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Human small intestinal and colonic tissue mounted in the Ussing chamber as a tool for characterizing the intestinal absorption of drugs

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ABSTRACT

The purpose of this study was to validate human small intestinal and colonic tissue mounted in the Ussing chamber as a tool for predicting the oral drug absorption in humans with the main focus on moderately and poorly permeable compounds. The obtained apparent permeability coefficient (P_{app}) of eleven test compounds was compared to their fraction absorbed (Fa) in humans taken from the literature. Beside the conventional P_{app} a new parameter, the apparent permeability coefficient total ($P_{app,total}$), involving both the apical-to-basolateral permeability and the time-dependent compound accumulation in the tissue was established. The permeability of lucifer yellow (LY), a fluorescent marker of the paracellular pathway and the test compounds showed no obvious differences between small intestine and colon. Furthermore, small intestinal and colonic tissue from a single donor showed similar permeability of both LY and a transcellularly transported compound metoprolol. All test compounds including low molecular weight hydrophilic compounds such as metformin, atenolol, sulpiride and famotidine showed adequate permeability reflecting human Fa values ($R^2 = 0.87$). The P_{app} values of digoxin, a P-glycoprotein (P-gp) substrate, were not significantly affected by the addition of verapamil, a P-gp inhibitor. In contrast, the $P_{app,total}$ values of digoxin increased approximately threefold in the presence of verapamil. In conclusion, both small intestinal and colonic tissue mounted in the Ussing chamber provide a good opportunity to predict the oral drug absorption rate in humans even for moderately and poorly absorbed compounds. The novel calculation of $P_{app,total}$ allows the study of the carrier-mediated drug–drug interactions in human intestine.

1. Introduction

The oral delivery is the most preferable administration route of drugs. It may be assumed that the principles of drug absorption from the gastrointestinal tract (GIT) are the same as for the passage across any other biological membrane. However, the gut wall is composed of several structures such as the monolayer of epithelial cells, lamina propria, lamina muscularis mucosae, submucosa and muscularis with blood and lymph supply. Interplay of many complex processes, e.g. passive diffusion, carrier-mediated uptake and excretion, paracellular transport via tight junctions or intestinal metabolism may influence the oral absorption of drugs. Therefore, it is of considerable consequence to predict the absorption rate of new drug candidates in a system, which mimics the *in vivo* conditions most closely.

At present, several screening methods serve as a prediction tool for drug absorption. Usually, the combination of parallel artificial membrane permeability assay (PAMPA) and cell models such as Caco-2 cell monolayers are used to assess the drug permeability in the early stages of drug discovery (Kerns et al., 2004). However, these *in vitro* systems lack the morphological and physiological features of the intestine. Therefore, the prediction obtained from these systems may not always be in accord with the drug permeability *in vivo* (Yee, 1997; Di et al., 2003). A simple lipid barrier such as the PAMPA cannot predict permeability involving

Abbreviations: BCS, biopharmaceutical classification system; BSA, bovine serum albumin; DDI, drug–drug interaction; Fa, fraction absorbed; FDA, food and drug administration; GIT, gastrointestinal tract; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; KRB, Krebs–Ringer bicarbonate; PAMPA, parallel artificial membrane permeability assay; P_{app}, apparent permeability coefficient; $P_{app,total}$, apparent permeability coefficient total; PD, transepithelial potential difference; PEG, polyethylene glycol; P-gp, P-glycoprotein; LY, lucifer yellow.

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transporter-mediated processes or the paracellular pathway (Avdeef et al., 2007). Caco-2 cells retain many morphological and functional properties of the enterocytes. However, the expression of drug transporters can be highly different compared to the expression in human intestine. The permeability data obtained from the Caco-2 system often vary between laboratories, even when standard protocols for transport studies are used (Englund et al., 2006; Hayeshi et al., 2008). Furthermore, the tight junctions formed in the Caco-2 cell lines appear to be tighter than those in enterocytes; a fact that may affect the permeability of drugs with contribution of the paracellular pathway (Artursson et al., 1993).

Hence, predicting the drug absorption requires additional evaluation in animal *in vivo* models. Nevertheless, data derived in other species do not always provide a reliable prediction of drug bioavailability in humans (Grass et al., 2002). To increase the predictive value of *in vitro* and animal *in vivo* studies, the sections of human intestinal tissue or mucosal biopsies mounted in the Ussing chamber can be used for the evaluation of drug absorption (Lennernas, 1998; Albin et al., 2001). The use of excised human intestinal tissue for *in vitro* permeation studies has also been recommended by the Food and Drug Administration (FDA, 2000). Major advantages of human intestinal tissue application are the maintenance of morphological structure, and functional expression of transporters and drug metabolizing enzymes reflecting the *in vivo* condition.

Our aim was to validate the human intestinal tissue mounted in the Ussing chamber as a tool for the prediction of oral absorption of drugs. Eleven model compounds representing all four classes of the Biopharmaceutical Classification System (BCS), a categorization of drugs based on their aqueous solubility and intestinal permeability, were chosen for the evaluation (Amidon et al., 1995; FDA, 2000). Besides drugs transported passively via transcellular diffusion, several compounds with contribution of the paracellular pathway or carrier-mediated transport were included in the study. To further validate the present model system, the functionality of the efflux transporter P-glycoprotein (P-gp) in the excised intestinal tissue was analyzed. The P-gp-mediated efflux is known to act as an absorption barrier of several drugs (Greiner et al., 1999). For this examination, the apical-to-basolateral permeability and the tissue accumulation of digoxin, a commonly used probe substrate for intestinal P-gp, was tested in the presence and absence of verapamil, a classic P-gp inhibitor (Pauli–Magnus et al., 2000; Oswald et al., 2011).

Usually, the permeability of test compounds is expressed by the apparent permeability coefficient (P_{app}), which represents the apical-to-basolateral permeability of drugs (Lennernas et al., 1997; Takahashi et al., 2008). However, the P_{app} neglects the accumulation of compounds in the intestinal tissue, which may serve as an "absorption reservoir" releasing the accumulated drugs into the portal circulation (Chiou, 1995). Thus, a new calculation of apparent permeability coefficient total ($P_{app,total}$) involving both the apical-to-basolateral permeability and the time-dependent drug accumulation in the tissue was developed. The $P_{app,total}$ values and conventionally calculated P_{app} values of the model compounds were compared to their fraction absorbed (Fa), a percentage of the drug dose absorbed in the portal circulation determined in human pharmacokinetic studies.

2. Material and methods

2.1. Chemicals

All compounds tested in this study are listed in Table 1. [^{14}C]Acetaminophen (77 mCi/mmol), [^{3}H]atenolol (5.10 Ci/mmol) and [^{3}H]metoprolol (36.80 Ci/mmol) were purchased from Hartmann Analytic (Braunschweig, Germany), [^{14}C]antipyrine (56 mCi/mmol) was provided by PerkinElmer, Inc. (Waltham, MA), [^{14}C]metformin (80 mCi/mmol), [^{3}H]pravastatin (15 mCi/mmol), [^{3}H]sulpiride (84 Ci/mmol) were obtained from BioTrend Chemikalien GmbH (Cologne, Germany), [^{14}C]PEG 4000 (50 mCi/mmol) was provided by GE Healthcare (Munich, Germany), [^{14}C]ibuprofen (55 mCi/mmol) was purchased from American Radiolabeled Chemicals, Inc. (St. Louis, MO) and [^{14}C]pravastatin (0.2 mCi/mmol) was provided by Daiichi Sankyo Co., Ltd. (Tokyo, Japan). Famotidine, sulfasalazine and all other compounds were purchased from Sigma–Aldrich GmbH (Steinheim, Germany).

2.2. Human tissue samples

Healthy sections of both human small intestine and colon were obtained from 25 patients (12 females, 13 males; median age 63 years) who underwent the gastrointestinal surgery for cancer. The most frequent additional diagnoses were hypertension (7) followed by diabetes mellitus (3) and hypercholesterolemia (2) with appropriate treatment. Experiments presented in this study were performed using tissue samples from all intestinal segments (duodenum, jejunum, ileum, ascending colon, transverse colon, descending colon and sigmoid colon). Exclusion criteria included inflammatory bowel diseases such as Crohn's disease, ulcerative colitis and celiac disease due to the altered intestinal permeability. Experimental procedures were performed according to the guidelines of the charitable state-controlled foundation, Human Tissue and Cell Research (HTCR, Regensburg, Germany), with informed patient's consent approved by the local ethics committee of the University of Regensburg, Germany. The intestinal samples were made anonymous. The sections of human intestine were provided by Hepacult GmbH (Regensburg, Germany) after being commissioned by HTCR and Daiichi Sankyo Europe GmbH (Munich, Germany).

2.3. Tissue mounting

After the resection, the intestinal tissue was transferred from the hospital within 30 min in ice-cold Krebs–Ringer bicarbonate (KRB) buffer supplemented with $NaHCO_3$ (25 mM), $CaCl_2$ (1 mM) and HEPES (10 mM) adjusted at pH 7.4. The adipose tissue and muscle layers were carefully removed and the mucosa was mounted in the Ussing chamber with an exposed tissue area of 0.46 cm^2 (Harvard Apparatus Inc., Holliston, MA). Before incubation with compounds, both compartments of the Ussing chamber were filled with 5 ml pre-warmed KRB buffer each and washed for 20 min. Afterwards, the KRB buffer was replaced by fresh pre-warmed KRB buffer and the tissue was equilibrated by another 20 min incubation. The KRB buffer was continuously oxygenated with 95% O_2 and 5% CO_2 gas mixture and maintained at 37 °C for the whole duration of the experiment.

2.4. Permeability studies

All transport studies were performed in the apical (donor; representing the intestinal lumen) to basolateral (receiver; representing the blood circulation) direction. After equilibrium period, the KRB buffer in the receiver compartment was replaced by 5 ml fresh KRB buffer containing 1% bovine serum albumin (BSA) to reduce the non-specific binding of the test compound to the plastic surface of the Ussing chamber. The KRB buffer in the donor compartment was replaced by an equal volume of fresh KRB buffer containing unlabeled test compounds at the final concentration of 50 μM or 0.4 or 2.0 μCi/ml as for radiolabeled compounds. To avoid the saturation of P-gp mediated efflux, unlabeled digoxin was applied at 10 μM with addition of 2.0 μCi/ml [^{3}H]digoxin and its transport was examined with or without the P-gp inhibitor