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A Residence–Time Distribution Analysis of the Hydrodynamics within the Intestine in Man during a Regional Single-pass Perfusion with Loc-I-Gut: In-vivo Permeability Estimation

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Abstract

The goal of this study was to determine the most appropriate hydrodynamic model for the Loc-I-Gut in-vivo perfusion system. The general mixing-tank-in-series model, which can approximate single mixing tank and laminar and plug-flow hydrodynamics, was fitted to the observed experimental residence-time distribution curves for the non-absorbable marker [^{14}C]PEG 4000. The residence-time distribution analysis shows that the hydrodynamics of the perfusion solution within the jejunal segment in man is well approximated by a model containing on average between 1–2 mixing tanks in series. The solution is well mixed when using perfusion rates of 2·0, 3·0 and 6·0 mL min^{-1}. The average mean residence time estimates from the fitted residence-time distribution were $12 \pm 7{\cdot}6$, $15 \pm 4{\cdot}2$ and $7{\cdot}7 \pm 4{\cdot}6$ min, respectively, at these three perfusion rates. The mean volumes of the segment (Vs) were 25 ± 15, 45 ± 12 and 46 ± 27 mL, respectively. There were no statistical differences between 2·0, 3·0 and 6·0 mL min^{-1} in respect of the number of mixing tanks (n) and mean residence times.

This residence-time distribution analysis indicates that the luminal fluid in the Loc-I-Gut perfusion system is well-mixed, and that permeability calculations based on the well-mixed assumption most closely approximate the actual local (average) membrane permeability within the perfused segment.

It is possible to determine in-vivo intestinal permeability values of drugs in man by recently developed perfusion methodology (Knutson et al 1989; Lennernäs et al 1992). This present regional in-vivo approach is based on single-pass perfusion of a 10-cm long segment of intestine localized between two inflated balloons (Fig. 1). The perfusion solution enters through one entrance in the middle of the segment and leaves through two holes, one at each end of the perfused intestinal region (Fig. 1). The effective intestinal permeability data (P_{eff}) obtained by this perfusion technique in man has previously been validated according to the criteria: mass balance in transport across the jejunal mucosa; sink conditions with regard to drug concentration across the intestinal barrier for a model drug (antipyrine); good correlation between the effective permeabilities (P_{eff}) and extent of drug absorption (from pharmacokinetic studies); almost complete recovery of PEG 4000—a non-absorbable volume marker; and the capacity of the apical membrane in the jejunal mucosa to discriminate between different molecular weights and sizes in the range 18–350 (Lennernäs et al 1992, 1993, 1994; Fagerholm & Lennernäs 1995b; Fagerholm et al 1995). The functional viability of the mucosa was also demonstrated by the rapid transmucosal transport of D-glucose and L-leucine from the regional jejunal segment (Lennernäs et al 1992, 1993, 1994). However, calculation of the most accurate P_{eff} is dependent on the hydrodynamic characteristics within the perfused intestinal segment, which requires knowledge of the intraluminal convection and diffusion processes occurring (Amidon et al 1980). The flow characteristics during a single-pass perfusion experiment can be studied by residence–time distribution analysis of the fluid elements of a non-absorbable tracer after a pulse-dose during continuous perfusion. The approach is to study the concentration–time profile of a non-absorbable tracer in the outlet intestinal perfusate during a single-pass perfusion experiment (Amidon et al 1980).

Our aim in this report is to describe the hydrodynamics of the solution during a single-pass perfusion with the Loc-I-Gut system by applying residence-time distribution-analysis (F-curve analysis). Furthermore, we will estimate the mean values of the volume of the jejunal segment (Vs) and the mean residence time (t*) during the equilibrium of the outlet concentrations. It is important to obtain a better understanding of the technical variables because these might influence the estimation of P_{eff}.

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Materials and Methods

Perfusion technique

In this study we used previously published perfusion data for man (Lennernäs et al 1992; Fagerholm et al 1995). The concentration–time data of [^{14}C]PEG 4000 in the perfusate leaving the jejunal segment were obtained at four different perfusion rates of 1·5, 2·0, 3·0 and 6·0 mL min^{-1} (Lennernäs et al 1992; Fagerholm et al 1995).

The instrument (Loc-I-Gut; Synectics AB, Sweden) is a 175-cm sterile polyvinyl tube with an outer diameter of 5·3 mm intended for intestinal perfusions in man (Fig. 1). It has six inner channels and is distally provided with two elongated latex balloons. A more detailed description of the tube and the positioning procedure can be found elsewhere (Knutson et al 1989; Lennernäs et al 1992). All perfusions were performed in the morning after a 10 h overnight fast; the

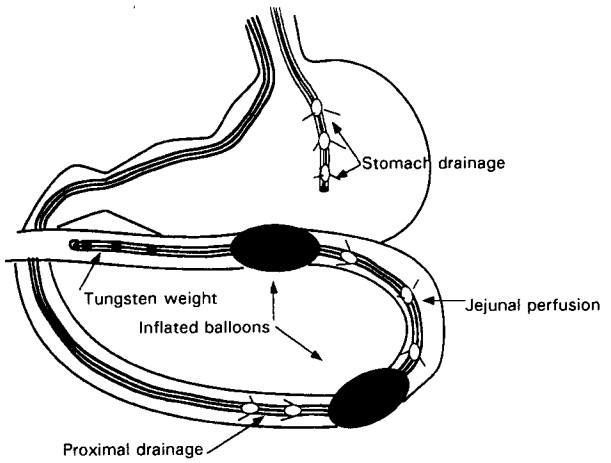

FIG. 1. The multichannel tube system with double balloons enabling segmental jejunal perfusion in man. The balloons are filled with air when the proximal balloon has passed the ligament of Treitz. The fluid flux is monitored by use of [^{14}C]PEG 4000, a non-absorbable volume marker (see Lennernäs 1992).

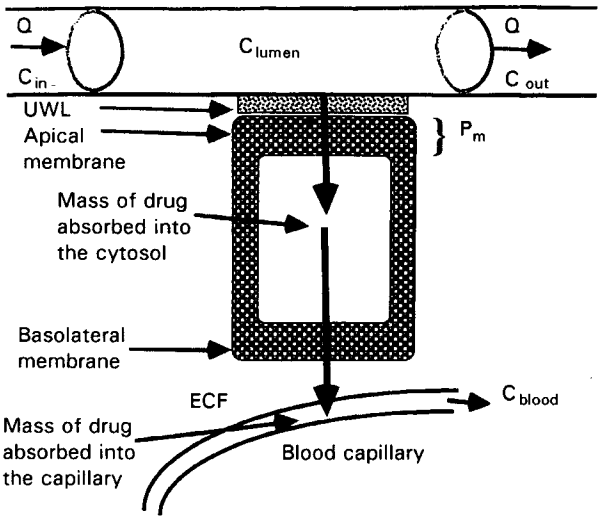

FIG. 2. The effective permeability (P_{eff}) describes both the partitioning of a drug molecule from the luminal fluid into the apical membrane (K) and thereafter the diffusion process (Dm) across the apical membrane (of a particular thickness) into the cytosol of the enterocyte. The effect of the unstirred water layer (UWL) is minimal because of extensive mixing within the small intestine in man, and P_{eff} is determined by the penetration through the membrane (Pm). Because the apical membranes have lower permeabilities than the basolateral membrane, it is likely that passage through the apical membrane is the rate-limiting step (Lande et al 1995). ECF, extra-cellular fluid; Q, fluid transport rate in the intestinal lumen.

subjects were recumbent during the whole experiment. Initially the enclosed intestinal segment was rinsed with isotonic saline (37°C) for at least 10 min using a calibrated syringe pump (model 355; Sage Instrument, Orion Research, Cambridge, MA). Thereafter the perfusion experiment (at 37°C) was started and the jejunal perfusate leaving the intestinal segment was quantitatively collected in tubes, kept on ice, at 10-min intervals. To minimize net fluid flux the perfusion solution used was isotonic.

Analytical methods

All chemicals used were of analytical grade. Perfusion solutions and perfusate samples were weighed (0·5 g) and the total radioactivity of [^{14}C]PEG 4000 was determined by liquid-scintillation counting for 10 min (Beckman model 244 instrument) after addition of Beckman ready safe (8 mL). By using the internal standard of the instrument the measured radioactivity was corrected for quenching.

General theory

Drug transport across the membrane wall of a tube is the relationship between the masses entering and leaving the tube:

$$dM/dt = Q_{in}C_{in} - Q_{out}C_{out} = Q \times (C_{in} - C_{out}) \quad (1)$$

where C_{in} and C_{out} are the inlet and outlet drug concentrations, respectively, and Q is the flow through the tube, usually assumed to be constant. The mass balance relationship can then be set to describe the rate of transport of the drug across the tube membrane (absorbed mass) according to Fick's first law:

$$dM/dt = A \cdot P_{eff} \times (C_{ref}^{lumen} - C_{ref}^{blood}) \quad (2)$$

where A is the surface area of the membrane, P_{eff} is an effective permeability coefficient and the reference concentrations C_{ref}^{lumen} and C_{ref}^{blood} are on the two opposite sides of the intestinal mucosa (Fig. 2). It is usually assumed that the reference blood concentration (C_{ref}^{blood}) is negligible in comparison with the lumen concentration. This has been directly shown to be valid for antipyrine in man (Lennernäs et al 1992).

The effective permeability (P_{eff}) in man represents both the partitioning of a drug molecule from the luminal fluid into the apical membrane (K) and thereafter diffusion (Dm) across the lipid membrane of thickness (d) (Fig. 2) (Stein 1986). Hence, together with the reference drug concentration within the intestinal segment it will determine the mass-transport rate per unit area ($J_{eff} = P_{eff} \times C_{ref}$) into the cytosol of the enterocyte across the apical membrane; this is considered the rate-limiting step (Fig. 2) (Lande et al 1994, 1995). The factors determining the extent of drug absorption (fa = M(t)/Dose), can be summarized by equation 3:

$$fa = M(t)/Dose = \int_0^t \iiint A \cdot P_{eff} \cdot C_{lumen} \cdot dAdT \quad (3)$$

where A is the available intestinal surface area, P_{eff} is the average value of the effective intestinal permeability along the intestinal region where absorption occurs, and C_{lumen} is the free reference concentration of the drug in the intestinal lumen (which is affected by dissolution, degradation, luminal metabolism, etc.) (Amidon et al 1995). According to this definition, absorption (fa) is all the processes affecting the disappearance of the drug from the intestinal lumen into the cytosol of the enterocyte; these are considered to include: the dose/solubility ratio; the rate of dissolution; chemical degradation/metabolism in the lumen; complex binding in the lumen; intestinal transit; and effective permeability (P_{eff}) across the intestinal mucosa.

The bioavailability (F) is a crucial pharmacokinetic variable determined by different processes as described in equation 4:

$$F = fa \times (1 - Eg) \times (1 - Eh) \quad (4)$$

where fa is the fraction absorbed into the intestinal mucosa across the apical brush-border membrane, Eg is the cytosolic

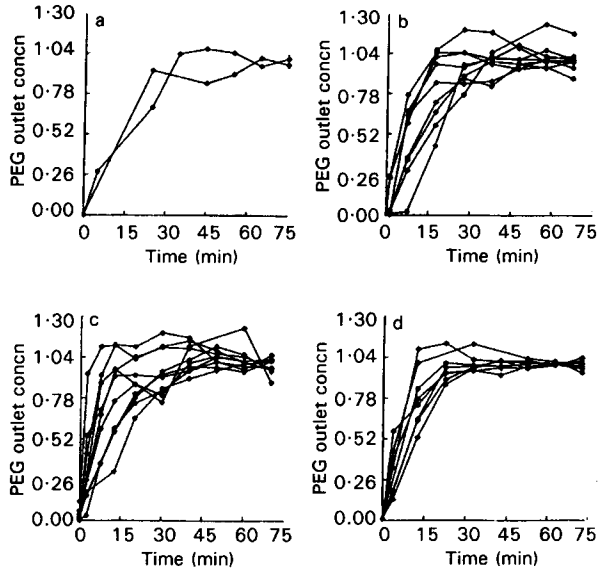

FIG. 3. The concentration–time profiles of [^{14}C]PEG 4000 in the perfusion solution leaving the jejunal segment during a single-pass experiment at different flow rates (a = 1·5; b = 2·0; c = 3·0 and d = 6·0 mL min^{-1}).

localized metabolism in the enterocyte and Eh is the extraction in the liver (including both metabolism and biliary secretion). This means that metabolism of drugs by enzymes located in the cytosol of the enterocytes (e.g. CYP 3A4) is not a part of the absorption process itself, instead it contributes to the first-pass effect and bioavailability.

Data analysis

It is obvious that P_{eff} is a key variable in drug absorption. To calculate an accurate value of jejunal P_{eff} in this in-vivo model, the hydrodynamics in the perfused segment have to be described. In this report an n-mixing tank model was used to analyse the residence–time distribution F-curve of the concentration–time curves of [^{14}C]PEG 4000 (a non-absorbable marker) (Fig. 3). The time-point used was obtained by subtraction of the time the perfusion solution spent in the tubes and plotting the concentration against the mid-point of the sampling interval. The model analysis used n mixing tanks in series plus a fractional tank (equation 5) (Amidon et al 1980):

$$F_1 = 1 - [r/(r-1)]^n e^{-[(n+r)t/rt^*]} - e^{(n+r)t/t^*} \times \sum_{I=1}^{n} [1 - \{r/(r-1)\}^{n-i+1}][1/(i-1)!]\{[(n+r)t]/t^*\}^{i-1} \quad (5)$$

where F is the fraction of PEG 4000, n is the number of tanks, r is the fractional tank, t is the time and t* is the mean residence time. The concentration–time profile is corrected for the lag time arising as a result of perfusion through the perfusion tube, because we are interested in the hydrodynamics through the jejunal segment only. The number of mixing tanks will be crucial to determination of whether well-stirred, plug, or laminar-flow model is most appropriate. The mean residence time (t*, eqn 6) was used to estimate the volume of the intestinal segment (Vs), and the number of tanks was used to characterize the intestinal hydrodynamics.

$$t^* = Vs/Q_{out} \quad (6)$$

The fraction of the dose absorbed (fa) can be estimated from the effective permeability data from man by use of equation 7:

$$fa(\%) = (1 - e^{-\alpha \cdot P_{eff}}) \times 100 \quad (7)$$

where α represents the correlation parameter for the permeability data in the respective model vs the fraction of the dose absorbed in man (Amidon et al 1995).

Variability is expressed as standard deviation (s.d.). Tests for significance of differences between number of tanks (n) and mean residence time (t*) between the perfusion rates were tested by one-way analysis of variance then by Scheffe's contrast test. The two perfusion experiments using the lowest flow rate (1·5 mL min^{-1}) were not included in the statistical analysis.

Results and Discussion

This is the first report describing the hydrodynamics of the perfused regional human jejunal segment between two balloons (Lennernäs et al 1992). Residence-time distribution-analysis shows that the hydrodynamics of the perfusion solution within the jejunal segment in man is best described by a well-mixed model containing, on average, between one and two mixing tanks in series. In addition, the concentration–time profiles of PEG 4000 in the perfusion solution leaving the jejunal segment rapidly increased to steady-state at each flow rate (2·0, 3·0 and 6·0 mL min^{-1}) and support our conclusion of extensive mixing in the perfused jejunal segment in man (Table 1 and Fig. 3). There was no statistical difference between 2·0, 3·0 and 6·0 mL min^{-1} with regard to the number of mixing tanks (n) or the t* values (Table 1). That this model was found to be most relevant is in accordance with the design of the perfused segment, where the perfusion solution enters the segment in the middle and leaves through two exit ports, one at each end of the intestinal segment (Fig. 1). Practically, it means that we perfuse the segment in two directions, which is similar to the fluid moving back and forth over short distances in normal segmental contractions in the intestine (Dillard et al 1965). The mixing seems to be instantaneous, and the effective permeability of a drug reflects its membrane permeability, because no significant unstirred water layer contributes to overall absorption resistance in this model. This was further confirmed by Fagerholm & Lennernäs (1995a), who observed no significant changes in the estimated effective permeability of two high-permeability compounds, D-glucose (10^{-3} cm s^{-1}) and antipyrine (4×10^{-4} cm s^{-1}), or in aqueous film thickness over a fourfold range of flow-rates (1·5–6·0 mL min^{-1}) (Lennernäs et al 1992; Fagerholm & Lennernäs 1995). In summary, this clearly indicates that unstirred layer effects are minimal for jejunal epithelial transport in-vivo in man, and therefore passage through the membrane is the rate-limiting process even for high permeability compounds (Lennernäs et al 1992; Fagerholm & Lennernäs 1995a). Because apical membranes of epithelial barriers have lower permeabilities than basolateral membranes, we consider the penetration of the apical membrane as the rate-limiting step for passive diffusion (Lande et al 1994, 1995).

Table 1. The hydrodynamic parameters in a jejunal segment in man during a single-pass perfusion.

Perfusion rate (mL min^{-1})	Number of subjects	Number of mixing tanks	Mean residence time (min)	Volume in the segment (mL)
1·5	2	4–11	14–22	21–33
2·0	10	2·1 ± 1·4	12 ± 7·6	25 ± 15
3·0	12	1·5 ± 0·7	15 ± 4·2	45 ± 12
6·0	8	1·1 ± 0·2	7·7 ± 4·6	46 ± 27

These parameters were calculated by residence-time distribution-analysis (F-curves). The data are presented as mean ± s.d. There were no statistical differences between the number of mixing tanks and mean residence time at 2·0, 3·0 and 6·0 mL min^{-1}.

The average values of the mean residence time (t*) calculated by use of equation 6 were 12 ± 7·6, 15 ± 4·2 and 7·7 ± 4·6 min, respectively, at these three perfusion rates. The mean volumes of the segment (Vs) were 25 ± 15, 45 ± 12 and 46 ± 27 mL, respectively (Table 1). These results are in good agreement with our previous estimates calculated by a different approach (Lennernäs et al 1992). The volume of a filled human jejunal segment of radius 1·75 cm and length 10 cm should be approximately 110 mL. The most likely explanation of the lower volume of the perfused segment is that the jejunal tissue in the loop contracts. This is caused by the motility of the upper small intestine which affects the location of the perfused intestinal segment, and is probably the main explanation of the variable t* values.

In a previous study we also showed a greater extent of inter-individual variability in the permeability coefficient at the perfusion rate of 6·0 mL min^{-1} (Lennernäs et al 1992). This might be because of a more variable filling volume (Vs) at the higher perfusion rate. Therefore, we suggest that perfusion studies should not be performed at 6·0 mL min^{-1}, even if the n-value indicates the existence of only one well-stirred compartment.

When the lower perfusion rate (1·5 mL min^{-1}) was used the number of mixing tanks was high (n > 4); this indicates that the hydrodynamics might be best described by the plug or laminar-flow model (Table 1 and Fig. 3) (Amidon et al 1980). There was also a tendency to reach the equilibrium level at a later time (Fig. 3). The average value of the mean residence time was 18 min.

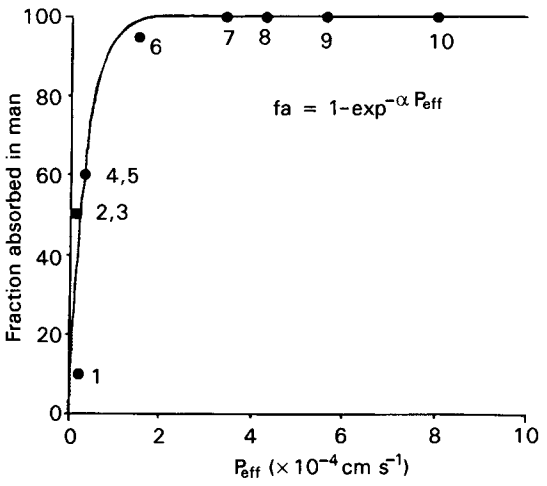

FIG. 4. The excellent prediction of the fraction absorbed (fa) in man by use of the effective permeability values obtained by use of the well-stirred hydrodynamic model. The correlation is valid for a broad range of different permeability values. Drug identities: 1, enalaprilat; 2, atenolol; 3, hydrochlortaizide; 4, terbutaline; 5, furosemide; 6, metoprolol; 7, L-dopa; 8, carbamazepine; 9, antipyrine; 10, naproxen. Permeability data were obtained from the literature (Lennernäs et al 1992, 1993, 1994, 1995a,b; Fagerholm et al 1995).

In this report we demonstrate that the measured outlet concentration is the most accurate reference drug concentration to use for calculation of P_{eff} from a regional single-pass perfusion with this double balloon approach in man. The accuracy of the well-mixed model is shown in Fig. 4, where the effective intestinal permeability coefficients for man enable excellent predictions of the fraction absorbed for drugs with a wide range of permeability values. These permeabilities have also been shown to correlate well with permeability data obtained by in-vitro and in-situ models (Lennernäs et al 1996, 1997; Fagerholm et al 1996).

In conclusion, we have demonstrated that the well-stirred model is most representative of the hydrodynamics in the intestinal segment at the perfusion rates usually used in man (2·0 and 3·0 mL min^{-1}). The mixing of a compound when it enters the jejunal segment is almost instantaneous and the mean residence time (t*) for an unabsorbed molecule in the segment at these flow rates (2·0 and 3·0 mL min^{-1}) is approximately 15 min. The rapid and extensive mixing in the jejunal segment also suggest that the P_{eff}-value obtained with this method for man reflects membrane permeability. This report also further justifies the use of the regional intestinal perfusion model for the in-vivo determination of drug-permeability in man.

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