Decrease in Plasma Concentrations of Antiangiogenic Agent TSU-68 ((Z)-5-[(1,2-Dihydro-2-oxo-3H-indol-3-ylidene)methyl]-2,4-dimethyl-1H-pyrrole-3-propanoic acid) during Oral Administration Twice a Day to Rats

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ABSTRACT:

TSU-68 ((Z)-5-[(1,2-dihydro-2-oxo-3H-indol-3-ylidene)methyl]-2,4-dimethyl-1H-pyrrole-3-propanoic acid) is a new drug under investigation that inhibits receptor tyrosine kinases involved in tumor angiogenesis. In clinical pharmacokinetic studies, lower plasma concentrations of orally administered TSU-68 are observed after the second dose given within 12 h after the first dose. We examined the cause of this observation through in vivo and ex vivo approaches using rats in which a rapid decrease in exposure was shown as in humans. In rats, the area under the concentration-time curve after the second dose was decreased to 26% of that after the first dose during administration of TSU-68 (200 mg/kg) twice a day. Plasma clearance of TSU-68 intravenously administered 12 h after oral administration was 1.5-fold higher and the half-life was 2-fold shorter compared with those after the single intravenous administration. The amount of absorbed TSU-68, as indicated by the radioactivity totally excreted in the bile and urine following oral administration of [14C]TSU-68, was unchanged by the prior oral administration. These results demonstrate that administered TSU-68 causes an increase in its elimination but not a decrease in its absorption after the subsequent administration. Furthermore, rat liver taken 12 h after administration of TSU-68 exhibited 6-fold higher activity of its microsomal oxidase than untreated liver. This result suggests that TSU-68 induced its own oxidative metabolism (i.e., autoinduction). In conclusion, the decrease in plasma concentrations of TSU-68 during the administration twice a day to rats was due to the rapid autoinduction. The same mechanism is probably at work in the clinical setting.

Vascular endothelial growth factor, platelet-derived growth factor, and fibroblast growth factor receptors are important for the growth and survival of endothelial cells during angiogenesis, which is a necessary step for tumor growth (Ferrara, 1999; Klint and Claesson-Welsh, 1999; Rosenkranz and Kazlauskas, 1999). TSU-68, a novel tumor angiogenesis inhibitor, was shown to inhibit tyrosine kinase activities of these receptors (Laird et al., 2000). Currently, the antiangiogenic activity of TSU-68 toward, and its antitumor effects on, diverse solid tumors are being evaluated in ongoing phase I/II clinical trials.

In clinical pharmacokinetic studies, repeated oral administration of TSU-68 twice daily for 28 days resulted in an approximately 50% decrease in the AUC seen after the first administration (Brahmer et al., 2002; Murakami et al., 2003; Sessa et al., 2006). Clarifying the cause of this phenomenon would lead not only to a better understanding of the pharmacokinetic characteristics but also to a prediction of drug-drug interactions. It is well known that a number of drugs cause an induction of liver enzymes, mainly the cytochromes P450, leading to lowered exposure to other coadministered drugs. If a drug induces the cytochrome P450 enzyme responsible for its own metabolism, which is referred to as autoinduction, the multiple-dose exposure to the drug becomes lower than the corresponding first-dose exposure to it (Nakata et al., 2000; Nicoll-Griffith et al., 2001; Shimizu et al., 2006). This autoinduction thus manifests as the same phenomenon as observed for TSU-68. This hypothesis is supported by a previous finding that the exposure to TSU-68 decreased after repeated intravenous administration for 12 days (Asad et al., 2003). However, the lowered exposure to TSU-68 was already observed after the second dose given within 12 h after the first dose on day 1, and this exposure level was similar to that found on day 28 (Kuenen et al., 2005). For cytochrome P450-inducing drugs, relatively little is known about such rapid induction causing a pharmacokinetic change within a day, because the induction has been generally evaluated after multiple-dose treatment over at least several days (Pelkonen et al., 1998; Thummel and Wilkinson, 1998; Ioannides, 2002; Lin, 2006). Besides the induction of metabolism, saturation and down-regulation of gastrointestinal absorption also have the potential to reduce the exposure. Drugs that induce the intestinal efflux transporter (i.e., P-glycoprotein) or affect

ABBREVIATIONS: TSU-68, (Z)-5-[(1,2-dihydro-2-oxo-3H-indol-3-ylidene)methyl]-2,4-dimethyl-1H-pyrrole-3-propanoic acid; AUC, area under the concentration-time curve; Cmax, maximum plasma concentration; CLp, plasma clearance; Vdss, steady-state distribution volume; t1/2, elimination half-life; HPLC, high-performance liquid chromatography; UGT, UDP-glucuronosyltransferase; UDPGA, uridine 5’-diphosphoglucuronic acid.
gastric emptying rate and gastric pH are known to limit the absorption of other coadministered drugs (Fleisher et al., 1999; Westphal et al., 2000; Lilja et al., 2004).

Owing to species differences in absorption and metabolism of drugs, it is generally difficult to predict clinical pharmacokinetics from the animal data. On the other hand, when clinical pharmacokinetic characteristics are extraordinary and inexplicable, retrospective animal studies as well as human in vitro studies become useful in investigating the characteristics in detail. In the case of TSU-68, rats and dogs also showed lower plasma concentrations after the second dose than after the first dose during administration twice a day. It would be reasonable to consider that in these animal species and humans a common cause for the pharmacokinetic change observed is shared. Therefore, the object of the present study was to reveal the cause through in vivo and ex vivo approaches by using rats, in which the decrease in plasma concentrations of TSU-68 was the most marked. We examined the effect of TSU-68 administered orally 12 h beforehand on its absorption and metabolism after the subsequent administration.

Materials and Methods

**Chemicals.** TSU-68, which is chemically (Z)-5-{[(1,2-dihydro-2-oxo-3H-indol-3-ylidene)methyl]-2,4-dimethyl-1H-pyrole-3-propanoic acid, was synthesized at SUGEN Inc. (South San Francisco, CA).\(^{14C}\)TSU-68 was synthesized at Dai-ichi Pure Chemical Co. Ltd. (Tokyo, Japan) with uniformly \(^{14C}\)-labeled indole moiety as shown in Fig. 1. The radiochemical purity was 96.8% and the specific activity, 1.37 GBq/mmol. All other chemicals were of reagent grade or of the highest purity available commercially.

**Animals.** Rats (male, Sprague-Dawley, 9 weeks old) were purchased from Charles River Japan Inc. (Kanagawa, Japan). Dogs (male, beagle, approximately 10 kg) and mice (male, BALB/c, 9 weeks old) were purchased from Covance Research Products Inc. (Kalamazoo, MI) and CLEA Japan Inc. (Tokyo, Japan), respectively. All animals were housed under a 12-h light/dark cycle with free access to food and water.

**Dose Preparations.** The oral dose of 40 mg/ml TSU-68 was prepared in 5 mg/ml aqueous carboxymethylcellulose containing 0.9% (w/v) benzyl alcohol, 0.4% (w/v) Tween 80, and 9 mg/ml sodium chloride. The intravenous dose of 8 mg/ml TSU-68 was prepared in 33 mM phosphate buffer containing 30% (w/v) polyethylene glycol 300 and 1% (w/v) benzyl alcohol (pH adjusted to 8.5 with HCl).

**Dosing of Animals and Blood Collection.** For the oral pharmacokinetics, mice, rats, and dogs were dosed twice a day (12 h apart) by gavage with 200 mg/kg TSU-68. This dose level was selected so that its exposure could be roughly similar to that for the clinical dose level (400 mg/m²). For the intravenous pharmacokinetics, rats were dosed with 30 mg/kg via the jugular vein after oral administration of 200 mg/kg TSU-68 given 12 h beforehand. A series of heparinized blood samples was withdrawn from the jugular vein (rat) or cephalic vein (dog) at 0, 1, 2 (dog only), 3, 5, 8, and 12 h after oral dosing, and 0, 0.25, 0.5, 1, 2, 4, 6, and 8 h after intravenous dosing. From anesthetized mice, heparinized blood samples were collected via the postcaval vein at 0.5, 1, 3, 5, 8, and 12 h after oral dosing. To determine rat plasma concentrations of radioactive TSU-68 and its total metabolites, the rats were dosed by gavage with 200 mg/kg TSU-68 and then 200 mg/kg \(^{14C}\)TSU-68 (6.9 MBq/kg), 12 h apart. Heparinized blood samples were withdrawn from the jugular vein at 2 and 4 h after dosing. Plasma samples were prepared from the blood by centrifugation (7000g, 5 min) and stored at −80°C until analysis.

**Biliary and Urinary Excretion.** Bile duct-cannulated rats were purchased from Charles River Japan, Inc. (Kanagawa, Japan), where the rats had undergone the surgery. The rats were dosed by gavage with 200 mg/kg TSU-68 and then 200 mg/kg \(^{14C}\)TSU-68 (3.6 MBq/kg), 12 h apart. After dosing, bile and urine samples were collected at the following intervals: 0 to 6, 6 to 12, 12 to 24, and 24 to 48 h, and stored at −80°C until analysis.

**HPLC Analysis of Plasma Samples.** Aliquots (50 μl) of the plasma samples were mixed with 75 μl of methanol, vortex-mixed, and then centrifuged at 10,000g. Aliquots (5 μl) of the supernatant were injected onto a Capcell Pak C\(_18\)UG120 column (5 μm, 15 cm × 2.0 mm; Shiseido Fine Chemicals, Tokyo, Japan) heated to 40°C. These analyses were performed using a Waters Alliance 2690 HPLC system (Waters, Tokyo, Japan). A mobile phase consisting of 20 mM sodium acetate buffer (pH 4) and acetonitrile (50:50 v/v) was delivered at a flow rate of 0.2 ml/min. The column effluent was monitored at a wavelength of 440 nm. This assay exhibited a linear dynamic range of 0.02 to 20 μg/ml.

Aliquots (0.5 ml) of the radioactive plasma samples obtained after administration of \(^{14C}\)TSU-68 were mixed with 1 ml of acetonitrile, vortex-mixed, and then centrifuged at 2000g. The supernatants were evaporated to dryness under nitrogen. The residues were reconstituted in 100 μl of 20 mM sodium acetate buffer (pH 4.0) and methanol (50:50 v/v). Aliquots (40 μl) of the samples were injected for radiometric HPLC analysis. These analyses were performed using a Shimadzu (Kyoto, Japan) LC-10AD HPLC system. Separation was achieved at 40°C on a Capcell Pak C\(_18\)UG120 column (5 μm, 15 cm × 4.6 mm; Shiseido Fine Chemicals). The mobile phase consisting of 20 mM sodium acetate buffer (pH 4.0, solvent A) and acetonitrile (solvent B) was delivered at a flow rate of 1 ml/min, starting at 30% solvent B and increasing to 70% solvent B as a linear gradient for 10 min. The radioactivity in the column effluent was monitored with a flow scintillation analyzer (Radiomatic FLO-ONE beta; PerkinElmer Life and Analytical Sciences, Boston, MA) using Ultima-Flo M cocktail (PerkinElmer Life and Analytical Sciences) at a flow rate of 3 ml/min.

**Analysis of Bile and Urine Samples.** Aliquots (50 μl) of the bile and urine samples were mixed with 10 ml of Hionic-Fluor scintillation cocktail (Packard), and the total radioactivity was measured with a liquid scintillation counter (Tri-Carb 2700TR; PerkinElmer Life and Analytical Sciences). Concentrations of radioactive TSU-68 in the bile and urine samples were analyzed by radiometric HPLC. Aliquots (100 μl) of these samples were mixed with 100 μl of methanol, vortex-mixed, and then centrifuged at 10,000g for 7 min. Aliquots (50 μl) of the supernatant were injected for the analysis (radiometric HPLC conditions as described for plasma samples).

**Liver Microsome Preparations.** Twelve hours after oral administration of 2000 mg/kg TSU-68, rats were sacrificed by decapitation. Livers were quickly removed, perfused with ice-cold saline, and subsequently homogenized in 1.15% KCl. The homogenates were centrifuged at 9000g for 20 min. The supernatant was then centrifuged at 100,000g for 60 min. Microsomal pellets were resuspended in 0.1 M Tris-HCl buffer (pH 7.4) containing 0.5 mM dithiothreitol, 0.1 mM EDTA, and 20% glycerol, and stored at −80°C until analysis. Microsomal protein concentrations were determined by using a BCA protein assay kit (Pierce Chemical Co., Rockford, IL).

**Cytochrome P450 and UGT Activities.** For determination of cytochrome P450 activity, after a 5-min preincubation of a mixture consisting of liver microsomes and TSU-68 at 37°C, the reaction was initiated by the addition of an NADPH-regenerating system. The final conditions were 0.5 mg of protein/ml of liver microsomes, 2 μM TSU-68, 1.3 mM NADP, 3.3 mM glucose 6-phosphate, 0.8 units/ml glucose-6-phosphate dehydrogenase, and 3.3 mM MgCl\(_2\). For UDP-glucuronosyltransferase (UGT) activity, a mixture consisting of 0.5 mg of protein/ml of liver microsomes (activated by 26 μg/ml alamethicin), 2 μM TSU-68, 8 mM MgCl\(_2\), and 5 mM saccharic 1,4-lactone (final concentrations) was preincubated for 5 min at 37°C, and the reaction was initiated by the addition of 2 mM UDPGA (final concentration). The reaction was then terminated by the addition of an equal volume of methanol after 0, 5, 10, 20, and 30 min. After centrifugation at 7000g for 5 min, the supernatant was applied to HPLC analysis, which was performed as described for the
the flow rate was 1 ml/min. The elimination half-life ($t_{1/2}$) was used, the acetonitrile ratio in a mobile phase was 40% v/v, and using linear trapezoidal approximation. The concentrations of the residual unchanged drug after the microsomal incubation were determined by liquid chromatography.

The area under the curve (AUC) was calculated from the data taken from 0 to 12 h, by using WinNonlin software (Pharsight Corporation, Mountain View, CA). The area under the plasma concentration-time curve (AUC) was calculated from the plasma concentration data.

**Pharmacokinetic Analysis.** Pharmacokinetic parameters were calculated from the plasma concentration data by using WinNonlin software. The elimination rate constant ($k_{el}$) determined by linear regression of the log concentration was observed. As shown in Table 1, the mean $C_{max}$ and AUC$_{0-12 h}$ decreased by 73% and 74%, respectively, without a change in the half-life ($t_{1/2}$) during the oral administration twice a day.

For other species, the 32% and 30% decreases in the AUC$_{0-12 h}$ and $C_{max}$, respectively, were observed in dogs, whereas no apparent changes in these parameters were observed in mice (Table 1).

**Results**

**Plasma Concentrations during Oral Administration Twice a Day.** Plasma concentrations of TSU-68 were determined during oral administration of the drug (200 mg/kg/dose) twice a day, 12 h apart. The plasma samples after the first dose and the second dose were obtained from distinct rat groups. At all time points, the plasma concentrations after the second dose were lower than those after the first dose (Fig. 2). No change in the time to reach the peak plasma concentration was observed. As shown in Table 1, the mean $C_{max}$ and AUC$_{0-12 h}$ decreased by 73% and 74%, respectively, without a change in the half-life ($t_{1/2}$) during the oral administration twice a day. For other species, the 32% and 30% decreases in the AUC$_{0-12 h}$ and $C_{max}$, respectively, were observed in dogs, whereas no apparent changes in these parameters were observed in mice (Table 1).

**Biliary and Urinary Excretion of Radioactivity.** Radioactivity excreted in rat bile and urine after oral administration of 200 mg/kg [14C]TSU-68 with or without prior oral administration of 200 mg/kg unlabeled TSU-68 12 h beforehand is shown in Table 2. Although both biliary and urinary recoveries of total radioactivity with the prior oral administration were higher for the first 6-h period than that without the prior oral administration, the total radioactivity excreted over 48 h was similar (~55% of the dose) after both dosings. The absorbed TSU-68 was found to be almost totally excreted from the body via the bile and urine over 48 h postdosing, as negligible radioactivity remained in the carcass (~0.2% of the dose). Consequently, the absorbed TSU-68 amount, which can be considered as the total amount of excretion, did not change during administration twice a day. The amount of unchanged drug excreted with and without the prior administration was quite low and comparable, accounting for

![Graph showing mean rat plasma concentrations of TSU-68 during oral administration of the drug (200 mg/kg/dose) twice a day, 12 h apart. Bars represent S.D.; n = 5 at each time.]
2.0% and 3.7% of the dose, respectively (Table 2). Thus the excretion process of the unchanged drug did not contribute significantly to TSU-68 elimination.

**Intravenous Pharmacokinetics.** The effect of the prior oral administration on the intravenous pharmacokinetics, in which the absorption process can be ruled out, was examined. As shown in Fig. 3, plasma concentrations of TSU-68 intravenously administered (50 mg/kg) to rats after oral administration of TSU-68 (200 mg/kg) 12 h beforehand declined more rapidly than those after the single intravenous administration. As to the intravenous pharmacokinetics, the plasma clearance (CL_p) with and without the prior oral administration was 245.9 ± 28.6 and 160.2 ± 27.0 ml/h/kg, respectively; and the half-life (t_1/2), 0.56 ± 0.11 and 1.06 ± 0.07 h, respectively (Table 3). Thus, the prior oral administration resulted in 1.5-fold increased plasma concentration of unchanged drug and its total metabolites after the second dose compared with that after the first dose, and this decrease in the AUC was greater than that after the first dose, and this decrease in the AUC was greater for rats than for dogs. A similar phenomenon was observed in the clinical pharmacokinetics of this drug (Kuenen et al., 2005). These species would be expected to share a common cause for the pharmacokinetic change. Therefore, our goal was to reveal the cause by using a computational approach.

**Plasma Concentrations of the Total Metabolites.** Radioactive plasma concentrations of unchanged drug and its total metabolites were determined at 2 h and 4 h after oral administration of 200 mg/kg [14C]TSU-68 to rats with and without prior oral administration of 200 mg/kg unlabeled TSU-68 12 h beforehand. The preliminary simulation experiment suggests that these sampling times are suitable for evaluating the metabolism. The concentration of total metabolites was calculated by subtracting the radioactive unchanged drug peak area from the total area of all the observed radioactive peaks. As shown in Table 4, the total metabolite concentration was nearly unchanged at 2 h and 4 h by the prior administration, despite the marked decrease in the unchanged drug concentration. Consequently, the metabolite-to-drug plasma concentration ratio with the prior oral administration was much higher than that without the prior administration. This result suggests that the lowered TSU-68 concentrations were due to an increase in its metabolism.

**TABLE 3**

<table>
<thead>
<tr>
<th>Prior Oral Dosing</th>
<th>AUC_0-24</th>
<th>CL_p</th>
<th>t_1/2</th>
<th>V_dss</th>
</tr>
</thead>
<tbody>
<tr>
<td>(-)</td>
<td>318.5 ± 47.8</td>
<td>160.2 ± 27.0</td>
<td>1.06 ± 0.07</td>
<td>0.24 ± 0.04</td>
</tr>
<tr>
<td>(+)</td>
<td>205.6 ± 24.3*</td>
<td>245.9 ± 28.6*</td>
<td>0.56 ± 0.11*</td>
<td>0.20 ± 0.03</td>
</tr>
</tbody>
</table>

* Significantly different from value without prior dosing (P < 0.05).

**TABLE 4**

<table>
<thead>
<tr>
<th>Time</th>
<th>Prior Dosing</th>
<th>Plasma Concentration (μg or μg-Eq/ml)*</th>
<th>Concentration Ratio (Metabolites/Unchanged Drug)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Total Metabolites</td>
<td>Unchanged Drug</td>
</tr>
<tr>
<td>2 h</td>
<td>(-)</td>
<td>3.3 ± 1.6</td>
<td>17.2 ± 5.5</td>
</tr>
<tr>
<td></td>
<td>(+)</td>
<td>6.0 ± 2.3</td>
<td>2.8 ± 1.6*</td>
</tr>
<tr>
<td>4 h</td>
<td>(-)</td>
<td>7.8 ± 1.7</td>
<td>18.5 ± 1.9</td>
</tr>
<tr>
<td></td>
<td>(+)</td>
<td>9.2 ± 2.6</td>
<td>3.8 ± 2.1*</td>
</tr>
</tbody>
</table>

* The total amount of the radioactive metabolites in plasma was expressed as μg-Eq of TSU-68/ml.

* Significantly different from value without prior dosing (P < 0.05).

**Discussion**

During the oral administration with TSU-68 (200 mg/kg/dose) twice a day to rats and dogs, the AUC after the second dose was lower than that after the first dose, and this decrease in the AUC was greater for rats than for dogs. A similar phenomenon was observed in the clinical pharmacokinetics of this drug (Kuenen et al., 2005). These species would be expected to share a common cause for the pharmacokinetic change. Therefore, our goal was to reveal the cause by using.
rats as the most appropriate animal model. We investigated the effect of TSU-68 orally administered 12 h beforehand on its absorption and metabolism after the subsequent administration.

The contribution of TSU-68 elimination processes can be evaluated by the intravenous administration, in which case the absorption process is excluded. The intravenous pharmacokinetics of TSU-68 with the prior oral administration exhibited a higher CL\(_p\) and a shorter t\(_{1/2}\) compared with those for the single intravenous administration. In addition, the metabolite-to-drug plasma concentration ratio after oral administration of TSU-68 was markedly increased by the prior oral administration. These findings suggest that prior administration of TSU-68 caused an increase in its metabolism after the subsequent administration, which can explain the lowered TSU-68 plasma concentrations. The distribution volume of TSU-68 was unchanged by the prior administration, thus suggesting that the lowered concentrations were probably not due to an increase in the tissue distribution.

Rat Liver Microsomes | t\(_{1/2}\) of TSU-68
--- | ---
| + NADPH | + UDPGA
Treated with TSU-68 | 9.0 ± 0.1* | 58.3 ± 16.8
Untreated | 57.7 ± 7.6 | 55.6 ± 13.7

* Significantly different from untreated microsomes (P < 0.05).

There were two quantitative differences in the effects of the prior oral administration on the oral and intravenous pharmacokinetics (Tables 1 and 3). First, the prior oral administration shortened the t\(_{1/2}\) of intravenously administered TSU-68, whereas the t\(_{1/2}\) was unchanged in the case of the oral administration twice a day. The lowered exposure by oral administration observed in humans is also achieved with little change in t\(_{1/2}\) (Xiong et al., 2004; Sessa et al., 2006). A possible explanation for the unchanged t\(_{1/2}\) is that the observed t\(_{1/2}\) after the second dose reflects the absorption rate rather than the elimination rate (i.e., flip-flop kinetics; Toutain and Bousquet-Mélou, 2004). Such kinetics is supported by the results showing that the intravenous t\(_{1/2}\) with the prior oral administration was 3-4-fold shorter than the oral t\(_{1/2}\). Second, the prior oral administration decreased the AUC of intravenously administered TSU-68 by 35%, whereas the AUC was decreased by 74% in the case of the oral administration twice a day. Thus, a discrepancy in the decrease was observed between the oral and intravenous AUC. A similar trend is also observed in the clinical pharmacokinetics (Sessa et al., 2006). The AUC after a low-dose infusion, which allows a concentration-time profile similar to that obtained with the oral dose, was decreased by the prior administration to an extent similar to that in the case of the oral administration twice a day (data not shown). In addition, the oral absorption process was demonstrated not to be affected as described below, and TSU-68 is classified as a low hepatic extraction drug. These findings support the hypothesis that the discrepancy may be attributed to the difference in the plasma concentration-time profile, which is dependent on the administration route, and not to decreased oral bioavailability.

Prior administration of TSU-68 did not change the total radioactivity recovered in bile and urine over 48 h after subsequent administration of [\(^{14}\)C]TSU-68. Because the amount of absorbed TSU-68 can be considered equal to the total amount of excretion, this result suggests that the absorption of TSU-68 was not changed during the administration twice a day and that, therefore, the absorption process of TSU-68 was not involved in the lowered plasma concentration. The total radioactivity recovered over the first 6-h period was only increased by the prior oral administration. Considering that most of the radioactivity was excreted as the metabolites, the accelerated recovery in this early period may have been due to the increased metabolite formation. It would be difficult to evaluate the ability to excrete TSU-68 as the unchanged drug based on its biliary and urinary recovery, which is governed by not only the excretory ability but also the oral bioavailability and the metabolic clearance. However, because of the much lower contribution of unchanged TSU-68 (<4% of dose) to the total excretion (~55% of dose), the plasma concentrations would not be changed so much, even if the excretory ability is regulated to some extent.

TSU-68 is metabolized by cytochrome P450 and UGT enzymes, which effect hydroxylation of the indoline ring and glucuronidation of the carboxyl group, respectively. Rat liver treated with TSU-68 exhibited much higher activity of its microsomal oxidase than untreated liver, although the UGT activity was not changed by the treatment. This ex vivo result indicates that TSU-68 induces its own oxidative metabolism (i.e., autoinduction). We therefore conclude that this autoinduction led to the lowered plasma concentrations. The autoinduction would also underlie the pharmacokinetic change clinically observed during the repeated administration of this drug. In a preliminary study, anti-CYP1A antibody incubated with rat liver microsomes markedly inhibited the microsomal oxidation of TSU-68, and TSU-68 administered to rats increased ethoxyresorufin O-deethylase (CYP1A) activity in the liver microsomes. These results suggest that TSU-68 induces CYP1A involved in its own metabolism in rats. In contrast to rats, the effect of the autoinduction was less pronounced in dogs and not observed in mice, even though the exposure to 200 mg/kg TSU-68 was comparable among these three species. The observed species difference, for which the basis has not yet been investigated, would be expected to result from not only the species-specific induction but also the species difference in cytochrome P450 isozyme involved in the oxidative metabolism.

In the present study, it is important to note the time dependence of the induction. As was shown in the ex vivo results, the oxidase activity was adequately induced as early as 12 h after the administration. Furthermore, the induced activity observed 12 h after the first dose was maximal during the repeated administration (data not shown). These findings are temporally consistent with the observation that the first dose had already adequately affected the AUC after the second dose given 12 h later. Most of the in vivo induction studies have adopted multiple administrations of an inducer over at least several days (Pelkonen et al., 1998; Thummel and Wilkinson, 1998; Ioannides, 2002; Lin, 2006), because it has been generally recognized that such a lag phase for the transcriptional and translational process is necessary to yield the maximal induction. Some reports on the time-dependent induction by typical CYP1A inducers have described a substantial increase in CYP1A mRNA and protein expression within 24 h after dosing (Zhang et al., 1997; Iba et al., 1999). However, it has not been investigated whether the inducers rapidly cause pharmacokinetic changes of other drugs metabolized by CYP1A. By contrast, although a rapid pharmacokinetic change similar to that seen with TSU-68 has been reported in which the clearance of all-trans-retinoic acid increases in a time-dependent manner and reaches its maximal level at 7 h during the intravenous infusion (Saaddeddin et al., 2004), there is no direct evidence that the rapid increase in the clearance is caused by the autoinduction. Therefore, further studies investigating...
the induced CYP1A at mRNA and protein levels will be required to fully explain the rapid in vivo induction by TSU-68.

In summary, the autoinduction of cytochrome P450 involved in oxidation of TSU-68 was shown through the rat in vivo and ex vivo studies to be a potent cause of the decrease in the plasma concentration of TSU-68 during the administration twice a day. Such rapid autoinduction would also be expected to account for the clinical observation that the exposure to TSU-68 was lower after the second dose given within 12 h after the first dose.

References


Brahmer JR, Kelsey S, Scigalla P, Hill G, Bello C, Elza-Brown K, and Donehower R (2002) A dose given within 12 h after the first dose. Observation that the exposure to TSU-68 was lower after the second autoinduction would also be expected to account for the clinical implication of TSU-68 during the administration twice a day. Such rapid studies to be a potent cause of the decrease in the plasma concentration of TSU-68 by chronic oral dosing.


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