Available online at www.sciencedirect.com
ScienceDirect
Toxicology in Vitro 22 (2008) 1070–1076
Toxicology in Vitro
www.elsevier.com/locate/toxinvit

In vitro cytotoxicity of eight β-blockers in human corneal epithelial and retinal pigment epithelial cell lines: Comparison with epidermal keratinocytes and dermal fibroblasts

Hoi I. Cheong, Juanita Johnson, Michel Cormier *, Kamran Hosseini

ALZA Corporation, Drug Device R&D, 1900 Charleston Road, Mountain View, CA 94043, USA

Received 3 October 2007; accepted 21 January 2008
Available online 2 February 2008

Abstract

β-Blockers are a class of agents that have been used extensively in topical preparations for the treatment of glaucoma. Recent evidence indicates that they may also be useful in a number of retinal diseases. Because biocompatibility is of utmost importance in the treatment of ocular-related diseases, we compared the in vitro cytotoxicity, using the MTT assay, of eight clinically available β-blockers (propranolol, alprenolol, atenolol, labetalol, metoprolol, pindolol, timolol, and bisoprolol) on human corneal epithelial and retinal pigment epithelial cell lines. Primary and immortalized corneal and retinal cell lines were compared for their susceptibility to the cytotoxic effect of the drugs. The cytotoxicity of β-blockers was also evaluated on human skin keratinocytes and fibroblasts in order to investigate susceptibility differences as a function of the tissue of origin. Results demonstrated large differences in cytotoxicity (about 60-fold) for these closely related drugs on the same cell line. Conversely, only relatively small differences in cytotoxicity were observed between the different cell lines for the same drug, indicating that the mechanism of cytotoxicity is not cell-specific. Calculation of the ratio between the cytotoxicity of β-blockers and their β-blocking constant is presented as a potential tool to help identify the least irritating, most potent drug.

Keywords: β-Blockers; Human corneal epithelial cell line; Human retinal pigment epithelial cell line; HRPEpiC; HCEpiC; ARPE-19; CRL-11515; Human keratinocytes; Human fibroblasts; Cytotoxicity; Glaucoma

1. Introduction

Glaucoma is a class of ocular diseases characterized by visual field loss as a result of optic nerve damage, with possible progression to blindness. Elevated intraocular pressure (IOP) not only raises the risk of developing glaucoma, but also worsens progression of glaucoma and visual field defects. The current foundation of glaucoma treatment is to lower the IOP, either with topical eye drops or surgery (Weinreb, 2001). β-Blockers are among the arsenal of topical compounds used to reduce the IOP level. Topical β-blockers lower IOP by inhibiting cyclic adenosine monophosphate (cAMP) production in the epithelium of corpus ciliary, thereby lowering aqueous humor production by 20–50% (2.5 μl/min to 1.9 μl/min), which can result in IOP reduction near 20–30% (Frishman et al., 1994; Brooks and Gillies, 1992). During the drug application, these topical compounds must cross the cornea and conjunctiva to reach the site of action in deeper layers of the eye. Thus, the epithelial cells of these ocular structures are the first tissue barriers exposed. We investigated the tolerance of these epithelial cells to topical β-blockers.

Several recent studies suggest that β-blockers have other effects on the neural retina and microvasculature of the posterior segment of the eye that may be beneficial in the treatment of other diseases. Nipradilol, proved to reduce retinal leukostasis in the retinal microcirculation in diabetic rats (Onoa et al., 2006), may have prophylactic indications for diabetic retinopathy. Nipradilol has also been reported to increase retinal blood flow (Kida et al., 2001). Metipra-

* Corresponding author.
E-mail address: michel.cormier@sbcglobal.net (M. Cormier).

0887-2333/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tiv.2008.01.013

nolol has shown antioxidant properties (Melena and Osborne, 2003), which proved to be efficacious in reducing oxidative injury to retinal pigment epithelium (RPE) cells and photoreceptors (Osborne and Wood, 2006). In another study, metipranolol lowered photoreceptor apoptosis induced by sodium nitroprusside (Osborne and Wood, 2004). Betaxolol has shown neural protection by suppressing glutamate-gated currents and sodium currents in ganglion cells (Gross et al., 1999). Neuroprotective effects have been reported with other β-blockers, such as timolol (Wood et al., 2003). The potential beneficial effects of β-blockers makes local application to the retina attractive, but the potential for toxicity to this tissue would require additional biocompatibility studies.

One of the first steps in drug development is toxicity screening. It is well documented that in vitro cytotoxicity is indicative of irritation potential (Bracher et al., 1987–1988; North-Root et al., 1985). As an alternative to in vivo toxicity testing, in vitro cell culture models were used in this study, as they are more ethical and conditions are better controlled.

Although there is a large body of work comparing the toxicity of drugs and surfactants using ocular-derived cells and tissue culture (Borenfreund and Borrero, 1984; Burgalassi et al., 2001; Grant et al., 1992), there is no documented work evaluating the cytotoxicity of a significant series of β-blockers in different ocular cells. This is surprising, considering that these compounds have been extensively used in eye drops for the treatment of glaucoma. Although most of the β-blockers have similar molecular sizes (molecular masses between 250 and 320 Da) and ionization constants (bases with pK_a∼9), their lipophilicity varies over a wide range (Schoenwald and Huang, 1983; Wang et al., 1991), which may affect biocompatibility.

We decided to compare the cytotoxicity of a number of these agents on primary and immortalized cultures of corneal epithelial cells and retinal pigment epithelial cells. The corneal epithelial cells are likely to be exposed to the highest drug concentrations following topical application and are therefore one of the best markers for ocular irritation. Since retinal pigment epithelial cells are essential for adequate neural retina attachment (Hornof et al., 2005) and are in close contact with the retinal layer itself, they may represent a good marker for retinal toxicity. In addition, it was of interest to understand if there were differences in susceptibility between primary and immortalized cell lines. Finally, the cytotoxicity of β-blockers was also evaluated on human skin keratinocytes and fibroblasts in order to evaluate susceptibility differences as a function of the origin of the tissue. Such comparisons are particularly relevant because keratinocytes and corneal epithelial cells function at an air-tissue interface, and fibroblasts reside in the skin and the cornea. The final goal of the study was to rank the cytotoxicity of the β-blocking agents which combined with potency data, could help identify the least irritating and most potent β-blocking agent for the topical treatment of ocular-related diseases.

2. Materials and methods

2.1. Cell culture

Four cell lines derived from ocular tissues were used in this study. They included primary and immortalized cultures of human retinal pigment epithelial cells and corneal epithelial cells. Primary human retinal pigment epithelial cells (HRPEpiC) and corneal epithelial cells (HCEpiC) were purchased from ScienCell (Carlsbad, CA). Culture flasks (Corning, Lowell, MA) for the primary cells were pre-coated with poly-L-lysine and sterile water the night before cell seeding. HRPEpiC were maintained in epithelial cell medium (EpiCM) supplemented with 1% epithelial cell growth supplement (EpiCGS), 2% fetal bovine serum (FBS), and 1% penicillin/streptomycin (P/S). HCEpiC were maintained in keratinocyte medium (KM) supplemented with 1% keratinocyte growth supplement (KGS) and 1% P/S. Immortalized human retinal pigment epithelial cells (ARPE-19) and corneal epithelial cells (CRL-11515) were purchased from American Type Culture Collection (ATCC, Manassas, VA). ARPE-19 did not require coating and were maintained in a 1:1 mixture of Dulbecco's Modified Eagle's Medium and Ham's F12 medium with L-glutamine supplemented with 10% FBS. Culture flasks for CRL-11515 were pre-coated with a solution of 0.01 mg/ml bovine plasma fibronectin, 0.03 mg/ml collagen solution, and 0.01 mg/ml albumin from bovine serum. CRL-11515 cells were maintained in KM supplemented with 1% KGS and 1% P/S. All cell lines were maintained at 37 °C, 100% relative humidity and 5% CO_2.

Two cell lines derived from human skin were also used in this study for comparison with the ocular cells. Adult human epidermal keratinocytes were purchased from Lonza (formerly Clonetics, Walkersville, MD). The cell line was maintained with KM supplemented with KGS and 1% P/S. Neonatal human skin fibroblasts (HDF 5474) were obtained from ATCC (Manassas, VA). The cells were propagated in RPMI 1640 medium (Cellgro, Manassas, VA) supplemented with 5% FBS, 300 mg/l L-glutamine and 1% P/S. The skin cell lines were maintained in the same conditions as the ocular cell lines.

2.2. β-blocker preparation

Eight β-blockers were tested in this study. They included propranolol hydrochloride (Sigma, St. Louis, MO), alprenolol hydrochloride (Sigma), atenolol (Sigma), labetalol hydrochloride (Sigma), metoprolol tartrate salt (Sigma), pindolol (Sigma), timolol maleate salt (Sigma), and bisoprolol free base (Chempacific, Baltimore, MD). Compounds were dissolved at the highest concentration tested in cell culture media. Bisoprolol, atenolol, and pindolol were salified by addition of 1 N hydrochloric acid (Ricca Chemical Company, Arlington, TX) to cell culture medium before further dilution. Timolol maleate salt was neutralized using 1 N sodium hydroxide (Ricca Chemical Com-