DETERMINATION OF THE ENZYME(S) INVOLVED IN THE METABOLISM OF AMIODARONE IN LIVER AND INTESTINE OF RAT: THE CONTRIBUTION OF CYTOCHROME P450 3A ISOFORMS

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ABSTRACT:

In humans, cytochrome P450 3A (CYP3A4) is a major enzyme involved in the metabolism of amiodarone (AM) to its major metabolite, desethylamiodarone (DEA). In rat, a commonly used animal model, metabolism of AM has not been well studied. To determine whether DEA is formed by CYP3A isoforms in the rat, microsomal protein was harvested from liver and intestine of male Sprague-Dawley rats. The metabolism of AM in each tissue was assessed utilizing chemical and immunological inhibitors. Ketoconazole, a presumed inhibitor of CYP3A1/2, significantly inhibited formation of DEA by hepatic and intestinal microsomes. However, based on the DEA formation kinetics in both microsomal preparations, it appeared that more than one cytochrome P450 enzyme was involved in the process. Coincubation of AM with microsomes and anti-CYP3A2 confirmed the role of CYP3A2 in the metabolism of AM in liver. DEA was also formed by rat recombinant CYP1A1 and CYP3A1, and was inhibited by ketoconazole; hence the participation of these enzymes in the intestinal DEA formation is likely. However, anti-CYP2B1/2 or -CYP1A2 antibodies had no effect on DEA formation. In rats given oral or intravenous AM, oral ketoconazole caused significant increases in area under the concentration versus time curve (AUC) of oral and i.v. treated rats and greater than 50% decreases in the total body clearance and V\textsubscript{max} of i.v. treated rats. Although low to undetectable concentrations of DEA were a limitation for determination of AUC of DEA in vivo, it was confirmed that ketoconazole could cause a significant increase in AM concentrations in rat.

Amiodarone (AM) is an iodinated class III antiarrhythmic benzofuran derivative with extensive clinical usage (Trivier et al., 1993; Soyama et al., 2002) in the treatment of life-threatening ventricular and supraventricular arrhythmias (Naccarelli et al., 2000). AM undergoes extensive hepatic biotransformation (Trivier et al., 1993; Soyama et al., 2002). Among the five pathways involved in the metabolism of AM (N-deethylation, hydroxylation, O-dealkylation, deiodination, and glucuronidation), N-dealkylation is most important in humans (Trivier et al., 1993; Soyama et al., 2002). The dealkylated metabolite, desethylamiodarone (DEA) shares some of the pharmacological and toxicological properties of AM. For instance, some of the electrocardiographic changes observed after long-term therapy with AM might be related to DEA (Kharidia and Eddington, 1996; Kodama et al., 1999). In addition, both AM and DEA inhibit the intracellular conversion of thyroxine to triiodothyronine. This inhibition may be related to some cardiotoxic effects of AM such as bradycardia and reduced myocardial oxygen consumption, and may explain the hypothyroid-like condition observed after chronic administration of AM (Hudig et al., 1994; Kodama et al., 1999).

AM possesses a very large pharmacokinetic volume of distribution (V\textsubscript{d}) and extensive tissue distribution, and, in turn, a long terminal half-life (t\textsubscript{1/2}) in rat and human plasma (Pollak et al., 2000; Shayeganpour et al., 2005). The drug is also extensively metabolized and has a low hepatic extraction ratio in human (Pollak et al., 2000). In rat, the pharmacokinetics of AM are comparable to that of humans, although clearance was higher in rats (Shayeganpour et al., 2005). Humans and rats show different plasma levels of DEA after AM administration, with lower levels being reported in rats compared with humans (Wyss et al., 1990; Meng et al., 2001).

In human liver microsomes, an important contribution of CYP3A4 and CYP2C8 isoforms has been demonstrated (Fabre et al., 1993; Trivier et al., 1993). In the human intestine, CYP3A4 is a predominant cytochrome P450 (P450) enzyme and plays a significant role in the first-pass metabolism of 50 to 70% of marketed drugs (Wacher et al., 1998). In addition, P-glycoprotein (P-gp) is also present at high levels in the villus tip enterocytes of the small intestine (Benet et al., 1999). The presence of CYP3A4 and P-gp in the intestine and their interaction can affect the disposition of drugs that are dual substrates for these proteins (Fabre et al., 1993). It is known that AM is a cosubstrate for both P-gp and CYP3A4 (Kato et al., 2001; Kalitsky-Szirtes et al., 2004). Therefore, oral bioavailability of AM would likely be influenced by presystemic activities of these proteins. Furthermore, inhi-
bition of either of these processes would be predicted to yield higher systemic concentrations of AM and, perhaps, lower levels of circulating metabolite(s).

Most current knowledge regarding the metabolism of AM has been obtained from human studies, although some data are available regarding the in vitro metabolism of the drug in rabbit and rat (Young and Mehendale, 1986). Nevertheless, few data are available regarding the drug-metabolizing enzyme(s) involved in AM biotransformation in the rat, which has been used as an animal model for AM pharmacokinetics (Young and Mehendale, 1986; Shayeganpour et al., 2005). The primary objective of this study was to investigate the hepatic and intestinal metabolism of AM to its presumed primary metabolite, DEA, in rat. The influence of ketoconazole (KTZ), a presumed inhibitor of CYP3A1/2 in the rat, was also assessed in vitro and in vivo for its effects on AM metabolism and plasma concentrations.

Materials and Methods

Chemicals. Amiodarone HCl, ethopropazine HCl, ketoconazole, and β-nicotinamide adenine dinucleotide phosphate tetrasodium were purchased from Sigma-Aldrich (St. Louis, MO). Desethylamiodarone was obtained as a gift from Wyeth Research (Monmouth Junction, NJ). Methanol, acetonitrile, and hexane [all high performance liquid chromatography (HPLC) grade], and triethylamine and sulfuric acid (both analytical grade) were purchased from EM Scientific (Gibbstown, NJ). Potassium dihydrogen orthophosphate, dipotassium hydrogen orthophosphate, potassium chloride, magnesium chloride hexahydrate, sucrose, and calcium chloride dihydrate (all analytical grade) were obtained from BDH (Toronto, ON, Canada). Haltone BP was purchased from MTC Pharmaceuticals (Cambridge, ON, Canada). Heparin sodium injection, 1000 U/ml, was obtained from Leo Pharma Inc. (Thorhull, ON, Canada). Amiodarone HCl (150 mg/ml) as a sterile injectable solution was prepared from Sandoz Canada (Boucherville, QC, Canada). Ketoconazole oral tablets (Nizoral) were purchased from the pharmacy of the University of Alberta Hospital (Edmonton, AB, Canada). Anti-rat polyclonal CYP3A2, anti-rat polyclonal CYP2B1/2, and rabbit, and anti-rat polyclonal CYP1A2 raised in mouse were purchased from Daiichi Pure Chemicals Co., Ltd. (Tokyo, Japan). Supersomes expressing either rat CYP1A1 or CYP3A1 with supplementation of cytochrome bs and P450 reductase were purchased from BD Gentest (Woburn, MA).

In Vitro Studies. Preparation of Rat Liver and Intestinal Microsomes. All experimental protocols involving animals were approved by the University of Alberta Health Sciences Animal Policy and Welfare Committee. For in vitro studies, eight male Sprague-Dawley rats (Charles River Canada) were used in in vivo drug interaction studies. Body weight ranged between 250 and 350 g and all of the rats were housed in temperature-controlled rooms with 12 h of light per day. The animals were fed a standard rodent chow (Lab Diet 5001; PMI Nutrition LLC, Richmond, IN). Free access to food and water was permitted prior to experimentation. Rats were allocated into four groups based on the route of administration and the prior treatment: control i.v. AM (n = 6), oral KTZ-i.v. AM (n = 6), control oral AM (n = 5), and oral KTZ-oral AM (n = 5).

The day before the pharmacokinetic experiment, the right jugular veins of all rats were catheterized with Silastic (Dow Corning, Midland, MI) laboratory tubing (0.64 × 1.19 mm) under halothane anesthesia. Each cannula was flushed with 100 U/ml heparin in 0.9% saline. After cannula implantation, the animals were transferred to regular holding cages and allowed free access to water, but food was withheld overnight. The next morning the rats were transferred to metabolism cages for conduct of the pharmacokinetic experiment.

Drug Administration and Sample Collection. AM injectable solution was used in both intravenous and oral dosing studies. The appropriate doses were prepared by dilution of AM solution in sterile normal saline to a final concentration of 12.5 mg/ml. On the morning of the pharmacokinetic study, i.v. treated rats received 25 mg/kg AM solution. These doses were injected over 60 s via the jugular vein cannula, immediately followed by injection over 1 min of approximately 1 ml of sterile normal saline solution. Orally treated rats received 50 mg/kg AM by oral gavage. Rats treated with KTZ received 17.1 mg/kg KTZ suspended in 1% methylcellulose by oral gavage, administered at the time of surgery the day before the experiment, and 0.5 h before and 6 h after AM administration on the day of the pharmacokinetic experiment. For i.v. studies, blood samples (150–300 μl) were collected at approximately 0.083, 0.33, 0.67, 1, 2, 3, 4, 6, 8, 10, 24, and 48 h after the dose administration. In these rats, at the time of first sample withdrawal, the first 0.2-ml volume of blood was discarded. This procedure was shown to have a negligible effect on AUC (area under the plasma concentration versus time curve) of drug in rats (Shayeganpour et al., 2005). The sampling times after oral doses were 0.25, 0.5, 1, 2, 3, 4, 6, 8, 10, 24, and 48 h after the dosing. After sample collection, each blood sample was centrifuged at 2500g for 3 min, and plasma was transferred to a new polypropylene tube and stored at −30°C until analyzed by HPLC (HPLC Assay). An HPLC method was used for analysis of AM and DEA. The assay had a validated lower limit of quantitation of 35 ng/ml for both AM and DEA based on 100 μl of rat plasma (Jun and Brocks, 2001; Shayeganpour and...
was reconstituted by adding 125 μl of microsomal incubation mixture and 1.5 ml of acetonitrile, 30 μl of internal standard (50 μg/ml ethopropazine HCl) was added. The tubes were vortex mixed for 30 s and centrifuged for 2 min at 2500g. The supernatant was transferred to new glass tubes, 7 ml of hexane was added, and the tubes were vortex mixed for 45 s and centrifuged for 3 min. The organic layer was transferred to new tubes and evaporated to dryness in vacuo. The dried residue was reconstituted by adding 125 μl of mobile phase, and aliquots of 50 μl were injected into the HPLC apparatus. For standard curve construction, drug-free microsomal preparations of liver or intestine were used and spiked with appropriate amounts of AM and DEA. The extraction efficiency of the assay was greater than 75%.

Data Analysis. The rate of DEA formation in both liver and intestinal microsomes was obtained by plotting the formed DEA at various substrate concentrations. Both single and multiple enzyme models for metabolism of AM to DEA were fitted to the formation rate versus time data using nonlinear curve-fitting routines using an in-house written program based on Microsoft Excel (Microsoft, Redmond, WA) and the Solver routine. The Michaelis-Menten model for a single enzyme was used (Venkatakrishnan et al., 2003) as follows:

$$\text{Rate of DEA formation} = \frac{V_{\text{max}} \times [\text{AM}]^n}{k_m^n + [\text{AM}]^n}$$  \hspace{1cm} (1)$$

where $V_{\text{max}}$ is the maximal rate of formation (capacity), $k_m$ is the affinity constant, $[\text{AM}]$ is the concentration of AM, and $n$ is the shape factor required to fit sigmoidal shapes. When $n = 1$, the model reduced to the simple Michaelis-Menten equation.

One of the two-enzyme models that was used consisted of a single saturable and a second linear component (Venkatakrishnan et al., 2003), as follows:

$$\text{Rate of DEA formation} = \frac{V_{\text{max}1} \times [\text{AM}]}{k_{m1} + [\text{AM}]} + E_1 \times [\text{AM}]$$  \hspace{1cm} (2)$$

where $k_{m1}$ and $V_{\text{max}1}$ were the kinetic constants for a high-affinity enzyme, and $E_1$ represented the $V_{\text{max}1}/k_{m1}$ ratio for the low-affinity enzyme. The optimal choice of enzyme model was judged by the residual sum of squares and the Akaike Information Criterion.

The simple competitive inhibition model was considered for the effects of KTZ as defined in the equation below, with $k_i$ as the dissociation constant of the inhibitor-enzyme complex and $[I]$ the inhibitor concentration.

$$k_{\text{m(i)}} = k_{\text{m(1)}} \times \left(1 + \frac{[I]}{K_i}\right)$$  \hspace{1cm} (3)$$

The $k_i$ value in liver was determined by Dixon plot analysis (Dixon, 1953; Cortes et al., 2001).

For the pharmacokinetic studies, noncompartmental methods were used to calculate the parameters of maximal plasma concentration ($C_{\text{max}}$) and time thereof ($t_{\text{max}}$), clearance (CL), volume of distribution at steady state ($V_{\text{ss}}$), terminal elimination rate constant, and half-life ($t_{1/2}$), as previously described (Shayeganpour et al., 2005). The log-linear trapezoidal rule was used to calculate the area under the plasma concentration versus time curve.

Statistical Analysis. Compiled data are expressed as mean ± S.D. unless otherwise indicated. One-way analysis of variance, Duncan’s multiple range post hoc test, and Student’s paired or unpaired $t$ tests were used as appropriate to assess the significance of differences between groups. Microsoft Excel or SPSS version 12 (SPSS Inc., Chicago, IL) were used in statistical analysis of data. The level of significance was set at $p < 0.05$.

Results

Metabolism of AM by Liver and Intestinal Microsomes. In both liver and intestine, as AM concentrations increased, there was an increase in the formation rate of DEA. It was found that in liver microsomes, the kinetic profile of DEA formation versus concentration conformed well to the Michaelis-Menten model with one enzyme (Fig. 1A). However, there did appear to be some deviation from the model with the highest mean concentration. The estimated $k_{m1}$, $V_{\text{max}1}$, and intrinsic clearance (CL_{int}) were determined in liver (Table 1). The variability assessed by coefficient of variation was substantially higher for $k_{m1}$ (74%) than $V_{\text{max}1}$ (21%).

In contrast to liver microsomes, over the range of concentrations studied, a linear model best fit the DEA formation versus AM concentration relationship ($r^2 > 0.99$) in intestinal microsomes (Fig. 1B). As a result, determination of $k_{m1}$, $V_{\text{max}1}$, and CL_{int} was not possible in these microsomes.

Identification of the P450 Isoenzymes Responsible for the Metabolism of AM to DEA. To identify the P450 isoenzymes involved
in the metabolism of AM to DEA, chemical inhibition, anti-P450 antibodies, and recombinant rat P450 enzymes were used. To further establish the role of CYP1A1 and CYP3A1 in the metabolism of AM to DEA, AM was incubated with recombinant rat CYP1A1 and CYP3A1 enzymes. As the concentration of KTZ was increased in the chemical inhibition study (Fig. 2A), a progressive decrease was noted in the formation rate of DEA in rat liver microsomes. In comparison to control incubations, all of the incubations in which KTZ was added yielded significantly lower DEA formation rates for each of the three AM concentrations studied (Fig. 2). In general, as KTZ concentrations were increased, there were significant decreases in formation rates of DEA.

In intestinal microsomes (Fig. 2B), increasing concentrations of KTZ did not seem to have the same effects on DEA formation. At the highest concentration of AM exposed to intestinal microsomes, no significant difference was observed in the formation of metabolite with increasing concentrations of inhibitor (Fig. 2B). Similar to liver, at lower concentrations of AM (23.5 and 47 μM), the formation of DEA in intestine was significantly lower than in control incubations when KTZ was present. There was, however, a difference in the pattern of effect of KTZ in intestine as compared with liver, because in intestine, KTZ did not result in a markedly progressive decrease in DEA formation at KTZ concentrations above 1.88 μM (Fig. 2B).

The inhibition constant, \( k_i \), was determined by the use of Dixon plot analysis in the liver microsomes (Fig. 3). The one-enzyme model did not fit well to the inhibition data; thus, a two-enzyme model was used, with involvement of a high-affinity and a low-affinity enzyme (eq. 2). This model provided an optimal fit to the data and was used to estimate the \( k_i \) for the inhibition of DEA by KTZ (Fig. 3). Based on this two-enzyme kinetic model, \( k_i \) was determined to be 2.74 ± 3.50 μM KTZ (range, 0.391–7.94 μM). This analysis was not possible in intestinal microsomes due to the poor quality of the fit of the model to the data.

Anti-CYP1A2, -CYP3A2, and -CYP2B1/2 antibodies were used to better identify the P450 enzymes involved in the metabolism of AM in liver and intestine microsomes. No significant differences were observed in the formation of DEA with CYP1A2 and CYP2B1/2 antibodies in both liver and intestine microsomes (Fig. 4). However, the formation of DEA was significantly decreased in using anti-CYP3A2 in liver, but not intestinal, microsomes.

**TABLE 1**

<table>
<thead>
<tr>
<th>Kinetic Parameter</th>
<th>Mean ± S.D. (Range)</th>
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<tbody>
<tr>
<td>( k_m ) (μM)</td>
<td>43.5 ± 32.1 (24.3–70.2)</td>
</tr>
<tr>
<td>( V_{max} ) (pmol/min/mg protein)</td>
<td>370 ± 78.4 (250–463)</td>
</tr>
<tr>
<td>( CL_{int} ) (μl/min/mg protein)</td>
<td>9.51 ± 3.42 (4.74–12.8)</td>
</tr>
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**FIG. 3.** Determination of \( k_i \) (2.74 ± 3.50 μM) in liver microsomes by Dixon plot. Inhibition data (see Fig. 2) were fitted to a two-enzyme model (combined eqs. 2 and 3), with results of model fits being represented by the solid lines.
When AM was incubated in the presence of rat CYP1A1 and CYP3A1 Supersomes, DEA was observed to be formed in both cases (Fig. 5). In addition, when KTZ (18.8 μM) was added, significant decreases were observed in the formation rate of DEA.

Pharmacokinetics of AM in Rats. KTZ pretreatment gave rise to a significant increase in plasma concentrations of i.v. administered AM in comparison with control rats. In rats given AM orally, significant increases were also detected in systemic disposition of AM upon administration of KTZ (Fig. 6). After i.v. doses of AM, the mean plasma AUC$_{0-48}$ and AUC$_{0-∞}$ were increased 1.67- and 1.60-fold, respectively, in KTZ-treated rats compared with the control animals (Table 2). The CL and V$_{dss}$ of AM in KTZ-treated rats were significantly lower than those of control rats. No significant difference was observed in the t$_{1/2}$ of i.v. AM in the presence and absence of KTZ (Table 2).

Compared with control animals, KTZ caused 3.07- and 2.06-fold increases in AUC$_{0-48}$ and AUC$_{0-∞}$, respectively, in the rats given AM orally. Administration of KTZ led to a substantial increase (137%) in C$_{max}$ of AM after its oral administration (Table 2; Fig. 6). No significant differences were observed in t$_{1/2}$ and t$_{max}$ of AM with or without treatment of KTZ (Table 2). Using AUC$_{0-∞}$, the oral bioavailability of AM (F) was estimated to be 37% and 47% in control and KTZ-treated rats, respectively. Similar estimates of F were obtained when partial AUC was used from 0 to 48 h (28% and 52% in control and KTZ-treated rats, respectively).

The mean C$_{max}$ of DEA was considerably lower than that of AM in each of the groups (Table 2). There were no differences between KTZ-treated and control animals given either i.v. or oral AM (Table 2). Although the amounts of DEA detected in some plasma samples were greater than the lower limit of quantification of the assay (35 ng/ml), many of the samples were not quantifiable for DEA. Hence, a complete plasma concentration versus time profile of DEA concentration was not obtained in most of the rats, and the AUC could not be reported.

**Discussion**

The role of P450 in the biotransformation of AM to DEA and its first-pass elimination in different tissues such as liver, kidney, intestine, and lung has attracted attention (Rafeiro et al., 1990; Kalitsky-Szirtes et al., 2004). In the present study, the role of hepatic and intestinal microsomes in biotransformation of AM to DEA was attempted. The study findings demonstrated a considerable difference between rat liver and intestinal microsomes in their ability to metabolize AM to DEA. Over the range of concentrations studied, liver microsomal protein showed a consistently higher rate of metabolism than intestinal microsomes (Fig. 1). Kinetic constants could be reasonably derived for the metabolism of AM in liver using the single-enzyme Michaelis-Menten model, but this was not the case for intestinal microsomes. However, it was of note that in liver microsomes, there appeared to be some deviation from the model with the highest mean concentration, and in three of the four livers, Eadie-Hofstee plots (Venkatarkrishnan et al., 2003) suggested the possibility of more than one enzyme being involved in liver formation of DEA. Given the linearity over the range of concentrations studied in intestinal microsomes (Fig. 1), it appears that the enzymes involved in metabolism of AM to DEA in intestine have a markedly lower affinity for AM than those in liver. This implied that there were tissue-specific enzymes involved in the formation of DEA. To our knowledge, there are no available reports describing the metabolism of human intestinal microsomes for comparison with our rat data.

In microsomes isolated from human livers, it has been reported that the mean V$_{max}$, k$_{m}$, and CL$_{int}$ were 68.7 pmol/min/mg protein, 38.9 μM, and 1.76 μl/min/mg protein, respectively (Trivier et al., 1993). In comparison, in our study, the V$_{max}$, k$_{m}$, and CL$_{int}$ in rat livers were observed to be 5.4-fold, 1.1-fold, and 5.4-fold higher than those values reported for human liver (Table 1). This is consistent with the higher total body CL noted in rats (23 ml/min/kg) (Shayeganpour et al., 2005) versus humans (range of means: 1.9 to 8.2 ml/min/kg) (Anastasiou-Nana et al., 1982; Riva et al., 1982; Vadiee et al., 1997).

Based on the differential findings in the kinetic profiles between
Fig. 6. Mean ± S.D. plasma concentration versus time profile of amiodarone with and without treatment with ketoconazole after single 25 mg/kg intravenous (A) or 50 mg/kg oral (B) doses of amiodarone HCl.

liver and intestinal formation of DEA from AM, it appeared there was more than one enzyme involved in the biotransformation, which warranted further study. Guengerich and Shimada (1991) proposed five different in vitro approaches for elucidation of catalytic activities of P450 in human liver. In our study, we selected three of these approaches to better identify the responsible enzymes. These included inhibition by a presumed chemical inhibitor of CYP3A in rat (KTZ), immunoinhibition, and Supersomes.

In liver, as the concentrations of KTZ were increased, there was a progressive decrease observed in the formation rate of DEA irrespective of the concentration of AM initially present (Fig. 2A). In intestinal microsomes containing either 23.5 or 47 μM AM, this pattern differed in that there was a decline in rate with a low concentration of KTZ, but with additional amounts of KTZ, there was no perceptible enhancement in the inhibition of DEA formation (Fig. 2B). Clearly, there was a difference in the involved P450 enzymes in this biotransformation between these two tissues, suggesting the involvement of different P450s in the formation of DEA from the parent drug, AM.

Although KTZ has been identified to be a selective inhibitor of CYP3A4 in human microsomes (Eagling et al., 1998; Turan et al., 2001), it has been reported that in rat, KTZ is not as selective an inhibitor of CYP3A (Eagling et al., 1998). Use of Dixon plots for determination of $k_i$ suggested that there are two P450 enzymes involved in the metabolism of AM in rat liver microsomes (Fig. 3). The inhibition data fit well to a two-enzyme model, where one enzyme had high capacity and high affinity, and the second had low capacity and low affinity for AM. Incubation of microsomes with anti-P450 antibodies indicated that in liver, but not intestine, CYP3A2 was involved in the formation of DEA from parent drug. There were no decreases noted in the formation of DEA in the presence of antibodies to CYP1A2 and 2B1/2, respectively, suggesting that these enzymes are not involved in this biotransformation (Fig. 4).

CYP1A1 is a metabolic enzyme responsible for chemical activation of xenobiotics to carcinogenic derivatives in some extrahepatic tissues such as lung (Zhao et al., 2004; Gharavi and El-Kadi, 2005). Use of Supersomes established a role for CYP1A1 and CYP3A1 in the formation of DEA (Fig. 5). In addition, both of these enzymes were significantly inhibited by the addition of KTZ. The inhibition by KTZ of CYP1A1 was more marked (96%) than that of CYP3A1 (78%). Due to high expression of CYP1A1 and CYP3A1 in rat intestine (Zhang et al., 1996; Turan et al., 2001; Kaminsky and Zhang, 2003), we can conclude that the aforementioned enzymes are involved in the metabolic biotransformation of AM to DEA in intestine. It is of note

| TABLE 2 |
| Pharmacokinetic parameters (mean ± S.D., range in parentheses) of amiodarone and desethylamiodarone after i.v. and oral administration in rats in the absence (controls) and presence of oral ketoconazole (17.1 mg/kg, three doses over 1 day) |
| Amiodarone | Desethylamiodarone |
| Control ($n = 6$) | KTZ ($n = 6$) | Control ($n = 5$) | KTZ ($n = 5$) |
| **AUC$_{0-48}$ (mg*h/l)** | 15.4 ± 2.90 | 25.6 ± 6.02$^a$ | 8.73 ± 1.98 | 26.8 ± 9.03$^a$ |
| (12.6–19.5) | (20.6–34.1) | (7.06–12.0) | (17.7–36.8) |
| **AUC$_{0-48}$ (mg*h/l)** | 18.5 ± 3.27 | 29.6 ± 7.49$^a$ | 13.6 ± 6.97 | 28.0 ± 9.46$^a$ |
| (13.4–23.0) | (22.2–41.5) | (8.11–24.1) | (18.3–39.3) |
| **CL (ml/h/kg)** | 1390 ± 269 | 889 ± 209$^a$ | 19.7 ± 4.6 | 12.6 ± 19.5 |
| (1086–1860) | (602–1126) | (2.18–10.1) | (20.6–34.1) |
| **V$_{ss}$ (l/kg)** | 40.9 ± 19.7 | 20.2 ± 9.47$^a$ | (9.01–37.0) | (2.18–10.1) |
| (22.1–51.5) | (9.01–37.0) | (2.18–10.1) | (2.18–10.1) |
| **$t_{1/2}$ (h)** | 39.4 ± 24.2 | 30.1 ± 21.5 | 32.2 ± 21.9 | 9.93 ± 1.31 |
| (17.2–78.8) | (10.1–59.5) | (16.9–69.8) | (8.21–11.3) |
| **C$_{max}$ (ng/ml)** | 723 ± 355 | 1716 ± 496$^a$ | (317–1123) | (1329–2296) |
| **Median $t_{max}$ (h)** | 3.93 | 8.00 | 2.18–10.1 | 2.08–10.3 |

$^a$ Significantly different from similarly dosed control rats.
that in addition to intestine, CYP1A1 is also an important enzyme found in lung (Seubert et al., 2002; Zhao et al., 2004). Furthermore, high expression of CYP1A1, CYP1A2, CYP2B1, CYP3A1, and CYP3A2 has been shown to occur constitutively within small hepatoctyes of adult rats. The ability of KTZ to inhibit intestinal CYP1A1 activity in humans has previously been demonstrated (Paine et al., 1999).

Our previously calculated high extraction ratio of AM reported in rat (Shayeganpour et al., 2005) incorporates both hepatic and extrahepatic elimination in other tissues such as intestine, lung, or kidney. Based on previous reports (Wyss et al., 1990; Shayeganpour et al., 2005), circulating DEA levels in the rat are much lower than those observed in human (Meng et al., 2001). This finding could be due to this pathway being of minor importance in the elimination of AM in the rat. Alternatively, the low concentrations of DEA observed in the rat may be due to a faster CL and perhaps larger Vd of DEA in comparison to AM. To our knowledge, there are no reports of DEA, CL, or Vd in rats after i.v. dosing. To assess the relevance of the in vivo findings, we undertook an in vivo experiment to determine the effect of KTZ inhibition on AM pharmacokinetics.

As previously observed (Shayeganpour et al., 2005), the concentrations of DEA were near the lower limit of quantitation of the assay in all groups of rats after i.v. and oral administration of AM. Nevertheless, administration of oral KTZ caused significant increases (1.60-fold) in the AUC of AM (Table 2; Fig. 6A). Furthermore, there was an unexpected decrease (2-fold) in the Vd of KTZ-treated versus control rats (Table 2). A similar finding has been observed in other studies involving nifedipine, docetaxel, and almotriptan coadministered with KTZ. The concomitant administration of oral KTZ and i.v. 0.5 mg/kg nifedipine to dogs showed an increased fold-in increase in AUC, and 1.73- and 1.2-fold decreases in the CL and Vd, respectively (Kuroha et al., 2002). Similarly, coadministration of 12.5 mg of almotriptan, a selective 5-HT1B/1D agonist, and 400 mg of KTZ to healthy volunteers generated a 44% decrease in the V/F of almotriptan (Fleishaker et al., 2004). The underlying reasons for this displacement interaction at the level of tissue binding, or an increase in the capacity and/or affinity of binding to plasma proteins, could explain the results.

After oral administration of AM, the effects of KTZ on exposure to AM were greater than those observed after its i.v. administration (Table 2; Fig. 6B). Given the demonstrated ability of AM to be metabolized by rat intestine (Fig. 1B; Table 1), this is perhaps not surprising. KTZ is recognized as a dual P-glycoprotein inhibitor (Ward et al., 2004), and AM is also identified as a substrate of P-glycoprotein (Kalitsky-Szirtes et al., 2004). In addition to inhibition of intestinal CYP3A1, the additional contribution of inhibition of intestinal CYP1A1 and reduced P-gp-mediated efflux are likely contributing factors to the increased oral bioavailability after coadministration of AM with KTZ.

In conclusion, the current results demonstrate that both liver and intestine play an important role in the first-pass metabolism of AM. Liver microsomal protein was more efficient at forming DEA than intestinal microsomes. Furthermore, we confirmed a role of CYP3A1, CYP3A2, and CYP1A1 in metabolism of AM to DEA in both liver and intestine of rat species. In addition, our pharmacokinetic studies demonstrated that oral administration of KTZ caused increased concentrations of AM, presumably by inhibition of CYP1A1 and 3A1.

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