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Research Article

Regional Jejunal Perfusion, a New *in Vivo* Approach to Study Oral Drug Absorption in Man

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Recently a new *in vivo* approach in man, using a regional intestinal perfusion technique, has been developed. The perfusion tube consists of a multichannel tube with two inflatable balloons, which are placed 10 cm apart. The tube is introduced orally and the time required for insertion and positioning of the tube is approximately 1 hr. In the present study eight healthy subjects were perfused in the proximal jejunum on three separate occasions. The first two perfusion experiments used the same flow rate, 3 ml/min, and the third experiment used 6 ml/min. Phenazone (antipyrine) was chosen as the model drug. The recovery of PEG 4000 in the outlet intestinal perfusate was complete in experiments 1 and 2, but slightly lower (90%) when the higher flow rate was used. The mean (±SD) fraction of phenazone absorbed calculated from perfusion data was 51 ± 12% (3 ml/min), 64 ± 19% (3 ml/min), and 42 ± 27% (6 ml/min) for the three experiments, respectively. The mean fraction absorbed estimated by deconvolution of the plasma data was 47 ± 16%, 51 ± 19%, and 38 ± 26%, respectively. The effective permeability of phenazone was 5.3 ± 2.5, 11 ± 6.8, and 11 ± 12 ($\times 10^4$) cm/sec, respectively. We have shown that it was possible to establish a tight intestinal segment which behaved as a well-mixed compartment. The low perfusion rate of 3 ml/min was preferred, since it resulted in the lowest variability in absorption. The absorption of phenazone and *d*-glucose were highly correlated, which was due partly to the mean residence time of the solution in the intestinal segment, but other factors such as variable mucosal surface area also seemed to be important. The new perfusion method was validated by the observed agreement found between absorption from the intestinal perfusate and the degree of absorption obtained by deconvolution of the plasma concentrations. Thus, the regional jejunal perfusion technique seems to have great potential for quantitative and mechanistic evaluations of drug absorption from the human intestine.

KEY WORDS: intestinal perfusion; oral absorption; intestinal permeability; phenazone; antipyrine; glucose.

INTRODUCTION

Intestinal perfusion techniques in man have been used for several decades to investigate absorption and secretion processes, motor activity, and gastric emptying (1–3) but have not been applied to investigate drug absorption except on rare occasions (4–7). In an intestinal perfusion experiment, a solution of the compound(s) of interest and a nonabsorbable marker is infused into the intestinal test segment. Samples of the perfusate are collected when the perfusion medium has passed through the segment. The nonabsorbable marker, often radiolabeled polyethylene glycol 4000 (^{14}C-PEG 4000) is present in the perfusion solution to correct for changes in the outlet drug concentrations due to fluid absorption and secretion. The difference between the inlet and the outlet concentrations of the solute is assumed to have been absorbed.

In earlier intestinal perfusion studies in man, mostly open or semiopen perfusion systems were used. Several factors diminish the reproducibility of the data obtained with these systems. These methods are hampered by the fact that proximal and/or distal luminal contents may enter the test segment to such an extent that the ability to control the absorption condition is attenuated. Moreover, such techniques generally require higher perfusion flow rates (10–20 ml/min) than normal jejunal flow rates, which range between 0.6 and 4.2 ml/min including both fasted and fed state (8,9). Further, the recovery of the perfusion fluid is low and variable, rendering quantitative and mechanistic studies of intestinal drug absorption unreliable. Moreover, in order to determine the absolute values of secretion clearance, absorption clearance, and permeability, the length of the intestinal segment studied has to be known.

Recently, an intestinal perfusion instrument has been

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developed (10) which consists of a multichannel tube with two inflatable balloons (Fig. 1). A 10-cm-long segment is created between these balloons, enabling perfusion of a defined and closed part of jejunum. This perfusion system has been used in previous studies where secretion of endogenous substances has been investigated. The leakage of intestinal contents into the segment over the proximal balloon has been reported to be approximately 2% using phenol red as a marker and the recovery of the nonabsorbable marker was almost complete (10–12). The occlusion of an intestinal segment between two intraluminal balloons offers one way of minimizing contamination of luminal contents both proximally and distally into the isolated segment. These qualities should permit the control of absorption conditions in the intestinal segment, to evaluate and quantify absorption mechanisms in human intestine. Further, the technique offers possibilities for precise and simultaneous kinetic studies of both intestinal and plasma compartments.

The main objective in this study was to evaluate the potential of the new regional perfusion tube as a tool for explicit investigations of drug absorption mechanisms in the human intestine. Phenazone (antipyrine) was chosen as a model drug on several grounds. The drug is neutral, is highly soluble in water, exhibits rapid and complete intestinal absorption, has no gut wall metabolism, has a low hepatic extraction ratio, is devoid of strong pharmacological activity, and can easily be measured in intestinal perfusate and plasma (13–18). The molecular weight is 188 and the partition coefficient of octanol:water, pH 7.4, is 0.4 (13). The presence of nutrients and electrolytes in the intestinal lumen and different osmotic pressures in luminal contents may affect oral drug absorption (19–22). Therefore we evaluated the absorption of *d*-glucose, alterations in concentrations of electrolytes, and osmolality of the outlet perfusion fluid in parallel with absorption studies of phenazone. This will provide us with reference data for future studies using this technique.

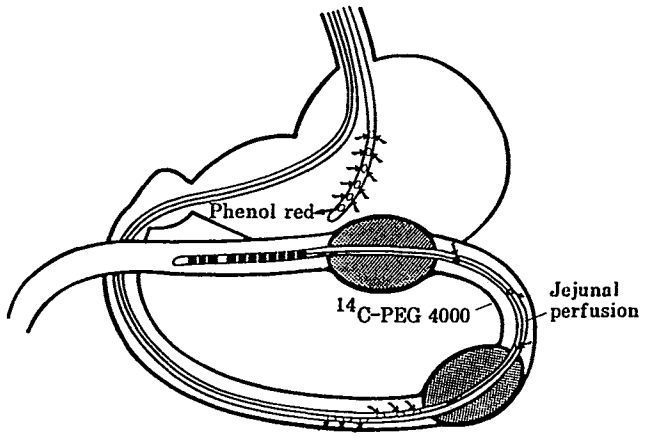

Fig. 1. The tube system with double balloons allowing segmental jejunal perfusion. The balloons are filled with air when the proximal balloon has passed the ligament of Treitz. Gastric suction is obtained by a separate tube. Phenol red is used as a marker in the stomach to detect proximal leakage into the isolated segment and ^{14}C-PEG 4000 is used as a volume marker.

MATERIALS AND METHODS

The Design of the Tube

The intestinal perfusions were performed with a newly developed, sterile and disposable perfusion tube for segmental intestinal perfusion. It is intended to be used for studies of absorption and secretion in the small intestine in humans (Loc-I-Gut, Kabi-Pharmacia, Sweden). The multichannel tube is 175 cm long and is made of polyvinyl chloride with an external diameter of 5.3 mm (16 French). It contains six channels and is provided distally with two 40-mm-long, elongated latex balloons, placed 10 cm apart, each separately connected to one of the smaller channels. The two wider channels in the center of the tube are for infusion and aspiration of perfusate. The two remaining peripheral smaller channels are used for administration of marker substances or for drainage. At the distal end of the tube is a tungsten weight attached in order to facilitate passage of the tube into the jejunum. Gastric suction is obtained through a separate gastric tube (Fig. 1).

Drug and Perfusate Composition

Phenazone (antipyrine) was supplied by Kabi-Pharmacia, Sweden. The concentration of phenazone in the perfusion fluid entering the intestinal segment was 2 mg/ml (10.5 m*M*) in all experiments. The perfusion solution infused into the segment consisted of 10 m*M* *d*-glucose, 5.4 m*M* KCl, 120 m*M* NaCl, 2 m*M* Na_2HPO_4, 1 g/L polyethylene glycol (PEG 4000), MW 4000, and 35 m*M* mannitol. Phenol red (50 mg/ml) was infused through the sump line of the gastric tube and detected as a marker of proximal leakage into the segment. The osmolality was approximately 290 mosm/L. Polyethylene glycol labeled with ^{14}C (^{14}C-PEG 4000) was purchased from Amersham Laboratories, Buckinghamshire, England, and added to the perfusion solution as a volume marker (2.5 μCi/L).

Study Design and Experimental Procedure

Eight healthy subjects (four male and four female, aged 22–30 years) gave informed consent to participate in the study. The study was approved by the Ethics Committee of the Medical Faculty, Uppsala University. The perfusion experiments were performed in the morning after a 10-hr overnight fast and each subject participated three times on 3 separate experimental days. The first two perfusion experiments (I and II) used the same flow rate (3 ml/min). In the third perfusion experiment (III) the flow rate used was 6 ml/min. The experiments were performed in the order mentioned in each subject and the time between the different occasions varied but was at least 4 days. The total time to complete the study was 4 months.

The tube was introduced orally after local anesthesia with lidocaine in the upper throat. A Teflon-coated guide wire was used during insertion of the tube since it facilitated passage through the stomach. The insertion and positioning of the tube were made under fluoroscopic guidance (Philips BV 21-S). The mean time required for the insertion was approximately 1 hr. When the tube had been positioned in the proximal jejunum, the balloons were inflated with 26–34 ml

of air, creating a 10-cm-long closed segment. The pressure of the intestinal balloons was 20–40 mm Hg.

The perfusion experiment started by rinsing the segment with isotonic saline (37°C) for at least 10 min, using a syringe pump (Model 355, Sage Instrument, Orion Research Inc., Cambridge, MA). After the rinsing period the segment was perfused for 40 min with the perfusion solution (free from drug), which was followed by the experimental period, where the intestinal segment was perfused with the medium containing phenazone. The jejunal perfusion fluid leaving the intestinal segment was collected on ice by gravity drainage and was fractionated at 20-min intervals when the perfusion flow rate was 3.0 ml/min and at 10-min intervals when the flow rate was 6.0 ml/min. All syringes and perfusion samples were weighed and the samples were then immediately frozen and stored at −20°C until analysis. The solution of phenol red (50 mg/L saline) was infused into the stomach at a flow rate of 1 ml/min. The subjects were recumbent during the whole perfusion period of 140–160 min. After the cessation of drug perfusion the intestinal segment was rinsed with approximately 150 ml saline during 3–5 min in order to prevent further drug absorption. Blood samples were withdrawn with a new needle for each sample into Na-heparinized test tubes. Blood samples were collected every 10 min up to 20 min after the cessation of the perfusion and then three samples were taken at half-hour intervals. The blood samples were centrifuged (1400*g* for 10 min). The plasma was frozen immediately and stored at −20°C until analysis.

Stability and Adsorption Test of Phenazone and *d*-Glucose

Incubation of phenazone and *d*-glucose in the perfusion medium at 37°C for 180 min caused no degradation of the compounds. The stability of phenazone and *d*-glucose in the intestinal perfusate was also tested. Luminal perfusate was incubated for 60 min at 37°C and a pH of 7.8, and no degradation of phenazone or *d*-glucose was detected. No adsorption of either phenazone or *d*-glucose to the material in the catheters was found.

Analytical Methods

All chemicals used were of analytical grade. Two previously published HPLC methods for determination of phenazone (17,18) were combined and slightly modified. The equipment used included a HPLC pump (Shimadzu LC 6A), a manual injector (Rheodyne 7125), and a UV spectrophotometer (Shimadzu SPD-6A). The mobile phase consisted of 70% phosphate buffer, 0.05 *M*, pH 7.0, and 30% acetonitrile. The separation column was 130 * 4.6-mm i.d. and the packing material was Nucleosil, 5 μm, with a precolumn (S5-ODS 2-10C5). The UV absorption was recorded at 254 nm at a flow rate of 1.0 ml/min and 4-dimethylaminopyrine was used as an internal standard. The retention times of antipyrine and 4-dimethylaminopyrine were 3.1 and 4.8 min, respectively. NaOH (50 μl, 0.5 *M*) was added to plasma and perfusate samples (500 μl), which were then extracted with 1.0 ml methylene chloride for 5 min. After centrifugation (3000*g* for 5 min), the organic phase (800 μl) was evaporated to dryness under a gentle stream of nitrogen. The residue was dissolved in 200 μl of mobile phase and 10 μl was injected onto the column. The regression coefficient of the standard curves was 0.99. The reproducibility, expressed as the coefficient of variation (CV) of repeated determinations, was 1.1, 1.0, and 2.9% for low, medium, and high concentrations, respectively. The CV between days was 10.0, 5.0, and 3.9%, respectively. The detection limit in plasma and intestinal perfusate was 0.1 and 1.0 μg/ml, respectively. Phenol red was measured spectrophotometrically (spectrophotometer Model PM 6, Zeiss) in intestinal perfusate after alkalinization to pH 11.8. Perfusate samples (0.50 g) were weighed and the total radioactivity of ^{14}C-PEG 4000 was determined by liquid scintillation counting (dpm) for 10 min (Beckman Instruments, Model 244) after the addition of 10 ml Beckman Ready Safe. The analysis of *d*-glucose and the electrolytes Na^+, K^+, and Cl^- was performed in an automatic multianalyzing instrument (Hitachi 717, Boerhinger Mannheim). The osmolality of the outlet perfusion solutions was measured by measuring vapor pressure (Vescor osmometer 5500).

Calculations

All calculations in this study were made from the steady-state concentrations of the perfusate leaving the intestinal segment. The volume of the intestinal segment during each sampling interval was estimated in the following manner:

$$\text{Volume of the segment} = \frac{PEG_{in} - PEG_{out}}{[PEG]_{out}} - \text{tube volume} \quad (1)$$

The volume of the intestinal segment was calculated by subtracting the accumulated amount of ^{14}C-PEG 4000, a nonabsorbable volume marker, in the perfusate leaving the intestinal segment (PEG_{out}) from the accumulated amount entering the segment (PEG_{in}) during the same sampling period. This difference represents the amount of ^{14}C-PEG 4000 left in the whole perfusion system (including both the tube and the intestinal segment). It is then divided by the outlet concentration of ^{14}C-PEG 4000, $[PEG]_{out}$, assuming that it reflects the concentration in the whole perfusion system, since the perfusion solution in the system is viewed as a well-stirred, (mixing tank model) and that ^{14}C-PEG 4000 is a nonabsorbable marker. Finally, the measured volume within the whole tube system was subtracted in Eq. (1) and the volume of the intestinal segment is obtained. The mean residence time (MRT) of ^{14}C-PEG 4000 during each sampling interval is readily estimated by dividing the total content of ^{14}C-PEG 4000 (dpm) left in the intestinal segment by the outflow rate of ^{14}C-PEG 4000 (dpm/min) for each sampling interval. The fluid flux in the segment was evaluated by comparing the total radioactivity per milliliter of the nonabsorbable volume marker ^{14}C-PEG 4000 in the inlet and outlet perfusate samples. The net water flux per centimeter in the isolated intestinal segment for each sample was calculated from

$$\text{Net water flux} = \left(1 - \frac{[PEG]_{out}}{[PEG]_{in}}\right) \times \frac{Q_{in}}{L} \quad (2)$$

where $[PEG]_{in}$ and $[PEG]_{out}$ are the entering and leaving ^{14}C-PEG 4000 (dpm/ml). Q_{in} is the perfusion rate entering

the intestinal segment and is obtained by measuring the inlet volume of the perfusate and dividing it by the sampling time interval, and L is the length of the segment (10 cm). The outlet perfusate concentrations of phenazone and *d*-glucose were corrected for any fluid change according to Eq. (3).

The fraction disappearing from the perfusate when it has passed through the intestinal segment is assumed to be absorbed. The fraction absorbed (FA) of phenazone and *d*-glucose was calculated from the ratio of the fluid-corrected concentrations leaving (C_{out}) and entering (C_{in}) the intestinal segment during steady state and is defined by

$$FA = \left(1 - \frac{C_{out} \times [PEG]_{in}}{C_{in} \times [PEG]_{out}}\right) \times 100 \quad (3)$$

This way of evaluating the absorption from the segment was compared with the average amount absorbed during the experiment (FA_{mean}) according to

$$FA_{mean} = (1 - \text{cumulative amount out/total amount infused}) \times 100 \quad (4)$$

where the cumulative amount out is obtained by multiplying the corrected volume leaving the intestinal segment (V_{out}) by the uncorrected leaving concentration $(C_{out})_{uncorr}$ during each sampling interval according to Eq. (5):

$$\text{Cumulative amount out} = \Sigma(V_{out}) \times (C_{out})_{uncorr} \quad (5)$$

One key factor affecting the extent of absorption of compounds is the effective intestinal permeability (P_e), a mass transfer (absorption) parameter. The solution in the intestinal segment is assumed to be described according to a mixing tank model (well stirred) and is then calculated from the following equation (23):

$$P_e = \frac{Q_{out} \times (C_{in} - C_{out})/C_{out}}{2\pi rL} \quad (6)$$

where Q_{out} is the perfusion flow rate leaving the intestinal segment obtained by dividing the measured leaving total volume by the sampling time (10 or 20 min); $2\pi rL$ is the area of the mass transfer surface (cylinder) of length (L) and radius (r) of 1.75 cm (24).

The fractions of phenazone absorbed calculated from Eqs. (3) and (4) were correlated with the absorbed amount derived from deconvolution of plasma concentration data, F_{plasma} (25). The mean values of the rate constants and the intercepts, obtained from the literature (14), were used in the weighting function.

Variability is expressed as the standard deviation (SD) throughout this paper. Tests of significance for differences in absorption parameters between the perfusion experiments were tested by one-way analysis of variance (ANOVA) followed by Scheffe's contrast test.

RESULTS

Segmental Recovery and Mean Residence Time of ^{14}C-PEG 4000

The mean recovery of ^{14}C-PEG 4000 in the perfusate leaving the intestinal segment was 97.2 ± 5.3% in 22 of the 24 perfusions but decreased in two perfusion experiments when the higher flow rate (6 ml/min) was applied (Table I). Proximal leakage of fluid into the segment according to phenol red detection in the perfusate leaving the segment was less than 2%. The measured flow rate leaving the intestinal segment was 3.0 ± 0.3, 2.9 ± 0.3, and 5.7 ± 0.6 ml/min (Table I). Applying the lower flow rate (3 ml/min), the mean residence time (MRT) of ^{14}C-PEG 4000 in the intestinal segment was estimated to be 15 ± 3 min for perfusion experiment I and 27 ± 13 min for perfusion experiment II. When the higher flow rate (6 ml/min) was used, a more variable MRT of 17 ± 15 min was obtained (Table I).

Water Flux and Transport of Electrolytes

The net flux of fluid into and out of the isolated segment occurred as both secretion and absorption, but to a relatively small extent (Table I). The mean steady-state concentrations of the electrolytes Na^+, K^+, and Cl^- in the perfusate leaving the intestinal segment were the same as the entering concentrations of the electrolytes. The coefficient of variation for the electrolyte concentrations was less than 5% (Table II). The osmolality in the perfusion solution leaving the intestinal segment was on average 288 ± 2.7, 289 ± 8.0, and 293 ± 11.6 mosm/L, respectively. The homeostasis of the electrolytes and, therefore, the isosmolality condition in the segment were maintained during the experiments. The mean pH in the outlet perfusate for the three perfusion situations was 7.8 ± 0.4, 7.9 ± 0.7, and 7.6 ± 0.4, respectively. The pH in the perfusate entering the intestinal segment was 7.4.

Absorption of Phenazone

The individual concentrations of phenazone in the perfusate leaving the intestinal segment for each perfusion are shown in Fig. 2. The increased interindividual variability in FA for experiment III (6 ml/min) was reflected by a simul-

Table I. Mean Values (±SD) of Technical Parameters Calculated from the Steady-State Level Following Intestinal Perfusion of the Proximal Jejunum in Humans[a]

Exp no.	Perfusion rate (in) (ml/min)	PEG recovery (%)	Net water flux (μl/min/cm)[b]	$V_{segment}$ (ml)	Perfusion rate (out) (ml/min)	MRT (min)
I	3.0	98.4 ± 6.9	7.4 ± 24.7	45 ± 8	3.0 ± 0.3	15 ± 3
II	3.0	97.3 ± 5.3	5.8 ± 28.2	74 ± 30	2.9 ± 0.3	27 ± 13
III	6.0	90.6 ± 9.0	18.9 ± 14.3	86 ± 71	5.7 ± 0.6	17 ± 15

[a] The inlet perfusion rate was 3 ml/min at experimental occasions I and II and 6 ml/min at experimental occasion III.
[b] Positive values indicate that fluid is secreted into from the lumen.

Table II. Mean Values (±SD) of Absorption Parameters of Phenazone and *d*-Glucose (d-glc) Calculated from the Steady-State Concentrations in the Perfusion Solution Leaving the Intestinal Segment During Perfusion of Proximal Jejunum in Humans[a]

Exp no.	Perfusion rate (ml/min)	P_e (phenazone) (cm/sec)[b]	P_e (d-glc) (cm/sec)[b]	FA (d-glc) (%)	FA_{mean} (d-glc) (%)	C_{ss} (Na^+) (m*M*)	C_{ss} (K^+) (m*M*)	C_{ss} (Cl^-) (m*M*)
I	3.0	5.3 ± 2.5	8.8 ± 4.4	63 ± 11	60 ± 15	132 ± 3.1	5.5 ± 0.2	127 ± 2.5
II	3.0	11 ± 6.8	19 ± 20	72 ± 19*	72 ± 20*	132 ± 2.1	5.3 ± 0.2	127 ± 2.3
III	6.0	11 ± 12	23 ± 30	50 ± 31*	52 ± 30*	131 ± 2.9	5.4 ± 0.2	126 ± 3.1

[a] Also given are the mean (±SD) steady-state concentrations in the perfusate of the electrolytes: Na^+, K^+, and Cl^-. The perfusion rate was 3 ml/min at experimental occasions I and II and 6 ml/min at experimental occasion III.
[b] Multiplied by 10^4.
* $P < 0.05$.

taneously increased variation of the plasma concentrations of phenazone (Fig. 3). Each perfusate concentration is normalized to the same inlet concentration, 2 mg/ml, and corrected for fluid flux according to Eq. (3). Mixing equilibrium of phenazone in the intestinal segment seemed to be reached at the 60-min sample when the flow rate was 3 ml/min and at the 40-min sample when the flow rate was 6 ml/min. Absorption parameters are shown in Table II. The FA of phenazone was 51 ± 12, 64 ± 19, and 42 ± 27%, respectively (Fig. 4). The significant decrease in absorption comparing experiments II and III was mostly due to the decreased residence time in the segment. The FA of phenazone based on steady-state values was in agreement with the average amount absorbed (FA_{mean}) during the experiment (Fig. 4). The effective permeability (P_e) was 5.3 ± 2.5, 11 ± 6.8, and 11 ± 12 ($\times 10^4$) cm/sec, respectively. No significant difference between the experiments could be observed in P_e (Table II). The interindividual variability in the absorption parameters was about 30% during the first experimental occasion but increased for the other two experimental occasions, due mainly to an increased variability in the residence time (Tables I and II). The intra- and interindividual variability in MRT is due mainly to a varied degree of filling volume in the segment. The linear correlation between the FA of phenazone and the MRT as described by r_{xy}^2 was 0.49 ($P < 0.05$) and the same correlation was found for the relation of P_e and MRT.

Plasma Levels of Phenazone

The mean (±SD) plasma concentration–time profile of phenazone is presented in Fig. 3. The mean fraction absorbed (F_{plasma}) for the three perfusion situations estimated by deconvolution of the plasma data was 47 ± 16% (3 ml/min), 51 ± 19% (3 ml/min) and 38 ± 26% (6 ml/min), respectively. The agreement among the three ways of calculating the degree of absorption is shown in Fig. 4. When the drug perfusion was terminated and the segment rinsed completely with saline, no further increase in the plasma concentrations of phenazone was observed (Fig. 3). The small decline in the plasma concentration profile is due to the half-life of phenazone, at least 10 hr (14–16).

Absorption of *d*-Glucose

The mixing equilibrium for *d*-glucose in the intestinal segment was reached at the same sampling time as for phenazone. In Table II absorption parameters of *d*-glucose are shown; the absorption of *d*-glucose was slightly more

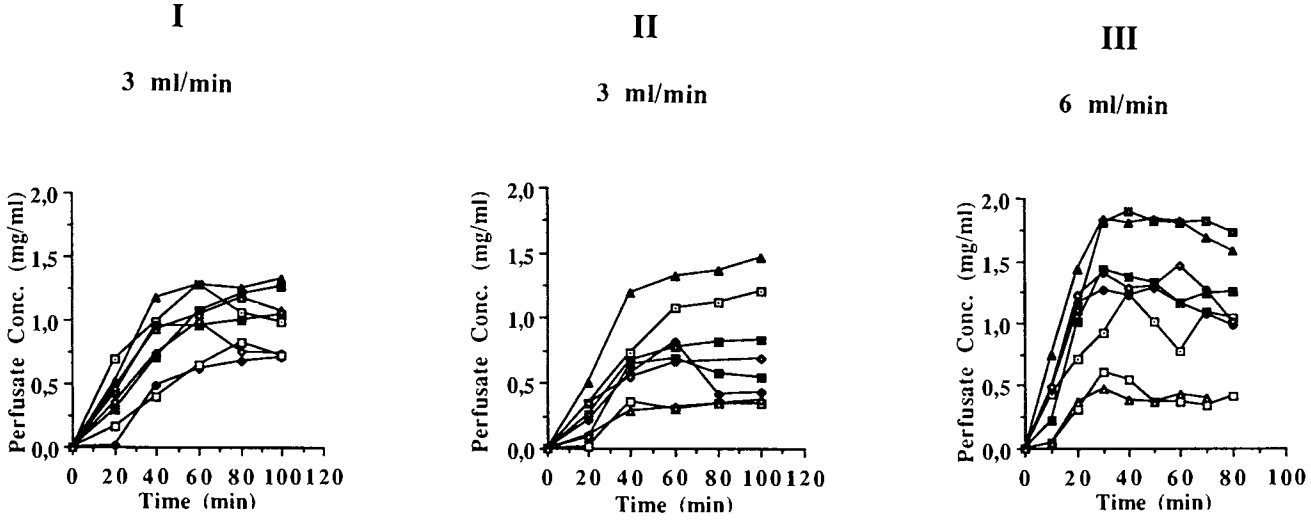

Fig. 2. Individual concentrations of phenazone in the perfusate leaving the intestinal segment. The concentration of phenazone in the perfusate entering the segment was 2 mg/ml (10.5 m*M*) in each experiment (n = 8 at each experimental occasion).

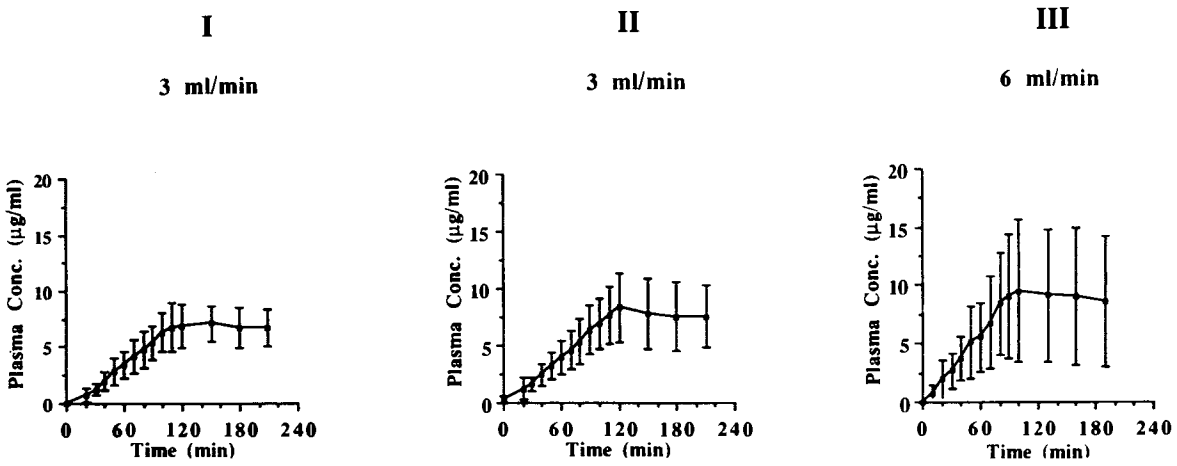

Fig. 3. Mean (±SD) plasma concentration–time curves of phenazone after intestinal perfusion with an inlet concentration of phenazone of 2 mg/ml. Perfusion time in experiments I and II was 100 min, and in experiment III it was 80 min (n = 8 at each experimental occasion).

efficiently absorbed compared to phenazone (Table II, Fig. 4). The FA of *d*-glucose was 63 ± 11, 72 ± 19, and 50 ± 31%, respectively. The effective permeability (P_e) for *d*-glucose was 8.8 ± 4.4, 19 ± 20, and 23 ± 30 ($\times 10^4$) cm/sec, respectively. During the high perfusion flow rate (6 ml/min) decreased absorption, unaffected permeability, and a higher degree of variability were observed for *d*-glucose, analogous to the findings for phenazone (Table II, Fig. 4). The FA values of phenazone and *d*-glucose, calculated from the 24 perfusion experiments (I, II, III), were highly correlated (r_{xy}^2 = 0.91, $P < 0.01$) (Fig. 5). The high correlation between the FA of both compounds (r_{xy}^2 = 0.80, $P < 0.01$) was also obtained only when results from the perfusion experiments which used the lower flow rate of 3 ml/min were compared (experimental occasions I and II; in total, 16 perfusion experiments). The linear correlation between the FA of *d*-glucose and the MRT was r_{xy}^2 = 0.49 ($P < 0.05$); the same correlation was found between P_e and MRT.

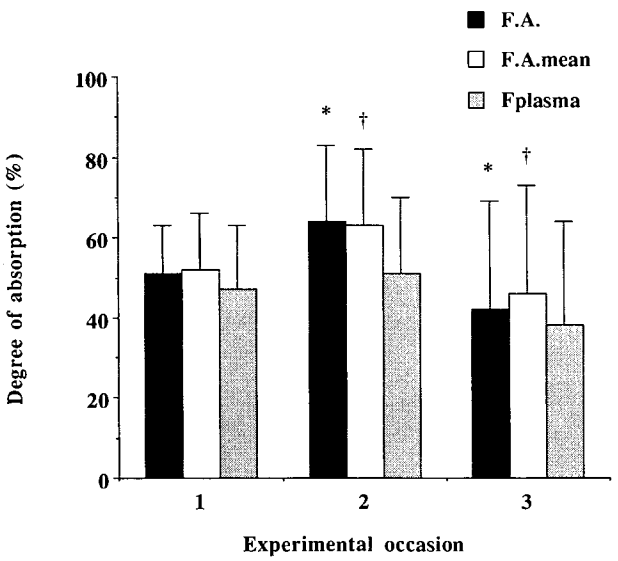

Fig. 4. The mean values (±SD) of the degree of absorption of phenazone calculated in three ways for each of the three experimental occasions (1, 2, and 3). Fraction absorbed (FA) and average amount absorbed (FA_{mean}) were calculated from the jejunal perfusion fluid concentrations of phenazone. The degree of absorption based on plasma concentrations of phenazone (F_{plasma}) was determined using the deconvolution technique. (*, †) $P < 0.05$.

DISCUSSION

The objective of this study was to evaluate the potential of a recently developed intestinal perfusion system (10) to investigate drug absorption from human small intestine. As discussed earlier, phenazone was chosen as a model drug due to its suitable absorption and disposition properties. A prerequisite for using intestinal perfusion as a tool for absorption studies is that the difference between the inlet and the outlet perfusate concentration is due to absorption of the drug molecules under investigation and that other processes that could lead to disappearance from the intestinal perfusate, e.g., instability, precipitation, adsorption to the tube wall, and luminal metabolism of the drug, can be ignored. Another crucial point is whether the solution in the intestinal segment can be viewed as well mixed. The good agreement between the fraction absorbed (FA) and the average amount absorbed (FA_{mean}) of phenazone based on our perfusate concentrations and deconvoluted plasma data (F_{plasma}) indicates a true absorption of the drug and validates our regional jejunal perfusion method (Fig. 4). The stable outlet concentrations of phenazone and *d*-glucose after reaching mixing equilibrium, together with the good agreement between FA and FA_{mean} and the gradual increase in the outlet perfusate concentrations of PEG 4000, support the hypothesis that the solution in the isolated intestinal segment was well mixed (23).

Another important criterion that should be fulfilled in our regional intestinal absorption system is that the normal physiological function of the isolated gut segment should not be affected by the presence of the tube. The absorption of phenazone and other compounds with a high membrane permeability, and therefore complete and rapid intestinal absorption (26,27), are sensitive to alterations in intestinal blood flow (28). The pressure in the inflated balloons of our perfusion instrument is considerably lower than the blood

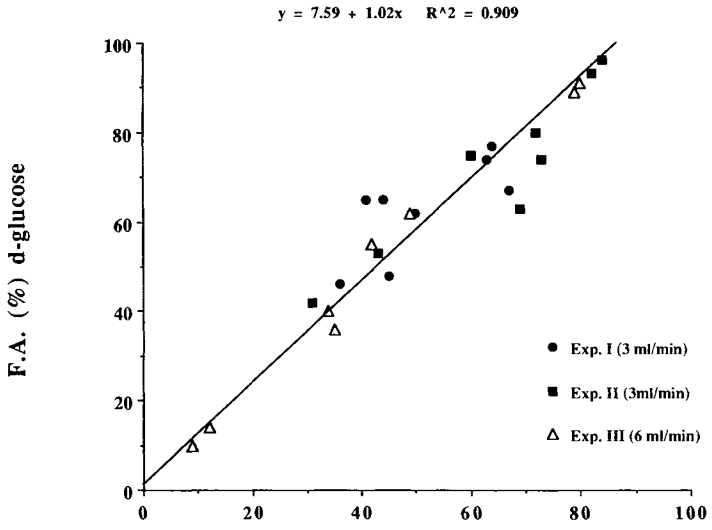

Fig. 5. Correlation between fraction absorbed (FA) of *d*-glucose and phenazone ($r^2_{xy} = 0.91$, $P < 0.01$) during regional jejunal perfusion in man. FA was calculated from the ratio between the inlet and the outlet fluid-corrected concentration of *d*-glucose and phenazone, respectively.

capillary pressure. However, it cannot be fully excluded that the blood flow could be disturbed to some extent by the pressure of the balloons.

When the low flow rate (3 ml/min) was applied, the residence time in the intestinal segment was about 15 min and about 50% of phenazone was absorbed. If the residence time in the intestinal segment had been increased to about 45–60 min, the estimated percentage absorbed of phenazone should be about 90%. After oral administration of 100-ml solution of phenazone to fasting volunteers, previous studies have found that the absorption of phenazone is complete ($F = 1$) and rapid, with a t_{max} of about 1 hr. The total time needed for nearly complete absorption was estimated to be about 60–80 min, assuming that the absorption is almost complete at t_{max} since the elimination rate constant of phenazone is slow (14–16). The time for total emptying of a 100-ml solution from the stomach is anticipated to be about 20–30 min in the fasted state (3). If the total gastric emptying time is subtracted from the total time for complete absorption, the time in the intestine needed for complete absorption is obtained and was estimated to be approximately 40–50 min.

In a perfusion study with a liquid meal consisting of homogenized oil, protein, and glucose, it was found that the absorption of *d*-glucose was complete within 100 cm of jejunum (1). The transit time for 100 cm of jejunum is approximately 50 min (24). The percentage *d*-glucose absorbed in our study was about 60% when the residence time in the closed gut segment was about 15 min. An increase in the residence time in the segment to 45–60 min should lead to complete absorption of *d*-glucose. The agreement of our absorption data for phenazone and *d*-glucose with a normal *in vivo* situation indicates that the perfusion instrument used in the present study has no major influence on the blood flow of the perfused segment or any other physiological function of importance for absorption of solutes from the perfused jejunal segment. Furthermore, the intestinal segment was able to maintain isosmolality and the homeostasis of the electrolytes.

The fraction absorbed of phenazone and *d*-glucose decreased significantly at a higher flow rate (6 ml/min) compared to that in experimental occasion II, which used the flow rate of 3 ml/min. One reason should be a shorter mean residence time in the intestinal segment (Table I). The mean residence time for a drug in an intestinal compartment is one critical factor for drug absorption. In our system the mean residence time may vary due to variability in the dynamic volume of the perfused segment and differences in perfusion flow rates. Our results confirmed that an increase in flow rate when the dynamic volume was similar induced a decreased fraction absorbed of phenazone and *d*-glucose. In contrast to the perfusion flow rate, the volume of the segment is dynamic and therefore difficult to control. Repeated experiments in the same individual (experimental occasions I and II) at the same flow rate showed an intraindividual variation in the volume, but without a significant influence on the absorption parameters. The volume of the segment was relatively constant during a perfusion experiment, which probably means that the intra- and interindividual variation in volume could be due to differences in the localization of the tube in the intestine. An additional explanation is cyclic fluctuations in the contractions of the small intestine, which are typical for the fasting gastrointestinal motility. However, the exact importance of the residence time is difficult to determine since it is difficult to calculate the intestinal segment volume with a high precision.

The interindividual variability of the absorption parameters increased significantly when the higher perfusion flow

rate was used. This is probably due to the highly variable filling volume in the intestinal segment and, consequently, the widely varying residence time. Physiological changes induced by the higher perfusion flow are probably not an important additional factor. Irrespective of the mechanism underlying this phenomenon, we can conclude that drug absorption studies during regional intestinal perfusions should be performed at a flow rate lower than 6 ml/min.

Intestinal permeability data for compounds in man are sparsely available, and for phenazone a direct comparison with data from other studies does not seem possible. The effective permeability coefficient (P_e) of phenazone was about 5–11 ($\times 10^4$) cm/sec. The permeability (P_e) of *d*-glucose in our study was about 9–23 ($\times 10^4$) cm/sec, which is higher than the permeability values in man of 1–2 ($\times 10^4$) cm/sec reported earlier (24). The permeability (P_e) of phenazone and *d*-glucose did not significantly change when the higher flow rate was applied. In several *in vitro* and *in vivo* studies the unstirred water layer has been assumed to be the rate-limiting step in absorption of compounds that have a high permeability over the intestinal mucosa (24). The reason why the permeability did not increase in this study is probably the fact that the luminal stirring, induced normally by the gut motility at this physiological flow rate (3 ml/min), is efficient to reduce the thickness of the unstirred water layer by itself. The increase in flow rate from 3 to 6 ml/min thereby increased stirring but did not further reduce the thickness of the unstirred water layer to any important degree. This suggests that the difference in thickness of the unstirred water layer (UWL) is probably of minor importance as a factor for variability in absorption rate in normal human intestine even for compounds with a high permeability such as phenazone and *d*-glucose. This has also been proposed previously from studies in conscious dogs and rats (29,30).

A significant difference ($P < 0.05$) was found between individuals in the fraction absorbed and effective permeability of phenazone and *d*-glucose. Most subjects maintained the same value of FA and P_e in all experiments. However, a few individuals deviated from a consistent value of the absorption parameters throughout the study, and this was related mainly to differences in the dynamic volume of the segment and, thereby, variable MRT. There was a high correlation ($r_{xy}^2 = 0.91$, $P < 0.01$) between the fraction absorbed (FA) of phenazone and that of *d*-glucose when data from all 24 experiments were compared (Fig. 5). This might be expected if phenazone and *d*-glucose were absorbed by the same mechanism in the gut. However, phenazone is passively absorbed over the intestinal mucosa and *d*-glucose is predominantly actively transported by a special carrier in the apical membrane of the enterocyte. Several factors exist that could explain the correlation in absorption between phenazone and *d*-glucose, such as residence time in the intestinal segment, variable thickness of the unstirred water layer (UWL), solvent drag, and variability in the intestinal surface area. Fraction absorbed was partly related to the residence time in the intestinal segment for phenazone ($r_{xy}^2 = 0.49$, $P < 0.05$) and *d*-glucose ($r_{xy}^2 = 0.49$, $P < 0.05$). The thickness of the UWL has previously been excluded as an important factor for intestinal absorption of solutes with a high permeability, such as phenazone and *d*-glucose. Solvent drag of the solutes cannot be a contributing factor in our study since no correlation was found between net fluid flux and FA. However, water flux across the intestinal mucosa is a potential factor that could contribute to the variability in absorption of drugs, especially when coadministered with food and fluids that generate higher concentrations of nutrients and produce changes in the osmolality in the lumen (19–22). Finally, the correlation between the absorption of phenazone and that of *d*-glucose can be explained by variability in the effective mucosal surface area due to nonspecific regulation of the intestinal mucosa by hypertrophy and changes in microvillus and villus dimensions (31,32). Intestinal surface area variation seems to be equally important for both passively and actively absorbed compounds, such as phenazone and *d*-glucose. The variable surface area theory is supported by the similar values for permeability obtained between experiments using different flow rates, which indicates that absorption of phenazone and *d*-glucose is membrane controlled, rather than influenced mainly by differences in thickness of the unstirred water layer (UWL).

We have shown that the new regional jejunal perfusion technique generates possibilities for isolating the absorption process from other factors affecting drug absorption following oral administration. The ability to control absorption conditions within the closed, regional intestinal segment results in a method that has great potential for leading to a better understanding of the basic mechanism(s) underlying drug absorption in man. Issues that could be studied are the interaction of actively absorbed drugs and nutrients (i.e., L-Dopa, amino acids), the influence that nutrients and variable osmolality in the lumen could have on water flux and therefore drug absorption, and the effect of absorption enhancers. Furthermore, the intestinal uptake of peptides, proteins, and other macromolecules could be investigated during well-controlled absorption conditions within the intestinal segment.

In conclusion, in this study we were able to validate the regional jejunal perfusion technique as a model for studies of true drug absorption in humans. The degree of absorption (FA) from the intestinal segment during the residence time of the solution in the intestinal segment agreed with the time estimated to achieve complete absorption of phenazone following oral administration. Further evidence for the validity of the method was found in the correlation between the FA and the degree of absorption (F_{plasma}) estimated from deconvolution of the plasma concentrations. Increased luminal stirring, achieved through an increase in perfusion rate, did not significantly increase the permeability of the test compounds. This suggests that the differences in thickness of the unstirred water layer are probably of minor importance as a factor for variability in absorption rate in normal human intestine, even for compounds with a high permeability such as phenazone and *d*-glucose. In our study a high correlation between the absorption of phenazone and that of *d*-glucose was found, which is partly explained by the MRT of the solution in the segment. An additional important factor is probably an interindividual variablility in mucosal surface area. Furthermore, it was possible to establish a tight intestinal segment which behaved as a well-mixed compartment. The low perfusion rate of 3 ml/min was preferred since it resulted in the lowest variability in absorption. Thus, the regional jejunal perfusion technique seems to have great po-

tential for quantitative and mechanistic evaluations of drug absorption from the human intestine. Phenazone should be a suitable reference drug in such experiments.

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