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LABORATORY BASED

Severe ocular infections with contact lens: role of multipurpose solutions

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Abstract

***Purpose*: To determine whether multipurpose solutions, widely used for contact lens disinfections, could be at the origin of ocular pathologies (contact lens intolerance and ocular infections).**
***Methods*: An observational cohort study (questionnaire analysis) was carried out to estimate the number of contact lens wearers, type of infection, and type of lens care regimen used by patients. Besides, multipurpose solutions cytotoxicity (necrosis and apoptosis) was evaluated on a conjunctival cell line using cytofluorometry.**
***Results*: In the general population, 59% of contact lens wearers use multipurpose solutions whereas 35% use oxidative products. Of the questioned contact lens wearers with ocular infections, 80% used multipurpose solutions. Multipurpose solutions are therefore not efficient enough against microorganisms, and cannot be considered as disinfectant solutions but only as preservatives. However, preservatives are known to be toxic to ocular surface, so apoptosis induced by multipurpose solutions could lead to ocular surface diseases. Our cytofluorometry study allowed us to demonstrate that contact lens multipurpose solutions containing preservatives are cytotoxic through caspase 3 induction, chromatin condensation and P2X7 cell-death receptor activation, in contrast with unpreserved sterile saline solutions that were found inert.**
***Conclusions*: Multipurpose solutions seem to be preservative but not disinfecting solutions. They are not adapted to the final rinse of contact lenses because of apoptosis induction. It could explain part of lens intolerance.**
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Introduction

Contact lens wear is liable to cause two pathologic events: severe ocular infection and ocular dryness. Multipurpose solutions, widely used as soft contact lens care regimen, are supposed to successively perform disinfection and rinse of contact lenses, with the same product. The question is: are these solutions efficient enough to disinfect contact lenses and are they not likely to induce tolerance problems? In March 2006, the Centers for Disease Control and Prevention (CDC) reported to the Food and Drug Administration (FDA) cases of *Fusarium* fungal keratitis among patients who used multipurpose solutions to disinfect their contact lenses. Bausch & Lomb then announced in April that consumers should immediately stop using and discard ReNu MoistureLoc multipurpose solution. Besides, in November 2006, Advanced Medical Optics Inc. recalled almost 3 million units of Complete Moisture Plus multipurpose solution due to sterility problems that could lead to eye infections. The link between Complete MoisturePlus contact lens solution and *Acanthamoeba* keratitis infection was identified as a result of an investigation by CDC in May 2007. Moreover, the XV–XX hospital, the first European ophthalmology centre, has reported five *Fusarium* infection cases since January 2006.

At the XV–XX hospital, the dispensation of fortified antimicrobial eye drops (antibiotic concentration 2–10 times higher than that used in commercially available eye drops), prescribed in severe ocular infections to treat keratitis, increased by 190% from 1996 to 2000. The less severe ocular infections can be treated

with commercial antibiotic eye drops, but few molecules are available and very often bacterial keratitis needs fortified antibiotics, which are produced in hospitals. Bacterial keratitis was rare until the widespread use of contact lenses. The incidence in the United States was about 11.0 per 100 000 persons per year in the 1980s.[1] A retrospective study carried out in the XV–XX hospital in 2003 showed that contact lens wear was the most important risk factor for developing bacterial keratitis,[2] but very little is known about the factors influencing severe infections due to contact lens wear. The aim of this study is to evaluate the proportion of contact lens wearers and lens care regimen in a sample of patients with severe ocular infections treated with hospital pharmacy-made fortified antibiotic eye drops. Our hypothesis is that contact lens multipurpose solutions are not disinfecting products but only preservatives.

The cytotoxic potential of benzalkonium chloride preservative, which is a quaternary ammonium like active constituents of most multipurpose solutions, has already been observed with antiglaucomatous (β-blockers) and fluoroquinolone eye drops.[3,4] The second aim of this work is to study the ocular tolerance of contact lens care regimens on a human conjunctival cell line whose sensitivity to preservatives such as benzalkonium chloride has been proved in many studies.[5–7]

We suggest that multipurpose lens care solutions containing preservatives could be at the origin of:

- severe ocular infection due to a lack of disinfection efficacy,
- contact lens intolerance due to a cytotoxic effect involving apoptosis by P2X7 receptor activation.

Methods

Patient characteristics and selection criteria

The study was carried out in the Centre Hospitalier National d'Ophtalmologie des XV–XX, Paris, France, from January 2000 to September 2001. A total of 91 adults suffering from severe ocular infection associated with visual acuity diminution were included in the study as they were prescribed hospital pharmacy-made fortified antibiotic eye drops. Patients were given a questionnaire in order to evaluate the aetiology and the origin of their severe ocular infection. Ethics Committee approval was not required for this study.

Multipurpose solutions in-vitro *toxicity evaluation*

Cell culture

A human conjunctival cell line (Wong Kilbourne derivative of Chang conjunctiva WKD, ECACC 93120839) was cultured under standard conditions (moist atmosphere of 5% CO_2 at 37°C) in Dulbecco's minimum essential medium (Eurobio, Les Ulis, France) supplemented with 10% fetal bovine serum (Dominique Dutscher, Brumath, France), 2 mM L-glutamine (Eurobio), 50 IU/ml penicillin (Eurobio), and 50 IU/ml streptomycin (Eurobio).

Confluent cultures were removed by trypsin (Eurobio) incubation and were seeded into 96-well culture microplates (Corning, Schiphol-Rijk, The Netherlands) at a density of 90 000 cells per ml for analysis. Cultures were kept at 37°C for 24 h.

Cell incubation

When cells reached approximately 80% of confluence, the culture medium was removed. Two multipurpose solutions were evaluated: a quaternary ammonium-containing solution (0.001% in Opti-free Express No Rub; Alcon, Fort Worth, TX, USA) and a biguanide (PHMB)-containing solution (0.0001% in Solo-Care Plus; Ciba Vision, Duluth, MN, USA). Sterile controlled ionization marine solutions (Serophta Lens, Goëmar, Saint-Malo, France/Lacrymer, Yslab, Quimper, France) were analysed as negative controls and cellular control was culture medium.

Experimental procedures

Experiments were conducted using a microplate fluorometer (Safire, Tecan France), which allows fluorometric detection (280–870 nm) with high sensitivity (pg-fg/ml). This technique allows the use of fluorescent probes directly on living cells and detects the fluorescent signal directly in the microplate in less than 1 min (for a 96-well microplate).

Caspase activation

Caspase 3 activity was evaluated using a caspase 3 kit assay (Z-DEVD-Rhodamine 110 fluorogenic substrate, Molecular Probes, PoortGebouw, The Netherlands) with anticancer drug camptothecin 10 μM as caspase-inducer positive control.

Apoptosis: chromatin condensation assessment (Hoechst 33342 test). Hoechst 33342 (Molecular Probes) was used to evaluate chromatin condensation in cells with propidium iodide in order to discriminate necrotic cells; UV fluorescent probe Hoechst 33342 (excitation = 360 nm; emission = 450 nm) enters living cells and apoptotic cells whereas propidium iodide enters necrotic cells much faster than Hoechst 33342.[8] The cells were exposed for 30 min to a Hoechst 33342 solution at a concentration of 10 μg/ml containing 1 μl of propidium iodide (1 mg/ml in water).

P2X7 cell-death receptor activation (YO-PRO-1 test). YO-PRO-1 (Molecular Probes), a DNA probe, only enters

apoptotic cells through P2X7 receptor.[9,10] Cells were exposed for 10 min (at room temperature in the dark) to a 2 μM YO-PRO-1 solution (excitation = 491 nm; emission = 509 nm).

Statistics

Results were obtained in fluorescence units and were expressed as a percentage of the control. Each drug concentration was tested in six wells, and each experiment was performed in triplicate. The mean values for each concentration were analysed with a one-way ANOVA test followed by Dunnett's test using Sigma Stat 2.0 software (Chicago, IL, USA) and the level of significance was fixed at 0.05.

Results

Clinical investigation

Figure 1 shows that 65% of patients with severe ocular infection were contact lens wearers. Most of these patients are women, who represent 70% of contact lens wearers (Table 1). This proportion is close to the general population as 75% of contact lens wearers are women. The same applies to age as 75% of female lens wearers in the general population are between 15 and 35 years old, which is equivalent to what we found in our survey (Table 1). Therefore, these two factors do not influence the infection risk. Table 1 shows that bactericidal corneal abscess and amoebic keratitis were respectively diagnosed in 61 and 29% of cases (the treatment served as an indicator of the diagnosis). Total 80% of contact lens wearers used multipurpose solutions (the main products being Opti-free Express No Rub, Solo-Care Plus, ReNu Multiplus, Complete) to disinfect their lenses (Figure 2a). Multipurpose solutions are supposed to improve patients' compliance and thus to reduce ocular infection risk. Nevertheless, multipurpose solutions are involved in 80% of severe ocular infections whereas oxidative products (hydrogen peroxide) were used by only 8.5% of patients with ocular infection. At the time of the study, the proportion of contact lens wearers using multipurpose solutions was 59% against 35% using oxidative products. Severe ocular infection (bacterial keratitis) can lead to severe visual disability, and even to cornea graft. Considering the possible serious consequences, we evaluated the proportion of multipurpose solution users among patients suffering from amoebic keratitis (29% of cases). Figure 2b shows that in our sample amoebic keratitis is seven times more frequent among multipurpose solutions users than among oxidative product users.

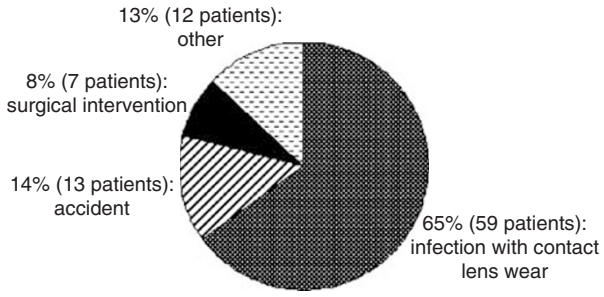

Figure 1 Ocular infection aetiology: 65% of patients with ocular infection were contact lens wearers.

Table 1 Characteristics of contact lens wearers with severe infection: sex, age, diagnosis

Sex	*Number of cases*
Female	41
Male	18
Total	59
Age (years)	*Number of cases*
15–25	20
26–35	24
36–50	12
>50	3
Total	59
Ocular infection diagnosis among contact lens wearers	*Number of cases*
Bacterial corneal abscess	36
Amoebic keratitis	17
Others and unknown	6
Total	59

Total 90% of contaminated contact lens storage cases were disinfected with multipurpose solutions, and a wide range of bacteria was found. We reported the microbiological analysis of 37 patients' cornea and contaminated storage cases disinfected with multipurpose solutions (Table 2). Microorganisms found in corneas are the same as those found in contact lens containers in 61% of cases.

In-vitro *apoptosis evaluation*

Assessment of apoptosis with the caspase 3 test showed that Opti-free and Solocare both significantly induced caspase 3 activity (4.2 × and 2.6 × compared to control, respectively; Figure 3a). Moreover, inductions of caspase 3 with Opti-free and with positive control camptothecin were similar.

A highly significant increase in chromatin condensation was observed with both multipurpose

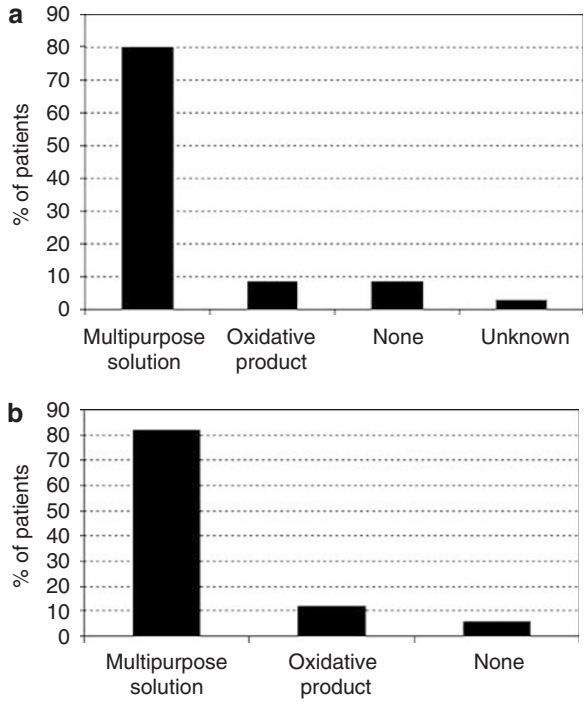

Figure 2 (a) Contact lens care regimens used by contact lens wearers with severe ocular infection: multipurpose solutions were mainly used (80%). (b) Contact lens care regimen used by contact lens wearers with amoebic keratitis: multipurpose solutions were mainly used (82%).

solutions (8.8 × and 2.6 × compared to control, $P<0.001$; Figure 3b). Apoptosis related nuclear morphological changes (apoptotic bodies) were observed using inverted fluorescence microscopy.

Apoptosis was then confirmed with P2X7 cell-death receptor activation assessed by YO-PRO-1 test; both multipurpose solutions induced its activation (9.8 × and 1.9 × compared to control with Opti-free and Solocare respectively, $P<0.001$; Figure 3b). Controlled ionization marine solution decreased P2X7 cell-death receptor activation (63.4% compared to control, $P<0.001$).

Discussion

Multipurpose solutions were introduced to replace oxidative products, which disinfect contact lenses by killing germs but do not rinse them. In 2001 in France, 59% of contact lens wearers used multipurpose solutions whereas 35% used oxidative products; 6% of wearers used unknown regimen (according to the French syndicate of contact lens manufacturers, Syffoc). Multipurpose solutions are far cheaper than oxidative products; the number of patients using oxidative products has consequently dropped in the last few years. The concentrations of active principles contained in multipurpose solutions are very low, about 500 times less than the usual concentration active against amoeba to cure keratitis. This is the reason why the concentrations used in multipurpose solutions may not be efficient against cysts and trophozoites usually responsible for contact lens infection. Indeed, Beattie *et al*,[11] published that several multipurpose solutions did not demonstrate efficacy against *Acanthamoeba castellanii* trophozoites and cysts. Besides, it has already been reported that Opti-free multipurpose solution was unable to disinfect contact lens inoculated with *Pseudomonas aeruginosa* and was found to fail to meet the FDA stand-alone disinfectant acceptance criteria for *Staphylococcus aureus*, *Serratia marcescens*, and *Candida albicans*.[12,13] Moreover, contact lens storage containers are frequently contaminated by Gram-negative and Gram-positive bacteria, which was revealed in particular with multipurpose solutions. Most of the germs found in contact lens storage cases are the same as those found in corneas. Contact lenses were contaminated during soaking time in containers and then contaminated the cornea. Moreover, 75% of contaminated contact lens containers were multicontaminated. The misuse of solutions by patients is often given as an explanation for ocular infection associated with contact lens wear. Packaging of contact lens care regimens include a list of instructions that patients should follow to avoid any risk of infection. The fact is that efficacy standards concerning multipurpose solutions are less exacting than those concerning oxidative products. The current bactericide requirements (ISO 14729 standard) are far lower than those concerning oxidizing disinfectants (EN 1040 standard): −1 to −3log *vs* −5log bacteria inhibition. The active constituents (quaternary ammoniums, polyhexanides) used at low concentration in multipurpose solutions should be considered as preservatives rather than disinfecting agents. This could explain the severe ocular infections that the CDC has observed lately.

Preservatives used in ophthalmology such as benzalkonium chloride are known to be toxic to ocular surface;[14] we thus evaluated the cytotoxic potential of different multipurpose solutions.

Controlled ionization marine solution, an unpreserved rinse solution, was not significantly different from cellular control, neither relative to caspase activity, nor to chromatin condensation. In contrast, controlled ionization marine solution slightly decreased P2X7 cell-death receptor activation, which could reflect a protection mechanism against P2X7 pore opening.

Both Opti-free and Solocare were thus found cytotoxic by an apoptotic mechanism; Opti-free containing quaternary ammonium induced apoptosis with high P2X7 receptor stimulation possibly leading to cytolysis, whereas Solocare containing biguanide rather induced

Table 2 Bacteria in cornea and contact lens cases disinfected with multipurpose solutions

Cornea	*Lens storage container*	*Cornea*	*Lens storage container*
Staphylococcus hominis	*Pseudomonas aeruginosa* *Serratia marcescens* *Candida fumata* *Hartmanella*	*Pseudomonas aeruginosa*	NC
Acanthamoeba	NC	*Pseudomonas aeruginosa*	*Pseudomonas aeruginosa* *Proteus mirabilis*
Serratia marcescens	NC	*Acanthamoeba*	*Acanthamoeba* *Klebsiella oxytoca* *Flavobacterium meningosepticum* *Penicillium* *Exophialia*
Acanthamoeba	NC	*Staphylococcus epidermidis*	NC
Pseudomonas aeruginosa *Staphylococcus hominis*	NC	*Staphylococcus aureus*	NC
Pseudomonas aeruginosa	*Pseudomonas aeruginosa* *Citrobacter freundii*	*Pseudomonas aeruginosa*	*Pseudomonas aeruginosa* *Proteus mirabilis* *Enterococcus faecalis* *Candida krusei*
Acanthamoeba	NC	NC	*Serratia marcescens* *Pseudomonas aeruginosa* *Klebsiella virosa* *Acanthamoeba*
Pseudomonas aeruginosa	*Pseudomonas aeruginosa* *Enterobacter cloacae*	*Staphylococcus epidermidis*	*Propionibacterium acnes*
Staphylococcus aureus *Streptococcus sanguis*	NC	*Pseudomonas aeruginosa*	*Pseudomonas aeruginosa* *Stenotrophomonas maltophilia*
Pseudomonas aeruginosa *Staphylococcus epidermidis*	*Pseudomonas aeruginosa* *Flavobacterium indologenes* *Serratia liquefaciens*	*Pseudomonas aeruginosa* *Staphylococcus capitis*	NC
Pseudomonas aeruginosa	*Pseudomonas aeruginosa* *Staphylococcus* coagulase	*Propionibacterium acnes* *Staphylococcus epidermidis*	NC
Staphylococcus simulans	NC	*Pseudomonas aeruginosa* *Propionibacterium acnes*	*Pseudomonas aeruginosa* *Serratia marcescens*
Staphylococcus epidermidis	NC	*Propionibacterium acnes*	NC
Pseudomonas aeruginosa	*Pseudomonas aeruginosa* *Proteus mirabilis* *Enterobacter cloacae*	*Staphylococcus epidermidis* *Propionibacterium acnes* *Alternaria alternata*	*Staphylococcus epidermidis*
Streptococcus cricetus	NC	*Propionibacterium acnes*	*Klebsiella oxytoca* *Stenotrophomonas maltophilia* *Candida krusei*
Pseudomonas aeruginosa	NC	NC	*Serratia liquefaciens* *Aeromonas hydrophila*
Staphylococcus epidermidis	*Serratia marcescens*	*Staphylococcus epidermidis*	*Pseudomonas putida*
Pseudomonas aeruginosa	NC	*Staphylococcus epidermidis*	*Enterobacter cloacae*
Propionibacterium acnes	*Klebsiella oxytoca* *Serratia plymuthica*		

Abbreviation: NC, not communicated.
Bacteria found after microbiological analysis of 37 patients' cornea and contaminated storage cases disinfected with multipurpose solutions were reported.

pure apoptosis with chromatin condensation and P2X7 cell-death receptor activation. Both solutions were dependent on caspase 3 pathway. Apoptosis is a cell death strongly different from necrosis. Necrosis is linked to inflammation, whereas apoptosis is theoretically not; this could explain the reason why many studies concluded that multipurpose solutions were not toxic due to the absence of acute inflammatory and necrotic reactions. Indeed, ISO 10993-5 standard, medical device biocompatibility assessment standard, only evaluates necrosis (using the neutral red test); unfortunately, it does not take apoptosis into account. As multipurpose solutions are the last products on contact with lenses, they can impregnate the lens during the soaking time and be released all day long. Indeed, soft contact lenses have the ability to absorb and release chemical

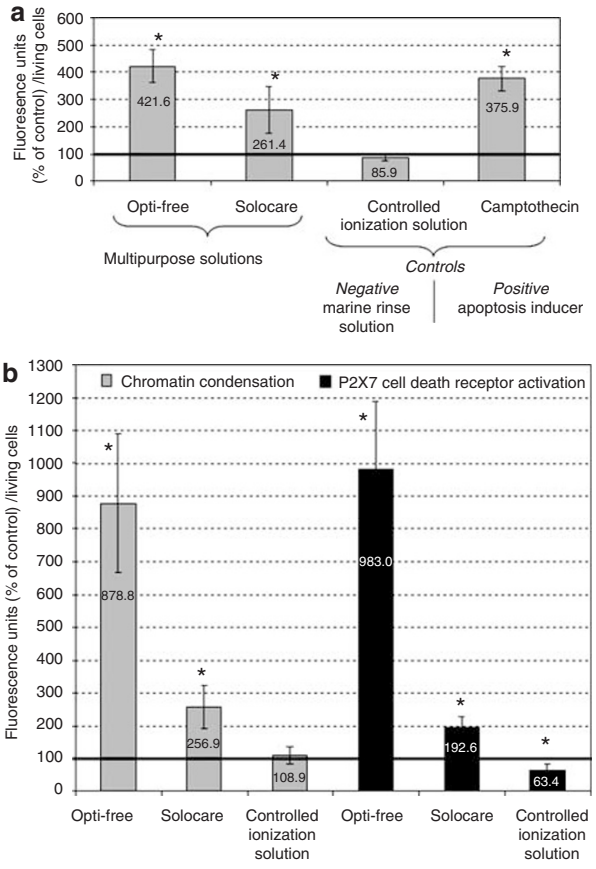

Figure 3 (a) Apoptosis evaluation: caspase 3 activity. Both multipurpose solutions induced caspase 3 on conjunctival cells. $^*P<0.001$ compared to control. (b) Apoptosis evaluation: chromatin condensation and P2X7 activation. Both multipurpose solutions induced chromatin condensation and P2X7 cell-death receptor on conjunctival cells. $^*P<0.001$ compared to control.

substances in aqueous solutions,[15] such as preservatives (quaternary ammonium and biguanide) contained in multipurpose solutions. The cytotoxicity of preservative-containing antiglaucomatous and fluoroquinolone eye drops has already been described.[3,4] Multipurpose solutions are apoptotic and could thus lead to long-term contact lens intolerance. This intolerance is generally associated with dry eye symptom resulting from chronic degenerative and mildly inflammatory changes of the conjunctiva and the corneal surface.[16] Apoptosis induced by multipurpose solutions could decrease the defence systems of the ocular surface, increasing the risk of infection.

Multipurpose solutions seem to be involved in contact lens wear iatrogenic pathologies: ocular infection and contact lens intolerance. Oxidative products (hydrogen peroxide 3%) should be preferred; nevertheless, there are two different oxidative products: two-step peroxides (disinfection and rinse are separated) and one-step peroxides (no rinse is needed as the peroxide is neutralized by catalase tablets or platinium disk). Disinfection and rinse steps should be separated; indeed, rinse solutions in contact with the eye have to be non cytotoxic such as sterile marine rinse solution. In medical hygiene, it is difficult to imagine that the available molecules can be effective against bacteria, viruses, and fungi and, at the same time, usable as rinse solutions without any consequences on epithelial cells. International standards concerning disinfection and cytotoxicity evaluation of multipurpose solutions should be revised.

We report the role of multipurpose solutions in two important and severe ocular pathologies. Oxidative products that are less associated with ocular infection than multipurpose solutions should be preferred for contact lens disinfection. Moreover, as disinfection by two-step oxidative products is followed by a rinse step with an inert saline solution, it could limit tolerance problem.

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