Impact of Luminal Fluid Volume on the Drug Absorption After Oral Administration: Analysis Based on *In Vivo* Drug Concentration–Time Profile in the Gastrointestinal Tract

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ABSTRACT: The objective of this study is to clarify the influence of fluid volume in the gastrointestinal (GI) tract on the oral drug absorption. *In vivo* rat luminal concentrations of FITC-dextran (FD-4), a nonabsorbable marker, and drugs (metoprolol and atenolol) after oral coadministration as solutions with different osmolarity were determined by direct sampling of residual water in each segment of the GI tract. The luminal FD-4 concentration after oral administration as hyposmotic solution was significantly higher than that after administration as isosmotic or hyperosmotic solution. As the change in FD-4 concentration reflects the change in the volume of luminal fluid, it indicated that the luminal volume was greatly influenced by osmolality of solution ingested orally. Then, fraction of drug absorbed (Fa) in these segments was calculated by comparing the area under the luminal concentration–time curve of FD-4 with those of drugs. Fa values of two model drugs in each GI segment decreased with increase in luminal fluid volume, and the impact of the fluid volume was marked for Fa of atenolol (a low permeable drug) than for that of metoprolol (a high permeable drug). These findings should be beneficial to assure the effectiveness and safety of oral drug therapy. © 2015 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 104:3120–3127, 2015

Keywords: biopharmaceutics classification system; gastrointestinal; luminal volume; oral absorption; osmolality; passive diffusion; permeability

INTRODUCTION

Fluid volume in the gastrointestinal (GI) tract is one of the important factors to determine the luminal drug concentration and thus the pattern of drug absorption. For estimating and/or simulating oral drug absorption, the volume of ingested water (250 mL in USA) has been often used.[1] However, it is obvious that the ingested volume differs from the effective volume in the GI tract for drug dissolution, because of the fluid secretion and absorption. In addition, fluid volume in the GI tract is fluctuated by the food and water intake. The human gastric volume in fasted state has been reported to be less than about 50 mL.[2] After food ingestion, the volume increased to 500–2000 mL.[2,3] Fluctuation of fluid volume by feeding also occurs in the small intestine.[4] However, there are few reports that demonstrate the relation between the fluid volume and drug absorption in the GI tract because of the difficulty to monitor the GI fluid volume *in vivo*.

Secretion or absorption of water across the GI membrane occurs through both the paracellular and transcellular routes. It has been reported that aquaporins, family of water channels, play important role for transport of water molecules via transcellular route.[5–8] Itoh et al.[9] reported that expression of aquaporin 3 was enhanced by vasoactive intestinal polypeptide under diarrheal state in human colonic epithelial cells, increasing the secretion of water and electrolytes into the intestinal lumen.

Intestinal fluid osmolarity in the fasted state has been reported to be 197 mOsm/kg.[10] Postprandial intestinal osmolarity elevated to 368–453 mOsm/kg based on human intestinal aspirates measurements.[10] Meal ingestion also increased the gastric osmolarity about 2.5 times.[11,12] As the movement of water through paracellular route and also via aquaporins is directed by osmotic gradients across the GI membrane,[7] the osmolarity of the GI fluid is one of the most important factors for fluid absorption and secretion.

It is well known that oral absorption of drugs categorized into biopharmaceutics classification system (BCS) class II, high permeable and low soluble drugs, or class III, low permeable and high soluble drugs, is greatly influenced by food intake. Generally, the solubility and dissolution rate of BCS class II drugs in the GI tract are improved by secretion of bile abundantly containing bile acids and phospholipids after meal ingestion, leading increase in their oral absorption.[4,13] On the contrary, the oral absorption of certain low-permeability drugs in BCS class III decreases after ingestion of foods (negative food effect).[4,14] One of the reasons for negative food effect is that dissolved drugs interact with

Abbreviations used: $AUC_{GI(drug)}$, area under the luminal concentration–time curve of drug; $AUC_{GI(FD\text{-}4)}$, area under the luminal concentration–time curve of FD-4; BCS, biopharmaceutics classification system; FD-4, FITC-dextran; Fa, fraction of drug absorbed; GI, gastrointestinal.

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mixed micelles of bile acids and phospholipids and/or food components, which reduces the free concentration of the drugs in the intestinal fluid.[4] However, the whole mechanisms of negative food effect is still unclear because, as described above, volume and osmolarity of GI fluid are considerably fluctuated after food intake, which definitely affect the oral absorption of drugs.

The aim of this study is to evaluate the influence of fluid osmolarity on the fluid volume in the GI tract, then clarify the effect of fluid volume on oral absorption of drugs. The concentration–time profiles of FITC-dextran (FD-4), a nonabsorbable marker, in the GI tract were monitored after oral ingestion as solutions with various osmolarity. Then, the impact of the fluctuation in the fluid volume on oral absorption of metoprolol (BCS class I, a high permeable and high soluble drug) and atenolol (BCS class III)[15,16] was evaluated based on the luminal drug concentration–time profile *in vivo*.

MATERIALS AND METHODS

Materials

FITC-dextran (MW 4400), metoprolol and atenolol were purchased from Sigma–Aldrich (St. Louis, Missouri). D-Mannitol was purchased from Nacalai Tesque, Inc. (Kyoto, Japan). All other reagents were of analytical-grade commercial products.

Preparation of FD-4 and Drug Solutions with Various Osmolalities

FITC-dextran, metoprolol, and atenolol were dissolved together in the purified water, 5% D-mannitol solution or 10% D-mannitol solution at a concentration of 200 μM. In addition, saline containing only 200 μM of FD-4 was also prepared. Saline and 5% D-mannitol solution are isosmotic. Purified water and 10% D-mannitol solution are hyposmotic and hyperosmotic, respectively.

Measurement of Luminal FD-4 and Drug Concentrations in Each GI Segment and Plasma Drug Concentrations

The sampling of residual fluid in each GI segment was conducted in the same way reported in our previous work.[17] Male Wistar rats were purchased from Japan SLC (Hamamatsu, Japan). Rats weighing about 220 g were fasted overnight prior to the experiments. After oral administration of 1 mL of various solutions containing FD-4, metoprolol, and atenolol, the rats were sacrificed at 5–10 min intervals over a 140-min period. The abdomen was then opened immediately to collect a sample of luminal water from each segment of the GI tract using a sponge by wiping the surface of the GI membrane. The sponge was immediately weighed and the amount of water was calculated by assuming a relative density of water equal to 1. The GI segments used in this study were stomach, duodenum (a 2-cm position distal to the stomach), upper jejunum (a 20-cm position distal to the stomach), lower jejunum (a 60-cm position distal to the stomach), and ileum (a 10-cm position proximal to the caecum).

Blood samples (about 1 mL) were taken from the jugular vein at the same time as the GI fluid sampling. Plasma was obtained by centrifugation and deproteinized by methanol precipitation. After centrifugation, the resulting supernatant was evaporated prior to resuspension in the solution consisted of 50 mM NaH_2PO_4 and acetonitrile at the ratio of 1:1.

Although the data of concentration–time courses in the rat GI tract following oral ingestion of FD-4, metoprolol, and atenolol with purified water has already been reported in our previous report,[17] the same experiment was conducted again to compare the results with those after oral administration of other solutions.

All procedures were performed in accordance with the Guidelines for the Care and Use of Laboratory Animals of the Committee for Animal Experiments of Setsunan University.

Calculation of Fraction of Drug Absorbed from Each Segment of the GI Tract

In accordance with our previous report,[17] the fraction of drug absorbed (Fa) from GI tract until the time when it passed through each segment was calculated by following equation.

Fraction of drug absorbed from GI tract (%):

$$= \frac{(AUC_{GI(FD-4)} \times CL_{fluid} - AUC_{GI(drug)} \times CL_{fluid})}{AUC_{GI(FD-4)} \times CL_{fluid}} \times 100$$

$$= \frac{(AUC_{GI(FD-4)} - AUC_{GI(drug)})}{AUC_{GI(FD-4)}} \times 100$$

where $AUC_{GI(FD-4)}$ and $AUC_{GI(drug)}$ are the area under the luminal concentration–time curve of FD-4 and drug, respectively, and CL_{fluid} means "the volume of drug solution cleared from each region of GI tract by fluid transit/unit time." The AUC_{GI}* CL_{fluid} in each GI segment corresponds to the amount of the drug or FD-4 that passed through each segment. As FD-4 is nonabsorbable, $AUC_{GI(FD-4)}$* CL_{fluid} is equivalent to the administered amount in all segments; hence, the difference between the AUC_{GI}* CL_{fluid} of FD-4 and the drug equals to the absorbed amount of the drug. In this study, as all drugs and FD-4 are dissolved in the same solution to be administered as a cassette, CL_{fluid} can be regarded as same for all drugs and be cancelled out in the above equation.

Calculation of Time Course of Oral Bioavailability of Drugs

To calculate the time course of drug absorption into the systemic circulation, plasma concentration of the drug after oral administration was deconvoluted with that after i.v. administration using decon.xls, a Microsoft excel incorporating a program for deconvolution, downloaded from http://www.geocities.co.jp/Technopolis-Jupiter/2752/new_page_2.htm.

Determination of FD-4 and Drug Concentration in Various Samples

The FD-4 in the luminal samples were determined by HPLC system (LC-10AT_{VP}; Shimadzu Corporation, Kyoto, Japan) with a fluorometric detector (RF-10A_{XL}; Shimadzu Corporation). An analytical column (TSK-GEL G6000 PWXL; Tosoh Corporation, Tokyo, Japan) was used at 40°C. The mobile phase prepared by mixing of 25.4 g/L of $NaH_2PO_4 \cdot 2H_2O$ and 44.1 g/L of $Na_2HPO_4 \cdot 12H_2O$ solutions at pH 7.4 was flowed at 1.0 mL/min. The wavelengths for excitation and emission were set at 495 and 514 nm, respectively.

The luminal and plasma concentration of drugs were quantified by HPLC system (LC-20AT; Shimadzu Corporation) with a LCMS detector (LCMS-2010A; Shimadzu Corporation). MercuryMS (Luna5μ C18, 10 × 4.0 mm^2 i.d.; Phenomenex, Torrance, California) were used as analytical column at 40°C. The