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Pharmacokinetics of gefitinib in humans: The influence of gastrointestinal factors

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Abstract

Purpose: To investigate whether differences in plasma pharmacokinetic profiles of gefitinib between healthy subjects having normal (N; $t_{1/2} > 20$ h) and altered (A; $t_{1/2} < 20$ h) pharmacokinetic (PK) profiles might be explained by inter-individual variability in gastric emptying and/or precipitation/dissolution of gefitinib in the proximal small intestine.
Methods: One hundred healthy male subjects were screened to enable identification of subjects with the two PK profiles. Twenty five subjects from the screening were subsequently enrolled in an intubation study where a 250 mg gefitinib dispersion preparation (IRESSA®, AstraZeneca) was administered directly into the stomach. Intestinal fluid samples were withdrawn continuously for 180 min post-dose using the Loc-I-Gut catheter positioned in the jejunum. The crystalline form of gefitinib was determined using Raman microscopy.
Results: There were no differences between normal and altered subjects with regard to gastric emptying or the precipitation/dissolution of gefitinib in jejunal fluid. Due to difficulties in crystalline identification in the jejunal fluid samples, only the same crystalline form as the dosed form was identified.
Conclusions: There was no pronounced difference in gastric emptying, precipitation and re-dissolution of gefitinib in proximal human jejunum between normal and altered subjects. Other mechanism(s) are also likely to be important in explaining the inter-individual differences in plasma exposure to gefitinib, such as polymorphism in various metabolic enzymes and/or transport proteins. However, the difference between altered and normal subjects cannot be easily explained and it is likely a multifactorial explanation including low jejunal pH, increased expression of enzymatic and transporter activity and rapid small intestine transit.

Keywords: Dissolution; Drug absorption; Gastric emptying; Gefitinib; Intestinal perfusion

1. Introduction

The epidermal growth factor receptor (EGFR) is over-expressed or dysregulated in a variety of solid tumours and plays a crucial role in their development, through involvement in increased cell proliferation and inhibition of apoptosis as well as enhancement of tumour vascularisation. Drug targeting of intracellular EGFR tyrosine kinase activity by gefitinib has resulted in a reduction in tumour growth and tumour cell death (Pao et al., 2004). Gefitinib (IRESSA®, AstraZeneca) is an anilinoquinazoline (4-quinazolinamine, *N*-(3-chloro-4-fluorophenylamino)-7-methoxy-6-[3-(4-morpholinyl) propoxy]) with a molecular weight of 446.9 Da. It is a lipophilic di-basic compound with a $\log D_{\mathrm{pH\,7.4}}$ of 3.9 and possesses two pK_a-values of 5.28 and 7.17, and accordingly exhibits a pH-dependent solubility in gastrointestinal fluid. The solubility of gefitinib in aspirated human gastric fluid (pH 5.0) and intestinal fluid (pH 7.0) was 4.98 and 0.085 mg/ml, respectively (data on file, AstraZeneca). The oral bioavailability following an single dose of 250 mg gefitinib (IRESSA® tablet) as a free base in healthy male volunteers was

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57% and manipulation of gastric pH to elevate it above five resulted in a 47% reduction of the relative bioavailability (data on file, AstraZeneca) (Swaisland et al., 2005). The maximum plasma concentration after a 250 mg dose gefitinib is typically observed after 5 h (Swaisland et al., 2005). As the in vivo intestinal permeability of gefitinib is predicted to be high based on data obtained in the Caco-2 model, we consider that the solubility and dissolution rate of gefitinib along the intestine might be the rate-limiting step in the absorption process. This is further supported by the observation that gefitinib undergoes rapid dissolution in acidic media but the solubility drops as pH increases to neutral intestinal pH (data on file, AstraZeneca). In summary, gefitinib is a Biopharmaceutical Classification System (BCS) class II compound, as the intestinal permeability is high and the solubility (and dissolution rate) is low along the intestine (Amidon et al., 1995).

Gefitinib is extensively distributed in the body and is mainly eliminated via metabolism and less than 0.5% (doses ranging from 1 to 75 mg) is excreted as unchanged gefitinib in urine (Swaisland et al., 2001). After a single oral dose of 50 mg radio-labelled gefitinib, $3.4 \pm 1.0\%$ was recovered in urine and $86.3 \pm 6.6\%$ (mean $\pm$ S.D.) was recovered in faeces of which only 12.1% was as parent drug in humans after sampling up to 240 h post-dose (Mckillop et al., 2004). Gefitinib and its metabolites are most likely eliminated by biliary and/or direct intestinal secretion.

The disposition kinetics of a 250 mg dose of gefitinib is characterized by a plasma half-life of about 39.7 h (range 26.9–83.2 h) in healthy volunteers (Swaisland et al., 2005). An analysis of the pharmacokinetic data from all healthy volunteer data generated during the clinical development of gefitinib showed that the majority of individuals orally dosed with gefitinib had post-absorptive plasma concentration time profiles that were biphasic with a terminal half-life in the order of 30 h. However, approximately 20% of the volunteer population had post-absorptive concentration–time profiles that were more monophasic in nature with an apparently shorter terminal half-life of about 10 h (data on file, AstraZeneca). Plausible explanations for the difference between these two groups with regards to plasma exposure might be differences in gastric emptying, drug dissolution and/or precipitation that affect existing absorption rate limitations and/or differences in metabolic capacity (i.e. clearance).

The Loc-I-Gut technique is a well established in vivo method for gastrointestinal intubation in humans and used for various biopharmaceutical and pharmacokinetic applications (Knutson et al., 1989; Petri et al., 2003; Lindahl et al., 1996; Tannergren et al., 2003; Sandstrom et al., 1998; Bonlokke et al., 1997, 2001, 1999; Lennernas et al., 1992). In the present study the Loc-I-Gut tube was introduced orally and once positioned in the jejunum a semi-open segment was created through inflation of the distal balloon, which offers well-defined conditions for in vivo investigations of dissolution and absorption of drugs (Fig. 1) (Bonlokke et al., 1997; Lennernas et al., 1992; Lindahl et al., 1997; Knutson et al., 1989). The present study was the first direct in vivo investigation in humans of the effect of precipitation and was aimed at studying changes of the drugs crystal structure on gastrointestinal absorption variables and the plasma exposure for a BCS class II drug such as gefitinib.

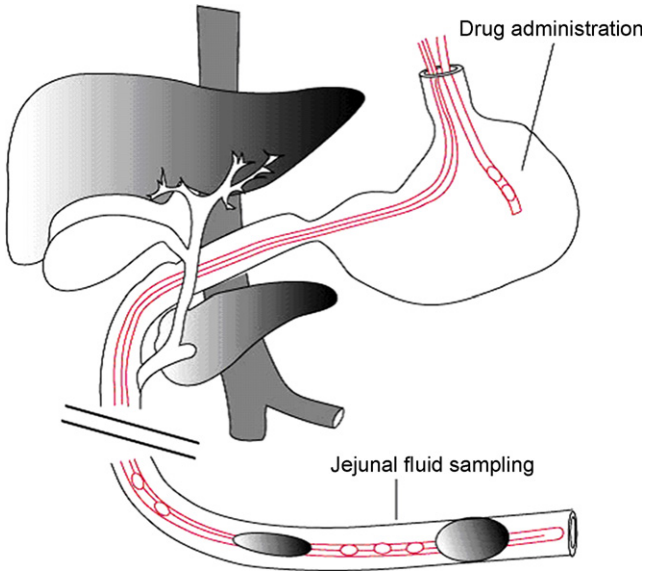

Fig. 1. A schematic view of the Loc-I-Gut tube positioned in the proximal human jejunum with a semi-open segment created by inflation of the distal balloon. The proximal balloon was kept deflated during the experiment. A water dispersion of gefitinib and ^{14}C-PEG 4000 (50 ml) was administered directly in the stomach followed by 190 ml of water for rinsing. Jejunal fluid samples were withdrawn continuously from the jejunum for 180 min after drug administration.

The main purpose of the study was to investigate whether the difference in plasma pharmacokinetic profiles between normal and altered healthy subjects might be explained by inter-individual variability in gastric emptying and/or precipitation/dissolution of gefitinib in the proximal small intestine by using a modification of an established in vivo intubation technique. The study also aimed to investigate the crystal form of the precipitated gefitinib in human jejunum by using Raman microscopy.

2. Material and methods

2.1. Subjects and study design

The study consisted of two separate consecutive study parts, study part I (SI) and study part II (SII). Both study parts were performed at the Clinical Research Department, University Hospital, Uppsala, Sweden and were separated by a minimum wash-out period of ten days. In SI, 100 healthy male subjects (aged 18–56 years and weighing 61–102 kg) were tested to determine and classify them on the basis of their pharmacokinetic (PK) profile of gefitinib following a single oral dose of 250 mg gefitinib (IRESSA®, AstraZeneca) in the fasted state. Blood samples were collected pre-dose and at intervals up to 120 h post-dose. After analysis of the plasma concentration–time data obtained, each subject was classified as having a "normal" (N; $t_{1/2} > 20$ h) or "altered" (A; $t_{1/2} < 20$ h) pharmacokinetic profile.

A total of 25 subjects from SI were enrolled in SII, based upon their pharmacokinetic profile, of which 20 subjects successfully completed SII. The group with normal PK profiles included 12

subjects (aged 21–26 years and weighing 63–100 kg) and the second group with altered PK profiles consisted of 13 subjects (aged 20–25 years and weighing 65–90 kg). Subjects in both groups were similar based on demographic variables (weight, height and age). In SII, after insertion of the Loc-I-Gut tube in the proximal jejunum and creation of a semi-open segment at fasted state an oral dispersion of 250 mg gefitinib in 50 ml water was administrated directly into the stomach followed by 190 ml of water to rinse the tube. Samples of intestinal fluid were withdrawn from the jejunal segment at time points up to 180 min after drug administration (Fig. 1). Blood samples were collected at the same time points as the intestinal samples and then at intervals up to 120 h post-dose. A subject was replaced in SII if the experimental procedures were technically incomplete for any reason.

All subjects underwent a full clinical examination in the 28 days prior to the first study day in SI and were required to have normal clinical and laboratory values (haematology, biochemistry, urine analysis, drug abuse screen). The physical examination included an examination of the cardiovascular and respiratory systems. A follow-up medical examination was performed within three weeks of the last pharmacokinetic assessment, confirming that all subjects were in good health. No regular use of prescription or herbal medications or recreational drugs were allowed and any medication that modified gastric pH was prohibited in the four weeks prior to the study day. Subjects that had received known CYP3A4 inducers or inhibitors within the past three months were excluded from the study. The subjects were restricted from consuming grapefruit, liquorice or Seville oranges and drinking excessive amounts of alcohol from 72 h pre-dose and until the last blood sample was collected.

The subjects gave written informed consent and the studies (SI and SII) were conducted in accordance with the Declaration of Helsinki and were approved by the Ethics Committee of Uppsala University.

2.2. Adsorption and stability test

The adsorption of gefitinib to the Loc-I-Gut catheter and its stability within the apparatus was investigated by an in vitro perfusion test in a glass tube for 120 min at 37 °C. Gefitinib (20 μg/ml) was dissolved in Fasted State Simulated Intestinal Fluid (FaSSIF) pH 6.5 and osmolality of 270 mosmol/kg. The FaSSIF consisted of 0.11 mM NaCl, 28 mM $NaH_2PO_4 \cdot H_2O$, 8.7 mM NaOH, 3 mM sodium taurocholate and 0.75 mM Lecithin. The drug solution was infused at a flow rate of 2 ml/min using a calibrated syringe pump (model 355; Sage Instrument, Orion Research Inc., Cambridge, MA, USA).

2.3. Study drugs

In SI the subjects received an oral dose immediate release (IR) tablet of 250 mg gefitinib (IRESSA®, AstraZeneca) taken with 240 ml water, with the subject in the upright position. In SII a single dose of gefitinib 250 mg (IRESSA®, AstraZeneca) was dispersed in 50 ml water and carbon 14-labeled polyethylene glycol 4000 (^{14}C-PEG 4000; Amersham Biosciences UK Limited, Little Chalfont, UK) was added to the dispersion to provide a final concentration of 2.5 μCi/l. The drug dispersion was administered through a separate tube directly into the stomach. After the drug administration, the dispersion vial and administration tube was flushed with an additional 190 ml of water into the stomach.

2.4. Experimental procedures

In SI each of the subjects remained at the hospital clinic from the evening before dosing up to 24 h post-dose. The subjects received a single oral dose of 250 mg gefitinib after fasting overnight. Venous blood samples (4 ml) were collected into tubes containing lithium heparin anticoagulant pre-dose and at 1, 3, 5, 7, 12, 24, 48, 72, 96 and 120 h after dosing. The blood samples were centrifuged within 30 min of collection at 4 °C, 1000 × *g* for 10 min to provide approximately 1 ml of plasma, which was then stored at −20 °C awaiting analysis.

In SII each subject was admitted to the study centre at the hospital on the evening prior to the study day. The Loc-I-Gut tube was inserted on the following morning after an overnight fast (10 h) (Knutson et al., 1989; Bonlokke et al., 1997). The tube was introduced orally after local anaesthesia with lidocaine spray (Xylocain®; AstraZeneca, Södertälje, Sweden) in the upper throat, and passage of the tube through the stomach was facilitated by the use of a Teflon-coated guide wire. Positioning of the tube in the proximal jejunum was performed within approximately one hour and was verified with fluoroscopy (Philips BV 21-S). The distal balloon was inflated with air (26–30 ml) creating a semi-open segment in the jejunum (Fig. 1) (Bonlokke et al., 1997, 2001, 1999). To facilitate jejunal fluid sampling a vacuum pump was connected to the Loc-I-Gut tubes inlet and outlet luminal drainage channels (Ameda suction pump type 23; Ameda AG, Zug, Switzerland). A Salem sump tube (Salem sump nasogastric drainage tube: Sherwood Medical, Unilever PLC, London, United Kingdom) was positioned in the stomach to enable dosing of the gefitinib dispersion and also in order to obtain gastric fluid samples. After positioning of the Loc-I-Gut tube a 2-h stabilisation phase followed during which jejunal fluid was collected. After the stabilisation phase the drug dispersion was administered directly into the stomach. Jejunal fluid was quantitatively collected on ice at the following time intervals: 10, 20, 30, 45, 60, 75, 90, 105, 120, 150 and 180 min after dosing. The volume, weight and pH of the jejunal samples were recorded and divided into four aliquots. To investigate the precipitation of gefitinib one aliquot of jejunal fluid was centrifuged at 13,000 × *g* for 5 min (Mini Spinn Plus Eppendorf centrifuge, Germany). The supernatant was immediately frozen at −20 °C and the precipitate was placed on a microscope slide and was left to dry over-night in a fume cupboard. After drying a cover slip was fixed and the sample was then subjected to Raman microscopy to enable determination of the crystalline form of the precipitated gefitinib. Each jejunal fluid sample was stored at −20 °C pending analysis.

Venous blood samples (4 ml) was collected into tubes containing lithium heparin anticoagulant prior to dosing and then at 10, 20, 30, 45, 60, 75, 90, 105 min, 2, 2.5, 3, 5, 7, 12, 24,

48, 72, 96 and 120 h after dosing. The blood samples were centrifuged as previously described for SI and stored at −20 °C until analysis.

2.5. Analytical methods

All plasma and jejunal perfusate samples were analysed by Analytico Medinet B.V., NL to determine gefitinib concentration.

2.5.1. Plasma

The plasma samples were analysed for gefitinib according to the method published in 2002 by Jones et al. (2002). Briefly, a liquid–liquid extraction with methyl-*t*-butyl ether was performed followed by reversed phase high performance liquid chromatography on an Intersil 150 mm × 4.6 mm ODS3 C18 column (protected by a guard column, 4.3 mm C18) and deuterated gefitinib (AstraZeneca, Alderley Park) was used as internal standard. The mobile phase was a mixture of 80% acetonitrile and 20% aqueous ammonium acetate (v/v) delivered at a flow rate of 1 ml/min. A volume of 10 μl of the extracted samples were injected onto the column and detection was performed using a Perkin Elmer SCIEX API 3000 triple quadrupole tandem mass spectrometer (PE series 200 auto sampler and fitted with a interface of a heated nebuliser source for atmospheric pressure chemical ionisation). The measurements for gefitinib were performed at *m/z* 447 and 128, respectively. The deuterated gefitinib measurements were performed at *m/z* 455 and 136, respectively. The concentration of gefitinib was calculated based on the peak area ratio (sample/internal standard) with reference to the calibration series. The calibration series was fitted by linear least-squares regression analysis of each standard using a weighting inversely proportional to the corresponding square of the concentration. The limit of quantification was 0.5 ng/ml.

2.5.2. Jejunal fluid

Jejunal fluid (250 μl) was diluted with acetic acid and deuterated gefitinib (AstraZeneca, Alderley Park) used as internal standard. Jejunal fluid was extracted and analysed as previously described for the plasma samples with the exception that the injected volume for the jejunal sample extracts was 25 μl. The concentration of gefitinib was calculated with reference to a calibration series prepared in jejunal fluid samples and the limit of quantification was 0.5 ng/ml.

In addition, the following analyses were performed at the Department of Pharmacy, Uppsala University. The total radioactivity ^{14}C-PEG 4000 in the dosing solution and the collected jejunal fluid samples were determined by liquid scintillation counting (Mark III; Searle Analytic Inc., Des Plaines, IL, USA). A volume of 500 and 50 μl of jejunal fluid sample and dosing solution, respectively, were added to 8 ml of scintillation fluid. The pH of the jejunal and gastric fluids were measured with a pH meter (Mettler Toledo MP225, CH 8603, Schwerzenbach, Switzerland). The osmolality of the jejunal fluid and gastric fluid was determined using the vapor pressure method (5520 vapor pressure osmometer; Wescor Inc., Logan, Utah, USA).

2.5.3. Analysis of precipitated gefitinib

The analysis of the precipitated gefitinib was performed by Pharmaceutical Analytical Research & Development, AstraZeneca, Alderley Park. The imaging system used for this study was a Perkin Elmer Spotlight NIR (near infrared) imaging microscope. Depending on the state of the sample, an area of between 0.5 and 3.0 mm^2 was imaged with a spatial resolution of 25 μm in all cases. For each image this gave between about 1000 and 10,000 spectra for analysis. From each subject two or three samples were examined by NIR imaging to give an indication for the presence of and any variation in gefitinib morphology with time. The spectra were examined for gross spectral features due to absorption bands characteristic of the drug molecule.

2.5.4. Bile acids and phospholipids analyses

The perfusate was analysed for bile acids and phospholipids by solid phase extraction followed by HPLC, with evaporation light scattering (ELS) detector (Persson et al., 2006). In brief, separation of bile acids from phospholipids was accomplished using pre-packed C18 columns (Isolute International Sorbent Technology, UK). The bile acids were eluted with methanol/H_2O and the phospholipids with combinations of methanol/methyltertbutyl ether/acetic acid. A Zorbax C18 Extend column (150 mm × 4.6 mm, 3.5 μm, Agilent Technologies, US) and a gradient of methanol/ammonium acetate buffer (pH 3.15) were used in combination with an ELS detector (PL-ELS 1000, Polymer Laboratories, Shropshire, UK) to characterise the concentration and type of bile acid. The concentrations of the following bile acids were determined; glycocholic acid (GCA), taurocholic acid (TCA), chenodeoxycholic acid (CDCA), glycochenodeoxycholic acid (GCDCA), taurochenodeoxycholic acid (TCDCA), deoxycholic acid (DCA), glycodeoxycholic acid (GDCA) and taurodeoxycholic acid (TDCA). To determine the concentration and type of phospholipids, a Diol C18 column (250 mm × 2.1 mm, 5 μm, YMC c/o Waters, Milford, USA) and a gradient of hexane/isopropanol/H_2O was used together with an ELS detector (ELS 1000, Polymer Laboratories, Shropshire, UK). The concentrations of the following phospholipids were determined; phosphatidylcholine (PC), lyso-phosphatidylcholine (LPC), phosphatidylglycerol (PG), diphosphatidylglycerol (DPG), phosphatidic acid/phosphatidylethanolamine (PA/PEA), phosphatidylserine (PS), phosphatidylinositol (PI) and sphingomyelin (SM).

The limit of quantification for the bile acids and phospholipids analyses were 0.1 and 0.04 mM, respectively.

2.6. Calculations

The recovery of ^{14}C-PEG 4000 was calculated according to equation one, i.e. as the accumulated amount of ^{14}C-PEG 4000 in the intestinal fluid sample leaving the segment divided by the total amount ^{14}C-PEG 4000 administered with the gefitinib dispersion in the stomach.

$$\text{PEG-recovery}\,(\%) = \sum \frac{V_{\text{sample}} \times C_{\text{sample}}}{V_{\text{adm}} \times C_{\text{adm}}} \times 100 \qquad (1)$$

The amount of precipitated gefitinib was calculated by assuming that all administered gefitinib had emptied from the stomach at the end of 180 min and therefore that the differences between the total amount of gefitinib present in a sample and amount of gefitinib in solution/dissolved was the precipitated or undissolved amount of gefitinib.

2.7. Pharmacokinetic data

The maximum detected peak plasma concentrations (C_{max}) and the time to reach maximum plasma concentration (t_{max}) for each subject were derived directly from their plasma concentration–time profiles. The plasma concentration–time data was analysed using non-compartmental methods (WinNonlin Professional Version 3.1 and WinNonlin Professional Version 4.0; Pharsight Corp., Mountain view, CA, USA). The rate constant of the slowest disposition phase (λ_Z) was calculated by log-linear regression of the terminal portion of the concentration–time profiles, and the terminal half-life ($t_{1/2}$) was calculated from the equation

$\ln 2/\lambda_Z$. The area under the plasma concentration–time curve up to the time of the last quantifiable plasma concentration, $AUC_{0\text{-}t}$, was calculated by the linear trapezoidal rule. The $AUC_{0\text{-}t}$ was then extrapolated to infinity using λ_Z to obtain the area under the plasma concentration–time curve from zero to infinity ($AUC_{0-\infty}$).

To ensure accuracy in the predicted $AUC_{0-\infty}$ the percent of the extrapolated AUC was not to exceed 15% of the total $AUC_{0\text{-}t}$.

2.8. Statistical analysis

The differences between altered and normal PK with respect of $t_{1/2}$, C_{max}, t_{max}, AUC, jejunal fluid pH, total concentration of bile acids and phospholipids and precipitation and recovery of gefitinib in jejunal fluid were evaluated with the Student *t*-test for unpaired data (MINITAB release 14, Minitab Inc., USA). Differences were considered to be significant at $P < 0.05$. All data presented in this report are expressed as arithmetic mean ± S.D. unless stated otherwise.

3. Results

3.1. Pharmacokinetic assessment in study part I (SI)

All the subjects showed similar demographic (weight, height and age). Gefitinib was well tolerated by all subjects when given as a single dose of 250 mg as a tablet. The plasma concentration–time profiles and the pharmacokinetic variables of gefitinib after oral dosing in the two groups (in total 100 subjects) are shown in Fig. 2 and in Table 1. Each male subject was classified into one of the two groups based on their elimination half-life of gefitinib: (a) the normal group (N; $t_{1/2} > 20$ h) and, (b) altered group (A; $t_{1/2} < 20$). The number of subjects in each group was 68 and 24, respectively. For 8 subjects the half-life and AUC could not be calculated due to the terminal phase being insufficiently well defined. In study part I (SI) there was a significant difference between the normal and altered groups with respect to plasma exposure ($AUC_{0-\infty}$). The difference between C_{max} and t_{max} was not significant between the two groups.

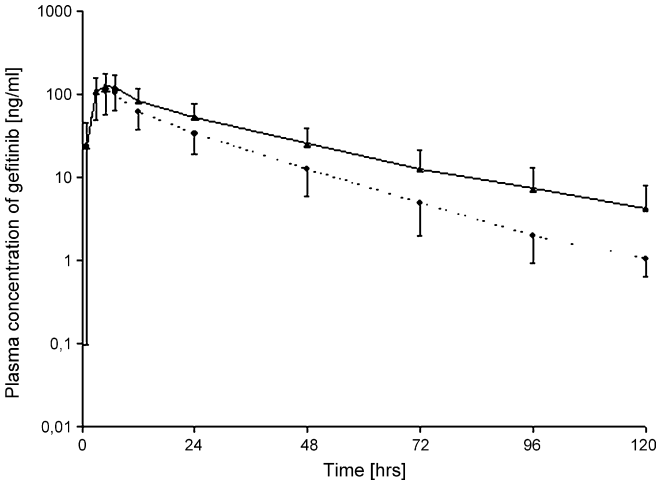

Fig. 2. The average plasma concentrations of gefitinib in 100 healthy male volunteers from SI were split into the two PK profiles, normal (N; $t_{1/2} > 20$ h) and altered (A; $t_{1/2} < 20$ h). The full lines represent normal PK profiles ($n = 68$) and dotted lines represent altered PK ($n = 24$). The data are presented as arithmetic mean ± S.D.

3.2. Pharmacokinetic assessment in study part II (SII)

Gefitinib was well tolerated by the healthy subjects when given as a single dose of 250 mg as a dispersion preparation administered via a gastric tube positioned in the stomach. Of the 25 subjects enrolled in SII, 20 subjects completed the study. Reasons for withdrawal were inability to insert the Loc-I-Gut in jejunum ($n = 1$), voluntary withdrawal of informed consent before study start ($n = 1$) and failure to return for study procedure ($n = 1$). For one subject the study was terminated after 75 min due to a low production of jejunal fluid, and for the fifth subject the dose was immediately expulsed upon drug administration, this outcome is likely to be due to intolerability of the Loc-I-Gut tube rather than intolerability of gefitinib. The plasma pharmacokinetic variables from study part II are presented in Fig. 3 and Table 2. The plasma exposure of gefitinib was lower in the intubation experiments (SII) compared to traditional oral administration (SI) in all subjects except two, which is most likely due to the continuous withdrawal of jejunal fluid samples from the intestine containing non-absorbed drug between 0 and 180 min post-dose. Of the selected subjects who completed SII, 10 had been pre-defined as having normal profiles (Subject 1, 2, 3, 4, 5, 13, 17, 19, 23 and 24) and had estimated half-lives in SII that ranged from 25.7 to 57.6 h. Relatively few subjects in SI had half-lives in the shorter range (i.e. <10 h), and because not all those selected from SI were available to participate in SII, the selection of subjects with altered profiles was from those with half-lives close to the defined 20 h cut-off value. In total 10 subjects had been pre-defined as having altered profiles (Subjects 6, 8, 10, 11, 14, 15, 20, 21, 22 and 25) and had estimated half-lives in SII that ranged from 11.6 to 27.6 h, but due to the intra-individual variability in the half-life, four had a half-life

Table 1
Pharmacokinetic variables after a single oral dose of 250 mg tablet of gefitinib in study I (SI)

PK profile	C_{max} (ng/ml)	t_{max} (h)	$t_{1/2}$ (h)	$AUC_{0-\infty}$ (ng h/ml)
Normal ($n=68$)	130 ± 45.9 (50.2–250)	5.42 (3.07–24.1)	26.9 ± 5.16† (20.00–45.60)	3850 ± 1690† (1730–9120)
Altered ($n=24$)	124 ± 65.8 (31.2–306)	5.08 (2.92–10.9)	17.5 ± 1.98† (12.1–19.7)	2380 ± 970† (862–5100)

Data are presented as arithmetic mean ± S.D. (range) except for t_{max} which are presented as median (range).
† Statistically different $P<0.05$.

Table 2
The plasma pharmacokinetic variables following administration of 250 mg gefitinib dispersion through a gastric tube (SII)

PK profile	C_{max} (ng/ml)	t_{max} (h)	$t_{1/2}$ (h)	$AUC_{0-\infty}$ (ng h/ml)
Normal ($n=7$)	79.9 ± 32.8 (30.4–113)	5.00 (2.00–7.00)	36.8 ± 10.1† (26.1–57.6)	2630 ± 1140 (1050–3680)
Altered ($n=6$)	73.6 ± 36.9 (41.2–142)	3.00 (2.00–5.00)	19.3 ± 6.09† (12.3–27.6)	1450 ± 923 (775–3230)

The data are presented as arithmetic mean ± S.D. (range) except for t_{max} which are presented as median (range).
† Statistically different $P<0.05$.

in SII that was longer than the pre-defined 20 h cut-off with a range from 22.2 to 27.6 h.

In SII we demonstrated that gefitinib exists in the undissolved state in the jejunal fluid once it empties from the stomach. The proportion of precipitated/undissolved and recovered gefitinib were not significantly different between subjects possessing altered or normal PK (Figs. 4 and 5, Table 3). The pH in the collected jejunal fluid was stable throughout the intubation experiment (Table 4). Unfortunately, due to assay limitations Raman spectrophotometer analysis was unable to determine the crystalline form of the precipitated/undissolved gefitinib obtained in the outlet jejunal perfusate for all but one sample. In this sample gefitinib was present as Form 1, the dosed form. The inability of Raman microscopy to identify the form of gefitinib in the collected jejunal samples was probably a consequence of the complex nature of human intestinal fluid samples. We were therefore unable to determine if there was a difference in crystalline form of the in vivo aspirated precipitate in altered compared to normal groups in this present study.

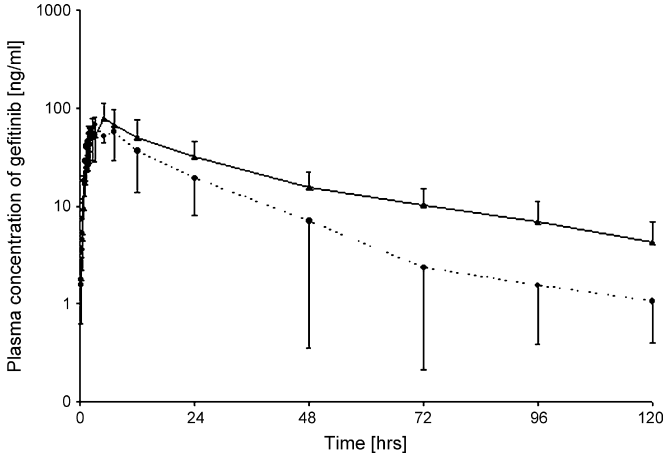

Fig. 3. Plasma concentration-time profiles from SII in normal ($n=7$) and altered ($n=6$) subjects after administration of a water dispersion of 250 mg gefitinib through a Salem sump tube positioned in the stomach and continuous sampling of jejunal fluid from the jejunum for 180 min. Full lines represent normal and dotted lines represent altered and the data are presented as arithmetic mean ± S.D.

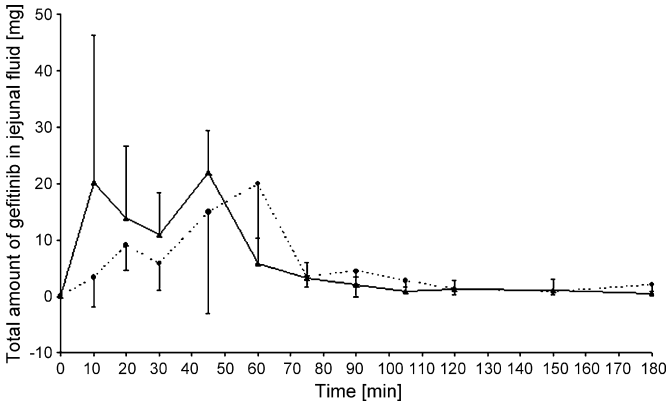

Fig. 4. The total concentration (i.e. in solution and precipitated/undissolved) of gefitinib in jejunal fluid collected every 20 min during 180 min. The data are presented as arithmetic mean ± S.D. The full lines represent normal PK profiles and dotted lines represent altered PK profiles.

The appearance of the non-absorbable marker ^{14}C-PEG 4000 in jejunal fluid over time illustrated that the gastric emptying processes was minimally affected by the presence of the gastrointestinal tube (Fig. 6). There was no significant difference in the total recovery of ^{14}C-PEG 4000 between normal and altered

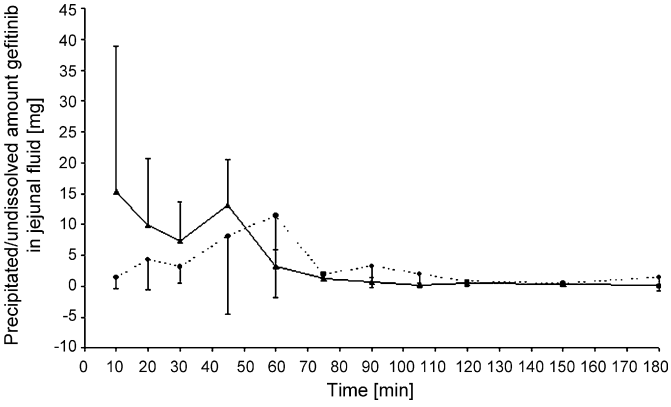

Fig. 5. The amount precipitated/undissolved gefitinib over time for subjects with normal and altered PK profiles presented as arithmetic mean ± S.D. The full lines represent normal PK profile and dotted lines represent altered PK profiles.

Table 3
The accumulated total amount (unabsorbed) gefitinib, in solution and in solid form (precipitated/undissolved)

PK profile	Accumulated precipitated/ undissolved gefitinib (mg)	Accumulated gefitinib in solution (mg)	Accumulated total gefitinib (mg)	^{14}C-PEG 4000 recovery (%)
Normal ($n=7$)	39.0 ± 28.1 (3.54–76.7)	23.3 ± 15.8 (1.57–46.2)	69.1 ± 36.1 (5.97–101)	76.9 ± 27.0 (27.1–104)
Altered ($n=6$)	32.2 ± 20.0 (8.03–58.4)	24.9 ± 13.2 (9.02–42.6)	57.0 ± 20.8 (30.2–91.7)	66.4 ± 32.9 (24.4–110)

The fraction recovered of the non-absorbable marker substance, ^{14}C-PEG 4000, was similar between the two groups, indicating that the gastric emptying was not the explanation for the difference in plasma exposure. The data are presented as arithmetic mean ± S.D. (range).

Table 4
The separate concentrations of bile acids and phospholipids in jejunal fluid leaving the intestinal segment in normal and altered subjects at 10, 90 and 180 min post-dose

	Normal PK profile, $N=7$			Altered PK profile, $N=6$		
Bile acids (mM)	10 min	90 min	180 min	10 min	90 min	180 min
GCA	0.66 ± 0.46	0.48 ± 0.24	0.58 ± 0.19	0.60 ± 0.45	0.84 ± 0.64	2.49 ± 2.69
TCA	0.29 ± 0.23	0.21 ± 0.01	0.25 ± 0.12	0.52 ± 0.30	0.40 ± 0.36	1.75 ± 1.97
GCDCA	0.31 ± 0.26	0.46 ± 0.14	0.44 ± 0.22	0.29 ± 0.12	0.45 ± 0.40	1.73 ± 2.20
TCDCA	0.29 ± 0.07	0.21 ± 0.07	0.20 ± 0.06	0.35 ± 0.13	0.38 ± 0.12	0.47 ± 0.39
GDCA	0.41 ± 0.11	0.34 ± 0.19	0.34 ± 0.17	0.42 ± 0.29	0.81 ± 0.97	1.13 ± 0.96
TDCA	0.26 ± 0.17	0.36 ± 0.29	0.18 ± 0.08	0.57 ± 0.64	0.43 ± 0.25	NQ
Phospholipids (mM)						
PC	NQ	NQ	NQ	NQ	0.11	0.06
LPC	0.06 ± 0.04	0.08 ± 0.08	0.08 ± 0.05	0.08 ± 0.09	0.05 ± 0.05	0.32 ± 0.55
PG	NQ	NQ	NQ	NQ	0.026	NQ
DPG	NQ	NQ	NQ	NQ	NQ	0.03
SM	NQ	NQ	NQ	NQ	NQ	0.07
pH						
Gastric pH	2.2 ± 1.1			1.8 ± 0.5		
Jejunal pH	7.1 ± 0.2	6.9 ± 0.5	7.1 ± 0.3	6.7 ± 0.3	6.0 ± 1.6	7.0 ± 0.4

Values for gastric pH pre-dose and jejunal pH at 10, 90 and 180 min are also presented. Data are presented as arithmetic mean ± S.D. Glycocholic acid (GCA), taurocholic acid (TCA), glycochenodeoxycholic acid (GCDCA), taurochenodeoxycholic acid (TCDCA), glycodeoxycholic acid (GDCA) and taurodeoxycholic acid (TDCA), phosphatidylcholine (PC), lyso-phosphatidylcholine (LPC), phosphatidylglycerol (PG), diphosphatidylglycerol (DPG), and sphingomyelin (SM). The following bile acids and phospholipids were below detection limit in all the collected jejunal fluid samples; chenodeoxycholic acid (CDCA), deoxycholic acid (DCA), phosphatidic acid/phosphatidylethanolamine (PA/PEA), phosphatidylserine (PS) and phosphatidylinositol (PI).

groups, even though the peak recovery occurred 15 min later in the altered compared to the normal group. A ^{14}C-PEG 4000 recovery less than 20% was considered as a technical failure and those subjects were excluded from the study ($n=5$) resulting in the total number of evaluable subjects being seven subjects with the normal PK profile and six subjects with the altered PK profile. A low ^{14}C-PEG 4000 recovery might reflect slower gastric emptying during the 180 min of the intubation and/or failure maintain a semi-open segment due to leakage of intestinal fluid passing the inflated distal balloon. The jejunal fluid contents of phospholipids and bile acids were determined at 10, 90 and 180 min and are presented in Table 4. The osmolarity of the collected gastric fluid was 191 ± 46 and 201 ± 44 mmol/kg (mean ± S.D.) for normal and altered groups, respectively.

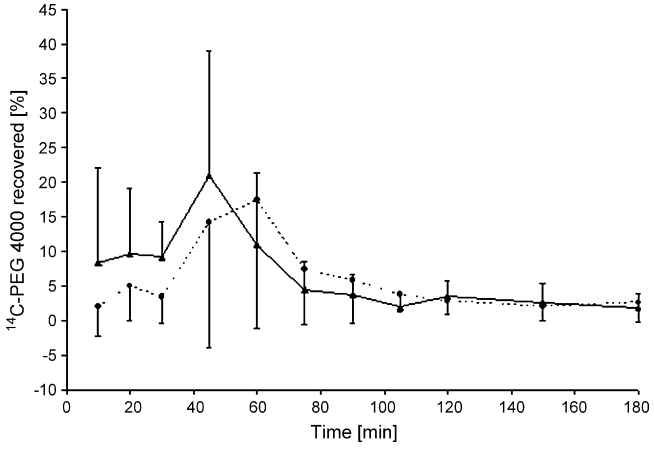

Fig. 6. The recovery of ^{14}C-PEG 4000 (mean ± S.D.) in jejunal fluid over time. The ^{14}C-PEG 4000 was collected in the fluid leaving the segment in the proximal human jejunum over 180 in 20 min fractions.

There was no adsorption of gefitinib (1.36% ± 6.19) to the Loc-I-Gut tube. Gefitinib was stable in the FaSSIF, respectively, during 120 min.

4. Discussion

In the present in vivo absorption study we investigated whether the differences in plasma pharmacokinetic profiles of gefitinib between normal ($t_{1/2}>20$ h) and altered ($t_{1/2}<20$ h) healthy subjects might be explained by inter-individual variability of gastric dissolution, gastric emptying and/or subsequent precipitation of gefitinib in the proximal small intestine. In the

screening part of the study, SI, we found that mean plasma exposure ($AUC_{0-\infty}$) was about 55% higher in the normal group compared to the altered group. We performed an in vivo examination of how biopharmaceutical variables affected the gastrointestinal absorption rate of gefitinib by using an established in vivo intubation technique. This method, Loc-I-Gut, has been extensively applied in drug absorption studies by our research group in numerous studies (Bonlokke et al., 1997, 2001, 1999; Lennernas et al., 1992; Lindahl et al., 1996; Petri et al., 2003; Sandstrom et al., 1998; Knutson et al., 1989; Tannergren et al., 2003).

In SI, 100 healthy male subjects were given an oral dose of gefitinib (250 mg) and 24 subjects were identified with an altered pharmacokinetics profile. The reason for the difference in plasma pharmacokinetics between these groups was hypothesised to be possibly due to pH-dependent gastrointestinal dissolution and precipitation processes of gefitinib. Hence, in SII gefitinib was administered directly into the stomach as a dispersion preparation and recovered jejunal fluid samples for each subject contained both soluble and precipitated/undissolved gefitinib (Figs. 4 and 5). The lower solubility of gefitinib in the human intestinal fluid compared to human gastric fluid is related to the pH-difference. The study also showed a considerable inter-individual variability in the fasting gastric emptying rate of fluids (Fig. 6), which is in accordance with its dependence on interdigestive motor activity (Oberle et al., 1990). Accordingly, the amount of precipitated/undissolved gefitinib in the aspirated human intestinal fluid also showed high inter-individual variability. However, there was no significant difference in gastric emptying rate between these two groups.

One criticism that has been levelled on perfusion techniques in general is that they may disturb the gastrointestinal physiology, especially the motility patterns (Wilding et al., 2001; Davis and Wilding, 2001). In a study reported by Read et al. (1983), the effect of a tube that reached the terminal ileum on gastrointestinal physiology was investigated by monitoring the gastrointestinal passage of a solid meal with gamma scintigraphy. They found that the presence of the tube significantly slowed the gastric emptying half-life from 1.2 ± 0.32 to 1.5 ± 0.35 h, and the small intestinal transit time decreased from 3.6 ± 1.33 to 1.3 ± 0.99 h (mean $\pm$ S.D.) (Read et al., 1983). Thus, the effects of the tube on gastric emptying are minimal and do not question the pharmaceutical relevance of drug absorption data collected using these perfusion methods. Further support for this conclusion is reported by Naslund et al. (2000) who clearly showed that there was no difference in gastric emptying between the following three methods: scintigraphic, oral dosing of paracetamol tracer and subsequent plasma sampling, and PEG dilution methods using intubation tubes

In SII, one subject was unable to tolerate the Loc-I-Gut insertion procedures, and another had a reflux expulsion of the dose immediately after drug administration. In a previous study, 17 healthy subjects received a 250 mg dispersion of gefitinib administered into the stomach via a nasogastric tube, and 18 subjects received a 250 mg dispersion of gefitinib as an oral solution with no episodes of vomiting reported after either administration route (Cantarini et al., 2004). Thus, the reflex expulsion of the dose in the present investigation may be a consequence of intolerance to the presence of the Loc-I-Gut tube rather than poor tolerability of the gefitinib dispersion. This is also the most likely reason for the withdrawal of one subject 75 min post-dose having a very low jejunal fluid production.

From Figs. 4 and 5 and Tables 2 and 3 it can be seen that differences in exposure between normal PK profile and altered PK profile subjects cannot be readily explained in terms of differences in the amount of gefitinib present in the jejunal fluid (either in total, in solution or precipitated/undissolved). Although other explanations are needed to explain the differences seen in plasma exposure between altered and normal PK profile subjects, data from individual subjects may offer some clues to the underlying mechanisms. For example, one subject originally defined as having a altered PK profile in SI had a low $AUC_{0-\infty}$ of 862 ng h/ml in SI and the AUC could not be calculated in SII due to plasma concentrations below detection levels at all time points. In this subject 161 mg of gefitinib was recovered in the jejunal fluid 10 min post-dose of which 139 mg was in solution. A possible explanation for the high amount of gefitinib present after only 10 min could be very rapid gastric emptying and a very rapid transit through the duodenum, which could explain the low jejunal pH observed in this subject (mean pH 2.4 ± 0.4, range 1.9–3.2). The low jejunal pH could indicate that the Loc-I-Gut catheter had been repositioned in the proximal duodenum, however, this individual had already in SI, a relatively low exposure of gefitinib. A low jejunal pH would cause gefitinib to be highly ionised, thereby reducing the ability for it to be absorbed across the intestinal epithelium during a limited GI-residence time, which would also explain the high recovery of gefitinib in solution in this individual. The subject was excluded from the study analysis since the plasma concentration of gefitinib was below detection levels at all time points. In common with this subject, another subject had a low $AUC_{0-\infty}$ in SII of 775 ng h/ml and the pH of the jejunal fluid after 45 min post-dose until 2 h post-dose was reduced from pH 6 to approximately pH 3. During this interval the collected jejunal fluid contained 87% of gefitinib in solution. Given the neutral pH of bile, enterohepatic circulation would probably not result in a low pH of the collected jejunal fluid sample. Thus, the most plausible explanations for the low jejunal pH are the Loc-I-Gut tube being relocated to the proximal duodenum by intestinal motility or these two subject having a gastrointestinal physiology that differ from the rest of the study population that might affect drug dissolution or intestinal permeability.

We also found that there was no difference between altered and normal groups regarding concentration of bile acids, phospholipids and pH in the jejunal fluid. These observations are in agreement with the observation that there was no difference in the recovery of gefitinib in solution and in solid (precipitated/undissolved) forms in the jejunal fluid leaving the intestinal segment.

Gefitinib is predominantly eliminated by metabolism and the hepatic extraction of gefitinib is estimated to be approximately 60% (49–68%) and therefore inter-individual expression of metabolic enzymes and various transporters (intrinsic clearance) will certainly influence the bioavailability of gefitinib

(Ranson and Wardell, 2004). A recently published study showed that the intrasubject and intersubject variability following a 250 mg dose of gefitinib was 2-fold and 10–15-fold, respectively, in healthy volunteers (Swaisland et al., 2005). Thus, data indicated that there is a correlation between plasma exposure for gefitinib and midazolam (a known CYP3A4 substrate) that would explain some of the observed inter-individual variability of gefitinib (Swaisland et al., 2006). The CYP3A4 activity in both intestine and liver is highly variable between individuals even if no bimodal polymorphism has been reported. The variable expression of CYP3A4 is due to the complex interplay between genetics and environmental factors (Westlind et al., 1999; Wojnowski, 2004). The difference between altered and normal subjects cannot be easily explained and it is likely a multifactorial explanation is required with several factors needing to be present. These include possibly low jejunal pH, increased expression of enzymatic and transporter activity and rapid small intestine transit.

In conclusion, there is no pronounced effect on gastric emptying, precipitation and re-dissolution of gefitinib in proximal human jejunum on the plasma concentration profile between the two groups. Instead other mechanism(s) are likely to be more important in explaining the inter-individual differences in plasma exposure to gefitinib, such as polymorphism in various enzymes and/or transport proteins, or possibly a combination of factors including GI-factors, none of which have a dominant effect in isolation.

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