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ORIGINAL ARTICLE

The relative bioavailability of gefitinib administered by granular formulation

Mireille V. Cantarini · Chris J. Bailey · Barbara Collins · Robert P. Smith

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Abstract
Background Gefitinib (IRESSA) is normally administered as a once-daily oral tablet. However, many patients with head and neck cancer have difficulty swallowing medication in a tablet form. A granular formulation has recently been developed to facilitate the administration of gefitinib to patients who are unable to swallow tablets.
Objectives The aims of this study were to determine the relative bioavailability of a single dose of gefitinib when administered as 250 mg of a new granular formulation compared with the standard 250 mg tablet, and to assess the intra-subject variability of the granular formulation, in healthy subjects.
Methods This was a randomized, open-label, three-period crossover study. Healthy male subjects ($n = 18$) received either a single gefitinib 250 mg tablet (once), or a 250 mg granular formulation of gefitinib (on two separate occasions) over the three dosing periods, in randomized order. Plasma concentrations of gefitinib were measured up to 240 h post-dose.
Results The treatment ratio estimates for area under the plasma concentration versus time curve (AUC) and peak plasma concentration (C_{max}) for the granular formulation when compared with the tablet were 1.05 (90% confidence intervals [CI] for the ratio 0.97–1.13) and 1.14 (90% CI for the ratio 1.01–1.28), respectively. The estimate for the intra-subject standard deviation for the granular formulation when given on 2 separate occasions was 0.143 for AUC and 0.165 for C_{max}, equivalent to a 1.4- and 1.7-fold intra-subject variability in AUC and C_{max}, compared with that observed for the tablet of two and threefold, respectively.
Conclusions There was little difference in exposure to gefitinib administered as the 250 mg granular formulation compared with the 250 mg standard tablet. The granular formulation of gefitinib could provide an alternative treatment regimen for patients unable or unwilling to swallow the standard tablet formulation, without compromizing exposure to gefitinib.

Keywords Relative bioavailability · Gefitinib · Granular · Pharmacokinetics

Introduction

Gefitinib (IRESSA) is, as an orally active inhibitor of the epidermal growth factor receptor (EGFR) tyrosine kinase, an enzyme which catalyzes phosphorylation reactions that are implicated in the proliferation and survival of cancer cells via intracellular signal transduction pathways. Expression of EGFR has been identified in a wide range of human cancers, and since this observation has frequently been correlated with poor prognostic features [8, 10], EGFR represents an important target for novel anti-cancer agents. EGFR overexpression occurs in 90–100% of head and neck cancers [5].

Pharmacokinetic studies in man show that gefitinib is rapidly cleared, has a high volume of distribution and the half-life in patients is about 40 h [9]. The absolute bioavailability of the 250 mg tablet in patients is 60% [12], and the plasma concentration profiles after oral dosing show it to be suitable for once-daily oral administration, with steady state being achieved by day 7 [1, 6, 9]. Gefitinib is cleared primarily by the hepatic route as parent compound plus metabolites, with less than 4% of the dose being cleared by the

M. V. Cantarini (✉) · C. J. Bailey · B. Collins · R. P. Smith
AstraZeneca, Alderley Park, Macclesfield,
Cheshire SK10 4TG, UK
e-mail: mireille.cantarini@astrazeneca.com

renal route [7]. The major cytochrome P450 enzyme involved in the metabolism of gefitinib is CYP3A4, although the formation of the major circulating human metabolite of gefitinib has been shown to be catalyzed primarily by the cytochrome P450 CYP2D6 [7].

Gefitinib is under investigation in patients with head and neck cancer. Gefitinib is normally administered as a once-daily oral tablet. However, many patients with head and neck cancer have difficulty swallowing medication in tablet form (particularly after surgery or radiotherapy), as do children [11], and patients with neuromuscular conditions [3].

For patients who are unable or unwilling to swallow gefitinib tablets, the drug can be administered as a liquid preparation via a nasogastric tube or as a drink. A study carried out in normal healthy subjects showed that dispersing the tablet in water, in order to administer gefitinib either as a drink or via a nasogastric tube, did not affect the pharmacokinetic profile of a single dose of gefitinib compared with a whole tablet swallowed [2]. In a phase II study of gefitinib 500 mg/day in patients with head and neck cancer some individuals received gefitinib via a gastrostomy tube, but there were no observed clinical or statistical differences in response, toxicity, or survival between those who received gefitinib via this method and those who received the drug orally as a tablet [4].

A granular formulation has recently been developed to facilitate the administration of gefitinib to patients who are unable to swallow tablets. The primary objective of this study was to determine the relative bioavailability of gefitinib administered using the new 250 mg granular formulation compared with the 250 mg standard tablet. A secondary objective was to estimate the intra-subject variability in exposure to gefitinib for the granular formulation.

Subjects and methods

Subjects

Eighteen healthy male Caucasian subjects were enrolled in this trial after giving written informed consent. Subjects were eligible if they had no clinically relevant conditions identified from their medical history, physical examination, laboratory investigations, or electrocardiogram. Major exclusion criteria included current or recent receipt (within the past 4 weeks) of any drug known to induce or inhibit the cytochrome P450 3A4 isozyme, or any drugs known to modify gastric pH. The trial was conducted in accordance with Good Clinical Practice and the Declaration of Helsinki. An independent ethics committee (Quorn Research Review Committee, Old Dalby, Leicestershire, UK) approved the protocol before the trial started.

Trial design

This was a randomized, open-label, three-period crossover study (D7919C00001), which was conducted at a single center (AstraZeneca, Clinical Pharmacology Unit, Alderley Park, Macclesfield, UK). In each trial period, subjects received one of the following treatments: Treatment A (1 × 250 mg gefitinib tablet); or Treatment B (1 × 250 mg gefitinib in the granular formulation). Each subject was expected to receive the tablet once and the granular formulation twice. There were three possible sequences of treatment: ABB; BAB; and BBA, with subjects randomized to one of the three sequences at the beginning of the study. There was a minimum of a 3-week washout between each treatment period to ensure that there was no carryover from the previous dose. Each gefitinib dose was administered with a total volume of 240 ml water. The granular formulation was supplied as the 250 mg dose pre-weighed in 25 ml clear glass bottles. It was prepared by mixing the granules with 50 ml purified water in a glass beaker, administering this dose as a drink, then rinsing the glass storage bottle and dosing beaker with the remaining volume of water, and administering the additional liquid as a drink. Blood samples (5.5 ml) for gefitinib assay were taken pre-dose and at 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 24, 36, 48, 72, 96, 120, 144, 168, 192, 216 and 240 h post-dose into tubes containing lithium heparin anticoagulant. Samples were centrifuged within 30 minutes of collection and plasma aliquots were stored at −20°C until analysis.

Subjects fasted for 12 h before receiving a dose of gefitinib. The consumption of grapefruit, grapefruit juice, liquorice, poppy seeds and Seville oranges, and strenuous physical exercise, were not allowed from 72 h prior to dosing until 168 h after each dose. Alcohol consumption was restricted from 24 h prior to dosing until 168 h after each dose. Concomitant mediations, other than paracetamol, were not allowed from 72 h before the first dose of trial treatment, until the post-trial medical examination.

Analytical methods

Plasma samples (0.5 ml) were analyzed for gefitinib after the addition of deuterated gefitinib internal standard and base, by liquid/liquid (methyl-*t*-butyl ether) extraction and reversed phase high performance liquid chromatography (Inertsil 150 × 4.4 mm ODS 3 [C18] column, 8:2, acetonitrile:26 mM ammonium acetate [v/v] mobile phase), followed by tandem mass spectrometric detection (precursor/product ions monitored for gefitinib = m/z 447/128, precursor/product ions monitored for deuterated gefitinib = m/z 455/136).

Concentrations of gefitinib were calculated with reference to a calibration series covering the concentration range

0.5–500 ng/ml, constructed by adding known amounts of gefitinib to control human plasma and processing these standards in parallel with the trial samples. Each 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 assay was validated according to the FDA's Guidance for Industry Bioanalytical Method Validation. Validation quality control (QC) sample precision (% relative standard deviation, SD) ranged from 6.3 to 10.3% with a mean of 8.2%, while accuracy (mean assay value/nominal value × 100) ranged from 90.6 to 98.3% with a mean of 95.4%. The limit of quantification for the assay was 0.5 ng/ml.

During the clinical study sample analysis, performance of the assay was monitored using QC samples alongside trial samples. Two QC samples at each of three different nominal concentrations were included in each analytical run. However, in analytical batches where real samples required dilution, additional QCs were included to reflect the dilution step employed. For an analytical batch to be accepted, two-thirds of the QC replicates had to lie within ±15% of their nominally spiked value, with at least one acceptable QC result obtained for each concentration. During the assay of study samples, QC precision (% relative SD) ranged from 6.2 to 8.1% with a mean of 7.3%, while accuracy (mean assay value/nominal value × 100) ranged from 94.5 to 98.1% with a mean of 96.2%.

Pharmacokinetic analysis

The primary pharmacokinetic variables for the single-dose pharmacokinetic profile of gefitinib following the granular formulation compared with the tablet were: area under the plasma concentration versus time curve (AUC) to infinity; and peak plasma concentration (C_{max}) of plasma gefitinib. Additional variables for the pharmacokinetic profile of gefitinib following the granular formulation compared with the tablet were: AUC from zero to the time of the last quantifiable concentration ($AUC_{[0-t]}$); time to C_{max} (t_{max}); terminal half-life ($t_{½}$); and the terminal rate constant (λz) of plasma gefitinib. Variables for the intra-subject variability of the granular formulation were: AUC; and C_{max} of plasma gefitinib.

The plasma concentration versus time data were analyzed using non-compartmental methods using WinNonlin Professional, Version 4 (Pharsight Corporation, NC, USA). The methodology determined C_{max} and t_{max} for each subject directly from their plasma concentration–time profiles. λz was calculated by log-linear regression of the terminal portion of the concentration versus time profiles where there were sufficient data, i.e. when the terminal phase was followed for at least three half-lives, and $t_{½}$ was calculated from the equation $0.693/\lambda z$. $AUC_{(0\text{-}t)}$ was calculated by the linear trapezoidal rule where t was the time of the last quantifiable plasma concentration of gefitinib. The AUC to infinity was determined from the $AUC_{(0\text{-}t)}$ plus the AUC extrapolated from the last quantifiable time point 't' to infinity by $Cp/\lambda z$, where Cp was the last quantifiable plasma concentration of gefitinib.

Statistical analysis

Previous experience has shown that gefitinib AUC and C_{max} conform to a log-normal distribution; therefore, these parameters were log-transformed before analysis. The study was sized to provide greater than 90% power to ensure that the width of the 90% confidence interval (CI) for the estimate of the treatment difference (between formulations) on the log scale would be $\leq$0.6. This indicated that 15 evaluable subjects would be required. Also, with 15 evaluable subjects completing the study, there was a >90% chance of showing a difference significant at the 5% level if the real difference was 50% higher (or 33% lower). Eighteen subjects were entered into the study to ensure that 15 evaluable subjects completed the study.

An analysis of variance model (ANOVA) was used to compare the AUC and C_{max} of the tablet and the granules, with factors fitted for the effect of sequence, subject within sequence, period, and treatment. The comparison was presented in terms of the geometric least square means (geometric LSM) for each formulation, the treatment effect (i.e. the ratio of the geometric LSM of gefitinib granular formulation:gefitinib tablet), and the 90% CI. For assessment of the intra-subject variability of the granular formulation, an ANOVA model for AUC and C_{max} was used with factors fitted for the effect of subject and period. The data from the period when the tablet was administered were not included in this analysis; only the data from the periods where the granular formulation was given were included. When interpreting the data, standard bioequivalent limits of 0.8–1.25 were used.

Results

Eighteen healthy male Caucasian subjects were enrolled. Their mean (range) age and weight was 41.2 years (23–56) and 78.8 kg (63–98), respectively. One subject was withdrawn prior to the third treatment period due to protocol non-compliance and consequently only received two doses, both of the granular formulation. For the primary pharmacokinetic analysis of the effect of the formulation on AUC and C_{max}, subjects who received both the tablet and at least one dose of the granular formulation were included in the analysis. For the secondary pharmacokinetic analysis, to obtain estimates for intra-subject variability of AUC and

Table 1 Gefitinib pharmacokinetic parameters for each formulation

Parameter (units)	Summary statistic	Gefitinib dose and formulation		
		250 mg tablet (n = 17)	250 mg granules 1[a] (n = 18)	250 mg granules 2[b] (n = 18)
AUC (ng h/ml)	gmean (CV %)	2,237 (122)	2,487 (122)	2,586 (115)
$AUC_{(0\text{-}t)}$ (ng h/ml)	gmean (CV %)	2,193 (121)	2,430 (122)	2,532 (114)
$t_{½}$ (h)	Mean ± SD	29 ± 16	31 ± 18	32 ± 17
C_{max} (ng/ml)	gmean (CV %)	89.0 (67)	104 (48)	103 (44)
t_{max} (h)	Median (range)	5 (1–12)	5 (1–12)	5 (2–8)

[a] First administration of granules for each subject (study period I or II)

[b] Second administration of granules for each subject (study period II or III)

AUC, area under the plasma concentration versus time curve; $AUC_{(0\text{-}t)}$, AUC from zero to the last quantifiable concentration; C_{max}, peak plasma concentration; *CV*, coefficient of variation; *gmean*, geometric mean plasma concentrations; t_{max}, time to C_{max}; $t_{½}$, terminal half-life

C_{max}, all 18 subjects received 2 doses of the granular formulation, and so all subjects were included in the analysis. A summary of the pharmacokinetic parameters for gefitinib after administration of each of the preparations is given in Table 1, where the first and second administration of the granular formulation is summarized, respectively.

Geometric mean (gmean) plasma concentrations of gefitinib over time were similar when gefitinib was administered as the 250 mg tablet and the 2 single doses of the 250 mg granular formulation (Fig. 1). The ratio of individual AUCs for the two granular formulation doses are given in Fig. 2. The gmean AUCs for both doses of the granular formulation were comparable, at 2,487 and 2,586 ng h/ml, and approximately 15% higher than that of the tablet (2,237 ng h/ml) (Table 1). The coefficient of variation (CV) values for the granular formulation doses and the tablet were similar. The range of individual AUCs observed for the granular formulation and tablet were also similar at 409–9,060 ng h/ml for the granular formulation and 457–9,260 ng h/ml for the tablet. Comparable gmean C_{max} were also achieved for the two doses of the granular formulation at 104 and 103 ng/ml, and approximately 17% higher than the gmean C_{max} for the tablet (89.0 ng/ml). The variability in C_{max} was greater for the tablet than the two granular formulations (CV of 67% compared with 48 and 44%), which was as a consequence of the smaller overall-range for the granular formulation (52.2–215 ng/ml) compared with that for the tablet (26.0–202 ng/ml). No apparent differences were seen in either absorption or elimination rates of the granular formulation of gefitinib 250 mg compared with the tablet as shown by similar values for t_{max} and $t_{½}$, respectively.

The estimate of the treatment ratio for AUC showed a 1.05-fold change in AUC for the granular formulation compared with the tablet (90% CI 0.97–1.13) (Table 2; Fig. 3). This suggests that the effect of the granular formulation on AUC of gefitinib 250 mg was small and was within the bioequivalence limits of 0.8–1.25. The estimated ratio of C_{max} for the granular formulation compared with the tablet was 1.14 (90% CI 1.01–1.28) (Table 2).

The intra-subject variability SD for the granular formulation was 0.143 for AUC, and 0.165 for C_{max} (CVs 14.3

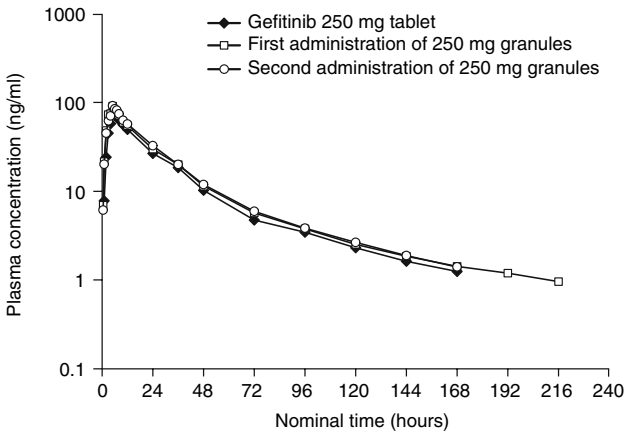

Fig. 1 Geometric mean plasma concentrations of gefitinib after a single dose of the 250 mg tablet and 2 single doses of the 250 mg granular formulation

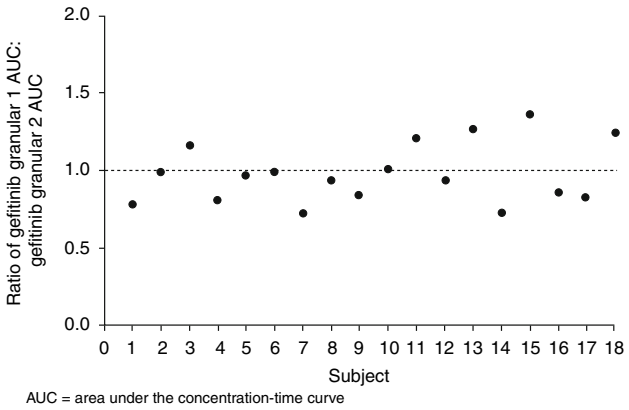

Fig. 2 Individual ratios of gefitinib granule 1 dose AUC/gefitinib granule 2 dose AUC by subject

Table 2 Analysis of area under the plasma concentration versus time curve (AUC) and peak plasma concentration (C_{max}) of gefitinib for granular and tablet formulations

Parameter (units)	Summary statistic	Gefitinib 250 mg granules (n = 17[a])	Gefitinib 250 mg tablet (n = 17[a])	Estimate of treatment effect ratio[b]	90% lower CI	90% upper CI
AUC (ng h/ml)	Geometric LSM	2,323	2,215	1.05	0.97	1.13
C_{max} (ng/ml)	Geometric LSM	102	89.0	1.14	1.01	1.28

Geometric LSM geometric least square means, *CI* confidence interval

[a] Excludes one subject who was withdrawn before receiving tablet formulation

[b] Treatment ratio = ratio of gefitinib 250 mg granules:gefitinib 250 mg tablet

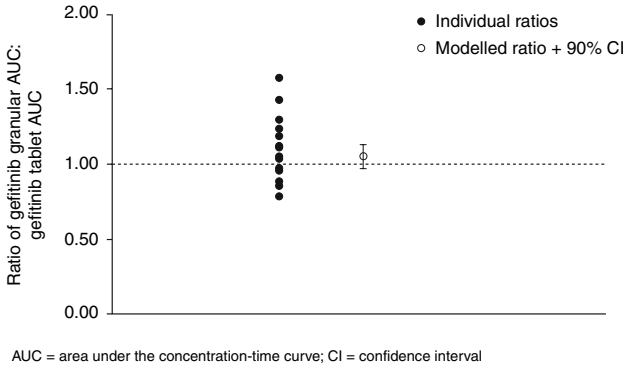

Fig. 3 Individual ratios of granular AUC (mean of gefitinib granule doses 1 and 2)/gefitinib tablet AUC, the modelled ratio and 90% CI

and 16.6%, respectively), equivalent to a 1.4- and 1.7-fold intra-subject variability in AUC and C_{max}.

Discussion

The aims of this study were to determine the relative bioavailability of a single dose of gefitinib when administered as 250 mg of a new granular formulation compared with the standard 250 mg tablet, and to assess the intra-subject variability of the granular formulation, in healthy subjects. Although this study was not designed with sufficient sample size to provide a definitive assessment of bioequivalence, the results did suggest that the two formulations were bioequivalent in terms of the AUC, such that the granular formulation may be used in patients who are unable to or have difficulty in swallowing the tablet. The C_{max} for the granular formulation was slightly higher than that following the tablet, although the difference was too small to be of clinical importance.

For the granular formulation, the ratios for repeated measures of AUC and C_{max} on individual subjects demonstrated a small intra-subject variability of 1.4-fold in AUC and 1.7-fold in C_{max}. This intra-subject variability is slightly better than that previously observed for the tablet in healthy male subjects of two and threefold for AUC and C_{max}, respectively [9], and is similar to that observed for the tablet in patients of 1.1- to 1.9-fold estimated from steady-state trough levels. Therefore, the results of this study suggest that the granular formulation will give similar exposure to gefitinib to the conventional tablet, with good reproducibility in individual patients.

The reproducibility of the results from this study have not yet been tested over a longer duration of treatment in a patient population, which is likely to have concomitant medications and adverse events all acting as confounding factors. Therefore, caution should be applied in extrapolating the results of this healthy volunteer study to the treatment of cancer patients in the absence of data from an appropriately designed clinical trial.

Conclusions

These results showed there was little difference in exposure to gefitinib administered as single doses of the 250 mg granular formulation compared with the 250 mg standard tablet, and the granule gave good reproducibility of exposure. Therefore, the granular formulation of gefitinib has the potential to provide an alternative treatment regimen for patients unable or unwilling to swallow the standard tablet formulation without compromising exposure to gefitinib.

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