

# Vascular Perfused Segments of Human Intestine as a Tool for Drug Absorption

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## ABSTRACT:

Blood-based vascular perfusion of isolated segments of human jejunum was developed as a tool for drug absorption studies before clinical trials. Acceptance criteria for viable human gut preparations included stable blood flow, arterial pressure, glucose utilization, active peristalsis, oxygen uptake, less than 3% absorption of a 70,000 mol. wt. dextran, and a ratio of first-order absorption rate constants ( $k_a$ ) of antipyrine to terbutaline of  $\geq 1.4$ . Mannitol absorption was less than that of antipyrine but larger than that of terbutaline and could not be used as a negative control in absorption studies with human intestine. In separate perfusions ( $n = 3$ ) a cassette of nine drugs was administered into the gut lumen, and the net absorption of each drug into the circulation was measured over 75 min. Using the mean values of  $k_a$ , the test compounds

could be ranked into four groups: group 1: sulfasalazine and furosemide,  $k_a = 3.9$  to  $4.0 \times 10^{-3} \text{ min}^{-1}$ ; group 2: cimetidine, timolol, nadolol, and ranitidine,  $k_a = 6.4$  to  $8.3 \times 10^{-3} \text{ min}^{-1}$ ; group 3: atenolol and metoprolol,  $k_a = 9.6 \times 10^{-3} \text{ min}^{-1}$ ; and group 4: theophylline,  $k_a = 17.5 \times 10^{-3} \text{ min}^{-1}$ . The rationale for evaluating yet another oral absorption system was as follows: first, a human gut segment with an intact vascular system is the closest system available to a clinical trial without performing one; and second, the data generated would be a direct measure of net drug transport from the gut lumen into the vascular circulation under near physiological conditions, which is not possible in models lacking a blood supply.

In drug development programs, knowledge of the range of oral absorption from gut lumen into blood in human subjects can be one of the key factors in drug candidate selection. Consequently, considerable resources and ingenuity have been applied to the development and validation of preclinical models, which are then claimed to predict oral absorption in vivo in human subjects. These diverse models span a broad range of biological complexity from isolated cells in culture (Artursson and Karlsson, 1991; Hilgendorf et al., 2000), mucosal sheets (Jezyk et al., 1992), everted gut sacs (Barthe et al., 1998), in situ lumen perfusion of animal intestine (Cao et al., 2006; Castella et al., 2006; Zakeri-Milania et al., 2007), and vascular perfused animal intestine (Roy et al., 1991) to whole animal absorption studies in vivo. Invariably, these model systems are validated by a quantitative comparison against standard human absorption data in vivo, such as percent fraction of dose absorbed (FA) obtained from oral versus intravenous pharmacokinetics in vivo or disappearance of native drug from the gut lumen in vivo (Lennernäs, 1998, 2007). However, the percent FA for a single compound, even in the same subject, is not a unique number because absorption is known to be affected by a variety of host factors including absorptive surface area, local pH, food effects, blood flow, intestinal transporter, and enzyme complement. These and other physiological factors make the measured values for percent FA in vivo less precise within and between individual

subjects. Hence, in the validation of absorption models using reference absorption standards in humans, one should be aware of the absorption range. For example, the percent FA for atenolol is frequently quoted as 50% but values range from 37 to 71% (Ingels et al., 2004), oral absorption of mannitol is 65% (Dowty and Dietsch, 1997) but ranges from 16 to 80% FA, with the lower values attributed to metabolism of mannitol to  $\text{CO}_2$ , furosemide absorption ranges from 50 to 61% FA (Ingels et al., 2004), and sulfasalazine absorption ranges from 7 to 17% FA (Ingels et al., 2004).

This study describes the development and qualification of isolated vascular perfused segments of human jejunum as a tool for drug absorption studies. The rationale for evaluating yet another system was as follows: first, human gut segments with an intact vascular system could be regarded not as a model system but as the “real thing”; second, this is the closest system available to a clinical trial without performing one; and third, the data generated are a direct measure of net drug transport from the gut lumen into the vascular bed under near physiological conditions, which are not possible in models lacking a blood supply. Once validated, such a system would also enable quantitative evaluation of gut metabolism, food effects, age, gender, and drug-drug interactions on absorption from the gut lumen.

## Materials and Methods

**Materials.** Texas Red dextran (mol. wt. 70,000, neutral) was purchased from Invitrogen (Carlsbad, CA), [ $^3\text{H}$ ]mannitol was from American Radiolabeled Chemicals (St. Louis, MO), RS-I buffer (AQIX) was from Res Del International (London, UK), and heparin was from Baxter (Deerfield, IL). The

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**ABBREVIATIONS:** FA, fraction absorption; HPLC, high-performance liquid chromatography;  $P_{\text{eff}}$ , permeability coefficient constant.

TABLE 1

## Experimental group details

Group 1: cassette dose containing antipyrine (21  $\mu\text{mol}$ ), terbutaline (20  $\mu\text{mol}$ ), and mannitol (21  $\mu\text{mol}$ ; containing 0.007  $\mu\text{mol}$  of [ $^3\text{H}$ ]mannitol as a tracer, with a specificity 6.6  $\mu\text{Ci}/\mu\text{mol}$ ) or Texas Red dextran (5 mg). Group 2: cassette dose containing 5  $\mu\text{mol}$  of sulfasalazine, furosemide, timolol, nadolol, ranitidine, atenolol, theophylline, and cimetidine and 10  $\mu\text{mol}$  of metoprolol.

Small Intestine No.	Age	Gender	Weight	Blood Group	A/T Ratio
	yr		kg		
Group 1					
1	21	M	77.0	O	1.76
2	19	M	70.4	O	1.88
3	31	M	93.0	O	1.63
4	36	F	88.9	A	1.40
5	38	M	62.6	A	1.87
6	18	M	80.0	O	1.48
Group 2					
7	23	M	54.4	B	
8	25	F	100.0	B	
9	39	M	84.8	O	

A/T ratio, antipyrine/terbutaline ratio.

preservation solutions UW (University of Wisconsin)-ViaSpan and Custodiol HTK (histidine-tryptophan-ketoglutarate) were purchased from Barr Pharmaceuticals (Montvale, NJ) and Essential Pharmaceuticals (Newtown, PA), respectively. Packed human red blood cells and human albumin serum were supplied by BioChemed Services (Winchester, VA). All other chemicals and reagents were obtained from Sigma-Aldrich (St. Louis, MO).

**Organ Retrieval and Perfusion.** Organ donors (Table 1) were identified by organ procurement organizations, and after obtaining informed consent from next of kin, the small intestine was acquired at the time of multiorgan retrieval in the same manner as for clinical transplant. Both ends of the intestines were sealed, the vasculature was flushed with cold UW or HTK solution, and the organs were transported immersed in this same solution on ice.

The study was approved by the institutional review board at Organ Recovery Systems and the procedures followed were in accordance with institutional guidelines. A single 45-cm proximal segment of the jejunum and the accompanying vascular bed was carefully isolated while submerged on an ice/saline slush, the primary branch of the mesenteric artery supplying the segment was cannulated, and both ends of the gut segment were sealed by suture. Immediately before perfusion the gut vasculature was gently flushed with 500 ml of RS-I buffer at room temperature to remove the storage solution and any debris. The jejunum segment was transferred to a perfusion apparatus, and the vasculature was perfused with RS-I buffer containing 15 to 20% (v/v) of washed matched human erythrocytes, 6% human serum albumin, 15,000 U/l heparin, 1  $\mu\text{M}$  L(-)-norepinephrine-(+)-bitartrate, 1  $\mu\text{M}$  dexamethasone, and 10 mM L-glutamine at 37°C and pH 7.4; the intestinal gut lumen remained sealed during vascular perfusion. For all perfused intestines the cold ischemia time (time from cross-clamp of the donor to beginning of perfusion) was  $15 \pm 0.9$  h. After a single-pass perfusion with 1 liter of perfusate, the preparation was switched to recirculating mode with 1 liter of fresh perfusate (Fig. 1). Once steady-state temperature (37°C) and perfusion pressure ( $37 \pm 3.8$  mm Hg) were reached and the intestinal wall showed active peristalsis, for the group 1 (donors 1–6; Table 1) a cassette dose containing antipyrine (21  $\mu\text{mol}$ ), terbutaline (20  $\mu\text{mol}$ ), and mannitol (21  $\mu\text{mol}$ , containing 0.007  $\mu\text{mol}$  of [ $^3\text{H}$ ]mannitol as a tracer, with a specificity 6.6  $\mu\text{Ci}/\mu\text{mol}$ ) was administered as a bolus injection into the gut lumen. In three perfusions mannitol was replaced by Texas Red dextran (5 mg). For the second group (donors 7–9; Table 1) a cassette dose containing 5  $\mu\text{mol}$  of sulfasalazine, furosemide, timolol, nadolol, ranitidine, atenolol, theophylline, and cimetidine and 10  $\mu\text{mol}$  of metoprolol was administered as a bolus injection into the gut lumen. Only one cassette dose was administered to each isolated gut segment. Samples of the recirculating perfusate were removed at 0, 15, 30, 45, 60, and 75 min after dosing, and blood chemistry and biochemical analyses were performed immediately. The plasma from each perfusate sample was separated by centrifugation and frozen at  $-70^\circ\text{C}$  until analyzed.

**Analytical Methods. Blood chemistry and biochemistry.** These were analyzed using a Stat Profile Critical Care Unit (Nova Biomedical Corp.,

Waltham, MA) and a Piccolo Clinical Chemistry Analyzer (Abaxis, Union City, CA), respectively.

**Antipyrine and terbutaline analysis.** Antipyrine and terbutaline were analyzed by reverse-phase HPLC on a Symmetry C18 column ( $4.6 \times 150$  mm, 5  $\mu\text{m}$  particles) using a Waters 2695 HPLC system (Waters, Milford, MA). Plasma samples (0.5 ml) containing internal standards bamethane (10  $\mu\text{M}$ ) and 4-dimethylamino-antipyrine (5  $\mu\text{M}$ ) were deproteinized with 1.5 ml of ice-cold acetonitrile. After centrifugation, the supernatant was evaporated to dryness in a vacuum centrifuge at 45°C and reconstituted in 125  $\mu\text{l}$  of water, and 20  $\mu\text{l}$  of this solution was subjected to HPLC. For antipyrine analysis, the mobile phase was 50 mM  $\text{NH}_4\text{Ac}$  buffer, pH 5 (solvent A) and acetonitrile (solvent B). The column was eluted with a linear gradient of 15 to 19.3% of solvent B for 13 min at a flow rate of 1 ml/min and column temperature of 25°C with the UV detector set at 254 nm. Under this condition, the retention times of antipyrine and internal standard 4-dimethylamino-antipyrine were 7.0 and 11.3 min, respectively. For terbutaline analysis, the mobile phase was 0.1% HAc buffer (pH 3) (solvent A) and acetonitrile (solvent B). The column was eluted with 100% of solvent A for 13 min, followed by a wash step of 60% buffer A-40% of solvent B from 13.5 to 16 min and then an equilibrium step using 100% of solvent A from 16.5 to 20 min. Flow rate was 1 ml/min, and the column temperature was 25°C. The fluorescence detector was set at excitation 274 nm and emission 315 nm. Under this condition, the retention times of terbutaline and internal standard bamethane were 5.3 and 7.8 min, respectively.

**Texas Red dextran analysis.** Texas Red dextran (mol. wt. 70,000) was analyzed using size exclusion HPLC with a TSK-GEL G3000 PW<sub>XL</sub> column ( $7.8 \times 300$  mm equipped with a Guard column; TOSOH Bioscience, Grove City, OH). Plasma (0.2 ml) was deproteinized in the presence of 10% trichloroacetic acid. After centrifugation, the supernatant was neutralized to pH 8 with 1 M  $\text{NaHCO}_3$ , and 20  $\mu\text{l}$  of this solution was subjected to HPLC. The column was eluted under isocratic conditions (mobile phase 50 mM phosphate buffer, pH 6.8) at flow rate of 1 ml/min and the column at room temperature. The fluorescence detector setting was at excitation 580 nm and emission 615 nm. Under this condition, the retention of Texas Red dextran was 8 min. The presence of dextran in the experimental samples was verified by incubating the plasma sample with dextrase at 37°C for 1 h before trichloroacetic acid precipitation. Digested Texas Red dextran appeared as a broad peak with a retention time large than 10 min.

**[ $^3\text{H}$ ]Mannitol analysis.** [ $^3\text{H}$ ]Mannitol was detected after combustion using a PerkinElmer 307 oxidizer and scintillation counter. The scintillation fluid Monophase S (PerkinElmer Life and Analytical Sciences, Waltham, MA) was used for  $^3\text{H}_2\text{O}$  collection.

**Sulfasalazine, Furosemide, Timolol, Nadolol, Ranitidine, Atenolol, Metoprolol, and Theophylline Analysis.** This cassette of compounds was analyzed by reverse-phase HPLC using the same column and system described above. Samples were prepared as described for antipyrine and terbutaline. For sulfasalazine, furosemide, timolol, nadolol, ranitidine, and metoprolol analysis, the mobile phase was 50 mM  $\text{NH}_4\text{Ac}$  buffer, pH 5 (solvent A) and acetonitrile (solvent B). The column was eluted under isocratic condition with 10% of solvent B for 10 min, followed by a two-step linear gradient elution consisting of 10 to 30% of solvent B from 10 to 30 min and 30 to 40% of solvent B from 30 to 35 min and then 40% of solvent B was kept from 35 to 38 min, followed by an equilibrium step with 10% of solvent B from 39 to 42 min. Flow rate was 1 ml/min and column temperature was 25°C with photodiode array UV detection. Sulfasalazine, ranitidine, and furosemide analyses were performed at 330 nm and timolol analysis at 275 nm. Nadolol and metoprolol analysis were carried out with a fluorescence detector with excitation/emission wavelength at 235 nm/310 nm. For atenolol and theophylline analyses, the mobile phase was 0.1% HAc buffer (pH 3) (solvent A) and acetonitrile (solvent B). The column was eluted with a four-step linear gradient elution consisting of 0 to 3% solvent B from 0 to 3 min, 3 to 15% solvent B from 3 to 15 min, 15 to 30% of solvent B from 25 to 35 min, and 30 to 40% of solvent B from 35 to 40 min, followed by an equilibrium step using 100% of solvent A from 41 to 42 min. Flow rate was 1 ml/min, and column temperature was 25°C. Atenolol analysis was carried out with a fluorescence detector with excitation/emission wavelength at 235 nm/310 nm. Theophylline analysis was carried out with the photodiode array UV detector at 275 nm.

**Statistics and Data Analysis.** Data were analyzed with a standard one-way analysis of variance for repeated measures followed by the Newman-Keuls

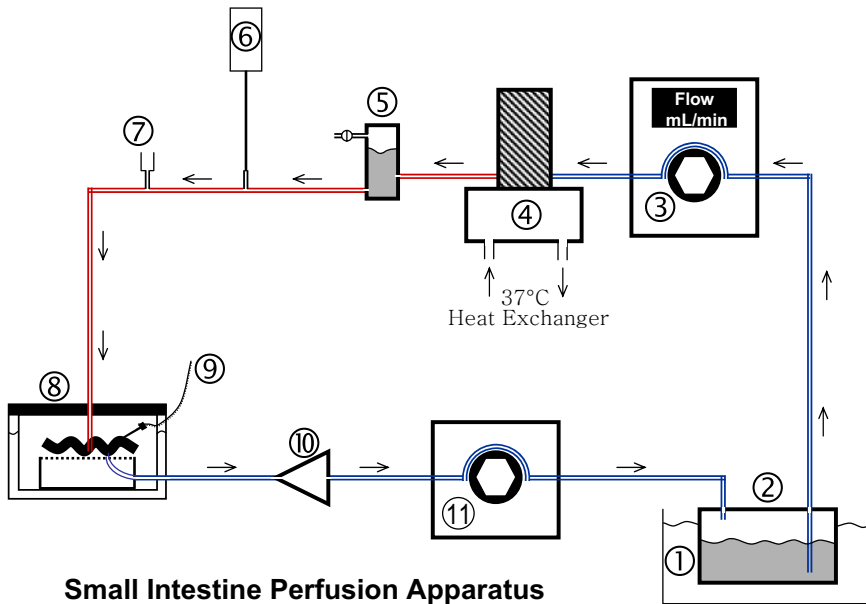


FIG. 1. Schematic diagram for ex vivo perfusion of isolated segments of human jejunum.

### Small Intestine Perfusion Apparatus

- |                            |                              |
|----------------------------|------------------------------|
| 1-Water Bath               | 7- Vacutainer                |
| 2-Perfusate Reservoir      | 8- Organ Incubator           |
| 3-Watson-Marlow Pump       | 9- Temperature Probe         |
| 4- Hollow Fiber Oxygenator | 10- Blood transfusion filter |
| 5- Bubble Trap             | 11- Peristaltic Pump         |
| 6- Pressure transducer     |                              |

post hoc test (GraphPad Software Inc., San Diego, CA). A value of  $p < 0.05$  was considered statistically different. All arithmetic means are presented  $\pm$  S.D. unless otherwise noted.

$k_a$  is calculated based on the first-order exponential equation  $N_t = N_0 (1 - \exp -k_a t)$ ; thus,  $\ln(N_t/N_0) = \ln[(N_0 - A_t)/N_0] = \ln(1 - A_t/N_0) = -k_a t$ , where  $N_t$  is the remaining amount of dosed compound (not absorbed) at time  $t$  (minutes),  $N_0$  is the total amount of dosed compound at initial time  $t = 0$  (minutes), and  $A_t$  is the amount of dosed compound absorbed at time  $t$ , which is obtained from recirculating plasma samples.  $k_a$  is the apparent absorption rate constant ( $\text{minutes}^{-1}$ ), obtained from plot of  $\ln(1 - A_t/N_0)$  via time  $t$ .

## Results

**Physiological Parameters Measured throughout the Perfusion of the Segments of Human Jejunum after Dosing with Antipyrine, Terbutaline, [ $^3\text{H}$ ]Mannitol, or Texas Red Dextran.** In all gut perfusions the predose perfusate flow was set and the perfusion pressure

was allowed to adjust to changes in the isolated tissue. As shown in Table 2, after dosing there was a slow but significant time-dependent reduction in perfusion pressure of 30 to 40% of the predose values ( $p < 0.05$ ). Temperature, osmolarity, partial pressure of oxygen, and the hematocrit did not change significantly during the postdosing period. Arterial and venous partial pressure of oxygen difference was higher than 100 mmHg and maintained throughout the perfusion period (data not shown). A slight, but significant, increase in the levels of pH ( $p < 0.05$ ) (Table 2) and a decrease in  $\text{CO}_2$  ( $p < 0.05$ ) (data not shown) were observed at the end of the perfusion period.

**Perfusate Chemistry and Biochemistry Measured throughout the Perfusion of the Segments of Human Jejunum after Dosing with Antipyrine, Terbutaline, [ $^3\text{H}$ ]Mannitol, or Texas Red Dextran.** In the recirculating perfusates delivered to each gut segment there were no significant changes in  $\text{Na}^+$ ,  $\text{Cl}^-$ , or  $\text{Ca}^{2+}$  concentra-

TABLE 2

Physiological and biochemical parameters of isolated vascular perfused segments of human intestine during predose and 15, 30, 45, 60, and 75 min after administration of antipyrine (21  $\mu\text{mol}$ ), terbutaline (20  $\mu\text{mol}$ ), and mannitol (21  $\mu\text{mol}$ ) or Texas Red dextran (5 mg) (group 1)

Data are mean  $\pm$  S.E.M.

Parameters	Mean Predose Values	Change at Each Time Point ( $n = 6$ )				
		15 min	30 min	45 min	60 min	75 min
		%	%	%	%	%
Perfusion pressure	38 $\pm$ 5.8 mm Hg	89.3 $\pm$ 3.7	75.5 $\pm$ 2.4*	69.7 $\pm$ 6.6*	65.9 $\pm$ 6.7*	64.3 $\pm$ 6.4*
Perfusion flow	59 $\pm$ 6 ml/min	100 $\pm$ 0.0	101 $\pm$ 1.2	101 $\pm$ 1.2	101 $\pm$ 1.2	101 $\pm$ 1.2
Temperature	35.9 $\pm$ 0.3°C	101 $\pm$ 1.2	101 $\pm$ 1.4	100 $\pm$ 1.6	102 $\pm$ 0.9	101 $\pm$ 0.9
Hematocrit	16.5 $\pm$ 0.7%	99.8 $\pm$ 2.6	93.8 $\pm$ 3.4	96.1 $\pm$ 2.0	96.1 $\pm$ 2.9	95.2 $\pm$ 3.1
pH	7.4 $\pm$ 0.1	100 $\pm$ 0.2	101 $\pm$ 0.4	101 $\pm$ 0.5*	102 $\pm$ 0.6*	102 $\pm$ 0.7*
Glucose	115.3 $\pm$ 2.6 mg/dl	97 $\pm$ 2.3	95 $\pm$ 3.5	91 $\pm$ 3.6*	86 $\pm$ 3.7*	79 $\pm$ 2.8*
Lactate	6.4 $\pm$ 0.9 mmol/l	114 $\pm$ 4.5	137 $\pm$ 11*	153 $\pm$ 15*	166 $\pm$ 18*	172 $\pm$ 16*
K <sup>+</sup>	6.1 $\pm$ 1.1 mmol/l	102 $\pm$ 0.9	104 $\pm$ 1.1*	107 $\pm$ 2.3*	110 $\pm$ 2.2*	111 $\pm$ 1.9*
Amylase	20.5 $\pm$ 7.4 U/l	N/A	159 $\pm$ 19*	N/A	186 $\pm$ 19*	N/A

N/A, not analyzed.

\*  $p < 0.05$  vs. predose (100%).

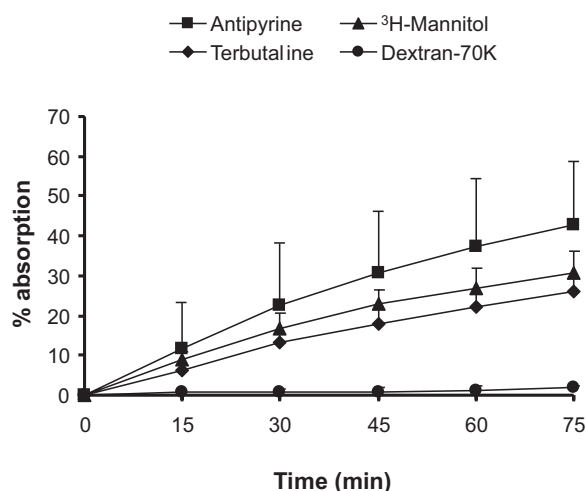


FIG. 2. Percent absorption of antipyrine ( $n = 6$ ), terbutaline ( $n = 6$ ), [ $^3\text{H}$ ]mannitol ( $n = 2$ ), and Texas Red Dextran (mol. wt. 70,000) ( $n = 3$ ) during perfusion of isolated segments of human intestine. Data are expressed as mean  $\pm$  S.D.

tions throughout the postdosing perfusion period (data not shown). In contrast, perfusate  $\text{K}^+$ , lactate, and amylase activity increased progressively and by 75 min postdose concentrations were elevated by approximately 10, 72, and 75%, respectively ( $p < 0.05$ ), whereas glucose concentration decreased progressively by approximately 30% of the predose value ( $p < 0.05$ ) (Table 2). Glucose consumption ( $\pm$ S.E.M.) was  $368 \pm 79 \mu\text{mol}/100 \text{ g}$  of intestinal segment in 1 h.

**Absorption of Antipyrine, Terbutaline, [ $^3\text{H}$ ]Mannitol, and Texas Red Dextran from Segments of Human Jejunum.** The time-dependent appearances of the four standards in perfusate are shown in Fig. 2 and Table 3, respectively. Within each of the three perfusions the relative amounts of antipyrine, terbutaline, and mannitol transported into the circulation was constant (Fig. 3). The ranking of first-order rate constants for transport from the gut lumen into perfusate was terbutaline  $<$  mannitol  $<$  antipyrine in the ratio of 1:1.2:1.9, which is consistent with the ratio of percent FA values observed in vivo (Table 3). The first-order rate constant was also consistent with the previously reported human permeability coefficient constant ( $P_{\text{eff}}$ ) (Table 3; Fig. 4). Of the Texas Red dextran,  $1.8 \pm 0.6\%$  was absorbed during the 75-min perfusion, indicating the integrity of the intestinal segments.

**Absorption of Sulfasalazine, Furosemide, Timolol, Nadolol, Ranitidine, Atenolol, Metoprolol, Theophylline, and Cimetidine from Segments of Human Jejunum.** In these perfusions the leakage of Texas Red dextran (mol. wt. 70,000) from the gut lumen into the circulation was low, less than  $2.3 \pm 0.9\%$  of dose (25% are fragments less than 10,000), confirming the integrity of the human jejunum segments. In terms of blood chemistry, results for this set of gut

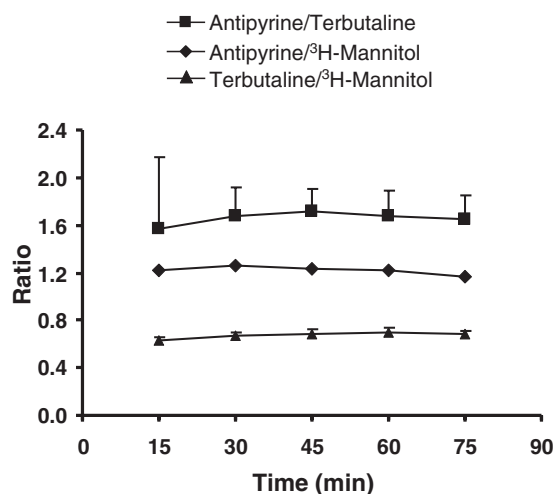


FIG. 3. Absorption ratio of antipyrine/terbutaline ( $n = 6$ ), antipyrine/[ $^3\text{H}$ ]mannitol ( $n = 2$ ), and terbutaline/[ $^3\text{H}$ ]mannitol ( $n = 2$ ) in the recirculating perfusate after dosing into the lumen of isolated segments of human intestine. Data are expressed as mean  $\pm$  S.D.

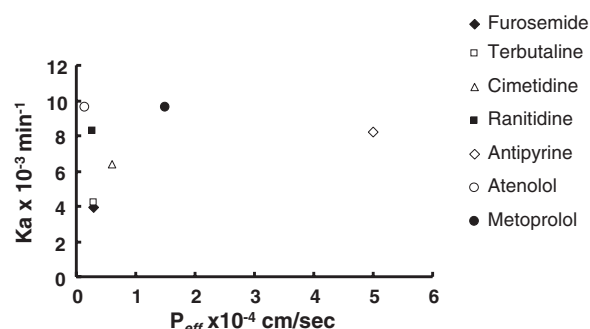


FIG. 4. Plot of in vivo human jejunum drug permeability versus apparent absorbance rate constant obtained from ex vivo human jejunum perfusion.

perfusions (listed in Table 4) were consistent with those in Table 2, after cassette dosing, with significant increases in perfusate lactate and amylase concentrations. The major difference was the consistent elevation of perfusion pressure after cassette dosing (Table 4) and higher glucose consumption of  $609 \pm 184 \mu\text{mol} \times (100 \text{ g})^{-1} \times \text{h}^{-1}$ .

Direct measurement of net drug appearance in the circulation showed a wide range (up to 11-fold) of first-order rates for the test compounds (Table 5). For seven of the nine drugs,  $k_a$  values for each compound in the three separate gut perfusions differed by up to 1.2- to 1.7-fold. One exception was furosemide for which the  $k_a$  value differed among three perfusions by up 3.6-fold. Taking the mean values of  $k_a$ , the compounds could be ranked into four groups with

TABLE 3

Absorption kinetics for antipyrine (22  $\mu\text{mol}$ ), terbutaline (20  $\mu\text{mol}$ ), and mannitol (21  $\mu\text{mol}$ ) after cassette dosing into the lumen of isolated vascular perfused segments of human intestine

Percent fraction absorbed in transit time of 2.5 h is derived from calculated  $k_a$  values.

Test Compound	$k_a \times 10^{-3}$ (Range) $\text{min}^{-1}$	$R^2$	%FA in Transit Time of 2.5 h	%FA in Vivo	Human $P_{\text{eff}}$ $\times 10^{-4} \text{ cm/s}$
Antipyrine ( $n = 6$ )	3.2–13.2	$>0.9460$	51–80	100 <sup>a</sup>	5 <sup>b</sup>
Terbutaline ( $n = 6$ )	2.2–6.2	$>0.9719$	34–60	60 <sup>c</sup>	0.3 <sup>b</sup>
[ $^3\text{H}$ ]Mannitol ( $n = 2$ )	4.9	$>0.9763$	36–55	16–80 <sup>d</sup>	

<sup>a</sup> Taken from Lennernäs (2007).

<sup>b</sup> Taken from Lennernäs (1998).

<sup>c</sup> Taken from Zhao et al. (2003).

<sup>d</sup> Taken from Dowty and Dietsch (1997).

TABLE 4

Physiological and biochemical parameters of isolated vascular perfused segments of human intestine during predose and 15, 30, 45, 60, and 75 min after administration of 5  $\mu\text{mol}$  of sulfasalazine, furosemide, timolol, nadolol, ranitidine, atenolol, theophylline, and cimetidine, and 10  $\mu\text{mol}$  of metoprolol

Data are mean  $\pm$  S.E.M.

Parameters	Mean Predose Values	Change at Each Time Point (n = 3)				
		15 min	30 min	45 min	60 min	75 min
		%	%	%	%	%
Perfusion pressure	34.1 $\pm$ 1.9 mm Hg	122.2 $\pm$ 10	147 $\pm$ 19*	150.2 $\pm$ 18*	145.6 $\pm$ 8.3*	138.3 $\pm$ 5.6*
Perfusion flow	42.9 $\pm$ 13.9 ml/min	100 $\pm$ 0.0	100 $\pm$ 0.0	100 $\pm$ 0.0	100 $\pm$ 0.0	100 $\pm$ 0.0
Temperature	35.9 $\pm$ 0.5°C	97.3 $\pm$ 0.1*	97.7 $\pm$ 1.0*	98.9 $\pm$ 0.7	99.3 $\pm$ 1.0	99.1 $\pm$ 0.9
Hematocrit	15.7 $\pm$ 0.7%	102.2 $\pm$ 2.2	100.3 $\pm$ 3.6	98.3 $\pm$ 5.4	103.9 $\pm$ 3.9	108.1 $\pm$ 5.1
pH	7.6 $\pm$ 0.2	100.3 $\pm$ 0.4	100.4 $\pm$ 0.6	100.2 $\pm$ 0.6	100.3 $\pm$ 0.7	100.3 $\pm$ 0.9
Glucose	115.7 $\pm$ 17.1 mg/dl	90.2 $\pm$ 2.1*	85.2 $\pm$ 4.0*	79.0 $\pm$ 4.8*	74.0 $\pm$ 5.9*	68.8 $\pm$ 6.9*
Lactate	11.3 $\pm$ 3.1 mmol/l	105.9 $\pm$ 1.6	113.9 $\pm$ 2.9	120.3 $\pm$ 3.7*	126.3 $\pm$ 7.6*	129.8 $\pm$ 11*
K <sup>+</sup>	5.8 $\pm$ 0.8 mmol/l	84.3 $\pm$ 19.1	85.3 $\pm$ 19.5	86.6 $\pm$ 19.5	88.3 $\pm$ 19.9	89.7 $\pm$ 20.0
Amylase	137.0 $\pm$ 103.4 U/l	N/A	134.1 $\pm$ 5.4	N/A	181.7 $\pm$ 21*	N/A

N/A, not analyzed.

\* $p < 0.05$  vs. predose (100%).

TABLE 5

First-order apparent rate constant ( $k_a$ ) of nine drugs in the circulation after cassette dosing into the lumen of isolated vascular perfused segments of human jejunum (n = 3)

Percent fraction absorbed in transit time of 2.5 h is derived from calculated  $k_a$  values.

Test Compound	$k_a \times 10^{-3}$ (Range)	Mean	%FA in Transit Time of 2.5 h	%FA from Clinical Studies	Human $P_{\text{eff}}$
	$\text{min}^{-1}$				$\times 10^{-4}$ cm/s
Sulfasalazine	3.2–4.7	4.0	38–51	7–17 <sup>a</sup>	
Furosemide	1.9–6.8	3.9	25–64	50–61 <sup>a</sup>	0.3 <sup>b</sup>
Cimetidine <sup>c</sup>	5.9–7.1	6.4	59–66	75 <sup>d</sup>	0.6 <sup>b</sup>
Timolol	6.8–9.3	7.9	64–75	95 <sup>e</sup>	
Nadolol	6.9–10.3	8.3	64–79	20/57 <sup>e</sup>	
Ranitidine	6.2–9.4	8.3	61–76	50–60 <sup>d</sup>	0.27 <sup>b</sup>
Atenolol	7.4–11.7	9.6	67–83	37–71 <sup>d</sup>	0.15 <sup>f</sup>
Metoprolol	7.9–11.2	9.6	69–81	95 <sup>d</sup>	1.5 <sup>f</sup>
Theophylline	12.5–20.9	17.5	85–97	96–100 <sup>e</sup>	

<sup>a</sup> Taken from Ingels et al. (2004).

<sup>b</sup> Taken from Zakeri-Milani et al. (2007).

<sup>c</sup> The values for cimetidine were obtained by liquid chromatography/mass spectroscopy analysis.

<sup>d</sup> Taken from Lennernäs (2007).

<sup>e</sup> Taken from Zhao et al. (2003).

<sup>f</sup> Taken from Lennernäs (1998).

increasing absorption rates: group 1: sulfasalazine and furosemide,  $k_a = 3.9$  to  $4.0 \times 10^{-3} \text{ min}^{-1}$ ; group 2: cimetidine, timolol, nadolol, and ranitidine,  $k_a = 6.4$  to  $8.3 \times 10^{-3} \text{ min}^{-1}$ ; group 3: atenolol and metoprolol,  $k_a = 9.6 \times 10^{-3} \text{ min}^{-1}$ ; and group 4: theophylline,  $k_a = 17.5 \times 10^{-3} \text{ min}^{-1}$ . These values are consistent with previous reported human  $P_{\text{eff}}$  and pharmacokinetics studies (Table 5; Fig. 4), with the exception of atenolol and nadolol, which are classified as low permeability and low absorption.

## Discussion

In drug development, the primary issue with any new or little used technology, such as blood-perfused human gut segments for absorption studies, is the question of validation. This widely used term often has different meanings under different experimental circumstances; however, in this study the meaning is quite specific, that is, how well will the absorption data from ex vivo vascular perfused segments of human intestine (Ex Vivo Metrics) predict drug absorption in vivo? The first major challenge to achieving this goal is the quality and consistency of the in vivo data itself, which are invariably obtained by indirect means, comparing pharmacokinetics after intravenous versus oral administration or the disappearance of drug from the gut lumen. None of these methods directly measure the rate of drug appearance in the portal blood. This fact coupled with the fact that absorption from the intestine is known to be affected by a variety of host factors

including absorptive surface area, local pH, food effects, blood flow, intestinal transporters, and enzyme compliment would account for the broad range of literature values for the amount of drugs absorbed in humans. Indeed, in some cases, so disparate are the quoted literature values for drug absorption in human subjects that it may be impossible to validate almost any new technology. For example, values for mannitol absorption range from 16 to 80% of the administered dose, those for atenolol from 37 to 71%, those for furosemide from 50 to 61% FA, those for sulfasalazine from 7 to 17% FA, and so on.

Another challenge to the validation of Ex Vivo Metrics arises from some of the consistent data recorded using high-throughput screens with less complex systems. In Caco-2 monolayers, for example, mannitol is often used as a marker for a tight-junction integrity check because its transport through the membrane is consistently low, and it is generally well accepted within the absorption scientific community that the mannitol absorption in humans is indeed low (<20%). However, careful studies in humans and animals suggest that mannitol is well absorbed from the intestine (65–80%) with less than 10% excreted in the feces (Nasrallah and Iber, 1969; Dowty and Dietsch, 1997). The early low values were attributed to metabolism to  $\text{CO}_2$  and difficulties encountered in mannitol analysis (Nasrallah and Iber, 1969). These findings do not diminish the role of Caco-2 cells in drug absorption studies, but the higher absorption of the biomarker mannitol across human intestine does not make it a good negative control

for absorption/permeability nor invalidate the perfused gut preparation as a meaningful way to obtain clinically relevant human absorption data.

In light of the heterogeneity of the donor population and the potential influence of host factors on absorption, it is evident that to predict absorption *in vivo* by Ex Vivo Metrics, each human gut preparation must be qualified as fit for use simultaneously with any evaluation of drugs and new chemical entities. The two internal passively absorbed standards antipyrine and terbutaline were chosen for this purpose because the literature values for percent FA after oral administration in humans is consistently different for the two compounds, with a percent FA ratio for antipyrine to terbutaline of 1.7 (Lennernäs, 1998). This absorption differential was reproduced in the present study of perfused human gut segments in which the ratio of the first-order rate constants for absorption of antipyrine to terbutaline was 1.9:1. In three unsuccessful human gut segment perfusions (data not shown), the differential rates of absorption between antipyrine and terbutaline were lost (ratio of 0.89, 0.91, and 0.89), and these two standards and mannitol were all transported at the same rate although the biochemical and physiological parameters were consistent with gut segments that demonstrate differential absorption between antipyrine and terbutaline. Taken together, these studies suggested that one of the important acceptance criteria for viable perfused human gut preparations should be that each gut perfusion must show a minimum antipyrine/terbutaline absorption ratio of 1.4.

The acceptance criteria for human gut perfusions have been met; the next concern is how the data from cassette dosing in human Ex Vivo Metrics correlate with the percentage FA from separate clinical studies and what weight should be given to each as a measure of absorption in humans. Two actively transported drugs, theophylline and sulfasalazine, stand out for consistently having the highest and lowest rates and extent of absorption, showing agreement with the facts that sulfasalazine has lower bioavailability and lower permeability (Watkinson, 1986; Mols et al., 2005) and that theophylline has high bioavailability. Likewise, metoprolol, ranitidine, timolol, and cimetidine consistently show midrange absorption, which is not surprising given the wide range. In contrast, both atenolol and nadolol dosed simultaneously with the other drugs, showed significantly higher extents of absorption in our method than have been reported in clinical studies (Table 5). This finding could suggest that absorption may be facilitated by cointeraction with other drugs. In the case of atenolol, this is not likely because reduced permeability has been reported by interaction with furosemide (Issa et al., 2003). Studies evaluating atenolol and nadolol absorption during single-drug administration are warranted.

In terms of its potential contribution to drug candidate selection,

even with cassette dosing, *ex vivo* perfusion technology is not high throughput. Nevertheless, in addition to the quantification of the entire absorption process from gut lumen into the circulation, the added benefits of defining the contribution of human gut to the metabolism of orally administered drugs plus the ability to evaluate the effects of formulations, prodrugs versus active drugs, drug-drug interactions, food effects, and age and gender differences in the target species should make this methodology a useful addition to the drug development process.

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