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VASCULAR PERFUSED SEGMENTS OF HUMAN INTESTINE

AS A TOOL FOR DRUG ABSORPTION

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Running title: *Ex Vivo* Metrics: A tool for Human Absorption Studies

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Abbreviations: **ka**: Absorption rate constant

% FA: Percentage of fraction absorption

UW: University of Wisconsin solution

HTK: Histidine-tryptophan-ketoglutarate solution

TCA: Trichloroacetic acid

PDA UV: Photodiode array ultraviolet-visible detector

P_{eff}: Permeability coefficient constant

Hct: Hematocrit

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ABSTRACT

Blood based vascular perfusion of isolated segments of human jejunum was developed as a tool for drug absorption studies prior to clinical trials. Acceptance criteria of viable human gut preparations included: stable blood flow, arterial pressure, glucose utilization, active peristalsis, oxygen uptake, less than 3% absorption of a 70K dextran, and a ratio of first-order absorption rate constants (ka) of antipyrine/terbutaline of ≥ 1.4. Mannitol absorption was less than antipyrine but large than terbutaline and could not be used as a negative control in absorption studies with human intestine. In separate perfusions (n=3) a cassette of 9 drugs was administered into the gut lumen and the net absorption of each drug into the circulation was measured over 75 minutes. Using the mean values of ka, the test compounds could be ranked into four groups: Group 1 - sulphasalazine and furosemide, ka = 3.9 - 4.0 x 10^{-3} min^{-1}; Group 2 - cimetidine, timolol, nadolol and ranitidine, ka =6.4-8.3 x 10^{-3} min^{-1}; Group 3 - atenolol and metropolol, ka = 9.6 x 10^{-3} min^{-1} and Group 4 - theophylline, ka = 17.5 x 10^{-3} min^{-1}. The rationale for evaluating yet another oral absorption system were, firstly, human gut segments with an intact vascular system is the closest system available to a clinical trial without performing one; secondly, 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 the blood supply.

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INTRODUCTION

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 has been applied to the development and validation of pre-clinical 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 (Arturrsson & 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 (Castella et al, 2006; Zakeri-Milania, et al., 2007; Cao X, et al, 2006), vascular perfused animal intestine (Roy et al.,1991), to whole animal absorption studies *in vivo*. Invariably, validation of these model systems is assessed by a quantitative comparison against gold standard human absorption data *in vivo*, such as percentage fraction of dose absorbed (% FA) obtained from oral versus intravenous pharmacokinetics *in vivo* or disappearance of native drug from the gut lumen *in vivo* (Lennernas, 1998, 2007). However, the % 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 compliment. These and other physiological factors make the measured values for % FA *in vivo* less precise within and between individual subjects. Hence, when validating absorption models using reference absorption standards in humans, it is as well to be aware of the absorption range. For example, the % FA for atenolol is frequently quoted as 50 % but values range from 37-71% (from Ingels et al., 2004), oral absorption of mannitol is 65%

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(Dowty and Dietsch, 1997) but ranges from 16 - 80 % FA, with the lower values attributed to metabolism of mannitol to CO_2, furosemide absoption ranges from 50 - 61 % FA (from Ingels et al., 2004), and sulphasalazine 7 - 17 % FA (from 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 were, firstly, human gut segments with an intact vascular system could be regarded not as a model system but the "real thing"; secondly, it is the closest system available to a clinical trial without performing one; thirdly, the data generated is 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 the blood - supply. Once validated, such a system would also enable a quantitative evaluation of gut metabolism, food effects, age, gender and drug-drug interactions on absorption from the gut lumen.

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MATERIALS

Dextran Texas Red (70K, neutral) was purchased from Molecular Probes (Eugene, OR); ^{3}H-Mannitol from American Radiolabeled Chemicals Inc. (St. Louis, MO); RS-I buffer (AQIX) from Res Del International (London) and heparin from Baxter (Deerfield, IL). The preservation solution UW-Viaspan[TM] (University of Wisconsin) and Custodiol HTK (Histidine-tryptophan-ketoglutarate) was purchase 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 (St. Louis, MO).

METHODS

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 multi-organ retrieval in the same manner as for clinical transplant. Both ends of the intestines were sealed and the vasculature was flushed with cold UW or HTK solution and the organs 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 of the proximal segment of jejunum and accompanying vascular bed was carefully isolated submerse on ice/saline slush, the primary branch of the mesenteric artery supplying the segment was cannulated and both ends of the gut segment sealed by suture. Immediately prior to perfusion the gut vasculature was gently flushed with

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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 perfused with RS-I buffer containing 15 - 20% (v/v) of washed matched

human erythrocytes, 6% human serum albumin, 15,000 U/L of heparin, 1 μM of L-(-)

Norepinephrine(+) bitartrate, 1 μM of 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 of 15±0.9 hrs. After a single-pass perfusion with 1L of

perfusate, the preparation was switched to re-circulating mode with 1 L of fresh

perfusate (Figure 1). Once steady-state temperature (37°C) and perfusion pressure

(37±3.8 mmHg) was reached and the intestinal wall showed active persistalsis, for the

group 1 (donors 1 - 6, Table 1) a cassette dose containing antipyrine (21 μMoles),

terbutaline (20 μMoles), mannitol (21 μMoles, containing 0.007 μMoles of ^{3}H-mannitol

as a tracer, with a specificity 6.6 μCi/μMole) was administered as a bolus injection into

the gut lumen. In three perfusions mannitol was replaced by Texas red 70 K dextran (5

mg). The second group (donors 7 - 9, Table 1) a cassette dose containing 5 μMoles of

sulfasalazine, furosemide, timolol, nadolol, ranitidine, atenolol, theophylline, cimetidine

and 10 μMoles of metropolol was administered as a bolus injection into the gut lumen.

Only one cassette dose was administered to each isolated gut segment. Samples of

the re-circulating perfusate were removed at 0, 15, 30, 45, 60 and 75 minutes post

dosing and blood chemistry and biochemistry was analyzed immediately. The plasma

from each perfusate sample was separated by centrifugation and frozen at -70 ºC until

analyzed.

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Analytical Methods

Blood chemistry and biochemistries: These were analyzed using a Stat Profile Critical Care Unit (Nova Biomedical) and a Piccolo Clinical Chemistry Analyser (Abaxis), respectively.

Antipyrine and terbutaline analysis*:* Antipyrine and terbutaline were analyzed by reverse phase HPLC on a Symmetry C18 column (4.6 x150 mm, 5 μm particles) using Waters 2695 HPLC system. Plasma samples (0.5 mL) containing internal standards bamethane (10 μM) and 4-dimethylamino-antipyrine (5 μM) were de-proteinated 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 μl of water and 20 μL of this solution subjected to HPLC. For antipyrine analysis, the mobile phase was 50 mM NH_4Ac buffer, pH5 (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/minute 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 minutes, respectively. For terbutaline analysis, the mobile phase was 0.1% HAc buffer (pH3) (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 min to 16 min, then an equilibrium step using 100% of solvent A from 16.5 to 20 min. Flow rate was 1 mL/minute and the column temperature was 25°C. The fluorescence detector was set at excitation 274 nm, emission 315 nm. Under this condition, the retention of terbutaline and internal standard bamethane were 5.3 and 7.8 minutes, respectively.

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Texas red dextran (70K) analysis: Dextran Texas Red was analyzed using size exclusion HPLC with TSK gel[TM] G3000 PW$_{XL}$ column (7.8 x 300 mm equipped with a Guard column, TOSOH Bioscience, Grove City, OH). Plasma (0.2 mL) was de-proteinated in the presence of 10% of trichloroacetic acid (TCA). After centrifugation, the supernatant was neutralized to pH 8 with 1 M NaHCO$_3$ and 20 µL of this solution was subjected to HPLC. The column was eluted with under isocratic condition (mobile phase 50 mM phosphate buffer, pH 6.8) at flow rate of 1 mL/min and column at room temperature. The fluorescence detector setting was at excitation 580 nm and emission 615 nm. Under this condition, the retention of dextran Texas red was 8 minutes. The presence of dextran in the experimental samples was verified by incubating the plasma sample with dextrase at 37°C for 1 hour prior to TCA precipitation. Digested dextran Texas red appeared as a broad peak with a retention time large than 10 minutes.

[3]H-Mannitol analysis: ^{3}H-Mannitol was detected after combustion using a PerkinElmer 307 Oxidizer and scintillation counter. The scintillation fluid Monophase S (PerkinElmer, Waltham, Massachusetts) was used for [3]H$_2$O collection.

Sulfasalazine, furosemide, timolol, nadolol, ranitidine, atenolol, metropolol, 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, metropolol analysis, the mobile phase was 50 mM NH$_4$Ac buffer, pH5 (solvent A) and acetonitrile (solvent B). The column was eluted with isocratic 10% of solvent B for 10 min, followed a two-step linear gradients elution consisting of 10 - 30% of solvent B from 10 to 30 minute, then 30 - 40% of solvent B from 30 to 35 minute,

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then 40% of solvent B was kept from 35 to 38 minutes, followed an equilibrium step with 10% of solvent B from 39 to 42 minute. Flow rate was 1 mL/minute and column at 25°C with PDA UV detection. Sulfasalazine, ranitidine and furosemide analysis were carried out at 330 nm, and timolol analysis at 275nm. Nadolol and metroprolol analysis were carried out with fluorescence detector with excitation/emission wavelength at 235 nm/ 310 nm. For atenolol and theophylline analysis, the mobile phase was 0.1% HAc buffer (pH3) (solvent A) and acetonitrile (solvent B). The column was eluted with a 4-step linear gradient elution consisting of 0 - 3% of solvent B from 0 - 3 min, 3 - 15% of solvent B from 3 to 15 minute, 15 - 30% of solvent B from 25 to 35 minutes, 30 to 40% of solvent B from 35 to 40 minutes, then followed an equilibrium step using 100% of solvent A from 41 to 42 min. Flow rate was 1 mL/minute and column temperature of 25°C. Atenolol analysis was carried out with fluorescence detector with excitation/emission wavelength at 235 nm/ 310 nm. Theophylline analysis was carried out with PDA UV detector at 275 nm.

Statistics and Data Analysis

Data was analyzed with a standard one way analysis of variance (ANOVA) for repeated measures followed by the Newman Keul's post hoc test (GraphPad Software, San Diego, CA). A p value of less than 0.05 was considered statistically different. All arithmetic means are presented ± SD unless otherwise noted.

Ka 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 $[(N_o -A_t)/N_o]$ = Ln $(1 -A_t/N_o)$ = - $k_a t$. Where N_t is the remaining amount of dosed compound (not absorbed) at time t (minute). N_0 is the total amount of dosed compound at initial time t=0 (minute). And, A_t is the amount of dosed compound

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absorbed at time t, which is obtained from re-circulating plasma samples. k_a is the

apparent absorption rate constant (min^{-1}), obtained from plot of Ln ($1-A_t/N_o$) via time t.

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RESULTS

Physiological parameters measured throughout the perfusion of the segments of human jejunum after dosing with antipyrine, terbutaline, ^{3}H-mannitol or Texas red dextran (70K)

In all gut perfusions the pre-dose perfusate flow was set and the perfusion pressure 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 30-40% of the pre-dose values ($p<0.05$,). Temperature, osmolarity, PO_2, and the haematocrit did not change significantly during the post dosing period. Arterial and venous PO_2 difference was higher than 100mmHg, 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 decrease in CO_2 ($p<0.05$, data not shown) was 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}H-mannitol or Texas red dextran (70K)

In the re-circulating perfusates delivered to each gut segment there were no significant changes in Na^+, Cl^- or Ca^{2+} concentrations throughout the post dosing perfusion period (data not shown). By contrast, perfusate K^+, lactate and amylase activity increased progressively and by 75 minutes post dose were elevated by approximately 10%, 72% and 75%, respectively ($p<0.05$), whereas glucose concentration decreased progressively by approximately 30% of the pre-dose value ($p<0.05$, shown in Table 2).

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Glucose consumption, expressed as µmoles per 100g of intestinal segment in one hour ±SEM, was 368 ± 79.

Absorption of antipyrine, terbutaline, ^{3}H-mannitol and Texas red dextran (70K) from segments of human jejunum

The time dependent appearance of the four standards in perfusate are shown in Figure 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 (Figure 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 % Fraction Absorbed values observed *in vivo* (Table 3). The first order rate constant was also consistent with previous reported human P_{eff} (Tabel 3 and Figure 4). 1.8 ± 0.6% of the Texas red dextran was absorbed during 75 minute perfusion, indicating the integrity of the intestinal segments.

Absorption of *sulfasalazine, furosemide, timolol, nadolol, ranitidine, atenolol, metropolol, theophylline and cimetidine* from segments of human jejunum

In these perfusions the leakage of Texas red dextran (70K) from the gut lumen into the circulation was low, less than 2.3 ± 0.9% of dose (25% are fragments less than 10K), confirming the integrity of the human jejunum segments. In terms of blood chemistry this set of gut perfusions (listed in table 4) was consistent with those in table 2, following 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 609 ± 184 µmoles x $(100g)^{-1}$ x h^{-1}.

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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 out of the nine drugs, ka values for each compound in the three separate gut perfusions differed by up to 1.2 to 1.7 fold. One exception was furosemide where ka value differed between three perfusions by up 3.6 fold. Taking the mean values of ka, the compounds could be ranked into four groups with increasing absorption rates, Group 1 - sulphasalazine and furosemide, ka = 3.9 - 4.0 x 10^{-3} min^{-1}: Group 2 - cimetidine, timolol, nadolol and ranitidine, ka = 6.4 - 8.3 x 10^{-3} min^{-1}; Group 3 - atenolol and metropolol, ka = 9.6 x 10^{-3} min^{-1} and Group 4 - theophylline, ka = 17.5 x 10^{-3} min^{-1}. This is consistent with previous reported human P_{eff} and pharmacokinetics studies (Table 5 and Figure 4), with exception of atenolol and nadolol that is classified as low permeability and low absorption.

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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 means something different 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 is 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 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 might be impossible to validate almost any new technology. For example, values for mannitol absorption range from 16 to 80% of the administered dose, atenolol ranges from 37 - 71%, furosemide 50 - 61 % FA, sulphasalazine 7 - 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 tight-junction integrity check because it's transport through the membrane is consistently low and it is

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generally well accepted within the absorption scientific community that the mannitol absorption in humans is indeed low (< 20%). But careful studies in humans and animals would suggest that mannitol is well absorbed from the intestine (65-80%) with less than 10% excreted in the faeces (Dowty and Dietsch, 1997; Nasrallah and Iber, 1969). The early low values have been attributed to metabolism to CO_2, difficulties encountered in mannitol analysis (Nasrallah & Iber, 1969). This does not diminish the role of Caco-2 cells in drug absorption studies but neither does the higher absorption of the biomarker mannitol across human intestine 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 in order 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 % Fraction Absorbed after oral administration in humans is consistently different for the two compounds, with a % FA ratio for antipyrine : terbutaline of 1.7 (Lennernas, 1998). This absorption differential was reproduced in the present study of perfused human gut segments where the ratio of the first order rate constants for absorption of antipyrine to terbutaline was 1.9 to 1. In three unsuccessful human gut segment perfusions (data not shown) the differential rates of absorption between antipyrine and terbutaline was lost (ratio - 0.89, 0.91 and 0.89) and these two standards and mannitol were all transported at the same rate although the biochemical and

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physiological parameters were consistent with guts segment that demonstrate differential absorption between antipyrine and terbutaline. Collectively, 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 to tebutaline 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 correlates with the % 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 sulphasalazine stand out in as being consistently the highest and lowest rates and extent of absorption, showing an agreement with the fact of sulfasalazine is lower bioavailability and lower permeability (Mos et al., 2005; Watkinson, 1986) and theophyline is high bioavailability. Likewise metoprolol, ranitidine, timolol and cimetidine are consistently mid-range absorption, which is not surprising given the wide range. By 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 could suggest that absorption may be facilitated by co-interaction with other drugs. In the case of atenolol, this is not likely since reduced permeability have 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,

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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, pro-drugs

versus actives, drug-drug interactions, food effects, age and gender differences in the

target species, should make this methodology a useful addition to the drug development

process.

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Acknowledgements

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Legends for figures

Figure 1:   Schematic for *ex vivo* perfusion of isolated segments of human jejunum.

Figure 2:   Percentage absorption of antipyrine (n = 6), terbutaline (n = 6), ^{3}H-mannitol (n = 2) and Texas red dextran-70K (n = 3) during perfusion of isolated segments of human intestine. Data is expressed as the mean ± SD.

Figure 3:   Absorption ratio of antipyrine/terbutaline (n = 6), antipyrine/^{3}H-mannitol (n = 2) and terbutaline/^{3}H-mannitol (n = 2) in the re-circulating perfusate after dosing into the lumen of isolated segments of human intestine. Data is expressed as the mean ± SD.

Figure 4:   Plot of *in vivo* human jejunum drug permeability vs. apparent absorbance rate constant obtained from *ex vivo* human jejunum perfusion.

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Table 1:   Experimental Groups Details

Sm. Intestine #	Age (years)	Sex	Weight (Kg)	Blood Group	A/T Ratio
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	-

Group 1 - cassette dose containing antipyrine (21 μMoles), terbutaline (20 μMoles) and mannitol (21 μMoles, containing 0.007 μMoles of ^{3}H-mannitol as a tracer, with a specificity 6.6 μCi/μMole) or Texas red 70 K dextran (5 mg). Group 2 - cassette dose containing 5 μMoles of sulfasalazine, furosemide, timolol, nadolol, ranitidine, atenolol, theophylline, cimetidine and 10 μMoles of metropolol. *A/T ratio- antipyrine/terbutaline ratio.*

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Table 2: Physiological and biochemical parameters of isolated vascular perfused segments of human intestine during predose and 15, 30, 45, 60 and 75 minutes after administration of antipyrine (21 μmoles), terbutaline (20 μmoles) and mannitol (21 μmoles) or Texas red dextran-70K (5mg) (Group 1).

Parameters	Mean Pre-dose Values	Mean % of change at each time point (min) ±SEM (n=6)				
		15	30	45	60	75
Perfusion Pressure	38 ± 5.8 (mmHg)	89.3 ± 3.7	75.5 ± 2.4*	69.7 ± 6.6*	65.9 ± 6.7*	64.3 ± 6.4*
Perfusion Flow	59 ± 6 (mL/min)	100 ± 0.0	101 ± 1.2	101 ± 1.2	101 ± 1.2	101 ± 1.2
Temperature	35.9 ± 0.3 (°C)	101 ± 1.2	101 ± 1.4	100 ± 1.6	102 ± 0.9	101 ± 0.9
Ht	16.5 ± 0.7 (%)	99.8 ± 2.6	93.8 ± 3.4	96.1 ± 2.0	96.1 ± 2.9	95.2 ± 3.1
pH	7.4 ± 0.1	100 ± 0.2	101 ± 0.4	101 ± 0.5*	102 ± 0.6*	102 ± 0.7*
Glucose	115.3 ±2.6 (mg/dL)	97 ± 2.3	95 ± 3.5	91 ±3.6*	86 ± 3.7*	79 ± 2.8*
Lactate	6.4 ± 0.9 (mmol/L)	114 ± 4.5	137 ± 11*	153 ± 15*	166 ± 18*	172 ± 16*
K$^+$	6.1 ± 1.1 (mmol/L)	102 ± 0.9	104 ± 1.1*	107 ± 2.3*	110 ± 2.2*	111 ± 1.9*
Amylase	20.5 ± 7.4 (U/L)	n/a	159 ± 19*	n/a	186 ± 19*	n/a

P<0.05 vs. Predose (100%). n/a- not analyzed.

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Table 3: Absorption kinetics for antipyrine (22 µmoles), terbutaline (20 µmoles) and mannitol (21 µmoles) after cassette dosing into the lumen of isolated vascular perfused segments of human intestine.

Test Compound	$ka \times 10^{-3}$ (min^{-1}) Range	R^2	% fraction absorbed in transit time of 2.5h	% fraction absorbed *in vivo*	Human P_{eff}, ($\times 10^{-4}$ cm/sec)
Antipyrine (n=6)	3.2-13.2	>0.9460	51-80	100[c]	5 [a]
Terbutaline (n=6)	2.2-6.2	>0.9719	34-60	60[b]	0.3[a]
[3]H-Mannitol (n=2)	4.9	>0.9763	36-55	16-80[d]	-

% FA in transit time of 2.5 h is derived from calculated Ka values. [a] *Taken from Lennernas, 1998.* [b] *Taken from Zhao et al., 2003.* [c] *Taken from Lennernas, 2007.* [d] *Taken from* Dowty and Dietsch, 1997.

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Table 4: Physiologic-al and biochemical parameters of isolated vascular perfused segments of human intestine during pre-dose and 15, 30, 45, 60 and 75 minutes after administration of 5 µmoles of sulfasalazine, furosemide, timolol, nadolol, ranitidine, atenolol, theophylline and cimetidine, and 10 µmoles metropolol

| Parameters	Mean Predose Values	Mean % of change at each time point (min) ± SEM (n=3)				
		15	30	45	60	75
Perfusion Pressure	34.1 ± 1.9 (mmHg)	122.2 ± 10	147 ± 19*	150.2 ± 18*	145.6 ± 8.3*	138.3 ± 5.6*
Perfusion Flow	42.9 ± 13.9 (mL/min)	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0
Temperature	35.9 ± 0.5 (°C)	97.3 ± 0.1*	97.7 ± 1.0*	98.9 ± 0.7	99.3 ± 1.0	99.1 ± 0.9
Hct	15.7 ± 0.7 (%)	102.2 ± 2.2	100.3 ± 3.6	98.3 ± 5.4	103.9 ± 3.9	108.1 ± 5.1
pH	7.6 ± 0.2	100.3 ± 0.4	100.4 ± 0.6	100.2 ± 0.6	100.3 ± 0.7	100.3 ± 0.9
Glucose	115.7 ± 17.1 (mg/dL)	90.2 ± 2.1*	85.2 ± 4.0*	79.0 ± 4.8*	74.0 ± 5.9*	68.8 ± 6.9*
Lactate	11.3 ± 3.1 (mmol/L)	105.9 ± 1.6	113.9 ± 2.9	120.3 ± 3.7*	126.3 ± 7.6*	129.8 ± 11*
K^+	5.8 ± 0.8 (mmol/L)	84.3 ± 19.1	85.3 ± 19.5	86.6 ± 19.5	88.3 ± 19.9	89.7 ± 20.0
Amylase	137.0 ± 103.4 (U/L)	n/a	134.1 ± 5.4	n/a	181.7 ± 21*	n/a

*$P<0.05$ vs. Predose (100%).   n/a- not analyzed.

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Table 5:   First-order apparent rate constant (ka) of nine drugs in the circulation following cassette dosing into the lumen of isolated vascular perfused segments of human jejunum (n = 3).

Test Compound	ka x10^{-3} (min^{-1}) Range	Mean	% fraction absorbed in transit time of 2.5h	% FA from clinical studies	Human P$_{eff}$, (x10^{-4} cm/sec)
Sulphasalazine	3.2-4.7	4.0	38-51	7-17[d]	-
Furosemide	1.9-6.8	3.9	25-64	50-61[d]	0.3[b]
Cimetidine[a]	5.9-7.1	6.4	59-66	75[f]	0.6[b]
Timolol	6.8-9.3	7.9	64-75	95[e]	-
Nadolol	6.9-10.3	8.3	64-79	20/57[e]	-
Ranitidine	6.2-9.4	8.3	61-76	50-60[f]	0.27[b]
Atenolol	7.4-11.7	9.6	67-83	37-71[d]	0.15[c]
Metoprolol	7.9-11.2	9.6	69-81	95[f]	1.5[c]
Theophylline	12.5-20.9	17.5	85-97	96-100[d]	-

%FA in transit time of 2.5 h is derived from calculated Ka values. [a] The values for cimetidine were obtained by LC/MS analysis. [b] Taken from Zakeri-Milani at al, 2007. *[c] Taken from Lennernas, 1998. [d] Taken from Ingels et al., 2007. [e] Taken from Zhao et al., 2003. [f] Taken from Lennernas, 2007.*
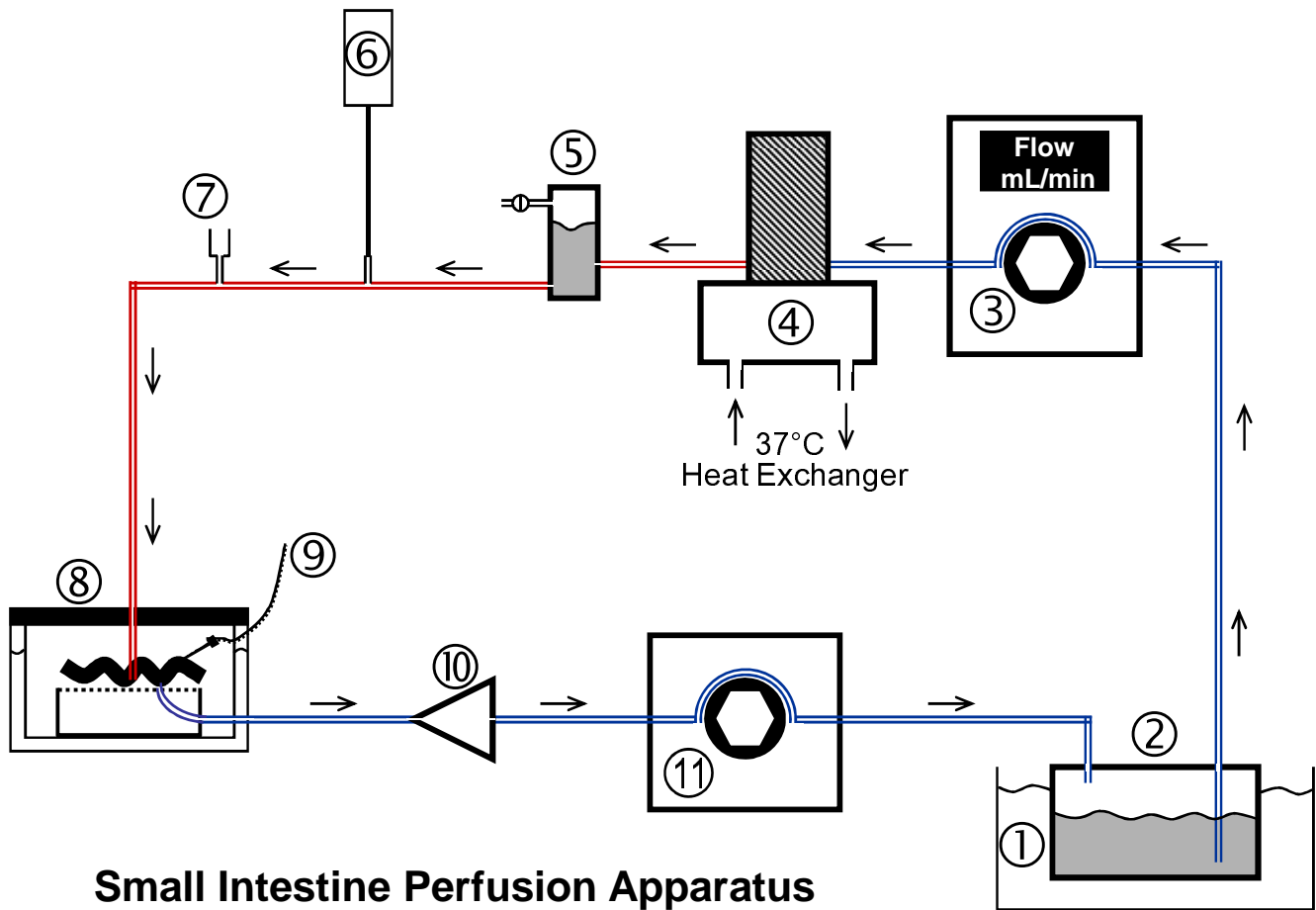
Small Intestine Perfusion Apparatus

1-Water Bath

2-Perfusate Reservoir

3-Watson-Marlow Pump

4- Hollow Fiber Oxygenator

5- Bubble Trap

6- Pressure transducer

7- Vacutainer

8- Organ Incubator

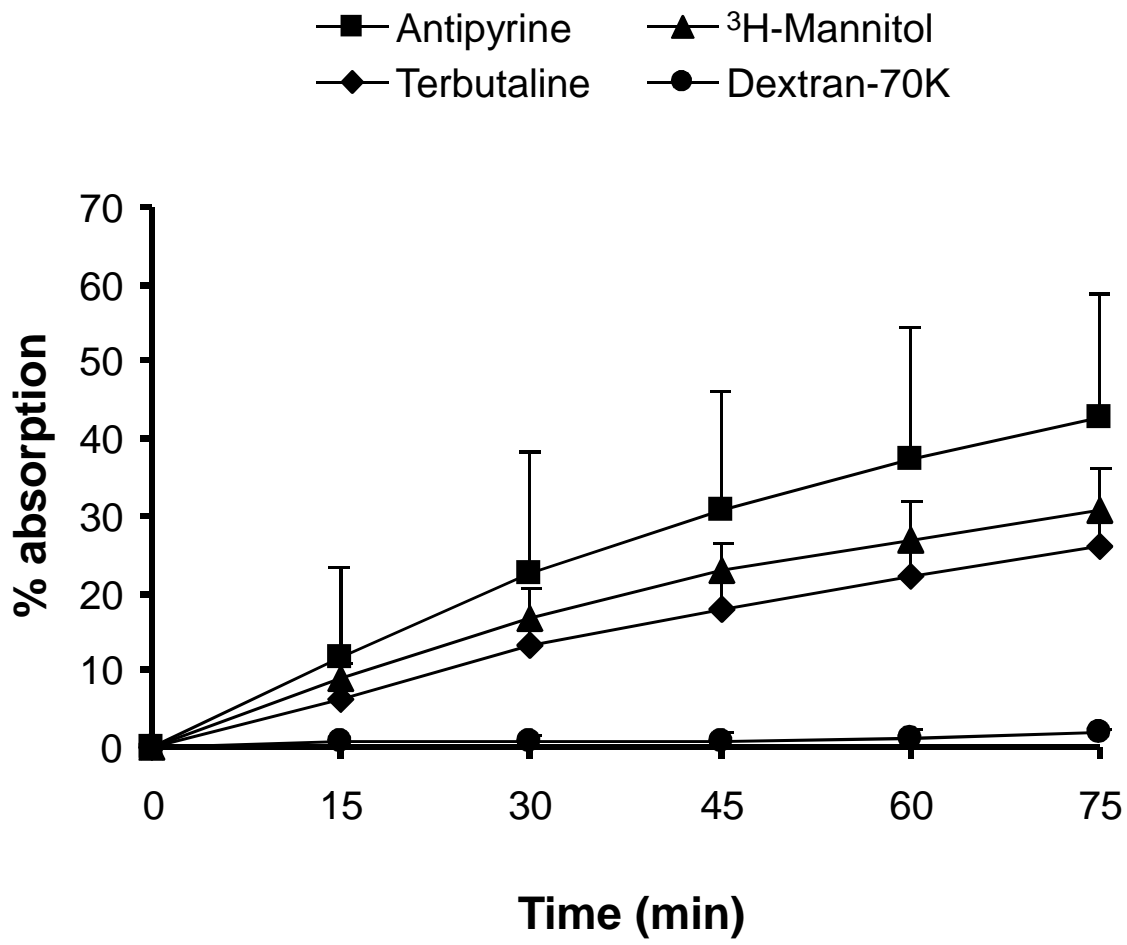9- Temperature Probe

10- Blood transfusion filter

11- Peristaltic Pump

Figure 1

Figure 2

Figure 3

Figure 4