Involvement of Influx and Efflux Transport Systems in Gastrointestinal Absorption of Celiprolol

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Received 16 May 2008; revised 28 August 2008; accepted 27 September 2008

Published online 9 December 2008 in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/jps.21618

ABSTRACT: Gastrointestinal absorption of several β-blockers is inhibited by citrus juices, although molecular mechanism(s) lying on their small intestinal absorption has not yet been identified. Here, we attempted to demonstrate involvement of both influx and efflux transporters *in vivo* in gastrointestinal absorption of celiprolol in mice. Plasma concentration of celiprolol (3 mg/kg) after oral administration was mostly under the limit of quantification in wild mice, whereas that in *mdr1a/b* knockout ($mdr1a/b^{(-/-)}$) mice was much more obvious, indicating P-glycoprotein-mediated efflux. Then, the oral absorption of celiprolol in $mdr1a/b^{(-/-)}$ mice was further examined to investigate influx transport mechanism with avoiding effect of P-glycoprotein. Coadministration of bromosulfophthalein (BSP), an inhibitor of various influx transporters including organic anion transporting polypeptide (OATP) reduced plasma celiprolol concentration. Inhibition by BSP of celiprolol uptake from apical membranes was confirmed in Ussing-type chamber of small intestinal tissues. Uptake of celiprolol by human small intestinal transporter OATP-A/1A2 was also confirmed in *Xenopus Laevis* oocytes. Interestingly, OATP-A/1A2 accepts various β-blockers including acebutolol, atenolol and sotalol, oral absorption of which is inhibited by coadministration of citrus juice or telithromycin in human. Taken together, these findings have suggested fundamental role of influx transport system(s) in oral absorption of celiprolol. © 2008 Wiley-Liss, Inc. and the American Pharmacists Association J Pharm Sci 98:2529–2539, 2009

Keywords: absorption; active transport; transporters; membrane transporter; organic anion-transporting polypeptide transporters; P-glycoprotein; intestinal secretion/transport

INTRODUCTION

β-Blockers have been clinically used for the treatment of various types of cardiovascular diseases including hypertension, myocardial infarction, angina pectoris and arrhythmia. They are also recently prescribed for chronic heart failure, although such application is still under the clinical trial in Japan.[1,2] In most cases, β-blockers are orally administered to the patients because of their adequate absorption. The mechanism for the gastrointestinal absorption of β-blockers has not yet been fully identified, but the earlier research done by Taylor et al.[3] has proposed two different types of absorption mechanism for hydrophilic (atenolol, nadolol, practolol, and sotalol with log *P* values from −0.79 to 0.76) and lipophilic (alprenolol, metoprolol, oxprenolol, pindolol, propranolol, and timolol with log *P* values from 1.75 to 3.65) β-blockers: The absorption rate constant for hydrophilic ones is almost identical among the

Abbreviations: OATP, organic anion transporting polypeptide; P-gp, P-glycoprotein; FD-4, FITC-dextran (Mw 4000); IS, internal standard; E3S, estrone-3-sulfate; BSP, bromosulfophthalein.

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Journal of Pharmaceutical Sciences, Vol. 98, 2529–2539 (2009)

compounds, whereas that for lipophilic ones depends on octanol-–water partition and is consistent with pH-partition theory.[3]

The unique properties in gastrointestinal absorption of β-blockers include possible involvement of efflux transporter(s) in their small intestinal absorption.

Bioavailability in human of acebutolol and celiprolol increases as the increase in dose,[4,5] probably due to the saturation of the efflux transport system in small intestine. Identification of the active efflux systems for β-blockers in small intestine was first proposed in our laboratory by the previous observation that cyclosporine A, an inhibitor for P-glycoprotein (P-gp) increased absorption rate constant of several β-blockers including acebtolol, atenolol, celiprolol and nadolol in rat *in situ* jejunum loop.[6] Among the β-blockers, celiprolol is one of the best characterized P-gp substrates and has also been suggested to be actively pumped out by P-gp in rat small intestine[7] and human intestinal Caco-2 cells.[8–10] In human, oral absorption of celiprolol was reported to increase by itraconazole,[11] and this effect could be accounted for by the inhibition of efflux transport system, possibly P-gp, if we consider minor contribution of metabolism to systemic elimination of celiprolol.

On the other hand, recent clinical investigations have clarified that grapefruit juice decreased oral absorption of acebutolol, atenolol, celiprolol, and talinolol.[11–14] Oral absorption of atenolol and celiprolol was also reduced by orange juice.[13,15] Coadministration of therapeutic agents including verapamil and telithromycin has also been reported to reduce oral bioavailability of talinolol and sotalol, respectively.[16,17] One of feasible hypotheses may be that a certain constituents in the juice inhibits uptake process of these β-blockers from apical side in the small intestinal epithelial cells, although such drug–food interactions may also be explained by other possibilities including inhibitory effect on gastric emptying rate and/or lowering effect on intestinal pH, leading to decrease in unionized form of β-blockers. Possible involvement of the influx transporters has already been suggested for gastrointestinal absorption of fexofenadine, which could be mediated by organic anion transporting polypeptide (OATP)-A (OATP1A2),[18,19] although the influx transporters for β-blockers has not yet been identified.

The drug–food interaction for β-blockers decreases their systemic exposure after oral administration. Therefore, molecular identification of intestinal transporter(s) responsible for the oral absorption should be important to avoid any unexpected drug–drug or drug–food interaction. However, limited information is available especially on influx transporters for β-blockers from apical membranes in small intestine. The purpose of the present study is to demonstrate involvement of influx (uptake) transporter(s) for celiprolol, since inhibitory effect of grapefruit juice on oral absorption of celiprolol was most obviously reported among the β-blockers.[11–14] After oral absorption, celiprolol is mainly excreted into urine as an unchanged form,[1,20,21] suggesting minor contribution of metabolism to the systemic elimination. Kirby and Unadkat[22] have already referred to *in vitro* data for celiprolol and talinolol being substrates for influx and efflux transporters in humans. However, there has been no direct demonstration regarding the involvement of both types of transporters in human small intestine possibly due to the limited availability of experimental systems in humans. On the other hand, the present study was aimed to use experimental animals to demonstrate the involvement of transporters in small intestinal tissues. For such purpose, involvement of the efflux transporter (such as P-gp) in small intestine may hinder the analysis of influx transporter since inhibition of the efflux transporter may compensate the inhibition of influx transporters. Then, the *mdr1a/b*$^{(-/-)}$ mice were further used to focus on the influx transporter(s) for celiprolol to avoid any interference by P-gp. To demonstrate involvement of influx transporter(s) in small intestine, bromosulfophthalein (BSP) was used as an inhibitor of various influx transporters including OATP in both *in vivo* and Ussing-type chamber system. To further clarify possible involvement of influx transporters, uptake of celiprolol and other β-blockers was demonstrated and characterized in *Xenopus laevis* oocytes expressing human intestinal transporters OATP-A and OATP-B (OATP2B1), both of which are the influx transporters localized on apical membranes of human small intestine.[19,23]

MATERIALS AND METHODS

Materials

Celiprolol hydrochloride was a gift from Nichi-iko Pharmaceutical Co., Ltd. (Toyama, Japan).

 DOI 10.1002/jps