Pharmacokinetics and Renal Tissue Distribution of Erlotinib in a Rat Model of Autosomal Dominant Polycystic Kidney Disease

Cinthia V. Pastuskovas¹, Hope Goldman², Noel Dybdal³ and Bert L. Lum⁴,#,*

¹Department of Preclinical and Translational Pharmacokinetics; ²Department of Angiogenesis and Tumor Biology; ³Department of Safety Assessment and Pathology; ⁴Department of Clinical Pharmacology, Genentech Inc., South San Francisco, CA, 94080, USA

Abstract: Background: Polycystic kidney disease (PKD) is a common hereditary disorder with an incidence of 1:700 to 1:1000 for the autosomal dominant polycystic kidney disease (ADPKD) and 1:10,000 for the autosomal recessive (ARPKD) forms. The epithelial growth factor (EGFR) axis may play a role in both forms by promoting epithelial cell proliferation and cyst formation.

Objectives: To characterize the plasma and tissue pharmacokinetics (PK), and cellular distribution of the oral EGFR inhibitor, erlotinib, in the Hanover-Sprague Dawley (Han:SPRD) heterozygote rat model of ADPKD to support preclinical development strategies.

Methods: Twenty-one Han:SPRD heterozygous male rats were administered a single erlotinib dose of 15 mg/kg with concentrations erlotinib and its’ major metabolite, OSI-420 determined in plasma and tissues using LC-MS/MS. To assess cellular distribution, microautoradiography was performed following a single oral dose of [14C] erlotinib (15 mg/kg, ~200 µCi/kg).

Results: Following dosing, erlotinib readily appeared in plasma and distributed into the renal tissue and PKD cysts at concentrations that were approximately 1.5-fold higher than the plasma compartment. Cellular distribution studies demonstrated that radioactivity associated with erlotinib was localized into the cyst lumen of the Han:SPRD rat model. The observed plasma concentrations are consistent with those observed in cancer patients.

Conclusion: Oral erlotinib in the Han:SPRD rat model demonstrated measurable concentrations in plasma and preferential distribution into renal tissue and cysts, suggesting a potential role in the treatment of PKD and further supports additional nonclinical studies including PK, target modulation, and PK/PD modeling to define the dose and schedules for clinical development in PKD.

Keywords: Erlotinib, pharmacokinetics, distribution, polycystic kidney disease, EGFR inhibition, Han:SPRD Rat, tissue pharmacokinetics, microradiography.

1. INTRODUCTION

Polycystic kidney disease (PKD) is one of the most common hereditary disorders, and its inheritance pattern results in either autosomal dominant (ADPKD) or autosomal recessive (ARPKD) forms, with rare cases of sporadic disease also described [1-4]. ADPKD has an incidence of 1:700 to 1:1000. ADPKD can cause an irreversible decline in kidney function and accounts for 8–10% of cases of end-stage renal disease. This is a multi-system disease characterized by the progressive bilateral development of renal cysts. Extrarenal cysts can also be found most often in the liver, pancreas, spleen, thyroid, and arachnoid membranes leading to a wide variety of extrarenal manifestations such as hepatocystic disease, intracranial aneurysms, and cardiac defects, among others. ADPKD is caused by mutations in the genes PKD1 or PKD2. 85% of cases are caused by mutations in the PKD1 gene on chromosome 16 while the other 15% are caused by mutations in the PKD2 gene on chromosome 4, which encodes the proteins polycystin-1 (PC1) and polycystin-2 (PC2), respectively [5-7].

Currently, treatment of ADPKD in the United States remains an unmet need, with no specific drugs approved by the Food and Drug Administration. However tolvaptan, an orally active, selective arginine vasopressin V2 receptor antagonist has been approved in Europe and Japan to treat ADPKD [6, 8-11]. The general approach to discovering new drug treatments has been through targeting signaling pathways with abnormal or impaired function. One pathway, the epithelial growth factor (EGFR) axis, has been observed to play a role in promoting epithelial cell proliferation and cyst formation. Although EGF expression is decreased in murine models of polycystic kidney disease [12-15], EGFR expression is increased in cystic renal and hepatobiliary epithelia [14, 16-19]. EGFR tyrosine kinase inhibitors have been successful in BPK mice (a model of –ARPKD) [20] and in the Han:SPRD rat (a model of ADPKD) [21] where
Erlotinib hydrochloride (CP-358774, OSI-774, Tarceva™, Genentech, Inc.) is an orally active EGFR inhibitor first approved in the USA for the treatment of patients with metastatic non-small cell lung cancer (NSCLC) [24-25]. Erlotinib inhibits the intracellular catalytic tyrosine kinase domain of EGFR by competing with adenosine triphosphate, leading to the inhibition of EGFR autophosphorylation and downstream signaling.

In vitro studies have shown that nanomolar concentrations of erlotinib inhibit EGFR-dependent proliferation of tumor cells and blocks cell-cycle progression in the G1 phase [26-27]. The PK of erlotinib is well characterized and plasma concentrations at clinically relevant doses are associated with inhibition of the EGFR receptor phosphorylation and downstream pathways [28-29]. In oncology patients, erlotinib is well tolerated. Commonly reported side effects include diarrhea and skin rash when administered at the approved dose of 150 mg/day dose [30]. Erlotinib is converted extensively into a number of oxidative metabolites in humans. The major circulating metabolite, OSI-420, is formed through desmethylation of one of the two side chains and is observed in plasma at concentrations that are about 5% of the parent [31].

In earlier studies using normal Sprague-Dawley rats, the kidney concentrations of erlotinib were several fold higher than that in plasma following single dose oral administration of erlotinib [3]. Thus, we sought to further assess erlotinib as a candidate for treating PKD. In our current study, plasma and tissue PK, and cellular distribution of erlotinib and its equally potent metabolite OSI-420 were measured in the Han:SPRD heterozygote male rat.

2. MATERIALS AND METHODS

2.1. Chemicals and Reagents

Erlotinib was obtained as the commercial tablet form (Tarceva™, Genentech, Inc., South San Francisco, CA). OSI-420 was obtained from OSI Pharmaceuticals (Boulder, CO). Captisol was obtained from CyDex (Lenexa, KS). [14C]erlotinib was obtained from Hoffmann-La Roche AG (Basel, Switzerland). Kodak NTB Emulsion (889 5666), Kodak developer (146-4593) and Kodak fixer (190-1875) were obtained from Eastman Kodak (Rochester, NY).

2.2. Species and Husbandry

Han:SPRD rats were obtained from the University of Kansas Medical Center (courtesy of Dr. J.J. Grantham) and bred in-house. Environmental controls were set to maintain a temperature of 19°C to 26°C (66°F to 79°F) with a relative humidity of 30 to 70%, and an approximate 12-hour light/dark cycle. Standard certified rodent diet (Purina rodent laboratory chow, Florham Park) or baby food (Gerber, Woodstock, Ontario) was provided, except during protocol-defined intervals of fasting. Water was provided ad libitum throughout the study. The studies were conducted in heterozygous (Cy/+?) and normal littermate control (+/+) Han:SPRD rats, aged 6-8 weeks with cyst formation in kidney detected by ultrasound. As male Han:SPRD rats develop more severe renal cystic disease than females, this study included only male rats. All in vivo studies were performed following approval of protocol number 05-0341 by the Institutional Animal Care and Use Committee (IACUC) at Genentech, Inc. in accordance with the National Institutes of Health guidelines on animal welfare.

The Han:SPRD rat is a frequently used animal model of inherited PKD with an autosomal-dominant inheritance pattern and has several features that resemble human ADPKD [22-23, 32-35]. Heterozygotes of Han:SPRD rats develop renal cysts and renal failure (in males) over several months, whereas homozygous animals develop rapidly progressive renal enlargement that leads to death in a period of a few weeks. Despite these facts, the genetic basis of PKD in the Han:SPRD rats is likely different from that of humans [36].

2.3. Erlotinib Dosing

For PK studies, 21 Han:SPRD heterozygous male rats were administered a single oral dose of erlotinib at...
15 mg/kg. This dose was selected based on experience in normal Sprague-Dawley rats in which the corresponding plasma concentrations of erlotinib were consistent with those achieved in clinical trials demonstrating efficacy (data not shown) [29]. Erlotinib tablets were ground into a fine powder, dissolved in 10% methylcellulose solution with the dose volume not to exceed 2 ml, and administered through oral gavage. All animals were fasted overnight (16 hours pre-dose) and food was returned 4 hours post-dosing to study the impact of food on the PK of erlotinib and OSI-420, the major active metabolite of erlotinib.

2.4. Erlotinib PK Sampling and Analytical Methods

At 0.5, 1, 2, 4, 8, 24 and 48 hours following the oral dosing three animals per time-point were euthanized via exsanguination under anesthesia. Blood, left kidney, liver, and lung as whole tissues, and shaved skin from the right lateral flank were collected within 1 min post euthanasia. Tissues were homogenized in 20 mM sodium phosphate buffer at pH 7.4 at a ratio of 1 g tissue to 3 ml buffer. Homogenates were centrifuged at 11,000 rpm for 15 min at 4°C. Supernatants were collected with erlotinib concentrations and OSI-420 concentrations, formed from the metabolism of erlotinib, determined using a liquid chromatography mass spectrometry (LC-MS/MS) assay developed and validated by MDS Pharma Services (MDS Inc., Montreal, Canada). Briefly, aliquots of the thawed samples were mixed with an internal standard and water and extracted into t-butyl methyl ether. The organic layer was evaporated to dryness under nitrogen and the residue reconstituted in mobile phase for analysis. Separation of analytes was accomplished by reversed-phase HPLC followed by mass spectrometric single-reaction monitoring, assay with triple quadrupole mass spectrometric detection (LC/MS/MS) for both erlotinib and its major metabolite OSI-420. The retention time for erlotinib was 1.4 minutes and for OSI-420, 1.10 minutes, with a total run time of 3.25 minutes. The lower limit of quantitation (LLOQ) of erlotinib and OSI-420 in plasma and tissue homogenates was 0.5 ng/ml. Pooled plasma and tissue time-concentration data were analyzed using non-compartmental analysis and nominal time (WinNonlin-Pro v3.2, Pharsight Corp, Mountain View, CA). Descriptive statistics were performed using Microsoft Excel (version 2007; Microsoft, Redmond).

2.5. Distribution

Four Han:SPRD heterozygous male rats aged 6-8 weeks and 4 male Sprague-Dawley rats (as control group) with an average body weight of ~400 g received a single oral dose of [14C]erlotinib mixed with radioinert erlotinib to complete a dose of 15 mg/kg dissolved in captisol. The radioactivity dose was equivalent to 200 µCi/kg. The high specific activity of the [14C]erlotinib stock solution (120.6 µCi/mg) was adjusted with radioinert liquid formulation of erlotinib to obtain a final specific activity of 13 µCi/mg. The ratio of unlabeled erlotinib to labeled erlotinib was 0.019. A volume of 800 µL dosing solution was administrated by gavage without a previous fasting period. Animals were euthanized at 2, 4, 8 and 24 hours after-dosing, with n = 1 per sampling time followed by transcordial perfusion with phosphate buffered saline (PBS) to remove blood, kidneys (left and right), liver, and skin from the dorsal thorax were collected, placed in plastic cassettes and stored in 10% formalin until processing for microautoradiography.

2.6. Microautoradiography

Paraffin sections (~4 µm thickness) of collected tissues were dipped in Kodak NTB Emulsion and allowed to develop in the dark at 4°C. At the end of the exposure period (40 days), sections were developed using Kodak developer and then fixed using Kodak fixer. After hematoxylin and eosin counterstaining, dehydration, clearing and mounting of coverslips, tissue sections were evaluated using bright- and dark-field microscopy.

3. RESULTS

3.1. Plasma and Tissue Pharmacokinetics

For PK studies, the Han:SPRD heterozygous male rats were given a single oral dose of erlotinib at 15 mg/kg. This dose level was selected based on previous preclinical studies in Sprague-Dawley rats demonstrating plasma concentrations of erlotinib consistent with those proven to be efficacious in clinical trials. The concentration of erlotinib and OSI-420 measured in plasma, kidney, liver, lung, and skin are shown in Figure 1 and Table 1. The plasma concentration of erlotinib reached its first peak of 2020 +/-390 ng/ml (mean +/- SD) at 1-hour post oral dose followed by a second peak of erlotinib observed at 4 hours (Figure 1A and B), and then decreased over time to below or around LLOQ (0.5 ng/ml). Concentrations of erlotinib in kidney, liver, lung, and skin demonstrated similar concentration-time profiles to that observed in plasma (Figure 1), suggesting that there was no apparent retention of erlotinib in the tissues analyzed.
The second peak coincided with the timing of the reintroduction of food. The effect of food here was consistent with that observed previously in normal Sprague-Dawley rats (data on file), which is consistent with food enhancement of enterohepatic recirculation, and consistent with a food-effect in humans [37].

To compare the tissue to plasma exposure of erlotinib and OSI-420, the area under the concentration-time curve (AUC\(_{0-48}\)) and concentrations of erlotinib and OSI-420 at the 1-hour time point were calculated. The results are presented in Table 1. The ratios between the concentrations of erlotinib and OSI-420 in the different tissues versus plasma are also shown in Tables 1A and 1B, respectively. The exposure of erlotinib in the Han:SPRD kidney tissue was ~1.5 fold higher than plasma, while OSI-420 demonstrated a higher uptake (~2.5 fold) in the cystic kidney than plasma. The concentrations of erlotinib and OSI-420 in the liver were higher than those observed in plasma (20,577 vs. 118,304 ng/ml*hr; 8,448 vs 53,949 ng/ml*hr for plasma vs. liver of erlotinib and OSI-420, respectively). In skin, erlotinib and OSI-420 exposure were consistent with plasma concentrations.

3.2. Cellular Distribution

To compare the cellular distribution of erlotinib in Han:SPRD versus normal Sprague-Dawley rats (control), kidneys (left and right), liver, and skin were collected and evaluated. In the cystic kidney of Han:SPRD rats, the predominant \[^{14}C\]erlotinib-associated signal was observed within the proteinaceous fluid within cyst lumens at 2 and 4 hours post oral dosing of \[^{14}C\]erlotinib (Figure 2). The radioactive signal was significantly weaker in the nephron tissue surrounding the cysts. In kidneys of normal control Sprague-Dawley rats, the radioactive signal was distributed uniformly throughout the renal interstitium at 2 hours post-dose; by 4 hours the radioactive signal, although weak, was predominantly distributed to the renal cortex and to a lesser degree in the tubule epithelium (Figure 3). These findings demonstrated erlotinib specific distribution to the renal cyst, the target tissue.
Table 1A: Summary of Erlotinib Pharmacokinetics in Plasma and Tissues

<table>
<thead>
<tr>
<th>Tissue</th>
<th>AUC_{0-48} hours</th>
<th>C_{1} hour</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ng/ml*hr</td>
<td>tissue:plasma ratio</td>
</tr>
<tr>
<td>Plasma</td>
<td>20,577</td>
<td>--</td>
</tr>
<tr>
<td>Kidney</td>
<td>29,733</td>
<td>1.44</td>
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<tr>
<td>Liver</td>
<td>118,304</td>
<td>5.75</td>
</tr>
<tr>
<td>Lung</td>
<td>29,714</td>
<td>1.44</td>
</tr>
<tr>
<td>Skin</td>
<td>23,759</td>
<td>1.15</td>
</tr>
</tbody>
</table>

Table 1B: Summary of OSI-420 Pharmacokinetics in Plasma and Tissues

<table>
<thead>
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<th>Tissue</th>
<th>AUC_{0-48} hours</th>
<th>C_{1} hour</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ng/ml*hr</td>
<td>tissue:plasma ratio</td>
</tr>
<tr>
<td>Plasma</td>
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<td>--</td>
</tr>
<tr>
<td>Kidney</td>
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<tr>
<td>Liver</td>
<td>53,949</td>
<td>6.39</td>
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<tr>
<td>Lung</td>
<td>13,306</td>
<td>1.58</td>
</tr>
<tr>
<td>Skin</td>
<td>14,378</td>
<td>1.70</td>
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Figure 2: Cellular distribution of [^{14}C]erlotinib in renal cysts of Han:SPRD rats. Representative tissue sections across the renal cortex of Han:SPRD rats at 4 hours post [^{14}C]erlotinib dose. Left panels: hematoxylin stained sections viewed by light field microscopy. Right panels: dark field microscopy of the same section showing the silver grains associated with [^{14}C]erlotinib radioactive signal. Arrows show distribution of radioactivity within the renal cysts.
Figure 3: Cellular distribution of $[^{14}C]$erlotinib in kidneys of control rats. Representative tissue section across the renal cortex of Sprague-Dawley (control) rats at 4 hours post $[^{14}C]$erlotinib dose. Right panels: hematoxylin stained sections observed by light field microscopy. Left panels: dark field microscopy of the same sections showing the silver grains associated with the $[^{14}C]$erlotinib radioactive signal. The renal cortex is shown in the upper panels and the renal corticomedullary junction is shown in the lower panels. The arrow indicates the glomerular structure.

The skin was included as tissue of interest as rash is a dose-limiting toxicity of erlotinib due to EGFR inhibition. Thus, distribution of $[^{14}C]$erlotinib-associated signal in the skin was weak and limited to the outer root sheath of hair follicles and epidermal epithelium while a scattered signal was found in the dermis and subcutis in Han:SPRD and control rats at all time points. At 8 hours and 24 hours, no radioactive signal was detected in the kidneys irrespective of strain and genotype and only a weak distribution was observed in the villous epithelium of the duodenum and the outer root sheath of hair follicles (data not shown). These findings were consistent with the PK tissue:plasma ratio results (Table 1).

4. DISCUSSION

The Han:SPRD rat is a frequently used animal model of inherited PKD with an autosomal-dominant inheritance pattern and has several features that resemble human ADPKD [22-23, 32-35]. Heterozygotes of Han:SPRD rats develop renal cysts and renal failure (in males) over several months, whereas homozygous animals develop rapidly progressive renal enlargement that leads to death in a period of a few weeks. Despite these facts, the genetic basis of PKD in the Han:SPRD rats is likely different from that of humans [36]. Han:SPRD rats display the pathophysiology and EGFR up-regulated apical expression pattern that closely mimics those in humans. The correlation between EGFR inhibition and delayed disease progression has been established within this model [21].

Erlotinib is an EGFR inhibitor first approved in 2004 for the treatment of non-small cell lung cancer demonstrating appropriate potency, bioavailability and PK, suitable for convenient once daily oral dosing [28, 30, 38]. In our study, we measured the plasma and tissue pharmacokinetics and cellular distribution of erlotinib and its equally potent metabolite OSI-420, in the Han:SPRD heterozygote male rat. Following single dose oral administration in this model, erlotinib drug
levels were readily detected in the plasma compartment and distributed into the renal tissue and PKD cysts at concentrations that were consistent with those demonstrating therapeutic activity in cancer patients at the approved dose of 150 mg orally per day [28]. Erlotinib is converted extensively into a number of oxidative metabolites in humans via metabolism in the in the human liver, primarily by the cytochrome P450 (CYP) isofrom 3A4, but also by CYP1A2 and to a minor extent by CYP2C8. The major circulating and active metabolite, OSI-420, is formed through desmethylation of one of the two side chains and is observed in plasma at concentrations that are about 5% of the parent [31]. Furthermore, erlotinib PK exposure in the kidney was approximately 1.5-fold higher than that achieved in the plasma. Consistent with these findings, cellular distribution studies demonstrated that erlotinib was specifically localized into the cyst lumen (Figure 2), supporting erlotinib as a potential candidate in the treatment of ADPKD. Exposure to erlotinib and metabolites was higher in rats in the fed versus fasted state [38-40]. The observations of increased erlotinib concentrations with food intake are similar to those observed in humans [37].

The overall objective of this study was to characterize the plasma and tissue PK and cellular distribution of erlotinib in the Han:SPRD heterozygote male rat model of ADPKD and to better guide future nonclinical translational studies with the ultimate goal of defining the dose selection for clinical trials. Eigenmann and colleagues demonstrated this approach for erlotinib, where nonclinical studies consisting of PK, biomarker target modulation, and PK/PD modeling using a direct inhibitory PD model and a tumor growth inhibition model, allowed accurate predictions of the human dose, dosing schedule and the development of resistance [42-43].

Oral erlotinib in the Han:SPRD rat model demonstrated measurable concentrations in plasma and preferential distribution into renal tissue and cysts, suggesting a potential role in the treatment of PKD. Although the overall PK uptake of erlotinib and OSI-420 to a cystic kidney in Han:SPRD rats appeared to be only mildly preferable when compared to plasma, it was revealed through microautoradiography that erlotinib primarily accumulates in the cyst lumen. It is reasonable to extrapolate that erlotinib and OSI-420 are the primary components in this pool of compounds, as renal metabolism of erlotinib is not expected to be either dominant or of a different pattern than hepatic metabolism. The cystic accumulation of erlotinib and OSI-420 exposes the epithelial cells lining the cyst lumen to a high concentration of these therapeutic compounds that in turn, inhibits epithelial proliferation caused by EGFR-mediated mitogenic stimulation. The enhanced distribution of erlotinib into renal tissue supports additional nonclinical studies including PK, target modulation, and PK/PD modeling to further define the dose and schedules for clinical trials in PKD. In light of reports of serious Interstitial Lung Disease (ILD)-like events, including fatalities, in patients receiving erlotinib for treatment of NSCLC, pancreatic cancer or other advanced solid tumors, further development of erlotinib in PKD was stopped, due to the risk-benefit in a non-oncology indication. [44]

5. CONCLUSION

Our studies demonstrated a nonclinical strategic approach for the study of drugs in PKD. Oral erlotinib in the Han:SPRD rat model demonstrated measurable concentrations in plasma and preferential distribution into renal tissue and cysts, suggesting a potential role in the treatment of PKD. The enhanced distribution of erlotinib into renal tissue which supported additional nonclinical studies including PK, target modulation, and PK/PD modeling to define the dose and schedules for clinical trials in PKD. However, in light of reports of ILD in oncology patients, further development of erlotinib in PKD was discontinued.

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LIST OF ABBREVIATIONS

\[\text{+/+} = \text{normal control}\]
\[^{14}\text{C} = \text{Carbon十四}\]
\[\text{ADPKD} = \text{autosomal dominant polycystic kidney disease}\]
\[\text{ARPKD} = \text{autosomal recessive polycystic kidney disease}\]
\[\text{AUC}_{0-48\text{hr}} = \text{area under the curve 0-48 hr}\]
\[\text{BPK} = \text{BALB/c polycystic kidney}\]
\[\text{Cy/+} = \text{heterozygous (Han:SPRD)}\]
EGFR = epidermal growth factor receptor
G1 = gap 1
Han:SPRD = Hanover Sprague-Dawley
IACUC = Institutional Animal Care and Use Committee
LC-MS/MS = liquid chromatography mass spectrometry
LLOQ = lower limit of quantitation
NSCLC = non-small cell lung cancer
PBS = phosphate buffered saline
PC = polycystin
PK = pharmacokinetics
PKD = polycystic kidney disease

CONFLICT OF INTEREST

Genentech, Inc., provided support for this research. At the time of the study all of the authors were employees of Genentech and were stockholders of Genentech, Incorporated.

REFERENCES


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