

Establishment of Novel Prediction System of Intestinal Absorption in Humans Using Human Intestinal Tissues

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ABSTRACT: The objective of this study was to establish a novel prediction system of drug absorption in humans by utilizing human intestinal tissues. Based on the transport index (TI), a newly defined parameter, calculated by taking account of the change in drug concentrations because of precipitation on the apical side and the amounts accumulated in the tissue and transported to the basal side, the absorbability of drugs in rank order as well as the fraction of dose absorbed (Fa) in humans were estimated. Human intestinal tissues taken from ulcerative colitis or Crohn's disease patients were mounted in a mini-Ussing chamber and transport studies were performed to evaluate the permeation of drugs, including FD-4, a very low permeable marker, atenolol, a low permeable marker, and metoprolol, a high permeable marker. Although apparent permeability coefficients calculated by the conventional equation did not reflect human Fa values for FD-4, atenolol, and metoprolol, TI values were well correlated with Fa values, which are described by $100 \cdot [1 - e^{-f \cdot (TI - \alpha)}]$. Based on this equation, Fa values in humans for other test drugs were predicted successfully, indicating that our new system utilizing human intestinal tissues would be valuable for predicting oral drug absorption in humans. © 2013 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 102:2564–2571, 2013

Keywords: human absorption; prediction; mini-Ussing chamber; human tissues; transport index (TI)

INTRODUCTION

Oral administration is the most convenient and common administration route of drugs and many new drug candidates are often developed as an oral dosage; therefore, estimation of the fraction of dose absorbed (Fa) or bioavailability (BA) in the early stage of drug development is very important, but often faces difficulties because of species differences and/or lim-

ited physiological relevance in *in vivo* and/or *in vitro* studies. As a tool for evaluating the membrane permeability of candidates, Caco-2 cell monolayers and the parallel artificial membrane permeability assay (PAMPA) are usually used to predict the absorbability of drugs in humans. PAMPA is useful to estimate the permeability of drugs across the lipid bilayer via passive diffusion and can provide results faster than studies using Caco-2 cells monolayer^{1,2}; however, the contribution of transport via the paracellular route and/or transporters to drug absorption cannot usually be ignored. Because Caco-2 cells have functional and morphological properties of small intestinal epithelial

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cells, they are often used to identify potential interactions with efflux transporters that could limit the intestinal absorption of drugs or prevent the access of drugs to the site of action.³ Cell-based assays utilizing Caco-2 cells and so on make it possible to evaluate the effect of the paracellular route and transporters on drug absorption; however, it is well known that transepithelial electrical resistance and the expression level of several transporters in Caco-2 cells are quite different from those in the human intestine, so predictions based on these *in vitro* systems cannot always successfully estimate the absorbability of drugs in humans.⁴ Preclinical pharmacokinetic studies performed during lead optimization give the first *in vivo* measurement of absorption and can highlight potential limitations of BA caused by intestinal processes; however, the use of mouse, rat, dog, and monkey species at this stage might give contradictory data, leading to significant difficulty in accurately predicting the situation in humans.

Ussing chamber studies have been performed in many laboratories to study the permeability, absorption potential, and/or absorption mechanisms of various compounds and/or new chemical candidates across excised sections of intestinal tissues. The advantage offered by Ussing chamber studies over cultured cell lines and PAMPA is the maintenance of the complex cellular heterogeneity and morphology of the mammalian intestine and structure of epithelial cells, which are not entirely mimicked by the relatively homogeneous cellular monolayer of Caco-2 cells and the artificial membrane of PAMPA. The presence of an extended myriad of transport proteins and drug metabolism enzymes, as well as an intact acid microclimate mucous layer adjacent to the villus tip of enterocytes, results in an experimental system that gives a more complete picture regarding the process that may impact drug absorption^{5–7}; however, species differences in permeability and metabolic capability are already known,^{8–11} suggesting that predictions based on studies using other species' tissues would not be necessarily in accordance with the absorbability of drugs in humans.^{12–15} The usage of human tissue, therefore, has a great advantage for more precise prediction of drug absorbability in humans.

In the present study, human intestinal tissues were used, sourced from inflammatory bowel disease (IBD) patients, such as ulcerative colitis (UC) and Crohn's disease (CD). Values of apparent permeability (P_{app}) calculated by the conventional equation were not in good agreement with results in humans in rank order. The transport index (TI) was developed to take account of tissue accumulation of drugs and changes in drug concentration on the apical side because of precipitation for the evaluation of drug permeability. The validity of TI and prediction of Fa of several drugs in humans based on TI were assessed.

Table 1. Patient Characteristics and Subject Numbers

Characteristics	Subject Number		
	Total	Small intestine	Large intestine
Sex			
Male	13	4	9
Female	4	0	4
Primary disease			
Ulcerative colitis (UC)	7	0	7
Crohn's disease (CD)	10	4	6

Age of subjects ranged between 22 and 70-year old (mean age, 37.1 years old).

MATERIALS AND METHODS

Materials

FD-4, metoprolol, atenolol, nadolol, propranolol, naproxen, ketoprofen, acebutolol, and spiperone were purchased from Sigma-Aldrich (Boston, Massachusetts). Cilostazol was obtained from Otsuka Pharmaceutical Company, Ltd. (Tokushima, Japan). All other reagents used were of the highest purity.

Human Intestinal Tissues

Human small or large intestinal tissues were taken from male or female patients, aged 22–70 years old, with UC and CD (Table 1), with permission from the patients in accordance with the Declaration of Helsinki. All experiments were approved by the institutional review board of all institutions, and written informed consent was obtained from all patients. Noninflamed tissues chosen from the isolated tissues were placed in ice-cold transport buffer immediately after removal from the patient's body in the surgical operation room and were transferred to the experimental room within 1 h. Transport buffer was composed of 128 mM NaCl, 5.1 mM KCl, 1.4 mM CaCl₂, 1.3 mM MgSO₄, 21 mM NaHCO₃, 1.3 mM KH₂PO₄, and 10 mM NaH₂PO₄, adjusted to pH 7.4.

Transport Experiment in Mini-Ussing Chamber

The mini-Ussing chamber shown in Figure 1 was used for transport experiments and the isolated human tissues were mounted following the method reported previously.¹⁶ Briefly, isolated intestinal tissues, from which the muscle layer had been removed with fine tweezers, were mounted vertically in mini-Ussing-type chambers, providing an exposed area of 0.07 cm² for permeation study. The volume of bathing solution on each side was 1.35 mL, and the solution temperature was maintained at 37°C with a water-jacketed reservoir. The bathing solutions on the apical and basal sides were transport buffer containing 5 mM mannitol and 5 mM D-glucose and 1% (w/v) bovine serum albumin, respectively, and the solution was gassed with 95% O₂/5% CO₂ before and during the transport experiment. Model drugs were placed on

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