Available online at www.sciencedirect.com
SCIENCE DIRECT®
International Journal of Pharmaceutics 294 (2005) 11–21
international journal of pharmaceutics
www.elsevier.com/locate/ijpharm

Absorption-enhancing effect of glycyrrhizin induced in the presence of capric acid

Teruko Imai [a,*], Michinori Sakai [b], Hiroshi Ohtake [b], Hidekazu Azuma [a], Masaki Otagiri [a]

[a] *Graduate School of Pharmaceutical Sciences, Kumamoto University, 5-1 Oe-honmachi, Kumamoto 862-0973, Japan*
[b] *Fundamental Research Laboratories, Hisamitsu Pharmaceutical Co. Inc., 1-25-11 Kannondai, Tsukuba, Ibaraki 305-0856, Japan*

Received 25 July 2004; received in revised form 8 December 2004; accepted 8 December 2004

Abstract

The absorption-enhancing effect of the simultaneous administration of sodium caprate (Cap-Na) and dipotassium glycyrrhizinate (Grz-K) was investigated to clarify an effect of Grz-K. A combination of 0.1% (w/v) Cap-Na and 2% (w/v) Grz-K had a rapid and long-lasting absorption-enhancing activity in Caco-2 cell monolayers under conditions where Cap-Na and Grz-K showed a weak and no activity, respectively. The simultaneous treatment of a Caco-2 cell monolayer with Cap-Na and Grz-K showed no change in intracellular calcium ion level, although a major mechanism of absorption-enhancing effect for Cap-Na was elevation of intracellular calcium ion level. On the other hand, the simultaneous enhancing effect of Cap-Na and Grz-K was inhibited by H7, a PKC inhibitor. Possibly, Grz-K showed an absorption-enhancing effect via PKC cellular signaling pathway after penetration into cell according to increasing membrane permeability by Cap-Na. The absorption of sCT by the rat colon was enhanced by a combination of 0.1% (w/v) Cap-Na and 2% (w/v) Grz-K, and its effect continued even 9 h after the onset of the experiment. Furthermore, the simultaneous treatment of 0.1% (w/v) Cap-Na and 2% (w/v) Grz-K showed a negligible histological changes to the colon mucosal membrane and a negligible toxicity on Caco-2 cell monolayer. A combination of Cap-Na and Grz-K shows a synergistic absorption-enhancing effect with little mucosal injury, which is applicable to colon-specific delivery.

Keywords: Absorption enhancer; Sodium caprate; Glycyrrhizin; Caco-2 cell; Colon delivery

Abbreviations: Cap-Na, sodium caprate; Grz-K, dipotassium glycyrrhizinate; TEER, transepithelial electrical resistance; Flu-Na, sodium fluorescein; FD-4, fluorescein isothiocyanate-dextran 4000; EDTA, sodium ethylenediaminetetraacetate; sCT, salmon calcitonin; IP_3, inositol 1,3,4-triphosphate; PKC, protein kinase C; HBSS, Hanks' balanced salt solution; HBSS/CMF, Hanks' balanced salt solution/calcium–magnesium-free; H7, 1-(5-isoquinolinesulfonyl)-2-methylpirerazide dihydrochloride

* Corresponding author. Tel.: +81 96 371 4626; fax: +81 96 371 4639.
E-mail address: iteruko@gpo.kumamoto-u.ac.jp (T. Imai).

0378-5173/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2004.12.029

1. Introduction

Oral drug delivery is still preferred by a majority of patients. Delivery systems in the colon are important in some cases, to minimize side effects and to maximize the therapeutic response of drugs. Colon delivery system can be improved in several ways. The timed-release system (Pulsinap®, Geomatrix®), pH-dependent coating (Hu et al., 1999), microbially degradable polymers (Prasad et al., 1998), intestinal pressure-controlled system (Jeong et al., 2001) and redox-sensitive polymer (Stubbe et al., 2001) are commonly used for this purpose. When drugs reach the colon via the delivery system, they should be effectively absorbed from the colon lumen. A successful way to improve drug absorption in the colon is the use of absorption enhancers. It is necessary to select a highly useful enhancer that induces potent and long-lasting absorption-enhancing activity without no or minimal mucosal injury.

We previously examined the absorption-enhancing activity of several representative absorption enhancers, i.e., sodium deoxycholate (Deo-Na), a bile acid, sodium caprate (Cap-Na), a fatty acid, and dipotassium glycyrrhizinate (Grz-K), a terpene (Sakai et al., 1997). In a study using an intestinal epithelial cell model, Caco-2 cell monolayer (Pinto et al., 1983; Hidalgo et al., 1989; Artursson, 1990), we found potent absorption-enhancing activity in the cases of Deo-Na and Cap-Na. Cap-Na induced a rapid response, while Deo-Na showed a relatively slow response. On the other hand, Grz-K that possessed low membrane permeability due to hydrophilicity rarely showed an effect on drug permeability through a Caco-2 cell monolayer, although it has been reported to enhance the in vivo transmucosal absorption of antibiotics and insulin (Tanaka et al., 1992; Mishima et al., 1989). Furthermore, we demonstrated that the discrepancy between the in vitro and in vivo study can be explained by the hydrolysis of Grz to its aglicon, glycyrrhetic acid, by bacterial β-glucuronidases in the intestinal lumen (Imai et al., 1999). Glycyrrhetic acid had a more potent enhancing activity than Cap-Na and Deo-Na at the same concentration. In addition, we examined the absorption-enhancing activity of simultaneous treatment with Deo-Na and Grz-K (Sakai et al., 1999). Consequently, the absorption-enhancing effect of a combination of Grz-K and Deo-Na was much greater than that of Deo-Na alone. However, the response of their simultaneous treatment was similar to Deo-Na. Moreover, we found that the simultaneous enhancing response was related to a cellular signaling system mediated by protein kinase C, the same mechanism as was found for Deo-Na (Qiao et al., 2000; Milovic et al., 2002). Therefore, two possible explanations for an effect of simultaneous treatment were considered. (1) The activity of Deo-Na was enhanced in the presence of Grz-K. (2) After transport into Caco-2 cell, Grz-K itself showed absorption-enhancing effect by a same mechanism as Deo-Na. If Grz-K itself possessed an absorption-enhancing response related to a cellular signaling system, in vivo activity of Grz-K was not induced by only a hydrolyzed product, glycyrrhetic acid, but also Grz itself. To demonstrate an absorption-enhancing effect of Grz-K itself is important for a pharmaceutical modification with Grz-K.

In this study, we have investigated an absorption-enhancing effect of Grz-K itself by means of a simultaneous treatment with Cap-Na in Caco-2 cell. It has been reported that Cap-Na is capable of opening the paracellular route, when intracellular calcium concentrations are increased via inositol 1,3,4-triphosphate (Tomita et al., 1995). Therefore, the absorption-enhancing effect of Grz-K could be characterized by a comparison of the simultaneous effect of Cap-Na and Grz-K and the effect of Cap-Na itself. Furthermore, the in vivo efficacy of a combination of Cap-Na and Grz-K has been confirmed by the absorption of salmon calcitonin (sCT) in the rat colon. The safety of a combination of Cap-Na and Grz-K has been evaluated by examining a morphological changes in the rat colon mucus membrane.

2. Materials and methods

2.1. Materials

Caco-2 cells were purchased from the American Type Culture Collection (Rockville, MD, USA). Sodium fluorescein (Flu-Na), fluorescein isothiocyanate dextran (FD-4), 1-(5-isoquinolinesulfonyl)-2 methylpiperazide dihydrochloride (H7), Dulbecco's modified Eagle's medium (DMEM), non-essential amino acids, benzylpenicillin G, streptomycin and sodium ethylenediaminetetraacetate (EDTA) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Fura-2 acetoxymethyl ester (Fura-2 AM) and