Tumor penetration of gefitinib (Iressa), an epidermal growth factor receptor tyrosine kinase inhibitor

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Abstract

The relative distribution of gefitinib-related material in nude mice bearing s.c. human tumor xenografts and in an orthotopic rat lung tumor model was investigated following oral administration (50 mg/kg) of [14C]-gefitinib. Selected tissue samples were monitored for radioactivity by liquid scintillation counting, whereas plasma and tumor extracts were assayed for gefitinib and its major metabolites (M523595 and M537194) by high-performance liquid chromatography with tandem mass spectrometric detection. Tissue distribution was also determined by whole body autoradiography. Gefitinib was extensively distributed into the tissues of tumor-bearing mice and unchanged gefitinib was shown to account for most of the tumor radioactivity. Concentrations of gefitinib in mouse s.c. tumor xenografts were similar to skin concentrations and substantially greater (up to 12-fold based on area under the concentration-time curve) than plasma. Concentrations of gefitinib-related material in an orthotopic rat lung tumor were similar to those in healthy lung tissue and were much higher than corresponding blood levels. Following treatment of breast cancer patients with oral gefitinib (Iressa) 250 mg/d for ≥14 days, gefitinib concentrations (mean, 7.5 μg/g, 16.7 μmol/L) in breast tumor tissue were 42 times higher than plasma, confirming the preferential distribution of gefitinib from blood into tumor tissue in the clinical situation. These gefitinib tumor concentrations are considerably higher than those reportedly required in vitro to achieve complete inhibition of epidermal growth factor receptor autophosphorylation in both epidermal growth factor receptor mutant (0.2 μmol/L) and wild-type cells (2 μmol/L). [Mol Cancer Ther 2005;4(4):641-9]

Introduction

Gefitinib (Iressa) is an orally active inhibitor of the epidermal growth factor receptor (EGFR; erbB1) tyrosine kinase, involved in signal transduction processes and implicated in the proliferation and maintenance of cancer cells (1, 2). Mutation or overexpression of EGFR, as well as erbB2, can lead to cellular transformation and has been linked to poor prognosis in cancer (3–5). These erb receptors have consequently been viewed as an attractive target for novel antitumor therapies and there are now a number of EGFR tyrosine kinase inhibitors in clinical development (1, 6, 7).

Gefitinib, a novel low molecular weight anilinoquinazoline, produces potent inhibition of EGFR/tyrosine kinase activity (IC50, 0.027 μmol/L) and inhibits EGF-stimulated tumor cell growth with an IC50 of 0.054 μmol/L (8). Gefitinib blocks autophosphorylation of EGFR in a number of cell lines and inhibits tumor growth (ED50, ~50 mg/kg) in mice bearing a range of human tumor xenografts (8, 9). Antitumor activity has been observed in a range of phase I and phase II clinical studies (10–13) and gefitinib is approved in a number of countries for the treatment of non–small cell lung cancer. Somatic mutations of the EGFR gene, which seem to confer enhanced sensitivity of some lung tumors to gefitinib, have recently been found (14, 15). These mutant receptors were more sensitive to gefitinib, being completely inhibited at 0.2 μmol/L, whereas wild-type EGFR required 2 μmol/L gefitinib for complete inhibition. Because the therapeutic dose (250 mg) of gefitinib results in mean steady-state trough plasma concentrations of 0.4 μmol/L, it was suggested that higher dose levels of gefitinib may be required to achieve complete inhibition of the wild-type receptor (14).

Gefitinib is cleared primarily by metabolism in rat, dog, and human (16), with morpholine ring oxidation and O-demethylation of the quinazoline methoxy group being the main routes of metabolism. Desmethyl-gefitinib (M523595) is the predominant metabolite observed in human plasma and is present at concentrations similar to gefitinib (17, 18). Gefitinib has a high volume of distribution in rats (8–10 L/kg), dogs (2–6 L/kg), and cancer patients (1,400 L), which is consistent with pronounced distribution of gefitinib into tissues. This was confirmed by a rat tissue distribution study where gefitinib-related material was found extensively distributed, achieving tissue/blood ratios of >10 in a number of organs, including lung, liver, and kidney (16).
The studies described here were designed to supplement this information by determining the relative distribution of gefitinib-related material into the tumors of mice bearing s.c. human tumor xenografts. These results formed the basis of poster presentations at the Second International Symposium on Signal Transduction Modulators in Amsterdam (October 2003) and the joint AACR-National Cancer Institute-European Organization for Research and Treatment of Cancer conference in Boston (November 2003). Further work to examine distribution into an orthotopic rat lung model is also summarized, together with gefitinib human tumor concentrations generated in a breast cancer study (18), originally presented as a poster at the 40th American Society of Clinical Oncology Annual Meeting in New Orleans (June 2004).

Materials and Methods

Chemicals

[14C]-gefitinib, N-(3-chloro-4-fluorophenyl)-7-methoxy-6-(3-morpholino-propoxy)-quinazolin-4-amine, was synthesized with the [14C]-label in the chlorofluoroaniline ring by the Isotope Chemistry Unit, AstraZeneca, Alderley Park. Initial radiochemical purity was in excess of 99% and specific activity was 115 μCi/mg. Unlabeled gefitinib (purity, 99.5%) was used to dilute the radiolabeled material where necessary and for dosing unlabeled compound in selected studies. Known metabolites (16), M523595 (desmethyl-gefitinib), and M537194 (morpholine ring-opened gefitinib), were also synthesized within AstraZeneca, with their structure and purity being assessed by high-performance liquid chromatography with UV detection (HPLC-UV), mass spectrometry, and nuclear magnetic resonance spectroscopy.

Dose Formulation

[14C]-labeled or unlabeled gefitinib was suspended at 5 mg/mL in 0.5% hydroxypropyl methylcellulose in 0.1% aqueous polysorbate 80 for administration by oral gavage.

Animal Studies

Mouse

Tumor Implantation. Female athymic (nu/nu genotype) Swiss mice, supplied by the Rodent Breeding Unit, Alderley Park, were housed in negative pressure isolators with 12-hour light/dark cycles and provided with sterilized food and water ad libitum. The mice weighed ~25 g and were at least 8 weeks of age at dosing. Studies were conducted using mice bearing three different tumor xenografts (LoVo, human colorectal adenocarcinoma; A549, human lung carcinoma; and Calu-6, human lung anaplastic carcinoma). These xenografts were established on the flank of the animal by s.c. injection of 1 × 10^7 cells in 100 μL serum-free medium for LoVo and A549, and 1 × 10^6 cells in 50% matrigel for Calu-6. When tumors reached a volume of 0.1 to 0.65 cm³ (5–24 days after the graft), mice were randomized into groups and treatment started.

Preliminary Study. Female nude mice (bearing LoVo tumor xenografts) received either a single or four daily oral doses of [14C]-gefitinib at a dose level of 50 mg/kg, incorporating 500 μCi/kg. In both dose groups, selected tissues, including tumor and blood samples, were taken at 2 and 8 hours for analysis of total radioactivity. Plasma and tumor extracts were also assayed for gefitinib and its major metabolites (M523595 and M537194) by HPLC with tandem mass spectrometric detection (HPLC-MS/MS). The distribution of radioactivity was also determined in a separate group of female nude mice (bearing LoVo tumor xenografts), which had received four daily oral doses of [14C]-gefitinib at 50 mg/kg (incorporating 500 μCi/kg), by whole body autoradiography of individual mice at various times after the final dose, as described previously (16).

Tumor Concentration Study. Three groups of female nude mice (bearing either LoVo, A549, or Calu-6 tumor xenografts) were dosed orally with gefitinib once daily for 4 days at a dose level of 50 mg/kg. Three mice were killed by inhalation of halothane at 2, 4, 8, 16, and 24 hours after the final dose and blood samples were collected into heparinized tubes. Blood was centrifuged to provide plasma, which was stored at ~20°C until analyzed. At the same time points, the tumor xenograft was excised from each animal, weighed, and placed into a preweighed tissue collection vial. The tumor samples were flash-frozen and stored at ~20°C before processing. Plasma and tumor samples were later assayed for concentrations of gefitinib by HPLC-MS/MS.

Rat

Tumor Implantation and Distribution Study. Female nude rats, weighing ~150 g and supplied by Harlan (Gannat, France), were housed in specific pathogen-free conditions with 12-hour light/dark cycles and provided with sterilized food and water ad libitum. Tumor xenografts were established by s.c. injection of 1 × 10^7 cells (human NCI-H460 cell line derived from a large cell lung carcinoma) in 200 μL serum-free medium into the flank of the animal, as described previously (19). When tumor size was about 1,500 mm³ (after about 2 weeks), the tumors were excised and cut into smaller fragments for orthotopic implantation. Further groups of nude rats were placed on their right flank under halothane anesthesia and a small (0.8 cm) incision made between the third and fourth intercostal ribs. A tumor fragment was grafted onto the left lung of each rat using suture thread and the incision of muscle and skin closed separately using suture thread. The animals were allowed to recover and maintained as before. Starting on day 14 after surgery, each animal received four daily oral doses of [14C]-gefitinib at a dose level of 50 mg/kg, incorporating 150 μCi/kg. The distribution of radioactivity was determined by examination of individual rats at various times after the final dose by whole body autoradiography, as described previously (16).

Sample Analysis

Determination of Radioactivity. Radioactivity in aliquots of plasma and dose formulation was determined by liquid scintillation counting using a Packard 2100TR counter. Tissues (except tumor) were homogenized in water and oxidized using a Packard 307 sample oxidizer before quantitation of radioactivity by scintillation counting.
Extraction of Tumor Samples. Tumors were transferred to microcentrifuge tubes, cut into small pieces using fine-bladed scissors and homogenized in water (1:1, w/v) using a hand-held motorized pellet pestle. Acetonitrile was added to the homogenate (1:1, v/v) and the tubes were vortex mixed and centrifuged. The supernatant was removed and retained, whereas the pellet was resuspended in a second aliquot of acetonitrile, vortex mixed, and centrifuged as before. The supernatant layer was removed and combined with the first portion for analysis of gefitinib and its major metabolites (M523595 and M537194) by HPLC-MS/MS, with measurement of radioactivity by scintillation counting.

Determination of Gefitinib and Metabolite Concentrations by HPLC-MS/MS. Plasma concentrations of gefitinib and its metabolites were determined by HPLC-MS/MS, essentially as described previously (17). Tumor extracts were processed in an identical manner, except that samples were diluted using acetonitrile/water (1:1, v/v). Assay performance was monitored using quality control samples and the limit of quantification for each analyte was 2 ng/mL for gefitinib or 5 ng/mL for M523595 and M537194.

Pharmacokinetic Analysis. The mean and SE of the three plasma or tumor tissue concentrations at each time point was calculated and noncompartmental pharmacokinetics variables determined from the mean concentration data using WinNonLin (version 3.1).

Results
Preliminary Tissue Distribution Study
Distribution of Radioactivity in Selected Tissues. Following administration of [14C]-gefitinib, radioactivity was well distributed into the tissues (Table 1), with the highest levels being found in liver (163 ± 26 µg equivalents/g at 2 hours), kidney (54 ± 4 µg/g), and lung (49 ± 1 µg/g). Peak concentrations of radioactivity in the LoVo tumor xenografts (13.8 ± 0.9 µg/g after a single dose and 16.1 ± 1.8 µg/g after multiple doses) were similar to those in skin (12.4 ± 3.5 and 10.4 ± 1.4 µg/g), the site of the s.c. xenograft implant, and these were considerably higher than plasma levels (5.7 ± 0.6 and 5.1 ± 0.8 µg/mL). Based on radioactivity concentrations, the tumor/plasma ratio was ~3 at 2 hours after dosing and had increased to 7 to 8 by 8 hours, reflecting a more rapid elimination of compound from plasma compared with tumor. There was no marked difference between radioactivity concentrations observed in plasma or tumor after single and multiple doses.

Distribution was also assessed by whole body autoradiography following four daily oral doses of [14C]-gefitinib (50 mg/kg). Radioactivity was well distributed into the tissues of the mouse and the profile of distribution was similar to that observed previously in the rat (16). In the transverse sections presented in Fig. 1, relatively high concentrations of gefitinib-related material were clearly observed in the tumor xenografts. Concentrations in these s.c. tumor xenografts were similar to those in skin but were considerably higher than levels in blood (observed within the heart) and skeletal muscle. High levels of radioactivity were also found in brown fat, bone marrow, and salivary gland in these sections. Other sections (not shown) confirmed the presence of high levels of radioactivity in liver, kidney, and lung, as well as in a number of other tissues.

Table 1. Tissue concentrations of radioactivity following single and multiple oral doses (50 mg/kg) of [14C]-gefitinib to female nude mice bearing subcutaneous LoVo tumor xenografts

<table>
<thead>
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<th>Tissue</th>
<th>Concentration (µg/g) ± SE</th>
<th>Time (h)</th>
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</thead>
<tbody>
<tr>
<td>Plasma</td>
<td>5.7 ± 0.6</td>
<td>2 h</td>
</tr>
<tr>
<td>Tumor</td>
<td>13.8 ± 0.9</td>
<td>1 h</td>
</tr>
<tr>
<td>Skin</td>
<td>12.4 ± 3.5</td>
<td>2 h</td>
</tr>
<tr>
<td>Lung</td>
<td>48.3 ± 9.4</td>
<td>8 h</td>
</tr>
<tr>
<td>Kidney</td>
<td>39.2 ± 2.6</td>
<td>2 h</td>
</tr>
<tr>
<td>Liver</td>
<td>90.0 ± 15.0</td>
<td>2 h</td>
</tr>
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</table>

NOTE: Results show the concentration of radioactivity (µg equivalents/g tissue) as the mean ± SE from groups of three mice, except where noted (*) when only two samples were obtained. The multiple dose groups received gefitinib as four daily oral doses.
Plasma and Tumor Concentrations of Gefitinib and Metabolites. Extraction of radioactivity from the homogenized tumor samples recovered 83% to 97% of the total radioactivity, indicating that the analytic method would provide satisfactory concentration data from tumor samples. HPLC-MS/MS analysis of plasma and tumor samples from the mice provided concentrations of gefitinib and its main metabolites, M537194 and M523595. These results showed that gefitinib accounted for most of the total radioactivity, with the measured metabolites comprising <10% (Fig. 2). Tumor concentrations of gefitinib (11.1 ± 0.6 μg/g after a single dose and 11.2 ± 0.9 μg/g after multiple doses) were also considerably higher than plasma (2.8 ± 0.3 and 2.5 ± 0.5 μg/mL), as observed with the radioactivity levels. The tumor/plasma ratio of gefitinib concentrations was 4 to 5 at 2 hours and 11 at 8 hours after both single and multiple doses. Although plasma and tumor concentrations of M537194 were low, this metabolite seemed to distribute preferentially into tumor tissue in a manner similar to gefitinib. In contrast, M523595 had lower tumor levels relative to its plasma concentrations.

Gefitinib Concentrations in Different Tumor Xenografts Types

Given the approved therapeutic target for gefitinib, further work was conducted to compare the distribution of gefitinib in LoVo with two lung-derived xenografts, A549 and Calu-6. Three groups of female nude mice (bearing either LoVo, A549, or Calu-6 tumor xenografts) were dosed orally with gefitinib once daily for 4 days at a dose level of 50 mg/kg. Comparative profiles of mean plasma and tumor concentrations of gefitinib are presented in Fig. 3. Systemic absorption of gefitinib was rapid, with mean peak plasma concentrations (2.51, 3.11, and 2.82 μg/mL for LoVo, A549, and Calu-6, respectively) in all three groups being observed at the first sample time of 2 hours. A relatively flat profile was observed with all three tumor types, with the mean peak tumor concentration (22.5, 13.3, and 9.7 μg/g, respectively) being achieved 2 to 8 hours after dosing. Gefitinib tumor concentrations were much higher than the corresponding plasma concentration at all time points, although there were marked differences in concentration across the three tumor types. At the end of the plateau (8 hours), tumor concentrations of gefitinib were 14.3, 11.5, and 4.9 times higher than plasma for the LoVo, A549, and Calu-6 xenografts, respectively, whereas overall exposure of tumor tissue, as measured by area under the concentration-time curve, was 12.4, 8.7, and 4.3 times greater than systemic exposure, respectively. The apparent elimination half-life of gefitinib was 3.1 to 3.3 hours in plasma and 4.7 to 5.8 hours in the range of tumor xenografts.

Distribution into Orthotopic Rat Lung Tumors

Female nude rats (bearing orthotopic NCI-H460 lung tumors) were dosed orally with gefitinib once daily for 4 days at a dose level of 50 mg/kg and tissue distribution was assessed by whole body autoradiography at 2, 8, and 24 hours after the final dose. Radiochromatograms of transverse sections through the lung region are presented in Fig. 4. Radioactivity was widely distributed throughout the tissues with the highest concentrations being observed at 8 hours. All tissues, except brain and spinal cord, had radioactivity levels higher than blood. Tumor concentrations were much higher than blood levels throughout the profile and were quite similar to those in healthy lung, although there clearly was an area of tissue at the tumor/lung junction that had much higher levels of radioactivity (Fig. 4).

Distribution into Human Breast Tumor Tissue

A neoadjuvant clinical study (BCIRG 103), conducted primarily to investigate molecular alterations in human breast cancer tissue after short-term gefitinib exposure, has

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**Figure 2.** Plasma and tumor concentrations of gefitinib and its metabolites following administration of a single or four daily oral doses (50 mg/kg) to female nude mice bearing LoVo tumor xenografts. Columns, mean from three animals; bars, ± SE. Concentrations of gefitinib, M523595, and M537194 in each sample were determined using HPLC-MS/MS detection.
provided the opportunity to generate the first gefitinib tumor concentration data in the clinical situation. Preliminary results of this trial were presented as a poster at the 2004 annual meeting of the American Society of Clinical Oncology (18) and are summarized here for comparison with the nonclinical data. Following daily oral administration of gefitinib (Iressa, 250 mg) to breast cancer patients for at least 14 days, the mean steady-state plasma concentration of gefitinib was 0.18 μg/mL (Table 2), whereas gefitinib concentrations in each tumor sample (mean, 7.5 μg/g; 16.7 μmol/L) were substantially higher (mean, 42-fold) than the corresponding plasma sample. However, comparison of gefitinib concentrations in plasma and tumor samples from each patient showed pronounced variability and it was difficult to conclude that there was an obvious correlation between concentrations in tumor and plasma (Fig. 5). Concentrations of M523595 were also monitored in this study and showed a mean plasma concentration of 0.24 μg/mL, quite similar to that of gefitinib (0.18 μg/mL). However, the mean tumor concentration of M523595 (0.11 μg/g) was much lower than that of gefitinib, representing only 40% of the corresponding plasma concentration of M523595.

**Discussion**

This series of studies has shown that gefitinib is extensively distributed into the tissues of tumor-bearing mice and rats. This was not unexpected because extensive tissue distribution of gefitinib was found previously in normal rats (16) and is consistent with the high volume of distribution of the compound observed across the species (17). This extensive tissue distribution of gefitinib is probably related to its physicochemical properties (aqueous solubility, 3.77 μmol/L; log P, 4.85), which allow the compound to distribute preferentially out of plasma and into tissues. There is no indication that gefitinib selectively targets tumor tissue, but it distributes well into tissues in general, particularly the highly perfused tissues such as liver, kidney, and lung; however, levels in brain were relatively low, probably as a result of P-gp–mediated efflux at the blood-brain barrier (16). As an EGFR tyrosine kinase inhibitor showing pronounced tumor growth inhibition in mouse tumor xenografts (8), gefitinib was also expected to distribute well into tumor tissue and the current work was designed to examine this aspect in greater detail.

With ~90% of the total radioactivity recovered, the tumor extraction procedure provides reliable gefitinib assay data and is considered suitable for use with nonradiolabeled tissue, such as clinical tumor samples. Initial work to examine concentrations of gefitinib and its main metabolites, M523595 and M537194, in mouse LoVo tumor xenografts showed extensive distribution into the tumors, but showed that most of the radioactivity was related to gefitinib, with only low levels of metabolites (M523595 and M537194) being present. Gefitinib concentrations were about 11 times higher than plasma concentrations at 8 hours after dosing. Although both plasma and tumor concentrations of M537194 were low, this metabolite seemed to accumulate in tumor tissue in a manner similar to gefitinib, whereas tumor levels of M523595 were lower than its plasma concentrations. Similar findings were also made in the recent human study, where breast tumor concentrations of M523595 represented only about 40% of its plasma concentration, whereas gefitinib showed pronounced (42-fold) tumor penetration (18). Previous *in vitro* work has shown that gefitinib and M523595 have a similar potency against EGFR tyrosine kinase activity in an isolated enzyme assay, but that M523595 has lower activity (14-fold less) than gefitinib.

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**Figure 3.** Plasma and tumor concentrations of gefitinib following four daily oral doses (50 mg/kg) to female nude mice bearing either LoVo, A549, or Calu-6 tumor xenografts. Points, mean concentration obtained from three animals; bars, ± SE. The study design and conditions were the same for each xenograft type.
in a cell-based assay. It was assumed that this was due to an inability of M523595, a phenol metabolite, to penetrate the cell, which is supported by the current clinical data and indicates that M523595 is unlikely to contribute significantly to the therapeutic activity of gefitinib, even when plasma concentrations of gefitinib and M523595 are comparable.

This preliminary tissue distribution analysis focused on the colorectal cancer–derived LoVo xenograft, which shows good sensitivity to gefitinib (8, 20). However, further work was conducted to compare the distribution of gefitinib in LoVo with two lung-derived xenografts, A549 and Calu-6. Whereas growth of s.c. A549 tumor xenografts in mouse is also markedly inhibited by gefitinib (8, 9), Calu-6 tumor xenografts are quite resistant to gefitinib treatment. In this study, gefitinib exposure (both $C_{\text{max}}$ and AUC) in all three tumor types was much higher than in plasma, which was quite similar in the three groups of mice. Concentrations of gefitinib, however, varied with tumor type, with LoVo xenografts having the highest exposure and Calu-6 having the lowest exposure. Because A549 xenografts were much larger than both LoVo and Calu-6 xenografts at the end of the study, the total amount of gefitinib in the A549 xenografts was actually quite similar to that in LoVo tumors. It is possible therefore that the overall concentration of gefitinib in A549 xenografts, a rapidly growing tumor, was restricted by some perfusion limitation. Whereas gefitinib concentrations of Calu-6 xenografts were markedly lower than the other tumor types, it is not yet clear whether this is related to the gefitinib resistance shown by the Calu-6 tumor.

Although the mouse s.c. xenograft model has been widely used to assess the antitumor activity of research compounds, it has a number of limitations which have been

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4 Unpublished data.

Figure 4. Whole body autoradiograms of transverse sections through female nude rats following four daily oral doses (50 mg/kg) of $[^{14}\text{C}]$-gefitinib bearing orthotopic NCI-H460 lung tumor xenografts. Sections from animals terminated at 2 and 8 h after the final dose, although animals terminated at other times were also examined. The selected sections enable a comparison of radioactivity concentrations in the tumor xenograft with blood (observed in the heart chambers). White, zero radioactivity; dark blue, lowest detectable level; progresses through light blue, green, yellow, and red with increasing concentration of radioactivity.
addressed to some degree in recent years by the development of an orthotopic mouse model (19, 21, 22). Whereas the preliminary mouse xenograft study showed that gefitinib concentrations in the s.c. xenografts were quite similar to those in skin, it was of interest to examine concentrations in an orthotopic lung model, particularly given the therapeutic target and because previous work had shown that distribution of gefitinib to the lung was also quite pronounced in normal rats (16). Analysis of sections from rats bearing orthotopic lung xenografts showed that levels of radioactivity (presumed to be largely unchanged gefitinib) in the tumor were similar to those in healthy lung; these were also clearly greater than skin and very much greater than blood levels. A region of intense radioactivity was observed at the junction of the tumor and the lung, and histologic examination indicated that this area of tissue resulted from peritoneal spread of the tumor xenografts, although there was no obvious explanation for the presence of high gefitinib concentrations. However, based on the limited amount of preclinical data generated in these studies, tumor concentrations of gefitinib seem more closely related to levels in the host tissue than to plasma concentrations.

With such a high volume of distribution (>1,400 L; ref. 23), it was anticipated that gefitinib would also be extensively distributed in humans. This was confirmed by data from a clinical study (BCIRG 103), in which gefitinib (Iressa, 250 mg) was given orally to breast cancer patients for at least 14 days (18). Gefitinib concentrations in each tumor sample (mean, 7.5 μg/g) were substantially higher (mean, 42-fold) than the corresponding plasma sample (mean, 0.18 μg/mL). Mean concentrations of M523595 (0.24 μg/mL), the major human plasma metabolite, were similar to those of gefitinib, but M523595 failed to distribute to the tumor (0.11 μg/g) in the same manner as gefitinib.

<table>
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<td></td>
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<td>Tumor (μg/g)</td>
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NOTE: Concentrations of gefitinib and M523595 in each sample were determined using the same HPLC method. Concentrations were below the assay limit of quantification, which was 5 ng/mL for M523595. Abbreviation: NQ, not quantifiable.

Figure 5. Comparison of plasma and tumor concentrations of gefitinib following daily oral administration of gefitinib (Iressa 250 mg) to breast cancer patients in study BCIRG 103.
Individual M523595 plasma concentrations showed pronounced variability (82-fold), consistent with the major role of the polymorphic enzyme, CYP2D6, in the formation of this metabolite, whereas the much lower variability in gefitinib concentrations is probably a reflection of the predominance of CYP3A4 in the overall metabolism of gefitinib (24).

Although gefitinib shows pronounced penetration into tumor tissue in mouse, rat, and human, this distribution profile seems compound specific and differs markedly from that of its metabolite, M523595, and the structurally related compound, erlotinib. In preclinical studies, erlotinib, given orally at 92 mg/kg, did not produce marked tumor penetration, achieving only a tumor/plasma ratio of 0.4 in mouse HN5 xenografts at 6 hours after dosing (25), compared with a gefitinib tumor/plasma ratio of 5 to 14 across a range of mouse tumor xenografts. A similar tumor/plasma profile has also been observed in a recent clinical study, where the mean concentration of erlotinib in lung or larynx tumors (2.9 μmol/L) of cancer patients treated daily with erlotinib (150 mg) for 9 days was about 55% of the mean plasma concentration (26). Although based on data from only four subjects, this limited distribution profile was consistent with the much lower volume of distribution (136 L) observed with erlotinib in man (27). These results clearly show that, although erlotinib achieves considerably higher plasma concentrations than gefitinib in cancer patients when given at their respective therapeutic doses (28), mean levels of erlotinib in human tumors (2.9 μmol/L) are substantially lower than gefitinib tumor concentrations (16.7 μmol/L) observed in the breast cancer study. Whereas the concentration of drug required for maximal EGFR inhibition and the level of inhibition required to achieve a clinical effect are still unclear, it is difficult to predict if higher tumor or plasma exposure would translate into greater clinical antitumor activity (28).

Whereas the mean gefitinib tumor concentration observed in the recent study (16.7 μmol/L) is considerably greater than the original in vitro estimates (IC_{50} 0.054 μmol/L) of EGFR inhibition (8), it is also higher than the concentration required for complete inhibition of mutant (0.2 μmol/L) and wild-type EGFR (2.0 μmol/L; ref. 14), indicating attainment of biologically relevant concentrations at the site of action. These findings are consistent with previous data demonstrating that gefitinib produced pronounced inhibition of EGFR in skin during early clinical trials with gefitinib (29). Although not examined in the trial, EGFR in skin is assumed to be wild type, because the EGFR mutations described recently have been shown to be somatic (14). However, given the current therapeutic indication for gefitinib and the lack of a clear relationship between tumor response and plasma pharmacokinetics observed in clinical trials (23), it would be interesting to determine gefitinib concentrations in lung tumors, where possible, and to investigate whether these show any correlation with changes in pharmacodynamic biomarkers or tumor response. Although adequate tumor concentrations of gefitinib are apparently achieved, a pronounced tumor response is generally only observed in about 10% of an unselected population (it may be substantially higher in Oriental subjects). These findings indicate that other signaling pathways, which are not susceptible to EGFR inhibition, may be available to maintain tumor growth in a large proportion of the population.

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References

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Tumor penetration of gefitinib (Iressa), an epidermal growth factor receptor tyrosine kinase inhibitor

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