EFFECT OF ITS DEMETHYLATED METABOLITE ON THE PHARMACOKINETICS OF UNCHANGED TAK-603, A NEW ANTIRHEUMATIC AGENT, IN RATS

YOSHIHIKO TAGAWA, KIYOSHI MIWA, RYOICHI TSUKUDA, YOSHINOBU YOSHIMURA, SHIGEHARU TANAYAMA, AND YUSUKE TANIGAWARA

Drug Analysis and Pharmacokinetics Research Laboratories, Pharmaceutical Development Division, Takeda Chemical Industries Ltd. (Y.T., K.M., R.T., Y.Y., S.T.) Osaka, Japan; and Department of Hospital Pharmacy, School of Medicine, Keio University (Y.Tan.), Tokyo, Japan

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ABSTRACT:

A factor in the dose-dependent pharmacokinetics of ethyl 4-(3,4-dimethoxyphenyl)-6,7-dimethoxy-2-(1,2,4-triazol-1-yl-methyl)-quinoline-3-carboxylate (TAK-603) acting on the immune system and has been shown to suppress the development of synovial lesions and joint destruction in adjuvant arthritic rats (Baba et al., 1996; Ohta et al., 1996). Thus, TAK-603 is currently in clinical trials as a new antirheumatic agent in Japan and the U.S. (Fig. 1). In metabolite identification, M-I (Fig. 1), a major metabolite in the plasma of animals and humans, has been shown to be pharmacologically active (Baba et al., 1998). In a nonclinical pharmacokinetic study, we investigated the disposition of TAK-603 in rats and dogs, using [14C]TAK-603 (Tagawa et al., 1998a). This study showed that [14C]TAK-603 administered orally to rats and dogs was absorbed well, and that the radioactive compounds were widely distributed in the tissues of rats. In both animals, TAK-603 was metabolized almost completely before being excreted predominantly into the feces via a hepato-biliary route.

Ethyl 4-(3,4-dimethoxyphenyl)-6,7-dimethoxy-2-(1,2,4-triazol-1-yl-methyl)-quinoline-3-carboxylate (TAK-603) acts on the immune system and has been shown to suppress the development of synovial lesions and joint destruction in adjuvant arthritic rats (Baba et al., 1996; Ohta et al., 1996). Thus, TAK-603 is currently in clinical trials as a new antirheumatic agent in Japan and the U.S. (Fig. 1). In metabolite identification, M-I (Fig. 1), a major metabolite in the plasma of animals and humans, has been shown to be pharmacologically active (Baba et al., 1998). In a nonclinical pharmacokinetic study, we investigated the disposition of TAK-603 in rats and dogs, using [14C]TAK-603 (Tagawa et al., 1998a). This study showed that [14C]TAK-603 administered orally to rats and dogs was absorbed well, and that the radioactive compounds were widely distributed in the tissues of rats. In both animals, TAK-603 was metabolized almost completely before being excreted predominantly into the feces via a hepato-biliary route.

During the oral ascending dose study in rats, the increase in area under the plasma concentration-time curve (AUC) of TAK-603 increased disproportionately to the dose (Tagawa et al., 1998b). A similar dose-AUC relationship was found in humans in a phase I study (K. Uebaba and M. Tei, unpublished results). In fact, TAK-603 showed dose-dependent pharmacokinetics in both rats and humans. Clinically, the pharmacological dose of TAK-603 has been proposed to be 100 mg/human. However, the disproportionate relationship of dose to AUC was confirmed from 25 mg/human. The phase I trials also indicated that TAK-603 absorbed by humans was metabolized almost completely before being excreted predominantly in the feces, as in rats. Therefore, the saturation of metabolic capacity was suspected to be a factor for the dose-dependent pharmacokinetics of TAK-603 in both rats and humans. In general, when a drug exhibits dose-dependent pharmacokinetics, it is difficult to design an accurate dosing regimen based on the dose-AUC relationship. Furthermore, the metabolism-related dose-dependent pharmacokinetics has the possibility to facilitate drug-drug interaction in clinical therapy. Therefore, it is necessary to elucidate the factors responsible for the dose-dependent pharmacokinetics of TAK-603 so as to establish a suitable dosing regimen and to predict the drug-drug interaction on the basis of pharmacokinetic theory.

In vitro metabolism studies using human liver microsomes indicated that the metabolism of TAK-603 and M-I was catalyzed by at least two enzymes with high and low affinities and that cytochrome P-450 3A4 played a major role in the high-affinity component of both TAK-603 and M-I (Tagawa et al., 1997). In vitro inhibition studies using human liver microsomes showed that both TAK-603 and M-I

Send reprint requests to: Dr. Yoshihiko Tagawa, Drug Analysis and Pharmacokinetics Research Laboratories, Pharmaceutical Development Division, Takeda Chemical Industries Ltd., 2-17-85, Juso-Honmachi, Yodogawa-ku, Osaka 532-8686, Japan. E-mail: Tagawa_Yoshihiko@takeda.co.jp
had high potency to inhibit competitively nifedipine oxidation (Tagawa et al., 1997), which is representative of cytochrome P-450 3A4-catalyzed reactions (Guengerich et al., 1986).

In our previous paper, to elucidate the factors for the dose-dependent pharmacokinetics of TAK-603 in humans, pharmacokinetic analysis of TAK-603 were carried out after i.v. injection and also for in vitro metabolic studies (Tagawa et al., 1998b). In these studies, the rat was selected as an animal model for humans because the metabolite composition in rat plasma resembled that in humans.

After i.v. injection of $[14\text{C}]$TAK-603 to rats at doses of 1, 5, and 15 mg/kg, disappearance of TAK-603 from the plasma showed dose-dependent first order elimination and did not show a typical capacity-limited elimination (Michaelis-Menten pattern). In vitro studies using rat liver microsomes showed that both TAK-603 and M-I were metabolized by at least two enzymes with high and low affinities and Michaelis-Menten constants ($K_m$) of the high-affinity component for TAK-603 and M-I were close. Furthermore, both TAK-603 and M-I inhibited nifedipine oxidation strongly and competitively and the inhibition constants ($K_i$) for TAK-603 and M-I were close to the respective high-affinity $K_m$ values. These results indicated that the enzyme catalyzing nifedipine oxidation was also concerned with the metabolism of both TAK-603 and M-I with high affinity in rat liver. Therefore, we considered that if TAK-603 was metabolized extensively to M-I in rat liver, there would be metabolic competition between TAK-603 and M-I and concluded that product inhibition by M-I could be a factor in the dose-dependent pharmacokinetics of TAK-603 in rats. These in vitro studies also indicated that the metabolic characteristics of TAK-603 and M-I in rats were similar to those in humans, and thus indicated that rats are a suitable animal model to estimate the dose-dependent pharmacokinetics of TAK-603 in humans.

The objective of this study is to confirm that the dose-dependent pharmacokinetics of TAK-603 are due to the product inhibition by M-I and to formulate a product inhibition model that describes this phenomenon using rats as an animal model.

**Materials and Methods**

**Chemicals.** TAK-603 and ethyl 4-(4-hydroxy-3-methoxyphenyl)-6,7-dimethoxy-2-(1,2,4-triazol-1-ylmethyl)quinoxaline-3-carboxylate (M-I, Fig. 1) were prepared by the Chemical Development Laboratories of the Production Division and the Pharmaceutical Research Laboratories II of the Pharmaceutical Research Division, respectively, in Takeda Chemical Industries, Ltd. (Osaka, Japan). Ethyl 4-(3,4-dimethoxyphenyl)-6,7-dimethoxy-2-(1,2,4-triazol-1-yl)[14C]-methyl)quinoxaline-3-carboxylate ($[14\text{C}]$TAK-603) with specific radioactivity of 4.33 to 4.55 MBq/mg was synthesized by Amersham International plc (Buckinghamshire, UK). The radio purity, verified by thin-layer chromatography (TLC), was more than 99%. All other chemicals and reagents of analytical grade were obtained from commercial sources.

**Animals.** The animals used were male Icl:Wistar rats (weight, 227–268 g; CLEA Japan Inc., Tokyo, Japan). They were fed laboratory chow (CE-2; CLEA Japan Inc.), had free access to water, and were housed for more than a week before use in temperature- and humidity-controlled rooms (23–26°C, 45 to 60% relative humidity) with 12-h light/dark cycles.

**Dosing and Sample Collection.** Pharmacokinetics of TAK-603 in M-I infused rats. $[14\text{C}]$TAK-603 was dissolved in a mixture of dimethyl sulfoxide and polyethylene glycol-400 (1:9, by volume) for i.v. injection at a dose of 1 mg/ml/kg. M-I was also dissolved in a mixture of dimethyl sulfoxide and polyethylene glycol-400 (1:9, by volume) for both bolus (loading) i.v. injection and continuous infusion. The loading doses of 2 and 20 mg/kg and the respective infusion rates of 5.3 and 16.0 mg/h/kg were calculated from the pharmacokinetic parameters of M-I in rats (Y.T., unpublished data) to attain two distinct targeted steady-state concentrations of M-I ($C_{ss}$).

All animals were fitted with cannula (PE50; 0.58 mm i.d.) in both the jugular vein (for drug administration) and the femoral artery (for blood sampling) on the day before the experiment (Harms and Ojeda, 1974; Ervine et al., 1996). Each rat was placed in a Bollman cage and was given a loading dose of M-I, followed by a continuous infusion into the jugular vein using an infusion pump (Harvard Pump 22, Harvard Apparatus, South Natick, MA) to achieve a targeted $C_{ss}$. A bolus of $[14\text{C}]$TAK-603 (1 mg/kg) was given 1 h after the infusion started. Vehicle-infused rats fitted with cannula were used as controls in this study.

Blood samples (300 µl) were obtained before and 5, 10, 15, and 30 min and 1, 2, 3, 4, and 6 h after dosing with $[14\text{C}]$TAK-603. Immediately after blood sampling, the heparinized blood was centrifuged for 10 min at 3000 rpm at 4°C and plasma was obtained. The plasma was divided into three samples; one (50 µl) was used to determine radioactivity, another (100 µl) was analyzed for $[14\text{C}]$TAK-603 by TLC (Tagawa et al., 1998a), and the third (50 µl) was analyzed for M-I by HPLC. The latter two samples were kept frozen at −20°C until analyzed.

**Pharmacokinetics of TAK-603 and M-I in bile-cannulated rats.** $[14\text{C}]$TAK-603, diluted appropriately with unlabeled compound, was dissolved in a mixture of dimethyl sulfoxide and polyethylene glycol-400 (1:1, by volume) for i.v. injection at doses of 1 and 15 mg/ml/kg. All animals were fitted with a cannula (PE50; 0.58 mm i.d.) in the jugular vein and the femoral artery (Harms and Ojeda, 1974). To interrupt the enterohepatic circulation, the common bile duct was also cannulated with 15 cm of PE50 tubing at 6 to 10 mm from the opening of the duodenum. The open end of the cannula was exited to the exterior through the incision of the right lateral abdominal wall and was passed above the animal’s back. The free end of the cannula was brought to the interior through the incision of the left lateral abdominal wall and was inserted into the opening of the duodenum to enable bile flow into the intestine overnight. This operation was performed on the day before the experiment. Just before the administration of $[14\text{C}]$TAK-603, the bile cannula was cut at 8 cm from the incision of the right lateral abdominal wall so as to divert the bile from the rat. Rats with intact cannulas connecting the bile duct to the duodenum were used as controls in this study. Each rat was placed in a Bollman cage and was given a bolus dose of $[14\text{C}]$TAK-603 through the cannula in the jugular vein. Blood samples (300 µl) were taken from the cannula in the femoral artery at 5, 10, 15, and 30 min and 1, 2, 3, 4, 6, 8, and 10 h after dosing with $[14\text{C}]$TAK-603. The plasma obtained by the above method was analyzed for TAK-603 and M-I by TLC (Tagawa et al., 1998).
The plasma obtained was divided into two samples; one (50 μl) for determination of the radioactivity and the other (100 μl) was kept frozen at -20°C until analyzed for composition of the radioactive materials.

In Vitro Plasma Protein Binding of TAK-603 and M-I in Rats. To examine the effect of M-I on the plasma protein binding of TAK-603, M-I was added in vitro at final concentrations of 0, 1, 10, and 20 μg/ml to rat plasma containing 1 and 10 μg/ml of TAK-603. In contrast, to examine the effect of TAK-603 on plasma protein binding of M-I, TAK-603 was added at final concentrations of 0, 1, and 10 μg/ml to rat plasma containing 1, 10, and 20 μg/ml of M-I. The protein binding of TAK-603 and M-I was determined by ultrafiltration. A portion (1.0 ml) of each plasma sample was transferred into an ultrafiltration device (Centrifree micropartition system, Grace Japan, Tokyo, Japan) pre-treated with 20% aqueous 3-[(3-cholamidopropyl dimethylammonio)-1-propanesulfonate solution and water to prevent nonspecific adsorption of TAK-603 and M-I on the ultrafiltration membrane, and then filtered by centrifugation at 3500 rpm for 15 min at room temperature. The filtrates were stored at -20°C and subsequently assayed for determination of the unbound fractions of TAK-603 and M-I. The total and unbound concentrations of TAK-603 and M-I were determined by HPLC.

Analytical Method for Unlabeled TAK-603 and M-I in Rat Plasma. The concentrations of unlabeled TAK-603 and M-I in samples were determined by HPLC with UV detection. Plasma samples (50 μl) were diluted with 50 mM KH2PO4 (0.5 ml) and the TAK-603 and M-I in the mixture were extracted with a mixture of diethyl ether and dichloromethane (3:1, v/v, 5.0 ml). After centrifugation, 10% of propylene glycol solution in methanol (100 μl) was added to the organic phase. After evaporating the organic phase to dryness under a stream of nitrogen gas, the residue was dissolved in the mobile phase (250 μl, described below) and 100 μl of the solution was injected into the HPLC system. The HPLC system consisted of a pump (model 510, Waters Associates, Milford, MA), a UV detector (Model SPD-10A, Shimadzu Co. Ltd., Kyoto, Japan), an integrator (model 820, Waters Associates), and an analytical column (Develosil ODS-HG-5, 150 × 4.6 mm i.d., Nomura Chemical Co. Ltd., Aich, Japan). The mobile phase was a mixture of 0.01 M KH2PO4 and methanol (1:1, v/v, adjusted to pH 7.0 with phosphoric acid). The flow rate was 1.0 ml/min and the absorbance was read at 253 nm. The retention times for TAK-603 and M-I under these conditions were 14 and 21 min, respectively.

Pharmacokinetic Analysis. Pharmacokinetics of TAK-603 in M-I infused rats. A two compartment open model was used for the pharmacokinetic analysis of TAK-603. Pharmacokinetic parameters (Vd, distribution volume of central compartment; λ, elimination rate constant) from each rat were obtained using the curve-fitting program “MULTI” (Yamaoka et al., 1981). The AUC was calculated summing AUC + Cλ/λ where AUC is the area under the curve for all measured plasma concentrations, Cλ is the last measured plasma concentration and λ is the rate constant estimated from the terminal phase of the plasma disappearance curve calculated by linear regression. The concentrations of TAK-603 at time 0 in plasma were estimated by back extrapolation of the fitted curve. The total body clearance (Cl tot) of TAK-603 was calculated by dividing the dose by the AUC.

An inhibition model (eq. 1) based on a general inhibition equation (Shaw and Houston, 1987) in which the rate of elimination was replaced by clearance, was fitted to the data by “MULTI” (Yamaoka et al., 1981).

\[
Cl_{tot} = Cl_1 (1 + CI/K_i) + Cl_2
\]  

(1)

where Cl tot is the total body clearance of TAK-603 in the presence of M-I as an inhibitor and Cl 1 and Cl 2 represent the component clearances that are affected and not affected by M-I, respectively. CI is the Cmin, in this study and K i is the inhibition constant for M-I.

Pharmacokinetics of TAK-603 and M-I in Bile-Cannulated Rats. For the kinetic parameters of both TAK-603 and M-I, λ was obtained by linear regression from the plasma concentration data, and the AUC was calculated as described above. Maximum concentration (Cmax) and time to reach Cmax (Tmax) for M-I were established directly from the plasma concentration data. AUC and Cmax of M-I were expressed as TAK-603 equivalent value.

To express, simultaneously, the plasma concentration-time profiles of TAK-603 and M-I, a kinetic model based on the product inhibition was developed for the bile-cannulated rats (Fig. 2). In this model, the disposition of TAK-603 in rats was assumed to ensure the following process. Intravenously administered TAK-603 is widely distributed in the rat body and is completely metabolized in the liver to only M-I. Pharmacokinetic studies of M-I in rats showed that M-I had almost the same distribution volume (Vd), dose/initial concentration) as TAK-603 (Y.T., unpublished data). Thus, Vd of M-I is assumed to be the same value as that for TAK-603 in this model. Because TAK-603 was almost completely metabolized before being excreted from the rat body (Tagawa et al., 1998a) and the sum of AUC values for TAK-603 and M-I accounted for most components of the total 14C (more than 80%), TAK-603 is assumed to be completely metabolized to only M-I. The results of in vitro studies using rat liver microsomes indicated that both TAK-603 and M-I are metabolized by the same enzyme (Tagawa et al., 1998b). Therefore, in the rat liver, the M-I yielded from TAK-603 is supposed to inhibit competitively the metabolism of unchanged TAK-603. Because of the bile cannulation, the M-I and its further metabolites are excreted into feces via bile without enterohepatic circulation.

According to the model in Fig. 2 with the above disposition process of TAK-603 and M-I, the following differential equations (eqs. 2 and 3) were formulated to represent the plasma concentration-time courses of both TAK-603 and M-I.

\[
dC_p/dt = -V_{max} \cdot C_p / (K_m (1 + C_{M-I} / K_i) + C_p) - \lambda_2 \cdot C_p
\]  

(2)

\[
dC_{M-I}/dt = V_{max} \cdot C_p / (K_m (1 + C_{M-I} / K_i) + C_p) + \lambda_2 \cdot C_p - \lambda_{M-I} \cdot C_{M-I}
\]  

(3)

Initial condition, when \( t = 0 \), \( C_p = 0 \), \( V_{max} = \) Dose/ \( V_d \), and \( C_{M-I} = 0 \), where \( C_p \) and \( C_{M-I} \) are the plasma concentrations of TAK-603 and M-I, respectively. \( V_{max} \) and \( K_m \) are the maximal velocity and Michaelis-Menten constant for the metabolism of TAK-603 to M-I, respectively. \( \lambda_2 \) represents the elimination rate constant of TAK-603 that is unaffected by M-I. \( \lambda_{M-I} \) is the elimination rate constant of M-I.
inhibition constant and the elimination rate constant of M-I, respectively. $V_d$ is the distribution volume of TAK-603 and M-I.

The mean values of the plasma concentration-time courses of both TAK-603 and M-I at doses of 1 and 15 mg/kg were simultaneously fitted to these equations. The optimum parameter values were calculated by a microcomputer with the program "MULTI(RUNGE)" (Yamaoka and Nakagawa, 1983).

Statistics. In the infusion study, comparison of pharmacokinetic parameters between control (vehicle infusion) and treatment groups was performed by one-way ANOVA followed by a post hoc Dunnett’s or Steel test. The unpaired Student’s $t$ test was used to detect the differences of pharmacokinetic parameters between control and bile-cannulated rats. The effect of saturation and competition on the plasma protein binding of TAK-603 and M-I was analyzed by two-way ANOVA (saturation $\times$ competition). In all analyses, a value of $p < 0.05$ was considered to be statistically significant.

Results

Pharmacokinetics of TAK-603 in M-I-Infused Rats. Figure 3 shows the concentration-time courses of TAK-603 given in a 1 mg/kg i.v. dose of $[^{14}\text{C}]$TAK-603 to rats i.v. infused M-I at rates of 5.3 and 16.0 mg/h/kg. The observed $C_{\text{AUC}}$ values and pharmacokinetic parameters of TAK-603 at specific $C_{\text{AUC-M-I}}$ levels are summarized in Table 1. Data in Table 1 are expressed as the mean values ± S.D. of the results from five rats. $C_{\text{AUC}}$ and $\lambda$ for TAK-603 decreased remarkably with increases in $C_{\text{AUC-M-I}}$, whereas $V_d$ was not altered by infused M-I. $C_{\text{AUC-M-I}}$ of TAK-603 was plotted against the observed $C_{\text{AUC-M-I}}$ in Fig. 4. In this figure, $C_{\text{AUC-M-I}}$ of TAK-603 declined hyperbolically with increases in $C_{\text{AUC-M-I}}$. The aforementioned inhibition model in which $Cl_{\text{tot}}$ of TAK-603 was divided into the two components, $Cl_1$ and $Cl_2$, is considered to be suitable for expression of this TAK-603-M-I interaction. Table 2 shows the optimum parameters calculated by a nonlinear regression method based on the above inhibition model. $Cl_1$ was approximately 5 times greater than $Cl_2$. A good correlation was shown between the simulated curve obtained by the optimum parameters and the observed data plot (Fig. 4).

Pharmacokinetics of TAK-603 and M-I in Bile-Cannulated Rats. The time courses of plasma TAK-603 and M-I in control and bile-cannulated rats given 1 and 15 mg/kg i.v. doses of $[^{14}\text{C}]$TAK-603 are plotted in Fig. 5, A and B, respectively. The pharmacokinetic parameters of TAK-603 and M-I in bile-cannulated and control rats are listed in Table 3. Data in Table 3 are expressed as the mean values ± S.D. of the results from four or five rats. With bile-cannulation, the disappearance of M-I from the plasma was accelerated (Fig. 5B). In the bile-cannulated rats, the $\Lambda$ of M-I increased significantly from control rats at both doses. No significant differences were found in $T_{\text{max}}$ or $C_{\text{max}}$ of M-I between control and bile-cannulated rats. Almost the same time course for plasma TAK-603 was found between both groups of rats at dose of 1 mg/kg. At 15 mg/kg, however, TAK-603 disappeared more rapidly in bile-cannulated rats than in control rats, as evidenced by a significant increase in $\lambda$ (Fig. 5A; Table 3).

The mean plasma concentrations of TAK-603 and M-I at each dose in the bile-cannulated rats were simultaneously fitted to eqs. 2 and 3. The calculated curves of both TAK-603 and M-I fitted well to the observed values with constant parameters over the two dosages (Fig. 6). The optimum parameters adopted to simulate the calculated curves are shown in Table 4.

In Vitro Plasma Protein Binding of TAK-603 and M-I in Rats.

The saturation in plasma protein binding for TAK-603 and M-I and the competitive binding between these compounds were examined in vitro to estimate the influence of changes in their plasma protein binding on the $V_d$ of these compounds (Table 5). The binding percentages of TAK-603 were about 70% at the concentrations of 1 and 10 $\mu$g/ml and these percentages were not affected by the addition of M-I to plasma at the concentration range tested. About 80% of M-I was bound to rat plasma protein over the concentration ranges of 1 to 20 $\mu$g/ml, however, the binding percentage of M-I was decreased slightly by the presence of TAK-603 at high concentration.

Discussion

In an ascending dose study in rats (Tagawa et al., 1998b) and also in a phase I study, the AUC of TAK-603 increased disproportionately with the dose. In vitro metabolic and inhibition studies using rat liver microsomes showed that both TAK-603 and M-I were mainly metabolized by the same enzyme that catalyzes nifedipine oxidation and that both TAK-603 and M-I inhibited nifedipine oxidation strongly and competitively with approximately the same $K_i$ values (Tagawa et al., 1998b). Therefore, we concluded that the dose-dependent pharmacokinetics of TAK-603 in rats could be due to metabolic inhibition of unchanged TAK-603 by M-I, representing product inhibition (Perrier et al., 1973; Lin et al., 1984). In this paper, we confirmed that the product inhibition by M-I is a factor in the dose nonproportionality of TAK-603-AUCs and formulated a product inhibition model to simultaneously represent the concentration-time profiles of TAK-603 and M-I in rat plasma at different dosages.

To examine the effect of M-I on $Cl_{\text{tot}}$ of TAK-603, the pharmacokinetics of TAK-603 after a bolus i.v. injection at a dose of 1 mg/kg was studied using rats infused continuously i.v. with M-I at rates of 5.3 and 16.0 mg/h/kg (Fig. 3). In this study, to minimize the influence of the M-I generated from dosing TAK-603 (1 mg/kg) on the concentration achieved by the M-I infusion, $C_{\text{AUC-M-I}}$ was targeted to be conspicuously higher than the $C_{\text{max}}$ of M-I generated from the infused TAK-603. In the M-I infused rats, the $Cl_{\text{tot}}$ of TAK-603 decreased with an increase in $C_{\text{AUC-M-I}}$. Because TAK-603 is almost completely metabolized before being excreted from the rat body (Tagawa et al., 1998a), this decrease in $Cl_{\text{tot}}$ indicates that infused M-I inhibited the metabolism of TAK-603. The relationship between $Cl_{\text{tot}}$ of TAK-603 and $C_{\text{AUC-M-I}}$ showed a hyperbolic curve (Fig. 4). In vitro metabolic studies using rat liver microsomes clarified that TAK-603 was mainly metabolized to M-I and the metabolism of TAK-603 was catalyzed by at least two enzymes with high and low affinities (Tagawa et al., 1998b). Therefore, the two metabolic component model was applied to explain this phenomenon. In this model, $Cl_{\text{tot}}$ of TAK-603 is
divided into two metabolic components in which $CL_1$ represents the component that is sensitive to M-I inhibition and $CL_2$ represents another component that is unaffected by M-I. In vitro studies for plasma protein binding of TAK-603 and M-I showed that the unbound fraction of TAK-603 was not altered by M-I (Table 5). Therefore, in this model, the influence of infused M-I on $V_d$ for TAK-603 via competitive plasma protein binding with M-I was ignored. The simulated curve fit the observed data well, suggesting that this model was appropriate to explain this phenomenon. The optimum parameter values listed in Table 2 showed that $CL_1$ was approximately 5 times greater than $CL_2$. This indicates that $CL_1$ plays a major role in the metabolism of TAK-603 and that $CL_{tot}$ of TAK-603 is easily influenced by M-I. From the results of this infusion study, it is concluded that M-I competitively inhibited the metabolism of unchanged TAK-603, thereby affirming the phenomenon of product inhibition.

In the disposition study of TAK-603 after oral administration to rats, it has been shown that most of the TAK-603 absorbed in rats is excreted into bile as M-I and its conjugate and that a portion of the metabolites excreted into bile undergo enterohepatic circulation (Tagawa et al., 1998a). In our preliminary study, the bile excretion and metabolic composition in the bile after i.v. dosing of $^{14}$C]TAK-603 to

### TABLE 1

<table>
<thead>
<tr>
<th>Loading dose of M-I</th>
<th>Infusion rate of M-I</th>
<th>Vehicle alone</th>
<th>2</th>
<th>20</th>
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<tr>
<td>C$_{ss}$M-I</td>
<td>µg/ml</td>
<td>0</td>
<td>2.94 ± 0.49</td>
<td>2.52 ± 0.14</td>
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<tr>
<td>$V_d$</td>
<td>l/kg</td>
<td>1.43 ± 0.15</td>
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<td>0.50 ± 0.05a</td>
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<tr>
<td>$CL_{tot}$</td>
<td>1/h/kg</td>
<td>3.96 ± 0.62</td>
<td>2.30 ± 0.25a</td>
<td>1.12 ± 10b</td>
</tr>
<tr>
<td>AUC$_{ss}$M-I</td>
<td>µg · h/ml</td>
<td>0.26 ± 0.03</td>
<td>0.44 ± 0.05a</td>
<td>0.90 ± 0.08a</td>
</tr>
</tbody>
</table>

Dose of TAK-603, 1 mg/kg. Mean ± S.D. (N = 5).

* Significantly different from the value for control rats ($P < 0.01$; ANOVA).

** Significantly different from the value for control rats ($P < 0.05$; ANOVA).

![Fig. 4](image)

**Fig. 4.** Relationship between TAK-603 clearance and M-I steady-state plasma concentrations in rats.

Dose of TAK-603, 1 mg/kg. Infusion rates of M-I, 5.3 mg/h/kg (C), 16.0 mg/h/kg (○), and vehicle alone (●). Solid line depicts predict curve based on the inhibition model (eq. 1) and the parameter values given in Table 2.

![Fig. 5](image)

**Fig. 5.** Effect of bile duct cannulation on pharmacokinetics of TAK-603 (A) and M-I (B) in rats after i.v. injection of $^{14}$C]TAK-603.

Doses of TAK-603, 1 and 15 mg/kg. Mean S.D. (1 mg/kg, N = 4; 15 mg/kg, N = 5). Solid and broken lines represent the bile-cannulated rats (●) and control rats (○), respectively.
bile-cannulated rats at doses of 1 and 15 mg/kg were determined. At both dosages, nearly 80% of the radioactivity administered was excreted into the bile over 24 h and much of the radioactivity was accounted for as M-I and its conjugate (nearly 35 and 45% of the dosed radioactivity, respectively). Unchanged TAK-603 was detected as a very minor component in the bile at both dosages (less than 2% of the dosed radioactivity). From these results, the residual time of

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**TABLE 3**

Effect of bile duct cannulation on pharmacokinetics of TAK-603 and M-I in rats after i.v. injection of [14C]TAK-603

<table>
<thead>
<tr>
<th>Compound</th>
<th>Parameters</th>
<th>U</th>
<th>Control (1 mg/kg)</th>
<th>Bile-cannulated (1 mg/kg)</th>
<th>Control (15 mg/kg)</th>
<th>Bile-cannulated (15 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAK-603</td>
<td>λ</td>
<td>1/h</td>
<td>1.22 ± 0.03</td>
<td>1.16 ± 0.14</td>
<td>0.60 ± 0.10</td>
<td>0.74 ± 0.08*</td>
</tr>
<tr>
<td></td>
<td>AUC&lt;sub&gt;∞&lt;/sub&gt;</td>
<td>µg · h/ml</td>
<td>0.32 ± 0.06</td>
<td>0.33 ± 0.04</td>
<td>13.58 ± 3.38</td>
<td>12.70 ± 2.17</td>
</tr>
<tr>
<td>M-I</td>
<td>T&lt;sub&gt;max&lt;/sub&gt;</td>
<td>h</td>
<td>0.75 ± 0.29</td>
<td>0.75 ± 0.29</td>
<td>1.60 ± 0.55</td>
<td>1.40 ± 0.55</td>
</tr>
<tr>
<td></td>
<td>C&lt;sub&gt;max&lt;/sub&gt;</td>
<td>µg/ml</td>
<td>0.266 ± 0.029</td>
<td>0.296 ± 0.025*</td>
<td>2.923 ± 0.32</td>
<td>3.323 ± 0.501</td>
</tr>
<tr>
<td></td>
<td>λ</td>
<td>1/h</td>
<td>0.40 ± 0.01</td>
<td>0.64 ± 0.15*</td>
<td>0.29 ± 0.04</td>
<td>0.47 ± 0.05*</td>
</tr>
<tr>
<td></td>
<td>AUC&lt;sub&gt;∞&lt;/sub&gt;</td>
<td>µg · h/ml</td>
<td>0.76 ± 0.17</td>
<td>0.70 ± 0.13</td>
<td>14.11 ± 3.58</td>
<td>12.75 ± 3.39</td>
</tr>
</tbody>
</table>

Mean value ± S.D. (Dose 15 mg/kg: N = 4; Dose 15 mg/kg: N = 5).
* Significantly different from the value for control (P < .05).
* Significantly different from the value for control (P < .01).

---

**TABLE 4**

Optimum parameters fitted to the product inhibition model of TAK-603 and M-I in bile duct cannulated rats after i.v. injection of [14C]TAK-603

<table>
<thead>
<tr>
<th>Parameters</th>
<th>U</th>
<th>Optimum value</th>
</tr>
</thead>
<tbody>
<tr>
<td>V&lt;sub&gt;max&lt;/sub&gt;</td>
<td>mg/h/l</td>
<td>41.33</td>
</tr>
<tr>
<td>K&lt;sub&gt;s&lt;/sub&gt;</td>
<td>µg/ml</td>
<td>36.70</td>
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<tr>
<td>K&lt;sub&gt;i&lt;/sub&gt;</td>
<td>µg/ml</td>
<td>2.08</td>
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<tr>
<td>A&lt;sub&gt;s&lt;/sub&gt;</td>
<td>l/h</td>
<td>0.05</td>
</tr>
<tr>
<td>A&lt;sub&gt;i&lt;/sub&gt;</td>
<td>l/h</td>
<td>0.75</td>
</tr>
<tr>
<td>V&lt;sub&gt;i&lt;/sub&gt;</td>
<td>l/kg</td>
<td>2.14</td>
</tr>
</tbody>
</table>

Mean plasma concentrations of TAK-603 and M-I at doses of 1 and 15 mg/kg were used for curve fitting. Simulated curves using Eqs. 2 and 3 with above parameters were shown in Fig. 6.

---

**M-I** in the rat body is considered to be lengthened by enterohepatic circulation after i.v. injection of TAK-603. Therefore, to confirm the occurrence of product inhibition by M-I generated from dosed TAK-603, the plasma concentration-time courses of TAK-603 and M-I were analyzed after i.v. injection of [14C]TAK-603 to bile-cannulated and control rats at doses of 1 and 15 mg/kg. The use of bile-cannulated rats had two objectives: 1) to examine the effect of interrupting the enterohepatic circulation of TAK-603, the plasma concentration-time courses of TAK-603 and M-I were fitted to the product-inhibition model (Fig. 5A), and 2) to ascertain if the kinetic model could be simplified by ignoring the influence of enterohepatic circulation M-I.

The disappearance of M-I from the plasma was accelerated by bile cannulation at both dosages (Fig. 5B). It was clearly shown that the enterohepatic circulation of M-I was interrupted by bile cannulation. Furthermore, in the time course of plasma TAK-603 concentration at dose of 15 mg/kg, a faster λ was found in bile-cannulated rats than in control rats (Fig. 5A; Table 3). It is reasonable to conclude that the efficient excretion of M-I from the body in the bile-cannulated rats lead to an increase in the λ of TAK-603. At a dose of 1 mg/kg, however, no differences were found in the time courses of the plasma TAK-603 concentrations between control and bile-cannulated rats (Fig. 5A; Table 3). The plasma concentration of M-I (C<sub>max</sub>; 0.296 µg/ml, Table 3) after i.v. dosing of TAK-603 as 1 mg/kg was about one-tenth of its K<sub>s</sub> value in the M-I infusion study (2.92 µg/ml, Table 2), so that M-I did not seem to significantly inhibit the metabolism of TAK-603.

The mean concentrations of TAK-603 and M-I for each dose in bile-cannulated rats were fitted to the product-inhibition model (Fig. 2, Eqs. 2 and 3). Because the plasma protein binding of TAK-603 and M-I did not saturate and hardly competed with each other in vitro (Table 5), the V<sub>d</sub> values of both compounds in this model were...
assumed to be constant over the two dosages tested in the bile cannulation study. The observed concentrations of TAK-603 and M-I were simultaneously fitted to this model with constant parameters over two dosages (Table 4). A reasonably good agreement between calculated curves and observed plasma concentration-time courses of both TAK-603 and M-I was found (Fig. 6). This model analysis using bile-cannulated rats indicates that product inhibition by M-I is the factor responsible for the dose-dependent pharmacokinetics of TAK-603 in rats.

In conclusion, we clarified that product-inhibition by M-I was responsible for the dose-dependent pharmacokinetics of TAK-603 in rats. The application of this product inhibition model to humans to design an appropriate dosing regimen in clinical therapy is under way in our laboratory.

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References


