Available online at www.sciencedirect.com
SCIENCE DIRECT®
International Journal of Pharmaceutics 290 (2005) 83–89
international journal of pharmaceutics
www.elsevier.com/locate/ijpharm

Synergistic effect of EDTA and boric acid on corneal penetration of CS-088

Takayuki Kikuchi[a,*], Masahiko Suzuki[a], Akira Kusai[a], Ken Iseki[b], Hitoshi Sasaki[c]

[a] *Pharmaceutical Development Laboratories, Sankyo Co., Ltd. 1-12-1, Shinomiya, Hiratsuka, Kanagawa 254-0014, Japan*
[b] *Department of Clinical Pharmaceutics and Therapeutics, Graduate School of Pharmaceutical Science, Hokkaido University, Kita-12-jo, Nishi-6-chome, Kita-ku, Sapporo 060-0812, Japan*
[c] *Department of Hospital Pharmacy, Nagasaki University School of Medicine, 1-7-1 Sakamoto, Nagasaki 852-8501, Japan*

Received 10 September 2004; received in revised form 18 November 2004; accepted 21 November 2004

Abstract

In order to investigate the effects of EDTA and boric acid (EDTA/boric acid) on the corneal penetration of CS-088, an ophthalmic agent, the apparent permeability coefficient of CS-088 in the presence of EDTA/boric acid across the isolated corneal membranes of rabbits was measured using an in vitro penetration chamber system. FITC-dextran (M.W. 4400) and an electrical method based on membrane resistance were used to provide a quantitative assessment of the enhancing effect of EDTA/boric acid.

The corneal penetration of CS-088 was significantly enhanced in the presence of EDTA/boric acid by approximately 1.6-fold. The permeability-enhancing effect of EDTA/boric acid was apparently synergistic and concentration-dependent on both EDTA and boric acid. The penetration of FITC-dextran, a paracellular marker, and electrical resistance of corneal membranes were not affected in the presence of EDTA/boric acid. Furthermore, no enhancing effect of EDTA/boric acid was observed in de-epithelialized corneas, although de-epithelialized corneas exhibited a markedly higher permeability of CS-088 that was 24-fold greater than that for intact corneas. In conclusion, EDTA/boric acid synergistically enhances the transcellular permeability of CS-088 in the outer layer but not in the inner layers of the corneal membrane.

Keywords: EDTA; Boric acid; Synergistic effect; Penetration enhancers; Corneal penetration

1. Introduction

Topical instillation is the drug application method most commonly used in ophthalmology. To improve the efficacy of drugs, various types of enhancers have

* Corresponding author. Tel.: +81 463 31 6442; fax: +81 463 31 6475.
E-mail address: tkikuc@sankyo.co.jp (T. Kikuchi).

0378-5173/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2004.11.019

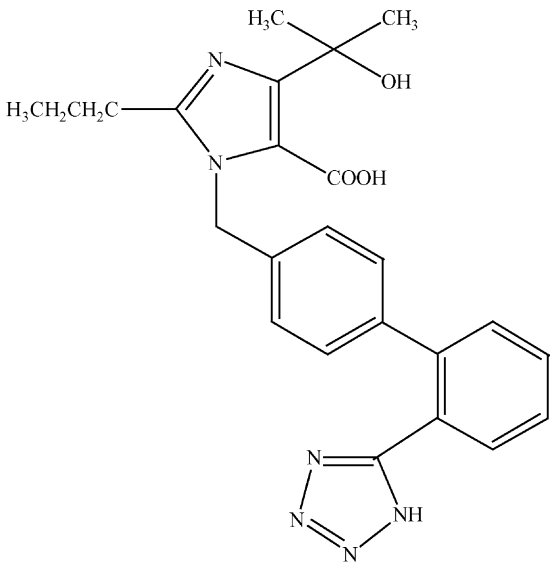

Fig. 1. Chemical structure of CS-088.

been added to drug formulations. Surfactants (Marsh and Maurice, 1971), bile salts (Sasaki et al., 1995a; Saettone et al., 1996), preservatives (Chamber and Edman, 1987; Ashton et al., 1990; Sasaki et al., 1995b; Madhu et al., 1996), or chelating agents (Sasaki et al., 1995c; Madhu et al., 1996; Saettone et al., 1996) are used to promote corneal penetration of the ophthalmic agents. However, these enhancers generally exhibit their effects by inducing morphological changes in the corneal membrane and occasionally lead to adverse effects such as irritation, in large doses (Durand et al., 1989; Rojanasakul et al., 1990; Grant et al., 1992; Jean et al., 2000; Meaney and O'Driscoll, 2000; Monti et al., 2002). Therefore, the amount of penetration enhancers should be minimized to prevent undesirable side effects.

CS-088 is a novel type of anti-glaucoma agent, an angiotensin AT_1 receptor antagonist (Inoue et al., 2001a,b), which is currently undergoing clinical studies (Fig. 1). CS-088 ophthalmic solution contains EDTA and boric acid as a stabilizer and a buffering agent, respectively. It was previously revealed that the in vivo pharmacological activity of CS-088 in rabbits was synergistically enhanced by a simultaneous application of EDTA and boric acid (unpublished data). This synergistic increase is advantageous for formula development because the amount of pharmaceutical additives required in a drug product can be reduced while maintaining the same pharmacological activity. This study was conducted to investigate the effects and mechanism of EDTA/boric acid on the corneal penetration of CS-088 ophthalmic agent using an in vitro penetration chamber system.

2. Materials and methods

2.1. Materials

CS-088 (4-(1-hydroxy-1-methylethyl)-2-propyl-1-1[[2′-[1H-tetrazol-5-yl]biphenyl-4-yl]-methyl] imidazole-5-carboxylic acid) was prepared in the Process Development Laboratories, Sankyo Co., Ltd. FITC-dextran (average molecular weight 4400 Da, FD-4K) was purchased from Sigma Chemical Company (St. Louis, MO). EDTA and boric acid were purchased from Kanto Chemical Co., Ltd. and Iwai Kagaku Co., Ltd., respectively. Methyl parahydroxybenzoate (MP) and propyl parahydroxybenzoate (PP) were purchased from Ueno Pharmaceutical Co., Ltd. All other chemicals used in this study were of reagent grade or of the highest possible grade.

2.2. In vitro penetration experiments

Male New Zealand White rabbits, weighing about 2.5–3.0 kg each, were sacrificed by administering an overdose of a sodium pentobarbital solution via the marginal ear vein. All experiments in the present study adhered to the "Principles of Laboratory Animal Care" (NIH publication #85-23, revised in 1985). The corneas were dissected and mounted in a penetration chamber (Iwata et al., 1980). For some experiments, the corneas were de-epithelialized by carefully scraping away the corneal epithelium with a scalpel until the stroma was exposed.

Test solution (4% CS-088 or 0.005% FD-4K, 2 mL) containing ophthalmic preservatives (0.033% MP and 0.018% PP) with or without additives (EDTA, boric acid) was added to the epithelial side (donor side) of the penetration chamber. Saline solution (2 mL) whose osmolarity was equalized to that of each test solution with sodium chloride was added to the endothelial side (receiver side). The solutions in each chamber were stirred gently with magnetic stirrers. The apparatus was maintained at 34 °C throughout the experiment. Sam-