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

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Received March 31, 1997; accepted July 17, 1997
KEY WORDS: in vivo dissolution; Loc-I-Gut; Carbamazepine; sparingly soluble drugs; intestinal perfusion.

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.
The perfusion solution (37°C) was administered through channel 1 (Figure 1) in the Loc-l-Gut tube with a flow rate of 2 ml/min, using a calibrated syringe pump (model 355, sage instrument, Orin 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 [14C]-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 jejunal was collected quantitatively on ice over 5 minute intervals. Collections were then weighed and centrifuged at 3000 rpm for 10 minutes. The supematant 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, Na2HPO4 and NaH2PO4 were all pharmaceutical grade. [14C]-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 [14C]-PEG 4000 was added.

**HPLC-Analysis**

Acetonitrile HPLC-grade, Na2HPO4 and NaH2PO4, 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-C18, 5 mm particle size (Sperisorb), 250 × 4 mm and a precolumn RP-C18, 5 mm particle size. The column temperature was 40°C and the column temperature was 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 [14C]-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 [14C]-PEG 4000 in the perfusion samples is calculated as:

\[
\text{Recovery} = \frac{\sum ([PEG]_{int} * V_p) * 100}{[PEG]_{in}}
\]

where [PEG]_{int} is the concentration of [14C]-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 [CBZ]_{in}, the concentration of dissolved carbamazepine in the supernatant samples [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:

\[
\text{Wout}_{tol}(t) = [CBZ]_{sup,t} * V_p
\]

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

\[
\text{Dose} = \text{CBZ}_{tol} + \text{CBZ}_{und} + \sum \text{Wout}_{tol} + \sum \text{Wout}_{sup} + \sum \text{CBZ}_{abs}
\]

Where Σ CBZ_{abs} is the accumulated amount of carbamazepine absorbed, CBZ_{und} is the amount of undissolved carbamazepine in the jejunal segment and Σ Wout_{sup} 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:

\[
\text{CBZ}_{abs} = \frac{\text{Dose} - (\sum \text{Wout}_{tol} + \sum \text{Wout}_{sup})}{\text{Recovery of PEG}}
\]

**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