Up-regulation of renal Mdr1 and Mrp2 transporters during amiodarone pretreatment in rats

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ABSTRACT

Although amiodarone (AMD) is known to produce drug–drug interactions through inhibition of transporter-mediated excretion of drugs, its impact on these mechanisms during chronic treatment has not been described yet. Therefore, the aim of this study was to investigate the influence of AMD pretreatment on the main multidrug transporting proteins, Mdr1 and Mrp2, in the liver and kidney. The expression of the transporters and pharmacokinetics of their substrates, rhodamine-123 (Rho123) and endogenous conjugated bilirubin (CB), were evaluated in rats after either AMD oral pretreatments (4–14 days) or single intravenous bolus. AMD pretreatment of all durations up-regulated renal Mdr1 and Mrp2 protein expression to 155–190% and 152–223% of the control values, respectively. In agreement, we observed a corresponding increase in renal clearance of both substrates. Hepatic expression was increased only for Mdr1 to 234–270% of controls, which was associated with increased biliary elimination of amiodarone without change in Rho123 biliary clearance. Interestingly, hepatic expression of another Mdr transporter, Mdr2, was progressively decreased by amiodarone administration. Acute administration of AMD reduced Rho123 biliary clearance by 64%. Our results indicate that repeated administration of AMD to rats is associated with significant increase in hepatic and renal expression of Mdr1 and Mrp2 transporters, which may contribute to variability in pharmacokinetics of AMD and simultaneously applied drugs.

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1. Introduction

Amiodarone is a potent drug used in the treatment of serious supraventricular and ventricular tachyarrhythmias. However, its wide clinical use is precluded by extensive adverse effects and drug–drug interactions. In order to limit these complications, careful monitoring of the therapy is recommended. In addition, prospective identification of the mechanisms involved in such therapeutic complications may allow their better prediction and even prevention.

The most frequently reported drug–drug interactions of amiodarone are those based on the inhibition of biotransformation [1]. As a highly lipophilic molecule, amiodarone is widely bound in the tissues with huge distribution volume and a correspondingly long serum elimination half-life of 40–60 days [2]. The main route of its elimination is the hepatic biotransformation to desethylamiodarone (DEA) by CYP3A4 and CYP2C8, with further metabolism by CYP3A4 followed by excretion into the bile [3]. Amiodarone was demonstrated as a potent inhibitor of CYP3A4, CYP1A2, CYP2C9, CYP2C19 and CYP2D6 [4,5]. Based on this mechanism, serious amiodarone drug–drug interactions were described with agents such as warfarin, simvastatin, and cyclosporine A [5]. In addition, amiodarone may affect excretion of drugs that either are poorly metabolized, e.g. digoxin [6], or those whose metabolism is not the rate-limiting step in their elimination, e.g. anthracyclines and vinca alkaloids [7]. Studies with cellular models identified that these interactions may occur in the liver via the inhibition of either canalicular multidrug resistance protein 1 (Mdr1) [8,9] or basolateral organic anion transporting polypeptide 2 (Oatp2) [10,11]. As a consequence, information about the principal underlying mechanism of these interactions has become less conclusive. In addition, it is well known that a long term treatment by Mdr1 inhibitors (e.g. cyclosporine A or ritonavir) may result in the transporter induction [12,13]. No such information is currently available for amiodarone.

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The present study aims to investigate amiodarone influence on the expression and function of two main drug transporting multidrug resistance proteins, Mdr1 and Mrp2, in the liver and kidneys. The function of the transporters was evaluated by elimination kinetics of two substrates, rhodamine-123 (Rho123 for Mdr1) and endogenous conjugated bilirubin (CB for Mrp2) in rats after either chronic (4–14 days) or acute (i.v. bolus) AMD administration. Detailed analysis of hepatic transport was evaluated using cultured primary hepatocytes. Changes in Mdr1, Mrp2, and Oatp2 protein expression were evaluated by Western blot.

2. Materials and methods

2.1. Materials

Rho123 and amiodarone were purchased from Sigma Chemical Co. (St. Louis, MO). Cyclosporine A (CsA) was a commercially available original formulation of Sandimmun (Novartis Ltd.). Mouse monoclonal antibodies C219, directed to the Mdr1 (150–170 kDa) and M2H-5, directed to Mrp2 (170–190 kDa) were purchased from Signet Laboratories, Inc. (Dedham, MA, USA). Rabbit anti-Oatp2 (75 kDa) polyclonal antibody was obtained from Millipore (Billerica, MA, USA). Rabbit anti-Mdr2 (170 kDa) and anti-Cyp3a2 (55 kDa) polyclonal antibodies were obtained from Abcam (Cambridge, UK) and Daiichi Pure Chemicals Co. (BD Gentest, Woburn, Massachusetts, USA), respectively. As a loading control for Western blot, rabbit polyclonal β-actin antibody (42–45 kDa) was purchased from Sigma (St. Louis, MO). Horseradish peroxidase-linked sheep anti-mouse and donkey anti-rabbit immunoglobulin G were purchased from GE Healthcare (Prague, Czech Republic). All other reagents were obtained from Sigma Chemical Co. (St. Louis, MO) and Bio-Rad laboratories (Hercules, CA, USA), respectively, and were of the highest purity available.

2.2. Animals and treatment

Adult male Wistar rats (Konárovice, Czech Republic) with initial weight of 270–280 g were used throughout the study. Animals were housed under controlled environmental conditions (12-h light–dark cycle; temperature, 22 ± 1 °C) with a commercial food diet and water freely available. All experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health (NIH Publication, 1996) and under the supervision of the Ethical Committee of the Faculty of Medicine in Hradec Kralove.

Rats were divided into six groups (n = 6, in each group). Amiodarone pretreated animals (25 mg/kg) received the drug once daily by stomach intubation for 4, 7, and 14 consecutive days. Corresponding three control groups were treated with an equal volume of vehicle alone (PBS, 2.0 ml/kg). Pharmacokinetic study with Rho123 was performed 24 h after the last dose administration. During this experiment, rats under light anesthesia with sodium pentobarbital (50 mg/kg) were fixed in supine position and cannulated in jugular vein, carotid artery, ductus choledochus and bladder for drug administration, blood, bile and urine sampling, respectively. The body temperature of the animals was maintained at 37 °C by a heating platform. Thereafter, a bolus dose of Rho123 (80 µg/kg) was applied intravenously, followed by a constant-rate infusion (Perfusor Compact; Braun, Prague) of a saline solution containing 4% mannitol delivering 400 µg (i.e. 1050 µmol)/kg of Rho123 per hour at a rate of 2 ml/h for 2 h. The loading and maintenance doses of Rho123 were determined by previous experiments [14]. Mannitol solution was used to maintain sufficient and constant urine flow rate. Bile and urine samples were collected in preweighed tubes at 20-min intervals from 60 to 120 min. Blood samples were collected at the midpoint of each collection period (70, 90 and 110 min after the infusion was started). Plasma samples were obtained from the whole blood by centrifugation at 1200 × g for 5 min at 4 °C. Organs for consequent evaluation of protein expression were immediately frozen in liquid nitrogen and together with plasma, bile and urine samples stored at −80 °C until analysis.

Acute effect of amiodarone, CsA (a known inhibitor of multidrug resistance transporters) or vehicle on Rho123 kinetics was tested similarly as described for pretreatments. After surgical preparations, the same loading and maintenance doses of Rho were introduced. Since the 60th min of Rho123 infusion, bile and urine samples were collected at 20-min interval for 40 min. At 100 min of Rho123 infusion, amiodarone (25 mg/kg), CsA (25 mg/kg) or isotonic saline was administered intravenously. Bile and urine samples were thereafter collected in preweighed tubes at 20-min intervals from 160 to 220 min. Blood samples were collected at the midpoint of each collection period (70, 90, 170, 190, and 210 min after the infusion was started). Samples were processed as described above.

2.3. Rho123 accumulation and efflux in primary rat hepatocytes

Hepatocytes were isolated as described previously [15], seeded in a density of 2 × 10⁶ cells per Petri dish (60 mm diameter), and cultured for one day. In Rho123 accumulation experiments, adherent cells were incubated in Williams E medium containing 1 µM Rho123 in the absence or presence of amiodarone (0.1 µM, 1 µM, and 5 µM) or CsA (1 µM and 10 µM), respectively, at 37 °C for 60 and 120 min. In dye efflux experiments, cells were preloaded with Rho123 by exposure to 1 µM Rho123 in the medium for 2 h and then incubated with Williams E medium without Rho123 in the absence (control efflux) or presence of amiodarone or CsA for up to 120 min. Subsequently, hepatocytes were washed three times with 4 °C cold PBS and intracellular dye was extracted by incubation with 0.5% Triton X (1.5 ml per dish) for 10 min at room temperature. The accumulated or excreted amount of Rho123 was normalized for the protein content per dish.

2.4. Drug analyses

The concentrations of amiodarone, and Rho123 were determined by high-performance liquid chromatography (HPLC) methods as described previously [14,16]. The concentrations of bilirubin and creatinine in plasma, bile, and urine were measured on Cobas Integra® 800 (Roche Diagnostics, Mannheim, Germany) according to manufacturer's instructions.

2.5. Pharmacokinetic analysis

Total plasma clearance (CLTOT) of Rho123 was estimated by dividing the constant infusion rate of Rho123 by the steady-state concentration in plasma (Cp). Biliary and renal clearance (CLB and CLR) of Rho123 during each collection period was calculated by dividing the respective excretion rate (BE, biliary; UE, urinary) by Cp determined for that collection period. Biliary excretion and clearance of AMD was calculated using the same method from measurements performed in bile and plasma samples obtained during the final collection periods (100–120 min in AMD orally pretreated rats, i.e. 24 h after last AMD administration; or 100–120 min after AMD intravenous bolus). Clearance of endogenous creatinine (CLCREAT) was determined as an indicator of glomerular filtration rate.

2.6. Immunoblot analysis

This method was performed as described previously [17]. Briefly, a membrane-enriched fraction (50 µg protein) was
separated on a 6.25% polyacrylamide gel. After being transferred to a nitrocellulose membrane, the proteins were blocked for 1 h at room temperature with 5% nonfat dry milk in Tris-buffered saline containing 0.05% Tween 20 (TBST). The membrane was then incubated with C219, M2-III5, anti-Mdr2, anti-Oatp2 (1:500) or anti-Cyp3a2 (1:5000) antibody for 1 h, washed four times with TBST, and incubated for 1 h with a peroxidase-conjugated secondary antibody (1:1000). After washing the membrane four times with TBST buffer, chemiluminescence was developed using an ECL reagents (GE Healthcare). The immunoreactive bands on the autoradiography films were scanned with calibrated densitometer ScanMaker i900 (UMAX, Prague, CZ) and quantified using the QuantityOne imaging software (Bio–Rad Laboratories, Hercules, CA). Equal loading of proteins onto the gel was confirmed by immunodetection of β-actin.

2.7. Statistical analysis

All quantitative data are presented as means and SDs. The results of the three control groups for AMD pretreatment are presented together because no statistically significant differences have been found among these groups. Differences between experimental and control values were assessed by one-way ANOVA followed by Tukey’s post hoc test using GraphPad Prism 5.0 software (San Diego, California).

3. Results

3.1. Protein expression

To determine the potential influence of amiodarone therapy on the protein expression of two transporters important for hepatic and renal drug excretion, Mdr1 and Mrp2, Western blot analysis was performed in total hepatic and renal membrane fractions obtained from rats treated for 4, 7 or 14 days either with amiodarone (25 mg/kg/day orally) or with corresponding volume of vehiculum (PBS) alone. As demonstrated in Fig. 1A/B, amiodarone pretreatment significantly induced Mdr1 expression in both liver and kidneys. The increase was most pronounced after 4 days (223% and 190% of controls in the liver and kidneys, respectively). To exclude a possibility of false results due to slight cross-reactivity of C219 antibody with Mdr2 phospholipid transporter, we analyzed also expression of this protein, which was progressively reduced throughout the AMD pretreatment (Fig. 1A). The expression of the main drug metabolizing enzyme co-operating with Mdr1, Cyp3a2 (rat orthologue of human CYP3A4), was unchanged in the liver, but increased in the kidney after 4 and 7 days of AMD administration (Fig. 1B). The expression of Mrp2 followed different patterns in the liver and kidneys. While in the latter (Fig. 1B) it was increased during the whole 14-day period of treatment by 234–270% compared to controls, the liver expression of the protein remained unchanged. Similarly, the expression of Oatp2, one of the main drug transporters localized at the basolateral membrane of hepatocytes, was also not influenced by amiodarone pretreatment.

3.2. Effect of amiodarone oral pretreatment on the kinetics of Rho123, AMD, and CB

Pharmacokinetic parameters of Rho123 are summarized in Table 1. Repeated administration of amiodarone produced progressive increase in urinary excretion rate of Rho123, which was associated with increased renal clearance of the dye. The bile excretion of Rho123 demonstrated tendency to increase, nevertheless the rise was not followed in biliary clearance parameter. Measurement of plasma and biliary concentrations of AMD showed that the drug was present in low concentrations in plasma of pretreated animals while bile concentrations and thus also excretions were comparable to those detected after single intravenous administration (Table 2). Importantly, there was statistically significant rise in biliary clearance of amiodarone, which reflected patterns of induced expression of Mdr1 in the liver (Fig. 1A, Table 2). The influence of oral amiodarone pretreatment (4, 7 and 14 days) on the renal and biliary clearances of conjugated bilirubin is shown in
Table 1
Steady-state pharmacokinetics of Rho123 in amiodarone (4, 7, and 14 days) pretreated rats. Control groups (4, 7, and 14 days of oral PBS, n=6 for each group) are presented together, because no significant difference was noted among them in any of evaluated parameter.

<table>
<thead>
<tr>
<th>Pretreatment control</th>
<th>Amiodarone 4 Days</th>
<th>Amiodarone 7 Days</th>
<th>Amiodarone 14 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine flow rate (μL/min)</td>
<td>9.4 ± 2.2</td>
<td>13.3 ± 2.2</td>
<td>14.7 ± 2.9</td>
</tr>
<tr>
<td>Urinary excretion rate (nmol/min/kg)</td>
<td>2.4 ± 0.6</td>
<td>3.7 ± 0.2</td>
<td>4.0 ± 1.6</td>
</tr>
<tr>
<td>Bile flow rate (μL/min)</td>
<td>18.8 ± 2.4</td>
<td>19.6 ± 4.2</td>
<td>17.5 ± 3.2</td>
</tr>
<tr>
<td>Biliary excretion rate (nmol/min/kg)</td>
<td>1.1 ± 0.2</td>
<td>1.8 ± 1.0</td>
<td>1.2 ± 0.3</td>
</tr>
<tr>
<td>Plasma (μM)</td>
<td>0.5 ± 0.05</td>
<td>0.6 ± 0.1</td>
<td>0.7 ± 0.2</td>
</tr>
<tr>
<td>C(shorter) (ml/min/kg)</td>
<td>4.7 ± 1.1</td>
<td>6.1 ± 0.9</td>
<td>7.3 ± 1.6</td>
</tr>
<tr>
<td>CLBile (ml/min/kg)</td>
<td>2.0 ± 0.3</td>
<td>2.9 ± 1.3</td>
<td>1.8 ± 0.6</td>
</tr>
<tr>
<td>CLTOT (ml/min/kg)</td>
<td>34.2 ± 2.4</td>
<td>28.7 ± 4.6</td>
<td>28.2 ± 7.7</td>
</tr>
<tr>
<td>CLcr (ml/min/kg)</td>
<td>7.7 ± 2.1</td>
<td>8.9 ± 2.1</td>
<td>9.2 ± 1.2</td>
</tr>
</tbody>
</table>

Values are shown as means ± SD (n=6).
* p<0.05.
** p<0.01—significantly different from control groups.

Table 2
Plasma concentrations, biliary excretions and biliary clearances of amiodarone after its either single intravenous dose or repeat oral (4, 7, and 14 days) dose administration (25 mg/kg/day).

<table>
<thead>
<tr>
<th></th>
<th>Pretreatment control</th>
<th>Amiodarone 4 Days</th>
<th>Amiodarone 7 Days</th>
<th>Amiodarone 14 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma (μM)</td>
<td>1.3 ± 0.2</td>
<td>0.14 ± 0.09</td>
<td>0.17 ± 0.15</td>
<td>0.14 ± 0.08</td>
</tr>
<tr>
<td>Biliary excretion rate (pmol/min/kg)</td>
<td>92 ± 35</td>
<td>132 ± 97</td>
<td>109 ± 41</td>
<td>58 ± 17</td>
</tr>
<tr>
<td>CLBile (ml/min/kg)</td>
<td>0.07 ± 0.02</td>
<td>0.9 ± 0.3***</td>
<td>0.8 ± 0.5***</td>
<td>0.5 ± 0.3</td>
</tr>
</tbody>
</table>

Values are shown as means ± SD (n=6).
** p<0.01.
*** p<0.001—significantly different from control groups.

Fig. 2. Biliary (CLBile; A) and renal (CLcr; B) clearance of conjugated bilirubin in amiodarone (4, 7, and 14 days) pretreated rats. Bar chart presents means ± SD (n=6); * p<0.05—significantly different from control groups. Control groups (4, 7, and 14 days of oral PBS, n=6 for each group) are presented together, because no significant difference was noted among them in any of evaluated parameter.

3.3. Effects of amiodarone intravenous administration on kinetics of Rho123

We investigated the effects of amiodarone (25 mg/kg) on the biliary and renal excretion of Rho123 under steady-state conditions during a continuous infusion, and the parameters are summarized in Table 3. Intravenous AMD reduced both biliary excretion and biliary clearance of Rho123 to 43% and 36% of control values, respectively, while renal elimination of the dye remained unaffected. The total clearance of Rho123 remained unchanged, however. In line with expectations, in the rats administered a bolus of known Mdr1 inhibitor, CsA, both biliary excretion and biliary clearance of Rho123 were significantly decreased compared to both control and amiodarone-administered groups (Table 3).

Table 3
Steady-state pharmacokinetics of Rho123 in amiodarone and cyclosporine bolus-administered rats.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Amiodarone</th>
<th>Cyclosporine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine flow rate (μL/min)</td>
<td>14.7 ± 4.2</td>
<td>16.9 ± 5.0</td>
<td>12.9 ± 5.7</td>
</tr>
<tr>
<td>Urinary excretion rate (nmol/min/kg)</td>
<td>5.7 ± 2.7</td>
<td>6.8 ± 1.6</td>
<td>3.8 ± 1.6</td>
</tr>
<tr>
<td>Bile flow rate (μL/min)</td>
<td>19.6 ± 2.3</td>
<td>17.4 ± 2.9</td>
<td>18.0 ± 3.4</td>
</tr>
<tr>
<td>Biliary excretion rate (nmol/min/kg)</td>
<td>2.1 ± 0.6</td>
<td>0.9 ± 0.3***</td>
<td>0.3 ± 0.07***</td>
</tr>
<tr>
<td>Plasma (μM)</td>
<td>0.7 ± 0.1</td>
<td>0.7 ± 0.05</td>
<td>0.7 ± 0.2</td>
</tr>
<tr>
<td>C(shorter) (ml/min/kg)</td>
<td>9.8 ± 3.8</td>
<td>10.2 ± 2.9</td>
<td>5.4 ± 2.2</td>
</tr>
<tr>
<td>CLBile (ml/min/kg)</td>
<td>3.6 ± 1.2</td>
<td>1.3 ± 0.4***</td>
<td>0.5 ± 0.2***</td>
</tr>
<tr>
<td>CLTOT (ml/min/kg)</td>
<td>29.6 ± 5.7</td>
<td>26.2 ± 2.2</td>
<td>25.2 ± 4.8</td>
</tr>
<tr>
<td>CLcr (ml/min/kg)</td>
<td>8.2 ± 0.8</td>
<td>11.5 ± 2.4</td>
<td>7.3 ± 2.6</td>
</tr>
</tbody>
</table>

Values are shown as means ± SD (n=6).
* p<0.05.
*** p<0.001—significantly different from control group.
3.4. Rho123 transport in primary rat hepatocytes

To determine localization of AMD– and CsA–Rho123 interaction in the liver, accumulation and efflux study was performed using primary rat hepatocytes (Fig. 3). After 60 min of incubation, amiodarone showed a significant concentration-dependent decrease in Rho123 accumulation, and 5 μM amiodarone inhibited accumulation of the dye down to 58% of the control, which was comparable to the effects of CsA in 1 μM concentration. The incubation for 120 min resulted in similar but more pronounced effects. No clear effects of amiodarone were observed in the efflux study, while CsA significantly inhibited also the efflux of Rho123 from hepatocytes.

4. Discussion

The principal findings of this study relate to the induction effects of amiodarone (AMD), a widely used antiarrhythmic agent, on the expression and function of the main efflux drug transporters in the kidney, Mdr1 and Mrp2. The data indicate that 4–14 days AMD administration to rats is associated with increased Mdr1 and Mrp2 protein expression with corresponding increased renal clearance of their model substrates, Rho123 and conjugated bilirubin. AMD pretreatment induced also Mdr1 expression in the liver but without significant influence on Rho123 biliary clearance. In contrast, acute AMD administration markedly reduced biliary clearance of Rho123 by blockade of its uptake to hepatocytes.

Induction of renal transporting protein expression, namely Mdr1, by its inhibitor has already been documented for CsA. This widely used immunosuppressive agent is a potent inhibitor of cytochrome P450 enzymes and Mdr1 transporter [18] as confirmed also by results of our study. However, repeated administration of CsA to patients with transplanted kidney results in increased expression of Mdr1 in the organ. Importantly, Mdr1 is not increased in patients with CsA nephrotoxicity. This indicates that CsA induces its own detoxification by Mdr1 and that inadequate up-regulation of the protein may contribute to the drug’s nephrotoxicity [19,20]. These data have been confirmed by preclinical studies in rats [21,22]. Regarding AMD, expression changes of any transporting protein during AMD treatment has never yet been described. Therefore we can only scale our results to a drug with very similar pharmacokinetic profile such as CsA. We detected similar increase in Mdr1 expression, which was further associated with higher expression of another important efflux transporter, Mrp2. Although the presence of AMD blocks Mdr1 with the lowest reported IC50 being 5.48 μM, the plasma concentrations measured in our study were deeply below this value. This may explain the observed increase in renal excretion of Mdr1 and Mrp2 substrates, Rho123 and CB, respectively. In addition, similarly to inducing effects formerly reported for two Mdr1 inhibitors, CsA and ritonavir [12,13], we also demonstrated an increase in hepatic Mdr1 expression after AMD pretreatment. The up-regulation was associated with increased biliary elimination of the drug, which indicates that AMD, apart from inhibiting Mdr1 function by direct binding to the transporter, may augment its own elimination. To improve the accuracy of Mdr1 detection, we analyzed also the expression of hepatic Mdr2 canalicular transporter, which exclusively mediates biliary excretion of phospholipids [23]. Unique information about amiodarone induced down-regulation of this transporter and its role in the pathophysiology of phospholipidosis [24] induced in clinical practice by the antiarrhythmic needs further evaluation. Moreover, Mdr1 as one of the main xenobiotic transporting proteins tightly cooperates with the main xenobiotic-metabolizing cytochrome P450 isofrom, Cyp3a2 (rat orthologue of human CYP3A4), and these two molecules share similar spectrum of substrates and regulatory mechanisms [25]. The liver expression of the enzyme was however not influenced by amiodarone pretreatment, so strong inhibitory potential of the drug against this isoform could be expected [4]. Amiodarone-induced transient increase in the kidney expression of Cyp3a2 must be interpreted in the view of its very low renal expression in comparison with hepatic tissue [26]. Thus the impact of this effect on overall metabolism of xenobiotics would be minimal.

Interesting information was a raised urine production in AMD-pretreated animals. Nevertheless, the systemic concentrations of amiodarone (Table 2) were well bellow the therapeutic limit (1–2.5 mg/L, i.e. 1.5–3.7 μM). Thus, only minimal systemic effect of amiodarone on heart rate and blood pressure could be expected. This complies with unchanged creatinine clearance, the parameter which is a reliable marker of glomerular filtration/kidney perfusion and rapidly drops during, e.g. hypotension. In agreement, recent reports demonstrated that significant reduction of blood pressure and heart rate with a subsequent reduction of glomerular filtration rate is achievable in rats only with amiodarone dose of 50 mg/kg given intravenously or intraperitoneally [27,28]. This may also explain why we have not observed the reduction of creatinine clearance after intravenous AMD administration. In contrast, increased urine production after oral pretreatment with AMD with unchanged creatinine clearance suggests that this increase may be associated with induction of tubular transporters. No such data are available for amiodarone yet, but we observed similar phenomenon when another Mdr1 inducing agent (dexamethasone) was applied to rats [14].

High potential of AMD to produce drug–drug interactions based on inhibition of cytochrome P450 enzymes has been demonstrated in numerous studies [5]. Moreover, its capability to increase plasma concentrations of digoxin, a poorly metabolized agent, has been known for a long time [29]. When digoxin was recognized as a
substrate of Mdr1 [30], and the ability of AMD to decrease multidrug resistance in tumors and to potentiate the intracellular accumulation of cytostatics [31,32] was demonstrated, inhibition of Mdr1 was suggested as the mechanism of this interaction. Consequently, experiments on Mdr1-transfected cells confirmed the inhibitory influence of AMD on Mdr1 with IC50 within the range of 5.48–45.6 µM. Further studies however suggested that the main mechanism of AMD–digoxin interaction is a blockade of digoxin uptake into the cells at the level of basolateral transporters Oatp2. The model employed was transfected Xenopus leavis oocytes, where AMD inhibited transport of digoxin with Ki of 1.8 µM [11]. AMD affinity to Oatp2 in therapeutic concentrations was thus shown to be much higher than to Mdr1. Subsequent in vivo study showed an increase in digoxin plasma concentration after an intravenous bolus of AMD, as a result of impaired biliary and intestinal excretion of digoxin [10]. The determination of digoxin tissue concentrations indicated that the impairment in biliary excretion was a result of its decreased accumulation in hepatocytes, i.e. at the level of basolateral uptake rather than canicular efflux.

Our results support the data that AMD-mediated interaction takes place rather at the basolateral membrane and not at the canicular Mdr1 [10,11]. The addition of AMD to primary hepatocytes in our study resulted in a decrease in cellular uptake of the dye without significant influence on the efflux velocity. Moreover, in vivo study showed that Rho123 biliary excretion was not influenced by AMD pretreatment despite the fact that biliary (and thus intracellular) concentrations of AMD were similar to those observed during i.v. bolus administration experiments. Because AMD plasma concentrations were very low in pretreated animals, we have recently demonstrated similar mechanism of alteration is basolateral membrane of hepatocytes. Indeed, using the same model substrate.

In conclusion, the present findings indicate AMD capability to modify elimination routes of Rho123, showing an increase in renal excretion of the dye along the pretreatment with this potent antiarrhythmic agent. The expression of the main drug efflux transporters in the kidney, Mdr1 and Mrp2, suggested that this increase may be related to the up-regulation of the transporters. Induction of Mdr1 in liver may speed the elimination of AMD from the body. In contrast, the drug’s plasma presence within the range of therapeutic concentrations resulted in marked decrease of Rho123 hepatic transport. As demonstrated during in vitro study, this effect originates from an inhibition of basolateral uptake of the dye into hepatocytes. Therefore a combination of the described mechanisms may be considered as the cause of AMD-mediated drug–drug and drug–endogenous compound interactions.

Acknowledgements

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References


