Effect of Food Intake on the Oral Absorption of Poorly Water-Soluble Drugs: *In Vitro* Assessment of Drug Dissolution and Permeation Assay System

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Received 21 November 2005; revised 25 April 2006; accepted 6 May 2006

Published online in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/jps.20691

ABSTRACT: The aim of the present work was to establish appropriate conditions for the dissolution/permeation system (D/P system) to estimate the effect of food intake on oral drug absorption. The D/P system is an *in vitro* assay system to evaluate the drug dissolution and permeation processes after oral administration. Caco-2 monolayer was used as a model membrane of the intestinal epithelium. In this study, two types of simulated intestinal fluid reflecting the fasted and the fed state conditions of the human gastrointestinal tract were used. Drugs were applied to the D/P system as a powder, then, permeated amounts of drugs into the basal side were monitored. A sigmoidal correlation was obtained between *in vivo* oral absorption (% absorbed of dose) and *in vitro* permeated amount (% of dose/2 h) under both states. From the D/P system, the estimated absorption of albendazole in both states was found to correspond well with *in vivo* observation. Moreover, the D/P system could estimate the effect of self-emulsifying formulation on the oral absorption of danazol, quantitatively. In conclusion, the D/P system was proved to be a useful assay system not only for the oral absorption of drugs, but also for the food effect on the absorption. J Pharm Sci 95:2051–2061, 2006

Keywords: absorption; biopharmaceutics classification system (BCS); Caco-2 cells; dissolution; food effects; *in vitro*/*in vivo* correlations (IVIVC); permeability; solubility

INTRODUCTION

The oral absorption of poorly water-soluble drugs, such as danazol and griseofulvin, is known to increase when they are administered after food intake.[1,2] As a main cause of the effect of food, the secretion of bile juice into the GI tract is accelerated by food intake, which enhances the solubility and dissolution rate and therefore the absorption of these drugs after oral administration. Within pharmaceutical companies, *in vivo* animal studies have been carried out to detect the effect of food on oral absorption of new drug candidates before advancing to clinical studies. However, animal studies often show large variations depending on the species and might result in an uncertain estimation on drug absorption in humans. Therefore, an *in vitro* screening system that can evaluate the effect of food intake on the oral absorption of drugs is strongly desired.

Galia et al.[3] have proposed the use of fasted and/or fed state simulated intestinal fluids (FaSSIF and FeSSIF) in the drug dissolution study to simulate the effect of food on drug dissolution, then on the bioavailability/bioequivalence of commercial drug products. FaSSIF and FeSSIF contain taurocholate (NaTC) and lecithin as bile acid and lipid to mimic the composition of human intestinal fluid. Dressman et al. have reported that FaSSIF and FeSSIF can be used in the drug dissolution test and are useful in formulation

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Journal of Pharmaceutical Sciences, Vol. 95, 2051–2061 (2006)

development.[4,5] On the other hand, Ingels et al. have reported that the use of FaSSIF as the apical medium in the Caco-2 permeation study may improve the biorelevance of the model to evaluate the oral absorption in humans.[6–8]

Both approaches with simulated intestinal fluids may not be sufficient to evaluate the effect of food intake on the total processes of oral drug absorption, because they consist of two processes, the dissolution and permeation of drugs, which occur sequentially in the GI tract. In order to evaluate the oral absorption of poorly soluble drugs, we have developed an *in vitro* system with which both dissolution and permeation processes can be analyzed simultaneously. In this system (dissolution/permeation system, D/P system), a Caco-2 monolayer is mounted in the side-by-side chambers. Both the apical and basal sides of the monolayer are filled with media and are constantly stirred. Drugs are applied to the apical medium as a solid form (powder or granule); then, dissolution in the apical side and permeation to the basal side of the drug are simultaneously monitored. In the previous report, it was demonstrated that the amount of drug that permeated to the basal side of the Caco-2 monolayer showed a sigmoidal correlation to their absorption *in vivo* in humans, regardless of the class of drugs in the Biopharmaceutics Classification System (BCS). Consequently, the D/P system is a useful tool to evaluate the oral absorption of poorly soluble drugs after oral administration as the solid dosage form.[9]

Ginski et al. have reported the use of the continuous dissolution/Caco-2 system to investigate drug absorption.[10,11] They observed the dissolution-absorption relationships and determined the rate limiting process of absorption in rapid and sustained dissolving formulations. Kobayashi et al.[12] have reported a system in which the effect of pH change in the GI tract on drug dissolution and permeation can be evaluated. Furthermore, Motz et al. have developed a flow through permeation cell system to simultaneously monitor the dissolution and permeability of drugs.[13,14] Those studies and devices have generated the useful information in considering the dissolution-permeation relationship in oral drug absorption. However, in order to predict the *in vivo* oral absorption of drugs quantitatively from the *in vitro* study, physiological conditions in the human GI tract, such as the fluid volume, fluid composition, and fluid pH must be taken into consideration, especially for the absorption of poorly water-soluble drugs. In our D/P system, the assay conditions were fixed carefully, based on the *in vivo* physiological conditions of the human GI tract to successfully obtain the IVIVC in oral drug absorption.[9]

In this study, we have tried to predict the effect of food intake on oral drug absorption in humans from *in vitro* experiments with the D/P system. Various kinds of simulated intestinal fluids were prepared and applied to the apical side of the D/P system to reflect *in vivo* fasted and fed state conditions in the GI tract. Then, we compared the results for the D/P system with *in vivo* observations in humans.

EXPERIMENTAL

Materials

The Caco-2 cell line was purchased from American Type Culture Collection (Rockville, MD) at passage 17. Dulbecco's modified Eagle medium (D-MEM) was purchased from Sigma-Aldrich (St. Louis, MO). Nonessential amino acids (NEAA), fetal bovine serum (FBS), L-glutamate, trypsin (0.25%)-EDTA (1 mM) and antibiotic-antimycotic mixture (10000 U/mL penicillin G, 10000 μg/mL streptomycin sulfate and 25 μg/mL amphotericin B in 0.85% saline) were purchased from Gibco Laboratories (Lenexa, KS). Danazol was purchased from Sigma-Aldrich. Albendazole, egg-phosphatidylcholine (lecithin), sodium taurocholate (NaTC) and bovine serum albumin (BSA) were purchased from WAKO Pure Chemical Industries, Ltd. (Japan). Gelucire® 44/14 was obtained from GATTEFOSSÉ (France). All other reagents used were of the highest purity.

Preparation of Caco-2 Monolayer

Caco-2 cells were grown in D-MEM supplemented with 10% FBS, 1% L-glutamate, 1% NEAA and 5% antibiotic-antimycotic solution as culture medium at 3°C in culture flasks (Nippon Becton Dickinson Co., Ltd., Tokyo, Japan) in humidified air with a 5% CO_2 atmosphere. Cells were harvested with typsin-EDTA and seeded on polycarbonate filters (0.3 μm pores, 4.20 cm^2 growth area) inside a cell culture insert (Nippon Becton Dickinson Co., Ltd., Tokyo, Japan) at a density of 3×10^5 cells/filter. The culture medium (1.5 mL in the insert and 2.6 mL in the well) was replaced every 48 h for the first 6 days and every 24 h thereafter. After