Journal of Toxicology: Cutaneous and Ocular Toxicology

ISSN: 0731-3829 (Print) (Online) Journal homepage: http://www.tandfonline.com/loi/icot19

Evaluation of the Toxicity of Benzalkonium Chloride on the Ocular Surface

CAROLINE DEBBASCH, PATRICE RAT, JEAN-MICHEL WARNET, MAGDA DE SAINT JEAN, CHRISTOPHE BAUDOUIN & Pisella Pierre-Jean

To cite this article: CAROLINE DEBBASCH, PATRICE RAT, JEAN-MICHEL WARNET, MAGDA DE SAINT JEAN, CHRISTOPHE BAUDOUIN & Pisella Pierre-Jean (2000) Evaluation of the Toxicity of Benzalkonium Chloride on the Ocular Surface, Journal of Toxicology: Cutaneous and Ocular Toxicology, 19:2-3, 105-115, DOI: 10.3109/15569520009051506

To link to this article: http://dx.doi.org/10.3109/15569520009051506

Published online: 27 Sep 2008.

J. Toxicol.—Cut. & Ocular Toxicol., 19(2&3), 105–115 (2000)

EVALUATION OF THE TOXICITY OF BENZALKONIUM CHLORIDE ON THE OCULAR SURFACE

*CAROLINE DEBBASCH, Pharm.D.**
PATRICE RAT, Pharm.D., Ph.D.
JEAN-MICHEL WARNET, M.D.
Departments of Cellular Pharmacotoxicology and Ophthalmology
XV-XX Hospital
Faculty of Pharmacy
University of Paris, V
Paris, France

MAGDA DE SAINT JEAN, M.D.
CHRISTOPHE BAUDOUIN, M.D., Ph.D.
PIERRE-JEAN PISELLA, M.D.
Department of Ophthalmology
Ambroise Paré Hospital, AP-HP
University of Paris V Boulogne, France

Abstract

Benzalkonium chloride (BAC), the most widely used preservative in ophthalmic solutions, acts on the ocular surface through toxic or immunoallergic reactions. Due to its surfactant properties, this quaternary ammonium strongly decreases lachrymal fluid stability. It also causes toxic effects to epithelial cells and increases corneal permeability. In vivo, strong histopathological changes are observed after topical treatments with preservatives, including infiltration by inflammatory cells, similar to those induced in humans by long-term topical treatments. We designed a series of in vitro experiments confirming the toxicity of BAC,

* Address reprint requests to: Caroline Debbasch, Pharm.D., Department of Cellular Pharmacotoxicology, XV-XX Hospital, 28, Rue de Charenton, 75012 Paris, France.

even at very low concentrations. In vitro, BAC induces cell necrosis at the concentrations found in most eye drops after a few minutes, and apoptosis when applied at lower concentrations. This apoptotic phenomenon is confirmed by 4′,6-diamidimo-2-phenylindole, dilactate (DAPI) coloration and with the use of flow cytometry. A decrease in cell size is observed with BAC at 0.001% and is confirmed by morphological assay. An overexpression of Apo 2.7 associated with an increase of sub G1 phase cells is also shown. BAC induces irreversible cytotoxic damages with some characteristics of apoptosis in a concentration-dependent manner.

Introduction

Long-term use of antiglaucoma drugs has been associated with toxic as well as inflammatory changes of the ocular surface. However, little is known concerning the accurate mechanisms of such toxic side effects in long-term and/or multitreated glaucoma patients. There is, however, growing evidence that long-term use of topical drugs may produce damage to conjunctival and corneal epithelial cells. Histopathological studies have confirmed that topically applied drugs may exert toxic effects to the corneoconjunctival surface and induce chronic infraclinical inflammation, as shown by the presence of immunological changes and inflammatory infiltrates in multitreated eyes.

The origin of topical inflammation has not yet been clearly determined but benzalkonium chloride (BAC), which is used as a preservative in almost all antiglaucoma preparations, has shown strong evidence of toxicity to the ocular surface. It may act on ocular surface through toxic[1] or immunoallergic reactions.[2] The toxicity can be direct on epithelial and goblet cells or indirect on the lachrymal film, resulting from its tensioactive properties. Furthermore, repeated doses of preserved eyedrops can lead to a cumulative effect and preservatives transferred with ophthalmic devices will have prolonged contact with the epithelium. An iatrogenic disorder may result that could lead to chronic irritation and subsconjuctival fibrosis, which could worsen keratoconjunctivis sicca or increase the risk of failure of trabeculectomy in patients with glaucoma.[3,4]

The effects of ophthalmic preservatives on corneoconjunctival cells have been investigated by scanning electron microscopy,[5,6] tandem scanning confocal microscopy,[7] in vitro studies of cytotoxicity of preservatives to cultured corneal epithelial cells,[8] quantitative evaluation of corneal epithelial permeability, and histological or cytological analyses after exposure to eyedrops of varying composition in humans[9–12] or animals. In vivo studies have been conducted after topical treatment with preservatives and in vitro experiments on human cultured conjunctival cells have also been done. Rare clinical studies have been conducted to compare preservative-free and preserved eyedrops. Conjunctival biopsies taken at the time of glaucoma surgery

have demonstrated a significant increase in immune cells and fibroblasts, possibly related to cumulative treatment with antiglaucoma drugs.[9–11] Impression cytological specimens from long-term-treated patients have shown abnormal induction of inflammatory markers by epithelial cells in about 50% of eyes.[12] These inflammatory and fibrotic anomalies may result in pseudopemphigoid or significant reduction of success rate following glaucoma surgery.[13]

To understand the relative role of preservatives such as benzalkonium chloride in the toxicity of long-term use of antiglaucomatous drugs, we performed a series of in vivo and in vitro experiments.

Different in vivo studies have been conducted. The preservatives most often currently used in topical ophthalmic preparations were tested in rats (cetrimide, benzalkonium chloride, benzododecinium bromide, thiomersal, methylparahydroxybenzoate). Topical drops were administered in both eyes, three times daily for 1 month. Histopathological evaluations and indirect immunochemistry studies were performed. This study demonstrated strong toxic effects of preserved solutions. Preservatives induced corneal epithelial damages and limbal and conjunctival infiltration by immunocompetent cells.[14] Comparison of the different preservatives showed no significant difference in the intensity of pathological effects between the five preservatives tested.

To address the eventual toxicity of beta blockers with or without preservative, another study was conducted in rats using immunochemistry. Benzalkonium chloride as well as unpreserved and preserved timolol were administered four times daily for

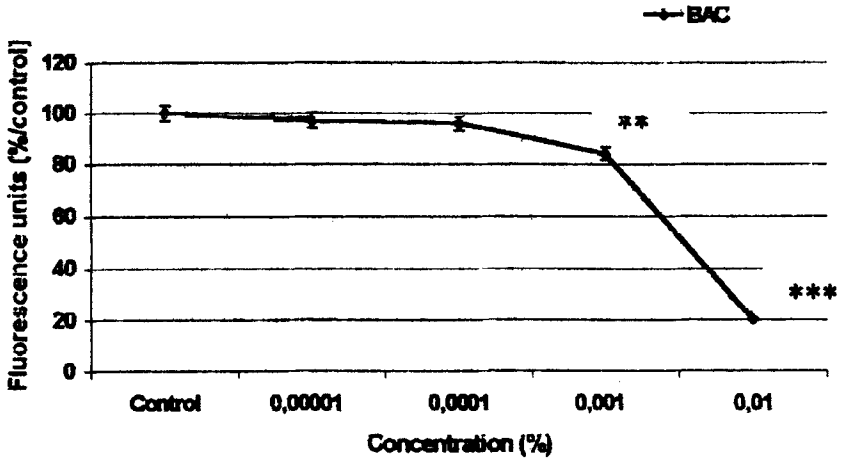

Figure 1. Evaluation of cellular viability after 15 min treatment with BAC. Results are expressed in variation from the control values: ** $p < 0.01$ as compared to control; *** $p < 0.0001$ as compared to control.

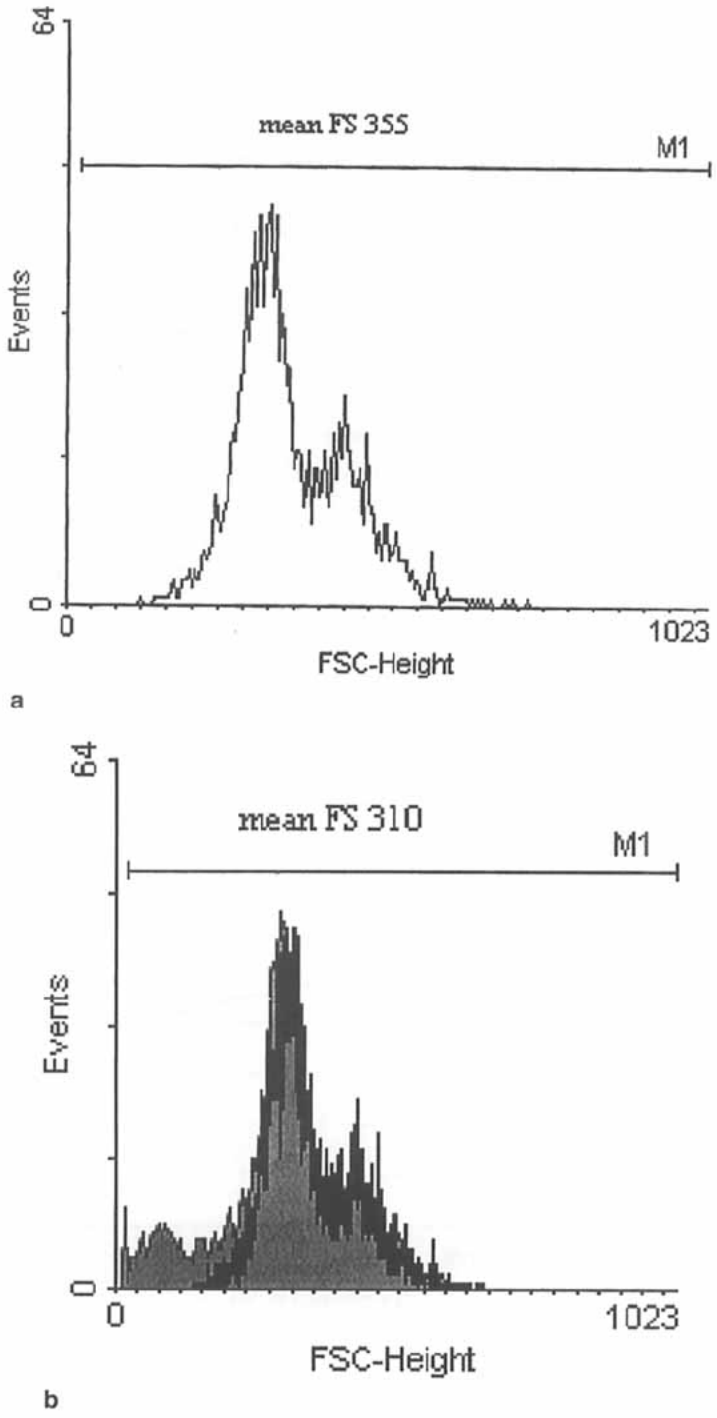

Figure 2. Cell size evaluation by flow cytometry after treatment with BAC. a. Control. b. BAC 0.001%. c. BAC 0.01%. d. BAC 0.1%.

DOCKET ALARM

Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts

Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research

With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips

Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS
Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS
Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS
Sync your system to PACER to automate legal marketing.