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Pharmacokinetics of Amiodarone in Hyperlipidemic and Simulated High Fat-Meal Rat Models

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ABSTRACT: The objective of this study was to examine the effect of a high fat meal and hyperlipidemia on the pharmacokinetic behavior of amiodarone. To evaluate these effects, single doses of amiodarone were administered to rats i.v. (25 mg/kg) or orally (50 mg/kg). Some rats were rendered hyperlipidemic by intraperitoneal doses of poloxamer 407 followed by amiodarone i.v. In other normolipidemic rats, amiodarone was administered i.v. in a fasted state or after the administration of 1% cholesterol in peanut oil. Amiodarone plasma concentrations were considerably (>11-fold) increased in hyperlipidemia. Substantial decreases were noted in the clearance, volume of distribution and unbound fraction (11.6, 23 and 24.7-fold, respectively) in plasma of hyperlipidemic rats. Oral lipid caused a significant increase in plasma $AUC_{0-\infty}$ (1.38-fold) and a significant decrease in clearance (1.5-fold) of amiodarone after intravenous doses. Oral consumption of 1% cholesterol in peanut oil significantly increased the plasma *AUC* (1.83-fold) and bioavailability of amiodarone (1.31-fold) after oral doses. In determining oral bioavailability of lipophilic drugs such as amiodarone in food effect studies, in addition to the increase in absorption of drugs, other factors such as a decrease in clearance due to increases in lipoprotein levels should be taken into account. Copyright © 2005 John Wiley & Sons, Ltd.

Key words: antiarrhythmic drugs; lipoproteins; protein binding

Introduction

Amiodarone (AM) was introduced as an antianginal drug in 1967 in Europe [1]. In addition to its antianginal effects, AM was also observed to produce a clinically important increase in action potential duration [2]. As a result, it found use as a class III antiarrhythmic drug in the treatment of life threatening ventricular and supraventricular arrhythmias [3]. AM has also been found to be safe for use in patients suffering from concomitant arrhythmia, and either post-myocardial infarction or congestive heart failure [4]. More recently, AM has been used in patients undergoing coronary artery bypass graft surgery to prevent post-operative atrial fibrillation [5]. Amiodarone, with or without beta blockers, and the implantable cardioverter-defibrillator have been the major therapeutic tools proposed to prevent sudden arrhythmic death in patients with organic heart disease, including those with poor left ventricular function [6]. A recent paper has emerged which suggests that AM does not offer an advantage in this respect [7]. Although AM is known to possess a narrow therapeutic range of concentrations [8], dosage was empirically determined based on body weight, and no mention was made of plasma concentration monitoring [7]. Due to the presence of a therapeutic range of concentrations, an

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understanding of the factors that may influence the pharmacokinetics of the drug is vital.

Amiodarone possesses a very large volume of distribution (661/kg) and extensive tissue distribution, and in turn, a long terminal phase half-life (25 days) in human plasma [9,10]. The drug is extensively metabolized, and has a low hepatic extraction ratio in humans [9]. One of its major active metabolites is the dealkylated metabolite, desethylamiodarone (DEA) [11,12]. Amiodarone has unpredictable absorption characteristics resulting in low and variable bioavailability after oral administration [13,14]. The chemical structure of AM, which confers high lipophilicity [15], may help to explain some of these pharmacokinetic behaviors [13]. The drug is also known to bind to circulating lipoproteins, which reportedly comprises 33.5% of its overall total protein binding (95.6%) in plasma [12,13,16].

The pharmacokinetics of AM may be dependent upon clinical status. For example, it has been demonstrated that the elimination half-life of AM is lower in normal volunteers than in cardiovascular patients [15]. Hyperlipoproteinemia is clearly identified as a major contributor towards an increased risk of athersosclerosis and coronary heart disease [17,18]. In addition to intrinsic hyperlipidemia, lipoproteins in plasma can be transiently increased following ingestion of a high fat meal. It is known that a fatty meal can increase the bioavailability of some lipophilic molecules such as AM [19,20], which displays incomplete or slow oral absorption [13,21]. The increased solubilization of lipophilic molecules in the digested fat content of food may facilitate an increase in drug absorption across the intestinal lumen. In addition to this increase in absorption, a postprandial decrease in systemic clearance as a result of a transient increase in binding of drug to lipoproteins may also contribute to an increase in *AUC* after ingestion of a high fat meal [22,23].

The present study sought to explore the effects of lipids on the pharmacokinetics of AM using the rat as an animal model. The poloxamer-407 rat model was chosen to mimic the hyperlipidemic state [24]. In addition, it examined the influence of dietary fat on the plasma concentrations and pharmacokinetics of AM in rat after oral and intravenous (i.v.) administrations.

Methods

Chemicals

Amiodarone HCL (AM), ethopropazine HCL, cholesterol, peanut oil and poloxamer 407 (P407) were obtained from Sigma (St Louis, MO, USA). Desethylamiodarone (DEA) was obtained as a gift from Wyeth Ayerst (Research Monmouth Junction, NJ, USA). Methanol, acetonitrile, hexane (all HPLC grade), triethylamine and sulfuric acid were purchased from EM Science (Gibstaun, NJ, USA). Potassium dihydrogen orthophosphate, sodium phosphate dibasic, sodium phosphate monobasic and sodium chloride were obtained from BDH (Toronto, Ontario, Canada). Halothane BP was purchased from MTC Pharmaceuticals (Cambridge, Ontario, Canada). Heparin sodium injection was obtained from Leo Pharma Inc. (Thornhill, Ontario, Canada). Amiodarone HCl as a sterile injectable solution was purchased from Sabex® (50 mg/ml) (Boucherville, Quebec, Canada).

Animals and preexperimental procedures

The study was approved by the University of Alberta Health Sciences Animal Policy and Welfare Committee. A total of 33 male Sprague–Dawley rats (Charles River, Quebec, Canada) were used in the studies. Body weight was 250–350 g and all the rats were housed in temperature controlled rooms with 12 h light per day. The animals were fed a standard rodent chow containing 4.5% fat (Lab Diet® 5001, PMI nutrition LLC, Brentwood, USA). Free access to food and water was permitted prior to experimentation.

Rats were allocated into five groups based on the route of administration and the prior treatment. Group A (n=7) (i.v. AM; control), Group B (n=7) (i.v. AM; oral lipid), Group C (n=7) (i.v. AM; hyperlipidemic), Group D (n=6) (oral AM; control), Group E (n=6) (oral AM; oral lipid).

About 36 h before the pharmacokinetic experiment, rats in group C were rendered hyperlipidemic by intraperitoneal administration of 1 g/kg poloxamer 407 (0.13 g/ml solution in normal saline). To ensure the proper injection of the dose, the animals were lightly anesthetized with halothane, injected with poloxamer and allowed

to recover. The day before the pharmacokinetic experiment, the right jugular veins of all rats were catheterized with Micro-Renathane tubing (Braintree Scientific, Braintree, MA) under halothane anesthesia. The cannula was filled with 100 U/ml heparin in 0.9% saline. After cannula implantation, 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 metabolic cages for conduct of the pharmacokinetic experiments.

Amiodarone administration and blood sample collection

AM injectable solution was used in both the i.v. and oral dosing studies. The appropriate doses were prepared by dilution of AM solution in normal saline to a final concentration of 12.5 mg/ml. On the morning of the pharmacokinetic study, rats in groups A, B and C received 25 mg/kg of AM solution i.v. The i.v. doses were injected over 60 s via the jugular vein cannula, immediately followed by injection of approximately 1 ml of sterile normal saline solution. Rats in groups D and E received 50 mg/kg of AM by oral gavage. The rats in groups B and E were treated with 2 ml/kg of lipid (peanut oil enriched with cholesterol 1% w/v) by oral gavage 0.5 h before and 2 h after the dosing.

For i.v. studies, blood samples were collected at approximately 0.083, 0.33, 0.67, 1, 2, 3, 4, 6, 8, 10, 24, 32 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 the area under the plasma concentration versus time curve (*AUC*) in rats. The sampling times after oral doses were after approximately 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 $2500 \times \mathbf{g}$ for about 3 min and the plasma was transferred to new polypropylene tubes and stored at -30°C until assayed for AM and DEA.

In all orally and i.v. dosed rats, where possible, if the cannula remained patent additional blood samples were obtained at approximately 3, 4, 5 and 6 days after administration of the dose.

Plasma protein binding

An erythrocyte vs buffer or diluted plasma partitioning method was used to determine the *in vitro* plasma protein binding of AM [25]. Briefly, normolipidemic and hyperlipidemic blood from P407 treated animals, was collected under halothane anaesthesia into heparinized tubes by cardiac puncture. The blood was divided into two equal parts into two different tubes and the plasma was separated from blood cells by centrifugation at $2500 \times \mathbf{g}$ for 10 min. Afterwards, the plasma was separated and the buffy coat layers were discarded. The blood cells were washed in an equal volume of isotonic Sorensen's phosphate buffer (pH 7.4) followed by centrifugation at $2500 \times \mathbf{g}$ for 8 min. These washing and centrifugation steps were repeated twice. After the last washing step, the volume of erythrocytes was determined in each tube and an appropriate amount of either diluted plasma or buffer was added to the tubes to yield a hematocrit of 0.3 (buffer) or 0.4 (diluted plasma) [22,25]. The normolipidemic plasma was diluted by a factor of 1:19, and the hyperlipidemic plasma was diluted by a factor of 1:29.

Amiodarone was added to each tube to provide for drug concentrations of 50 μg/ml. Then, the tubes were incubated in a 37 °C shaking water bath for 1 h. This duration of incubation had been established to be sufficient for equilibration.

Assay

A high performance liquid chromatography (HPLC) method was used for analysis of AM. The assay had a validated lower limit of quantitation of 35 ng/ml for both AM and DEA in 100 μl rat plasma [26,27]. Briefly, to 100 μl plasma was added 0.3 ml of acetonitrile. After centrifugation the supernatant was transferred to tubes containing 0.3 ml of pH 5.9 phosphate buffer. The contents were extracted using 3 ml hexane, the supernatant was dried and reconstituted with mobile phase. The final solution was injected into the HPLC using reverse phase chromatography and a C_{18} analytical column. For assay of oral lipid and P407 treated animals, blank drug-free plasma from similarly treated

animals was used for construction of the standard curves.

Plasma samples were assayed within 1 week of collection. Based on the results of a stability study (data not shown) there was no evidence of degradation of AM or DEA in rat plasma over this time period.

Data analysis

Noncompartmental methods were used to calculate the pharmacokinetic parameters [28]. The elimination rate constant (λ_z) was estimated by subjecting the plasma concentrations in the terminal phase to linear regression analysis. The terminal elimination phase half-life ($t_{1/2}$) was calculated by dividing 0.693 by λ_z.

The concentration at time 0 h after i.v. dosing was estimated by back extrapolation to time zero using the first two measured concentrations (5 min and 20 min) after dosing. The $AUC_{0-\infty}$ after dosing was calculated using the combined log-linear trapezoidal rule from time 0 h post-dose to the time of the last measured concentration, plus the quotient of the last measured concentration divided by λ_z.

Clearance (CL) was calculated as $CL = \frac{Dose}{AUC_{0-\infty}}$, and steady-state volume of distribution (Vd_{ss}) was calculated as $Vd_{ss} = CL \times \frac{AUMC}{AUC}$ [28] where $AUMC$ is the area under the first moment plasma concentration vs time curve.

The absolute oral bioavailability (F) of AM, assuming linear kinetics, was calculated by dividing the AUC_{oral} by $AUC_{i.v.}$ correcting for dose, as follows

$$F = \frac{(\text{mean oral } AUC \times Dose_{i.v.})}{(\text{mean i.v. } AUC \times Dose_{oral})}$$

The calculation was repeated using partial AUC_{0-24h} and for $AUC_{0-\infty}$. In making these calculations, care was taken to ensure that for fasted F, the oral and i.v. AUC of fasted animals were used, and that for oral lipid-fed animals, the oral and i.v. AUC of oral lipid-fed animals was used.

The calculation of unbound fraction in plasma (f_u) was determined by a series of equations outlined by Schumacher *et al*. [25]. The erythrocyte concentrations of AM in the erythrocyte-diluted plasma (C_E) were determined by the following equation

$$C_E = \frac{C_B - C_P \times (1 - HCT)}{HCT}$$

where C_B is the concentration of AM in blood cell-diluted plasma suspension, C_P is the concentration of AM in the diluted plasma and HCT is the hematocrit in the erythrocyte-diluted sample.

To estimate the erythrocyte concentration of AM in the erythrocyte-buffer samples (C_{E*}), the concentration of AM in the blood cell-buffer concentration was substituted for C_B, and the buffer concentration (C_{buffer}) of AM was substituted for C_P. The unbound fractions were determined by the following equation

$$f_u = \frac{\alpha \times \frac{P_p}{P_b}}{1 - \left[\frac{P_p}{P_b} \times (1 - \alpha)\right]}$$

where α is the plasma-dilution factor. The partition coefficients for erythrocyte: plasma (P_p) and erythrocyte: buffer (P_b) of AM are represented by the quotients $\frac{C_E}{C_{Plasma}}$ and $\frac{C_{E*}}{C_{buffer}}$, respectively.

Statistical analysis

Compiled data were expressed as mean $\pm$ SD, unless otherwise indicated. One-way ANOVA, 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 (Microsoft, Redmond WA) or SPSS version 12 (SPSS Inc., Chicago, IL) were used in analysis of data. The level of significance was set at α=0.05

Results

In the hyperlipidemic rats pretreated with P407, the plasma concentrations of AM were substantially higher than those in control rats (Figure 1). Oral lipid also appeared to increase the plasma concentrations of AM after i.v. dosing, particularly in the first 24 h post-dose (Figure 1). The increase in plasma concentrations of AM was most marked in the hyperlipidemic rats. Plasma

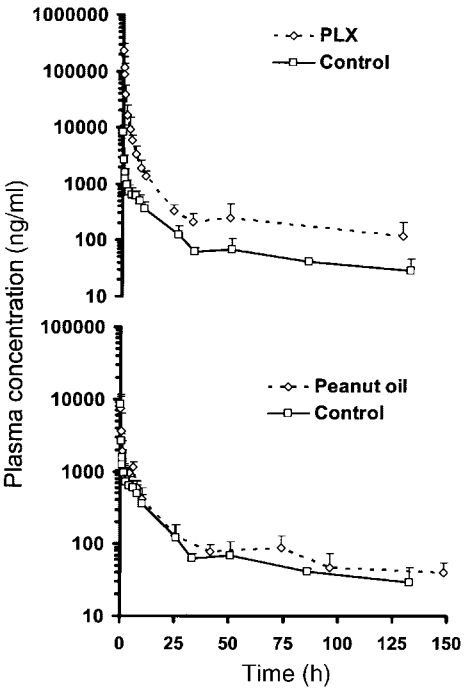

Figure 1. Mean ± SD plasma concentration versus time profiles of amiodarone after 25 mg/kg i.v. doses of amiodarone HCl given to normolipidemic (n=7; Control), hyperlipidemic (n=7; PLX) and oral lipid-fed (n=7; Peanut oil) rats

concentrations declined in a shallow manner in the terminal phase of all rats administered AM.

After i.v. doses of AM, the plasma *AUC* was increased substantially ($p < 0.05$) in the presence of hyperlipidemia (Table 1). The *AUC* in hyperlipidemic animals were significantly higher than both groups of normolipidemic animals. Compared with the fasted animals, the mean $AUC_{0–24}$ and $AUC_{0-\infty}$ were 16- and 11-fold higher in the hyperlipidemic rats, respectively. In a direct statistical comparison (Student's *t*-test) between fasted animals and those fed with lipid, a significant increase in AUC_{0-24} and $AUC_{0-\infty}$, was detected. However, when the post hoc Duncan's test was used in combination considering all three groups of animals given i.v. AM, *AUC* of the postprandial animals was not different from the fasted animals (Table 1).

The *CL* and Vd_{ss} of hyperlipidemic animals were lower than that of both normolipidemic groups of animals given i.v. drug (Table 1). The calculations of *CL* and Vd_{ss} revealed that hyperlipidemia was associated with significant reductions of 12- and 23-fold, respectively, compared with fasted normolipidemic animals. Although the *CL* of AM was significantly higher (1.5-fold) in the normolipidemic fasted than in the lipid-fed animals, there was no difference in Vd_{ss}. The half-life was not observed to be different between any of the groups given i.v. drug.

After oral lipid, significant increases were detected in systemic exposure of AM when given orally (Figure 2). Compared with fasted animals, there were increases of 2.1, 1.8 and 2.7-fold in the AUC_{0-24}, $AUC_{0-\infty}$ and C_{max}, respectively, of lipid fed animals (Table 1). There was, however, no change observed in t_{max} or terminal phase $t_{1/2}$ of oral AM after the administration of oral lipid (Table 1). There was variability observed in the plasma concentration vs time profiles, particularly during the absorption phase, leading to secondary C_{max} values in some of the rats (Figure 2). Using $AUC_{0-\infty}$, the oral *F* of AM was estimated to be 35% and 46% in control and oral lipid treated animals, respectively. The partial *AUC* from 0–24 h was also used to calculate *F*. In this case, the estimates of *F* in fasted and oral lipid-fed animals were 28% and 44%, respectively.

Extensive binding of AM to plasma proteins was observed in rat plasma. Plasma protein binding of AM in normolipidemic plasma indicated that the unbound fraction was 0.0853% ± 0.0319% (n=8 samples). This was reduced significantly in hyperlipidemic plasma to 0.00345% ± 0.00114% (n=4 samples). The unbound fraction in normolipidemic plasma was thus 25-fold higher than hyperlipidemic plasma. A high concentration of spiked AM was required in order to permit quantitation of the drug in the buffer samples. Because after oral lipid the lipoprotein concentrations are in a state of flux after ingestion, the postprandial unbound fraction in pooled plasma was not determined. It was not possible to measure the unbound concentrations in actual plasma samples from the pharmacokinetic study, due to assay sensitivity limitations.

The mean C_{max} of DEA was considerably lower than that of AM in each of the groups (Table 1). Although the detection limit of DEA in plasma was more than its quantifiable concentration (35 ng/ml), a complete plasma concentration vs

Table 1. Mean ± SD (range in parentheses) of pharmacokinetics of amiodarone after administration to rats in control (fasted) rats and in animals pretreated with 1% cholesterol in peanut oil or poloxamer 407

Group A Control (n=7)	Group B Oral lipid (n=7)	Group C Poloxamer (n=7)	Group D Control (n=6)	Group E Oral lipid (n=6)
Intravenous (25 mg/kg amiodarone HCl)			Oral (50 mg/kg amiodarone HCl)	
Amiodarone				
AUC_{0-24} (mg·h/l)				
12.6 ± 2.42 (8.40–15.5)	16.8 ± 3.37 (12.4–20.9)	197 ± 58.8[a,b] (99.0–255)	6.95 ± 1.73 (5.36–9.17)	14.9 ± 3.11[a] (10.2–18.8)
$AUC_{0-\infty}$ (mg·h/l)				
20.3 ± 7.98 (11.8–35.8)	28.1 ± 4.15 (22.5–33.1)	231 ± 66.9[a,b] (134–295)	14.3 ± 5.06 (8.11–22.0)	26.1 ± 6.61[a] (17.1–33.9)
$t_{1/2}$ (h)				
44.0 ± 16.5 (26.0–74.8)	46.6 ± 13.8 (29.2–64.7)	50.8 ± 24.2 (19.7–93.7)	49.1 ± 28.0 (20.7–95.5)	103 ± 83.7 (17.5–254)
CL (ml/h/kg)				
1378 ± 465 (698–2121)	907 ± 141[a] (755–1111)	119 ± 43.1[a,b] (84.6–186)	ND	ND
Vd_{ss} (l/kg)				
47.0 ± 14.9 (28.2–70.0)	41.5 ± 15.1 (19.7–68.3)	2.04 ± 2.75[a,b] (0.349–8.09)	ND	ND
C_{max} (ng/ml)				
ND	ND	ND	662 ± 301 (317–991)	1776 ± 759[a] (828–2903)
t_{max} (h)				
ND	ND	ND	8.09 ± 8.51 (2.07–23.9)	5.73 ± 2.57 (2.93–10)
Desethylamiodarone				
C_{max} (ng/ml)				
135 ± 158	150 ± 126	405 ± 286	52.0 ± 36.6	32.7 ± 34.8
t_{max} (h)				
0.801 ± 1.30	2.94 ± 4.00	12.9 ± 25.3	9.13 ± 8.00	7.09 ± 2.44

[a] Significantly different from control rats (Duncan's multiple range post hoc test comparing all groups).
[b] Significantly different from control and oral lipid treated rats (Duncan's multiple range test).
ND, not determined.

time profile of DEA concentration was not obtained in most of the rats, and hence, *AUC* could not be reported.

Discussion

Poloxamer 407, a hydrophilic non-ionic surface active agent, has been shown to cause significant elevation in plasma cholesterol and triglycerides in various rodent models, including rat [29]. It has been demonstrated that poloxamer 407 exerts its actions by inhibiting plasma lipoprotein lipase and probably cholesterol 7α-hydroxylase [29]. In the present study, the pharmacokinetics of AM in rats were markedly affected by the large increases in plasma lipoprotein levels induced by P407 [24,29–31]. Notable decreases in *CL* and Vd_{ss}, and increases in *AUC* were observed (Table 1). It is likely that this increase in plasma concentrations was attributable to a change in

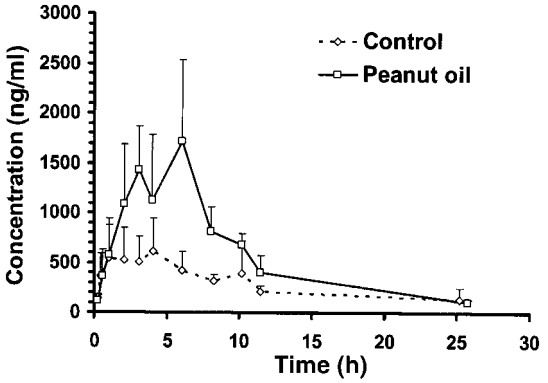

Figure 2. Mean ± SD plasma concentration versus time profiles of amiodarone after 50 mg/kg oral doses of amiodarone HCl given to normolipidemic (n=6; Control) and oral lipid-fed (n=6; Peanut oil) rats

the unbound fraction of the drug, which was observed to decrease by 25-fold in P407 plasma. Changes of this magnitude in the P407 hyperlipidemic rat model have been observed for the enantiomers of halofantrine [22]. After administration of oral lipid to the rats, the changes were not as great as those observed in P407 treated animals. Nevertheless, a significant change in *CL* was detected between the oral lipid-fed and fasted groups (Table 1). A significant increase was also observed in the *AUC* compared with fasted normolipidemic rats in a direct 2-way comparison. These were the expected results on *CL* and *AUC* if the oral lipid were to cause an increase in the fraction bound to chylomicrons and VLDL. Similar to these results, similar changes were previously observed in halofantrine pharmacokinetics in rats administered single oral doses of peanut oil [22].

Oral consumption of lipid caused significant increases in AUC_{0-24} (2.1-fold) and $AUC_{0-\infty}$ (1.8-fold) of orally administered AM compared with fasted rats (Table 1). The increase in C_{max} was also significant (2.7-fold; Table 1). Quantitatively, these findings in rats were very similar to those seen in humans given AM in the presence and absence of a high fat meal [19,20]. In human studies, the *AUC* and C_{max} of AM were observed to be 2.4- and 3.8-fold higher, respectively, in the fed compared with the fasted state [19]. These increases in exposure of the animals to AM are probably best explained by an increase in the delivery of the drug to the blood circulation, secondary to an increase in the solubility of the drug in the gastrointestinal tract.

In humans, after food ingestion, bile output and luminal concentration of bile components peak within 30 min in the proximal small intestine, due to constriction of the gall bladder [19]. The net effect is an increase in micellization of lipophilic drugs in the duodenum, an increase in solubility and ultimately oral bioavailability. In rats there is no gall bladder, and the flow of bile into the duodenum from the common bile duct is continuous. This raises the question; why does rat behave so similarly to humans with respect to the effect of lipid on the bioavailability of amiodarone and halofantrine? Although the bile flow is continuous in rat, it is known that in fasted rats both bile flow and rate of excretion of bile acids into the intestinal tract is diminished [32]. Further, ingestion of food is known to stimulate the rate of bile flow into the duodenum [33]. In addition to an increase in bile flow, food components (glucose or triglyceride) infused intraduodenally cause an increase in biliary output of bile acid and phosphatidylcholine in the rat [34], as is the case in humans upon contraction of the gall bladder. To our knowledge, however, there are no data available that have been focused on the output of bile and its composition following a bolus of lipid, as was performed in our study.

It has been demonstrated that in the postprandial state, failure to take into account the decreased *CL* of halofantrine can result in an overestimate of the impact of a high fat meal on the increase in bioavailability [22]. In a typical food effect study, the dose normalized oral *AUC* after food is compared with that in the fasted state. The *AUC* of i.v. administered drug in the fasted and fed states is not usually available from the same study subjects. The impact of not having the i.v. data can be simulated using our results. If the *F* were determined as in a typical study, the oral fed *AUC* would be divided by the fasted *AUC*. Using the $AUC_{0-\infty}$ data (Table 1), the relative bioavailability was calculated to be 1.83-fold higher in the fed rats. However, the actual increase in bioavailability in the fed state is calculated as absolute F_{fed} divided by absolute F_{fasted}, which from our results show only a 1.31-fold higher extent of AM absorption after lipid.

Thus, the effect of food on oral bioavailability in the absence of the appropriate *AUC* data after i.v. doses would be overestimated by 40%.

The hepatic extraction ratio of AM was calculated using the average value of hepatic blood flow in 250 g rats [35] and the *CL* of drug in our rats calculated after i.v. doses (Table 1). Assuming complete metabolism by the liver, significant differences were determined between the fasted (0.72 ± 0.24), oral lipid-fed (0.47 ± 0.14) and hyperlipidemic rats (0.062 ± 0.023). The estimated extraction ratio in the fasted animals was higher than that directly found (0.49 ± 0.013) in studies involving isolated perfused rat liver [36], which suggests the involvement of extrahepatic elimination in the rat after administration of AM.

This study tried to characterize the complete profile of DEA but the concentrations were in many cases below the lower limit of quantitation of the assay. This may indicate a more rapid *CL* or perhaps a more extensive Vd_{ss} of the formed metabolite, compared with the parent drug. It is also possible that in the rat, DEA is formed to a lesser extent than in human. Wyss *et al*. had reported a similar finding to ours, in that concentrations of DEA (expressed as radioactive equivalents) measured after i.v. doses of 50 mg/kg AM were much lower in plasma than parent drug [10]. Recently it was shown that not only DEA, but also a hydroxylated benzofuran metabolite, is formed in the rat after administration of AM [37].

In conclusion, increased levels of lipoproteins due to hyperlipidemia or high fat food can alter the pharmacokinetics of AM. This may have consequences in the interpretation of AM plasma levels and bioavailability estimates.

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