced mixed aldehydes that could discolor protein deposits on the lenses [179]. Consequently, the useful life of the lens was shortened. An expiration date is thus necessary for contact lens solutions.

Preservatives can also induce undesirable effects on the ocular surface. Indeed, hydrophilic polymeric materials used for soft lenses can adsorb chemicals such as preservatives. Although this adsorption is not harmful per se, the later desorption presents a toxicity problem for the eye [180]. The lens acts as a reservoir for the chemicals, thereby providing an opportunity for prolonged contact between these chemicals and the ocular surface. For this reason, substances that are not toxic in eye drops may become toxic when used with soft lenses. Allergic and toxic reactions to preservatives in soft contact lens solutions have been extensively reviewed [107]. For instance, 0.004–0.005% thiomersal may induce delayed ocular hypersensitivity, patients developing superior limbic keratoconjunctivitis, conjunctival hyperemia, limbal follicles, giant papillary conjunctivitis, corneal infiltrates, superficial punctate keratitis, pseudo-dendritic corneal lesions, epithelial opacities and even neovascularization [108,172,173,181–184]. Generally, upon discontinuation of lens wear and hence exposure to thiomersal, all signs of conjunctivitis slowly resolve without permanent sequel [185,186]. Chlorhexidine that binds very well to soft contact lenses and is slowly released has been associated with complications in contact lens wearers such as intraepithelial microcysts, corneal desquamation and photophobia [125,187–190]. Positive patch test reactions have been found with thiomersal and chlorhexidine, confirming their allergenic potential [168]. When used with soft lenses, chlorobutanol has been reported to cause mild conjunctivitis [90]. Finally, BAK, whose uptake by soft contact lenses is high, has been

demonstrated to increase corneal epithelial desquamation and cause ocular inflammation [191–193]. BAK has also been reported as an allergenic compound [169]. Despite its rather widespread use to chelate calcium from lens deposits, EDTA only occasionally results in ocular allergic evidence [1,107].

Hard lenses have the same problems as soft lenses, but to a much smaller extent because they have a lower degree of adsorption.

6. Alternatives to irritant preservatives

Alternatives to the use of irritant preservatives exist and include the development of better tolerated preservatives and special packaging devices.

6.1. New preservatives

The research and development of new preservative chemical entities that are better tolerated and less toxic is a challenge. Polyquad and Dymed are quaternary ammonium preservatives which appeared in the 1990s and seem to provide promising properties [194]. Polyquad (polyquaternium-1) is a high molecular weight compound used both in contact lens solutions and artificial tears. It is very effective in preventing microbial growth, especially fungi, and seems to be well tolerated by patients. Sodium perborate is a relatively new preservative. In the bottle, it generates hydrogen peroxide, a highly effective antimicrobial agent, and once the solution is in contact with the tear film, the hydrogen peroxide is converted to water and oxygen by endogenous enzymes [6]. Sorbic acid is a widely used food preservative that has been proposed as an alternative to the more toxic effects of BAK if formulation compatibility is possible [195].

Finally, the newest preservative is a stabilized oxychloro complex called Purite. When the patient releases the drops from the opaque bottle, and the solution is exposed to light, Purite breaks down into water and sodium chloride.

Though the new chemicals may be promising, it should be kept in mind that it is very difficult to obtain a preservative that is both efficient against microorganisms and safe toward human tissues, this difficulty arising from the non-specific activity of preservatives.

6.2. Non-preserved unit dose eye drops

When eye drops are prescribed without preservatives, e.g. for injured eyes or for use during eye examination or surgery, or when it is preferable to have no preservatives present, for instance in the treatment of dry eye, single-dose containers are required by the European pharmacopoeia. Unit dose containers appeared in 1965 in Great Britain [6].

The unit dose packages are usually made of low density polyethylene and consist of a tubular, compressible body and a pointed cone with a twist-off closure or a closure cap [196]. The package whose capacity can vary between 0.1 and 1 ml is often prepared in form-fill-seal equipment where the melted resin forms plastic containers which are aseptically filled and sealed.

The main advantage of a unit dose package is the elimination of the risk of solution contamination, making the addition of preservative unnecessary, because an opened package has to be discarded after application of a drop or at the end of the day. This dosage form is well suited to intermittent use, for instance in ophthalmic diagnosis products or in pre- or postoperative treatments. Although single-dose containers are ideal for preservative-free ophthalmic solutions, they present some drawbacks. Firstly they are relatively expensive. Day to day use in the chronic treatment of eye diseases would mean a waste of solution and plastic packaging [197]. The cost of the unit dose package is five to ten times higher than that of multidose eye drops [6]. Secondly, a clear-cut opening of the container is difficult to obtain even when scissors are used, so that the size of the eye drops, and hence the dose, can vary depending on the dimensions of the orifice created [197]. Furthermore, a barb of plastic can remain around the orifice when twisting off the closure, causing irritation when the eye is touched during administration of a drop. This could be of relevance in the case of elderly patients. Creating a preformed fracture on the pointed cone where the tab is to be twisted off can avoid this drawback [198]. Thirdly, some plastic containers lack suppleness, making squeezing the bottle to extrude a drop of solution difficult. Finally, if the risk of contamination is theoretically nil, in actual practice, patients tend to keep the unit dose container longer than for a single use, even for several days, with the ensuing risks of contamination. Indeed, the package always contains excess solution that the patient is susceptible to use since he thinks he is fighting waste.

6.3. Non-preserved multidose eye drops

To avoid preservative-induced ocular problems while maintaining eye drop sterility, the packaging in multidose bottles of non-preserved eye drop formulations that contain antibiotics or alkaloids has been suggested as the drugs showed good inherent efficacy against microbes [199]. However, once opened by patients the in-use storage life is limited to 7 days, provided the preparations are stored in a refrigerator. This approach has some drawbacks. Firstly, it is restricted to drugs that have intrinsic antibacterial activity; thus, it is not suitable for preparations like conventional artificial tears. Secondly, the use of refrigerated non-preserved eye drops in multidoses requires very good patient compliance, and elderly people may find it cumbersome and uncomfortable to instill a chilled eye drop [195]. Thirdly, refrigeration is not an absolute protection against microbial contamination. Indeed, some mesophilic and many psychrophilic bacteria can multiply at temperature

between 4 and 8 °C, especially since home refrigerators are hardly sterile [195].

6.4. Novel multidose devices for eye drops

An interesting alternative to unit dose containers consists of a packaging in multidose bottles with a special filter device [6,200]. Either the device contains a sterile preservative-free solution protected against microbial contamination by a 0.2 micron porosity filter or a preservative (BAK for instance) is contained in the solution and retained by a filter (adsorbing resin) upon instillation [201]. In both cases, a preservative-free eye drop is delivered to the eye. A first ophthalmic preparation has been marketed in France under the trade name Naabak®. Other antiglaucomateous medications are now commercially available (Timabak®) [202]. The cost of this new packaging system, though higher than the traditional multidose bottle, is lower than the unit dose packages.

These devices, though they keep the ophthalmic preparations sterile, are in plastic squeeze containers with screw-on caps, a design reported to be prone to bacterial colonization of the space between the bottle tip and the cap or along the threads of the container cap [26,27,195].

7. Conclusion

The prevention of infectious diseases is certainly preferable to their treatment. The use of preservatives is the easiest way to prevent microbial spoilage of ophthalmic medications. However, ophthalmic preservatives are a double-edged sword. Indeed, they constitute a necessary compromise between what is legally required and necessary, and what is microbiologically efficacious on the one hand and possibly toxic on the other [51]. In other words, preservatives are meant to destroy microorganisms across a broad spectrum and to protect the eye against possible (secondary) infection, but unfortunately their action is non-specific and they can damage ocular tissues [203]. Medical reports have described ocular damage due to preservatives with surface-active properties like BAK and mercurial preservatives are well known to induce severe hypersensitivity reactions or mercurial lens deposits. As a consequence, patients using preserved eye drops are at risk of developing ocular surface disorders. However, a total suppression of preservatives would neither be desirable nor justified as it would mean risking severe ocular infection. Yet the use of preservatives in eye drops should be restricted to solutions that are used selectively during a short period of time, for instance in ophthalmic solutions for diagnosis. In others cases, caution is urged in the use of preservative-containing topical ocular medications over an extended period in patients with extensive ocular surface diseases [103]. Indeed, weakened eyes, i.e. eyes that have undergone surgery or have chronic ocular surface disease (glaucoma, dry eye or allergy) or have been abraded by unfitted contact lenses, are certainly more sensitive to preservatives. Preservatives should be avoided, if possible, in these cases, because the risk of worsening patient symptoms, of compromising the issue of the affection or of impairing wound repair after surgery cannot be excluded. The use of preservative-free tear substitutes should be promoted as they are as effective as preserved artificial tears, and avoid adverse ocular effects induced by preservatives [204,205]. Some promising alternatives have been proposed recently in the form of original new packaging systems that are now available. Economic and pharmaceutical considerations must be taken into account as well as patient compliance in the choice of these new alternatives to the use of preservatives.

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