A New Approach for Direct *In Vivo* Dissolution Studies of Poorly Soluble Drugs

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INTRODUCTION

Intestinal absorption of drugs given in a solid dosage form is in general determined by the following four factors: available surface area, intestinal transit time, membrane permeability, and concentration-time profile of the drug in the lumen. The free drug concentration available for transport across the intestinal mucosa is determined by solubility, dissolution rate, degradation, metabolism, and complex binding of the drug. Studies of solubility and dissolution rate *in vivo* are, therefore, crucial for a better understanding of the absorption and bioavailability for water insoluble drugs.

The recently proposed biopharmaceutical classification system (BCS) suggests that drugs might be classified according to the aqueous solubility and permeability. For class II and IV drugs—low solubility with high or low permeability, respectively—the dissolution rate in the luminal liquids will most likely be the rate limiting step in the absorption process (1). The dissolution rate of a drug is normally determined *in vitro* in an artificial buffer solution. However, sometimes these *in vitro* systems fail to predict drug dissolution *in vivo*, which probably is due to a poor understanding and simulation of the physiological factors in the gastrointestinal tract.

Previously, *in vivo* dissolution in the lumen has been investigated by indirect methods such as deconvolution of plasma concentration-time profiles of the drug (2,3). No reports on methods for direct determination of the *in vivo* dissolution in humans are available in literature. Therefore the characteristics of the human jejunal fluid in fasted state has recently been investigated in order to build up knowledge about these processes *in vivo* (4). Another report provides data from human duodenum in fed state (5). The Loc-I-Gut tube has previously been used for studies of jejunal transport mechanism and metabolic first pass effects (6–8). The aim of the present study was to evaluate whether the regional perfusion technique Loc-I-Gut can be used for on-line studies of the dissolution rate of drugs during *in vivo* conditions.

MATERIALS AND METHODS

Study Design

In this preliminary methodological study, two healthy male volunteers participated. The study was performed according to the Helsinki declaration. The subjects were not receiving any other medication, and the perfusion experiment was performed after 10 hours fasting.

The perfusion tube is a disposable polyvinyl chloride tube (Loc-I-Gut®, Synectics AB, Sweden) 175 cm in length and with an external diameter of 5.3 mm (16 French) (7). A tungsten weight is attached to the distal end of the tube to facilitate its passage into the jejunum. The tube contains six channels; 4 narrow and 2 wider. Distally, it is provided with two 40 mm long elongated latex balloons 10 cm apart, each connected to one of the smaller channels (6). The two wider channels (inner diameter 0.9 mm), are used for infusion of the suspension and aspiration of the perfusate respectively. One of the smaller channels was used for perfusion. Gastric suction was applied through a separate tube, located in the antrum region. The Loc-I-Gut tube was introduced orally after local anaesthesia of the upper throat with lidocaine, a teflon-coated guide wire was used during insertion of the tube to facilitate the passage through the stomach. The insertion and the positioning of the tube were made under fluoroscopic guidance (Phillips BV 21-S) (7). The time required for the insertion of the Loc-I-Gut was approximately 1 hour. After the Loc-I-Gut reached its position in the upper jejunum, the proximal balloon had passed the ligament of Treitz, 24–30 ml air was inflated into the distal, balloon and a semi-open intestinal segment was created (Figure 1).

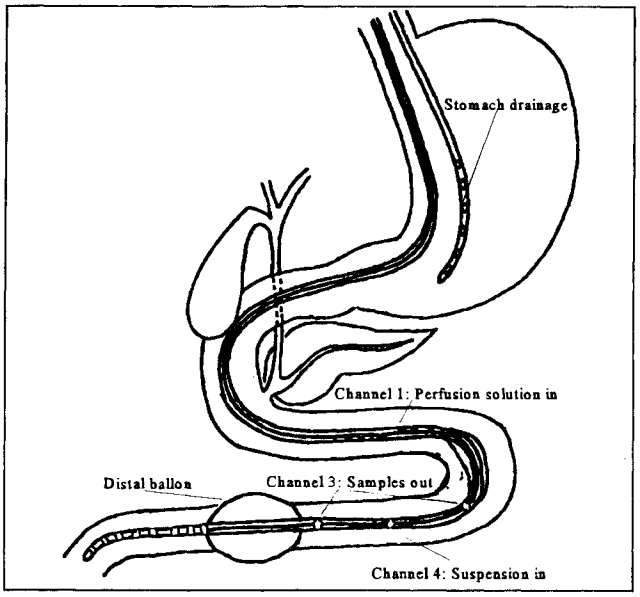

Fig. 1. The multichannel tube system Loc-I-Gut in the human jejunum. Air is filled in the distal balloon to create a semi-open segment. Gastric suction is applied by a separate tube placed in the antrum region of the stomach.

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The perfusion solution (37°C) was administered through channel 1 (Figure 1) in the Loc-I-Gut tube with a flow rate of 2 ml/min, using a calibrated syringe pump (model 355, sage instrument, Orion Research Inc., Cambridge, MA, USA). That flow rate is within the normal flow rate in the human jejunum (0.6–4.2 ml/min) (9,10). After 10 minutes of perfusion with perfusion solution, 10 ml of a carbamazepine suspension containing 60 mg Carbamazepine and the radioactive labelled marker [^{14}C]-PEG 4000 was administered at a flow rate at 5 ml/min in channel 4 (Figure 1). Channel 4 (a wider channel) was then rinsed with 2 ml of perfusion solution to assure that none of the carbamazepine particles were left in the channel. Immediately thereafter the perfusion with perfusion solution through channel 1 recommenced and was continued for 55 minutes. Perfusion samples were withdrawn by gravity drainage. Perfusion solution leaving the jejunum was collected quantitatively on ice over 5 minute intervals. Collections were then weighed and centrifuged at 3000 rpm for 10 minutes. The supernatant and the sediment were separated, and thereafter immediately frozen and stored at −20°C until analysis.

After the study, the semi-open segment was rinsed with isotonic saline (60 ml), the perfusate was collected and treated in the same way as the other perfusate samples.

Chemicals

Perfusion Study

Carbamazepine crystals was a gift from Orion-Farmos Pharmaceuticals, Finland. Eighty-five percent of the crystals were ≤20 μm and all crystals were ≤ 50 μm according to the manufacturer. KCl, NaCl, mannitol, D-glucose, Na_2HPO_4 and NaH_2PO_4 were all pharmaceutical grade. [^{14}C]-PEG 4000 was purchased from Amersham Labs, England. The perfusion solution was an isoosmotic 70 mM phosphate buffer (pH = 6.5) containing 5.4 mM KCl, 48 mM NaCl, 35 mM mannitol and 10 mM D-glucose.

Carbamazepine 6 mg/ml was prepared as a suspension in the perfusion solution and 0.625 μCi of the non-absorbable volume marker [^{14}C]-PEG 4000 was added.

HPLC-Analysis

Acetonitrile HPLC-grade, Na_2HPO_4 and NaH_2PO_4 Ph. Eur. Quality were purchased from E. Merck, Darmstadt, Germany.

Analytical Assay

The perfusate samples were allowed to thaw, and a 500 μl sample was mixed with 500 μl acetonitrile (supernatant samples) or 1000 μl acetonitrile (sediment samples). The samples were centrifuged for 2 min. at 9500 g. The supernatant was transferred to a new tube and centrifuged again for 30 sec. to ensure that no particular matter was injected into the chromatographic system. Carbamazepine in the perfusate was determined using a reverse-phase liquid chromatographic system with UV-detection at 290 nm (Merck Hitachi L-7110, L-7200 and 7400), using a slightly modified version of a published HPLC-method (12). The mobile phase consisted of 50/50, Acetonitrile/Sodium phosphate buffer pH = 7.4 (ionic strength 0.05) with a flow rate of 1.5 ml/min. The stationary phase consisted of an analytical column RP-C_{18}, 5 mm particle size (Sperisorb), 250 × 4 mm and a precolumn RP-C_{18}, 5 mm particle size, 40 × 4 mm using a column temperature at 40°C. The retention time for carbamazepine was approximately 2.7 min. The accuracy of the assay at sample concentrations of 1.044, 42.58 and 163.86 μg/ml was 97.9%, 99.6% and 96.1%, respectively. The limit of quantification was 1.64 μg/ml.

The activity of [^{14}C]-PEG 4000 was determined by liquid scintillation counting (dpm) for 10 min. (Beckman instrument, model 244) after the addition of 5 ml of Beckman Ready Safe®. The radioactivity was corrected for quenching using the internal standard of the instrument.

Data Analysis

The recovery of ^{14}C-PEG 4000 in the perfusion samples is calculated as:

$$\text{Recovery} = \frac{\sum ([\text{PEG}]_{out} * V_p) * 100\%}{\text{PEG}_{in}} \quad (1)$$

where $[\text{PEG}]_{out}$ is the concentration of ^{14}C-PEG 4000 in the outlet perfusate sample, V_p is the volume of the fractionated perfusate sample and PEG_{in} is the amount of PEG administered in the suspension.

Carbamazepine

The following variables were calculated: The concentration of dissolved carbamazepine in the carbamazepine suspension $[\text{CBZ}]_0$, the concentration of dissolved carbamazepine in the supernatant samples $[\text{CBZ}]_{sup}$, and the amount of carbamazepine in the sediment of the perfusate sample CBZ_{sed}.

The accumulated amount of dissolved carbamazepine washed out from the segment is:

$$\text{Wout}_{sol}(t) = [\text{CBZ}]_{sup,t} * V_{p,t} \quad (2)$$

The mass balance for carbamazepine in the system during the perfusion, can be described as follows:

$$\text{Dose} = \text{CBZ}_{sol} + \text{CBZ}_{undis} + \sum \text{Wout}_{sol} + \sum \text{Wout}_{par} + \sum \text{CBZ}_{abs} \quad (3)$$

Where $\Sigma\ \text{CBZ}_{abs}$ is the accumulated amount of carbamazepine absorbed, CBZ_{undis} is the amount of undissolved carbamazepine in the jejunal segment and $\Sigma\ \text{Wout}_{par}$ is the accumulated amount of carbamazepine leaving the segment as particles.

Assuming that the tightness of the segment is illustrated with the PEG recovery, the amount carbamazepine absorbed during the perfusion and rinsing can be calculated as:

$$\text{CBZ}_{abs} = \frac{\text{Dose} - (\Sigma\text{Wout}_{sol} + \Sigma\text{Wout}_{par})}{\text{Recovery of PEG}} \quad (4)$$

RESULTS AND DISCUSSION

The accumulated amount of PEG 4000 in the outlet perfusate over time is shown in Figure 2. The recovery of PEG 4000 was 81.5 and 76.8% in the two subjects, after rinsing the segment with 60 ml of saline.

Carbamazepine was chosen as a model drug for the present study since the absorption upon oral administration has been shown to be dissolution rate limited (12,13). According to the

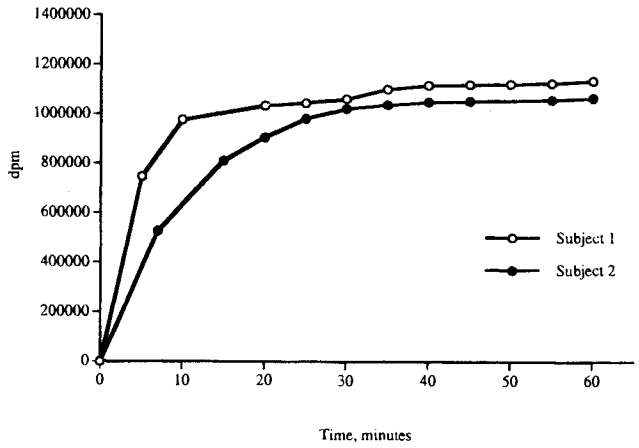

Fig. 2. The accumulation of the amount of non-absorbable volume marker, 14C-Peg 4000, in the perfusate samples leaving the jejunal segment in subject 1 and 2 during the *in vivo* dissolution experiment.

Biopharmaceutical Classification System, carbamazepine is a class II drug, low solubility—high permeability. It has been estimated that it requires 770 ml water to dissolve a 200 mg dose of carbamazepine (1).

Individual profiles of the accumulated amount of carbamazepine as dissolved, undissolved and total carbamazepine over time in various fractions in the perfusate are shown in Figures 3a–b. Interestingly, undissolved carbamazepine is found in the perfusate samples until 40–45 minutes after administration, which is in accordance with dissolution rate limited kinetics.

In total 21.5 and 63.5% of the carbamazepine dose administered to the two subjects were recovered in the perfusate samples. The lower recovery of carbamazepine in the perfusate samples versus the PEG 4000 recovery is due to a more extensive absorption of carbamazepine in subject 1. The amount absorbed was about 38 mg and 17 mg for subject 1 and 2, respectively (equation 4).

CONCLUSIONS

In this report, a novel method for the direct determination of the *in vivo* dissolution of drug particles has been developed.

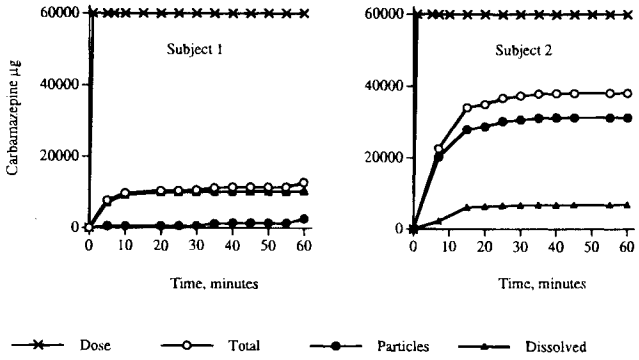

Fig. 3a–b. Individual (subject 1 and 2) accumulation of carbamazepine during an *in vivo* dissolution test in the human jejunum.

This approach to study *in vivo* dissolution is promising as there is a need for improvements in the understanding of the *in vivo* dissolution of slightly soluble compounds. The dissolution testing in this semi-open segment is in spite of the artificial perfusion solution and placement of a balloon in the jejunum, under *in vivo* conditions considering motility, hydrodynamics, and endogenous secretion in the jejunal segment. However, further optimization, especially for determining the amount of drug absorbed from the jejunum is needed.

In a separate study carbamazepine has been determined to be a high permeability drug ($4 * 10^{-4}$ cm/s) according to the Biopharmaceutical Classification System (Lennernäs, AAPS). Interestingly, in that study subject 2 also had a lower jejunal P_{eff} for carbamazepine (P_{eff} at $1.7 * 10^{-4}$ cm/s) than average (Lennernäs et al. unpublished data), which agrees with the lower degree of absorption found in the present study. This might also explain the higher degree of wash out of solid carbamazepine particles as a consequence of slower dissolution rate, due to poor luminal sink condition. The difference between the subjects can be due to the interindividual differences in intestinal luminal content (4) and thereby different solubilization of carbamazepine, as well as highly variable surface and physiological status of the jejunal villus tips (14,15).

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REFERENCES

1. G. L. Amidon, H. Lennernäs, V. P. Shah, and J. Crison. *Pharm. Res.* **12**:413–420 (1995).
2. W. R. Gillespiee and P. Veng-Petersen. *Biopharm. Drug. Disp.* **6**:351–355 (1985).
3. M. Niklasson, C. Graffner, and M-J. Nilsson. *Int. J. Pharm.* **40**:165–171 (1987).
4. A. Lindahl, A-L. Ungell, L. Knutson, and H. Lennernäs. Characterization of Fluids from the Stomach and Proximal Jejunum in Men and Women. *Pharm Res.* In Press (1997).
5. O. Hernell, J. E. Staggers, and M. C. Carey. *Biochemistry* **29**:2041–2056 (1990).
6. L. Knutson, B. Odlind, and R. Hällgren. *Am. J. Gastroenterol.* **84**:1278–1284 (1989).
7. H. Lennernäs, Ö. Ahrenstedt, R. Hällgren, L. Knutson, M. Ryde, and L. K. Paalzow. *Pharm. Res.* **9**:1243–1251 (1992).
8. A. Lindahl, R. Sandström, A-L. Ungell, B. Abrahamsson, T. W. Knutson, L. Knutson, and H. Lennernäs. *Clin. Pharmacol. Ther.* **60**:493–503 (1996).
9. P. Kerlin, A. Zinnmeister, and S. Phillips. *Gastroenterology.* **82**:701–706 (1982).
10. K. H. Soergel. Flow measurements of test meals and fasting contents in the human small intestine. In L. Demling and R. Ottenjann (eds.) *Gastrointestinal motility*, Georg Thieme, New York, 1971, pp. 81–92.
11. S. A. Mira, Y. M. El-Sayed, and S. I. Islam. *Analyst* **112**:57–60 (1987).
12. R. H. Levy, W. H. Pitlick, A. A. Troupin, J. R. Green, and J. M. Neal. *Clin. Pharm. Ther.* **17**:657–668 (1975).
13. H-H. Frey and W. Löscher. *Arch. Int. Pharmacodyn. Ther.* **243**:180–191 (1980).
14. E. B. Chang and M. C. Rao. Intestinal water and electrolyte transport: mechanisms of physiological and adaptive responses. In: L. R. Johnson (editor) Physiology of the Gastrointestinal Tract. Raven Press, New York, pp. 2027–2081 (1994).
15. A. Strocchi and M. D. Levitt. *Dig. Dis. & Sci.* **38**:729–734 (1993).