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Effects of intestinal constituents and lipids on intestinal formation and pharmacokinetics of desethylamiodarone formed from amiodarone

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Abstract

To model the impact of intestinal components associated with a high fat meal on metabolism of amiodarone, rat everted intestinal sacs were evaluated for their ability to metabolize the drug to its active metabolite (desethylamiodarone) under a variety of conditions. The preparations were obtained from fasted rats or rats pretreated with 1% cholesterol in peanut oil. After isolation of the tissues, the intestinal segments were immersed in oxygenated Krebs Henseleit buffer containing varying concentrations of bile salts, cholesterol, lecithin and lipase with or without soybean oil emulsion as a source of triglycerides. Amiodarone uptake was similar between the five 10-cm segments isolated distally from the stomach. Desethylamiodarone was measurable in all segments. Based on the metabolite-to-drug concentration ratio within the tissues, there was little difference in metabolic efficiency between segments for any of the treatments. Between treatments, however, it appeared that the lowest level of metabolism was noted in rats pretreated with 1% cholesterol in peanut oil. This reduction in metabolic efficiency was not observed in gut sacs from the fasted rats to which soybean oil emulsion was directly added to the incubation media. Despite the apparent reduction in intestinal metabolism, there was no apparent change in the ratio of metabolite-to-drug area under the plasma concentration versus time ratios of fasted rats and those given 1% cholesterol in peanut oil, suggesting that the intestinal presystemic formation of desethylamiodarone is not substantial.

Introduction

The enterocytes lining the lumen of the gastrointestinal tract possess a number of phase I and II drug metabolizing enzymes that can potentially contribute towards a reduction in drug oral bioavailability (Catania & Carrillo 1990; Lin et al 1999; Doherty & Charman 2002; Kaminsky & Zhang 2003). Among the cytochrome P450 isoenzymes (CYP), the gastrointestinal tract possesses CYP1A1, CYP2B1, CYP2C6, CYP2C11, CYP3A, CYP2D6 and CYP2J4 activity (Kaminsky & Zhang 2003). Although it is contributory, intestinal metabolism generally imparts a much lower impact on bioavailability than hepatic metabolism due to the greater number and breadth of metabolizing enzymes in the liver (Kaminsky & Zhang 2003).

High-fat meals can affect the oral bioavailability of many lipophilic drugs, and this is most often attributed to an increase in solubilization within the gastrointestinal tract. Orally administered lipids may also facilitate a bypass of first-pass hepatic metabolism of lipoprotein-bound drugs via the intestinal lymphatic pathway (Caliph et al 2000). Lipids may also potentially interfere with drug transport protein activity (Custodio & Benet 2005) and hepatic metabolizing efficiency (Yoshinari et al 2006). There is some indirect evidence suggesting that intestinal metabolism can also be affected by oral lipids. In the presence of a high-fat meal to dogs, the oral bioavailability of halofantrine was increased by 12 fold (Humberstone et al 1996). However, the increase in the level of the main metabolite, desbutylhalofantrine, was not proportional with the increase (~2-fold) in the parent drug plasma concentrations. The authors suggested that lymphatic shunting had diminished the amount of first-pass hepatic metabolism. However, halofantrine possesses a low hepatic extraction ratio and, as such, lymphatic shunting is unlikely to be the sole explanation for the observed reduction in metabolite–halofantrine plasma AUC ratio. Although not as striking, a similar observation was noted for amiodarone in man, where a high-fat-content meal caused the concentration of amiodarone to be increased

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to a larger extent than that of its primary circulating metabolite, desethylamiodarone (DEA) (Meng et al 2001).

Both halofantrine and amiodarone share a number of similarities from a pharmacokinetic perspective. Both are highly lipophilic, highly metabolized, and highly lipoprotein bound drugs (Brocks & Wasan 2002; Shayeganpour et al 2005). To further explore this issue, the ability of the gastrointestinal tract to metabolize amiodarone to DEA in-vitro was examined using rat everted intestinal segments in the presence and absence of components (bile and lipid) that would be present in the gut lumen after a high-fat meal. It was hypothesized that the metabolite-to-drug ratio would be decreased in the presence of lipid components. To assess the consequence of altered presystemic metabolism in-vivo, the pharmacokinetics of DEA were also assessed in the presence and absence of oral lipid after administration of an oral dose of amiodarone.

Materials and Methods

Chemicals

Amiodarone hydrochloride, ethopropazine hydrochloride, β-nicotinamide adenine dinucleotide phosphate tetrasodium (NADPH), sodium chloride and sodium hydroxide were purchased from Sigma-Aldrich (St Louis, MO, USA). DEA was obtained as a gift from Wyeth Research (Monmouth Junction, NJ, USA). Methanol, acetonitrile, hexane, water (all high-performance liquid chromatography (HPLC) grade), formic acid 88% and hydrochloric acid (37%) were purchased from Fisher Scientific (Fair Lawn, NJ, USA). Magnesium sulfate, sucrose and calcium chloride (all analytical grade) were obtained from BDH (Toronto, ON, Canada). Peanut oil, sodium potassium tartrate, cupric sulfate anhydrous, bovine serum albumin (BSA), Folin-phenol reagent, lecithin, lipase, porcine bile extract, cholesterol and an LDH assay kit were obtained from Sigma (St Louis, MO, USA). Isoflurane USP was purchased from Halocarbon Products Corporation (River Edge, NJ, USA). Heparin sodium injection, 1000 U mL^{-1}, was obtained from Leo Pharma Inc. (Thornhill, ON, USA). Amiodarone hydrochloride (50 mg mL^{-1}) as a sterile injectable solution was purchased from Sabex (Boucherville, PQ, Canada). Soybean oil emulsion was obtained from Baxter (Mississauga, ON, Canada) as Intralipid.

Simulated bile solution

Simulated rat bile solution (SBS) was prepared as a mixture of 4.9 g L^{-1} bile extract (providing approximately 10 mmol L^{-1} conjugated bile acids), 12.5 mg L^{-1} lipase, 0.1 mmol L^{-1} cholesterol (0.6 g L^{-1}) and 0.8 g L^{-1} lecithin in Krebs-Henseleit bicarbonate buffer (KH) (Kido et al 2003). To permit solubilization of the components, the mixture was sonicated for 4–6 h at 37°C. Lipase was added to permit partial hydrolysis of the triglycerides present in the soybean oil constituents of Intralipid.

Animals

All experimental protocols involving animals were approved by the University of Alberta Health Sciences Animal Policy and Welfare Committee. A total of 35 male Sprague–Dawley rats (Charles River, CRC, Quebec, Canada) were used in the studies. Body weight ranged from 250 to 350 g and all of the rats were housed in temperature-controlled rooms with 12 h light per day. The rats were fed a standard rodent's chow containing 4.5% fat (Lab Diet 5001; PMI nutrition LLC, Brentwood, USA). For the pharmacokinetic and in-vitro intestinal metabolism studies, free access to water was permitted before experimentation although food was withheld overnight before experimentation. In the everted intestinal metabolism studies free access to food and water was permitted before experimentation.

In-vitro everted intestinal metabolism

All rats received rodent chow up to the time of experimentation, although in one subgroup the rats (n = 4) were pretreated with two doses of peanut oil enriched with cholesterol 1% w/v (2 mL kg^{-1}) given 2.5 h and 30 min before the harvesting of the intestinal segments.

Intestinal segments were isolated as previously described (Brocks et al 1993). At the time of experiment rats were anaesthetized under isoflurane–oxygen delivered by anaesthetic machine. A single vertical incision was made along the midline of the abdomen to gain access to the intestines. The bile duct of each rat was closed using a single thread knot. Distal to the pyloric sphincter and the ligament of Treitz, the intestinal tissue was cut into 5 segments of approximately 10-cm length. Segments were sequentially labeled G1 to G5. Segment G1, the most proximal segment, represented mostly the duodenum. Segments G2 to G5, extending towards the ileum, represented a continuum of intestinal composition, ranging in series, from jejunum to ileum. Each segment was rinsed with ice-cold KH (pH 7.5). Each segment was everted using a glass rod. One end of the everted gut sac was ligated with a silk thread and tied. The other end was left open for filling with KH (37°C) and then tied with silk thread to form the sac. Each segment was then placed into a jacketed tissue bath containing 14.7 μM of amiodarone hydrochloride. The total volume of buffer solution in each chamber was 40 mL to which was added 8 μL of amiodarone hydrochloride for injection (50 mg mL^{-1}) per chamber.

The segments were incubated with amiodarone in the presence of a number of different compositions of buffer solutions. These were pH 7.4 KH (n = 5 rats), KH+5% SBS (n = 4 rats), and KH+25% SBS (n = 4 rats). The intestinal segments from the rats pretreated with peanut oil were incubated with KH+25% SBS. In one of the groups (n = 4 rats), gut sacs were exposed to KH+25% SBS plus 5% soybean oil emulsion.

Each chamber was continuously aerated with oxygen–carbon dioxide (95:5) at 37°C for 2 h. After the end of the incubation time, a needle attached to a syringe was inserted into the sac and a sample of the solution was collected for analysis of amiodarone and DEA. Each segment was then fully removed from the incubation chamber, blotted dry with tissue paper and weighed, then subjected to homogenization and assay for amiodarone and DEA.

In-vitro intestinal microsomal metabolism

Rats (n = 4 per group) were sacrificed under anaesthesia and their whole small intestine was harvested. Intestinal tissues

were washed in ice-cold KCl (1.15% w/v), cut into pieces and homogenized separately in cold sucrose solution (5 g of tissue in 25 mL of sucrose 0.25 M). Microsomal protein from homogenized tissues was isolated by differential ultracentrifugation. The final pellets were collected in cold sucrose and stored at −80°C. The Lowry method was used to measure the total protein concentration in each microsomal preparation (Lowry et al 1951).

Each 0.5-mL incubate contained 1 mg mL^{-1} protein from each of the microsomal preparations from the four individual rats, 25 μM of amiodarone hydrochloride, 1 mM of NADPH and 5 mM of magnesium chloride hexahydrate dissolved in 0.5 M potassium phosphate buffer (pH 7.4). The substrate was added to the intestinal microsomal suspension and the oxidative reactions were started with the addition of NADPH after a 5-min pre-equilibration period. All incubations were performed in quadruplicate in a 37°C water bath shaker for 30 min. Incubation conditions were optimized so that the rate of metabolism was linear with respect to incubation time and microsomal protein concentration.

Pharmacokinetics

For the pharmacokinetic study, 12 rats were given oral amiodarone hydrochloride at a dose level of 10 mg kg^{-1}, allocated into two groups of 6 each based on their previous treatment. These groups comprised a control and a set of rats given 2 doses of peanut oil (2 mL kg^{-1}) enriched with cholesterol 1% w/v by oral gavage 30 min before and 2 h after the drug was given.

The day before the experiment, the right jugular vein of each rat was catheterized with Micro-Renathane tubing (Braintree Scientific, Braintree, MA, USA) under isoflurane anaesthesia. The cannula was filled with 100 U mL^{-1} heparin in 0.9% saline. After implantation, the rats were transferred to regular holding cages with food withheld overnight. The next morning the rats were transferred to metabolic cages and dosed with amiodarone. Each rat received 10 mg kg^{-1} of amiodarone hydrochloride suspension by oral gavage. The dose was prepared by dispersion of the powdered amiodarone hydrochloride in 1% methylcellulose to a final concentration of 4.5 mg mL^{-1}. In the lipid pretreated group the dose of amiodarone was given 30 min after the first dose of lipid.

The blood nominal sampling times after oral doses were 0.5, 1, 2, 3, 4, 6, 8, 10, 24 and 48 h after the amiodarone dose. After sample collection, each blood sample was centrifuged at 2500 *g* for about 3 min and plasma was transferred to new polypropylene tubes then stored at −30°C until assayed for amiodarone and DEA.

Assays

A validated LC-MS method was used to measure the concentrations of both amiodarone and DEA in the intestinal tissue, serosal fluid, microsomal incubations and plasma (Shayeganpour et al 2007a). The assay had a validated lower limit of quantification of 2.5 and 5 ng mL^{-1} (or ng g^{-1}) of amiodarone and DEA, respectively, in rat plasma and tissues, and interday coefficient of variation of less than 20%. The procedures involved in the extraction and assay of amiodarone and DEA from serosal solutions (100 μL) and intestinal homogenates were similar to plasma and tissue homogenates, respectively. Denatured microsomal media, KH and blank intestine were used for standard curve preparation as appropriate, in line with the specimens being assayed.

The method was modified to assay both amiodarone and DEA in microsomal preparations. Briefly, to each tube containing 500 μL of microsomal incubation mixture and 1.5 mL of acetonitrile, 30 μL of internal standard (2.5 μg mL^{-1} ethopropazine hydrochloride) was added. The tubes were vortex mixed for 30 s and centrifuged for 2 min at 2500 *g*. 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 1 mL of pure methanol and volumes of 5–10 μL were injected into the LC/MS apparatus. For standard curve construction, drug-free microsomal preparations of intestine without NADPH were used and spiked with appropriate amounts of amiodarone and DEA.

Viability of the everted gut sacs was verified by measuring lactate dehydrogenase activity in the incubation media using a commercially available kit (Sigma, St Louis, MO, USA).

Pharmacokinetic and statistical analysis

Non-compartmental methods were used to calculate the pharmacokinetic parameters of amiodarone and DEA, including maximal plasma concentration (C_{max}) and time of its occurrence (t_{max}). Elimination rate constant (λz) was estimated by subjecting the log-transformed plasma concentrations in the terminal phase to linear regression analysis. The terminal elimination phase half-life (t½) was calculated by dividing 0.693 by λz. The log-linear trapezoidal rule was used to calculate the area under the plasma concentration versus time curve.

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 (Microsoft, Redmond, WA, USA) or SPSS version 12 (SPSS Inc., Chicago, IL, USA) were used for statistical analysis of data. The level of significance was set at $P < 0.05$.

Results

In-vitro everted intestinal metabolism

The lactate dehydrogenase activity in the serosal and mucosal sides of the everted gut sacs did not increase over the time frame of the incubations, indicating viability of the tissue preparations. No amiodarone or DEA was detected in the serosal solutions within the everted intestinal segments. On the other hand, both amiodarone and DEA were present within the gastrointestinal tract segments (Figure 1), thus indicating the ability of the intestine to absorb and metabolize the drug.

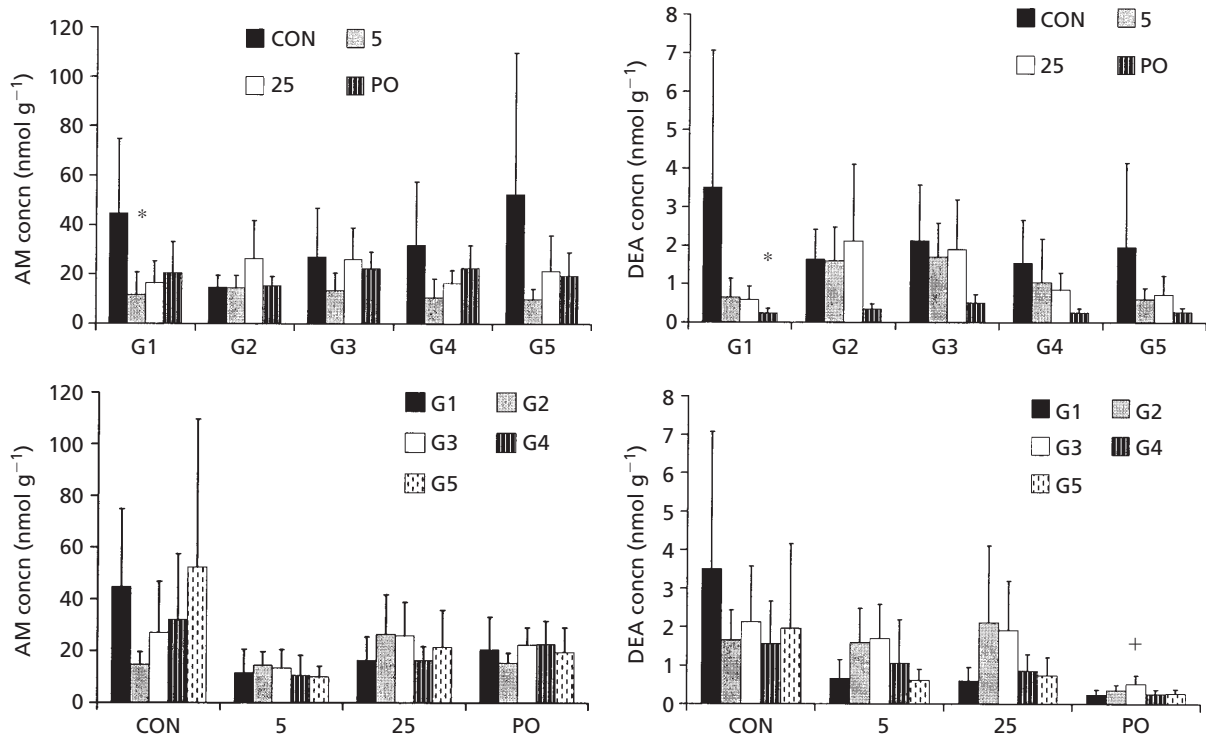

Figure 1 Concentrations of amiodarone (AM) and desethylAM (DEA) in rat intestinal segments after incubation with everted gut sacs (G1–G5) for 2 h. Treatments were normal diet, with sacs incubated with control (Krebs-Henseleit, CON), 5% bile in Krebs-Henseleit (5) and 25% bile in Krebs-Henseleit (25). 25% Krebs-Henseleit was also incubated with control sacs in the presence of 5% Interalipid (LP), or sacs from rats pretreated with peanut oil (PO). $^{*}P < 0.05$, compared with control incubation; $^{+}P < 0.05$, compared with G1, G4 and G5.

For each segment, there was little evidence of significant difference in the uptake of amiodarone between treatments (Figure 1, upper panels). Only in the first duodenal segment was there a difference detected, in which the KH+5% SBS had significantly lower amiodarone than KH alone. In general this was the case for DEA as well, in which in the first segment the peanut oil pretreated group had a significantly lower DEA concentration than the KH control treatment. With respect to the treatment groups themselves, each segment in general seemed to take up amiodarone to an equivalent degree (Figure 1, bottom left panel). Within treatments, there was also little significant difference in the DEA concentration between segments (Figure 1, bottom right panel). The lone exception was in the peanut oil-pretreated group, in which segment G3 had more DEA within it than some of the other segments.

More differences were discernible when the ratio of metabolite to drug within the segments was evaluated (Figure 2, left panel). When viewed by segment, the rank order of mean ratios of the treatment groups was generally peanut oil pretreated < KH+25% SBS < KH < KH+5% SBS. In segments G1 and G3 no significant differences were noted. In each of these groups the peanut oil pretreatment had the lowest ratio. In segment G4 and G5, the KH+5% SBS had the highest ratio ($P < 0.05$). When viewed by treatment group, there was little difference in this ratio between segments (Figure 2, right panel).

Because of the regional similarities in the uptake and generation of drug and metabolite, respectively, between the segments (Figure 1, upper panels), to evaluate overall effects of the treatments on intestinal metabolism all data were combined for segments G1–G5. For amiodarone the KH control group had the highest overall uptake of drug and presence of metabolite. For DEA the lowest concentrations were present in the peanut oil pretreated group. Similarly, the lowest ratio of metabolite to drug was present in the peanut oil pretreated group.

In total there were three groups of everted gut sacs exposed to 25% SBS. These included KH+25% SBS, KH+25% SBS+5% Intralipid, and the peanut oil pretreated groups (Figure 4). In the KH+25% SBS+5% lipid group there was a significant decrease in the uptake of amiodarone in the gastrointestinal tract tissues. With respect to DEA concentration, the highest amount was measured in the KH+25% SBS exposed segments. The DEA formation, expressed as a percent of amiodarone, was lowest in the peanut oil pretreated rats. In this parameter, there was no difference between the 25% SBS and 25% SBS+5% Intralipid segments.

In-vitro microsomal intestinal metabolism

No significant differences were observed between the intestinal microsomal formation rates of DEA of control untreated rats (2.30 ± 0.707 pmol min^{-1} (mg protein)$^{-1}$) and rats pretreated with oral lipid (2.83 ± 1.23 pmol min^{-1} (mg protein)$^{-1}$)) (n = 4 in each group).

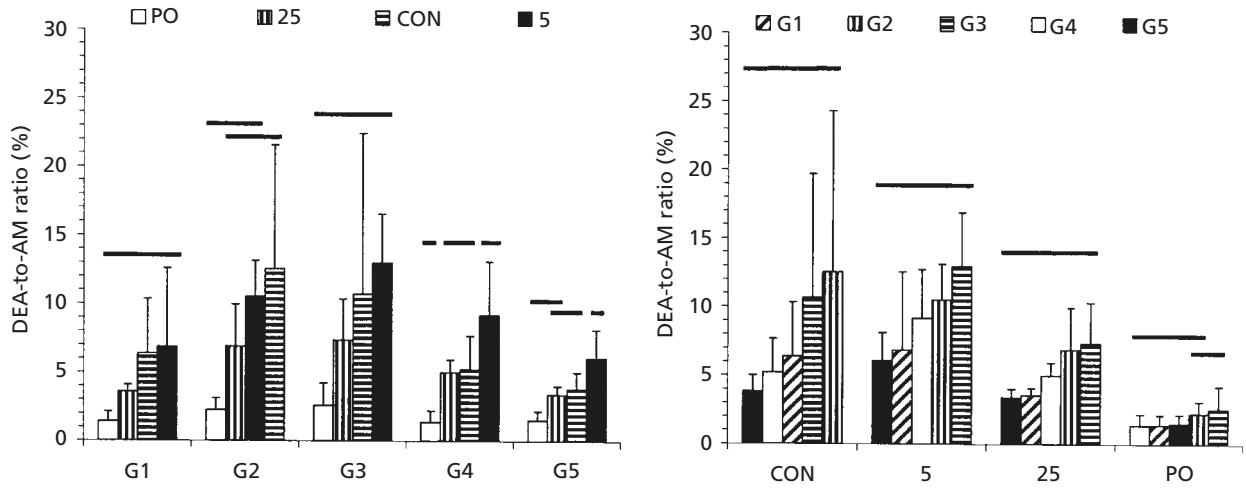

Figure 2 The ratio of desethylamiodarone (DEA) to amiodarone (AM) in each rat intestinal segment expressed as a percentage of the total amount of AM present in each segment. Continuous lines over each bar indicate no significant difference between the encompassed groups. See Figure 1 for abbreviations. Above the columns, continuous lines represent no difference between groups under those lines. Groups not encompassed by the continuous lines are significantly different.

Pharmacokinetics

As previously reported, amiodarone concentrations were much higher than those of DEA in all rats at all time points. After administration of the oral lipid there were significant increases in concentrations of both amiodarone and DEA. As measured by AUC_{0-24h} and C_{max} values, amiodarone increased by approximately 2.8 and 3.1 fold, respectively. In contrast, the mean AUC_{0-24h} and C_{max} of DEA increased by 4.1 and 6.1, respectively. The AUC_{0-24h} ratios of DEA to amiodarone after normalization for molecular weight were 0.086 ± 0.033 and 0.13 ± 0.052 for the fasted and oral lipid-treated rats, respectively. These values were not significantly different.

Discussion

The in-vitro rat everted gut sac model has been successfully used in the characterization of intestinal metabolism of a number of drugs (Brocks et al 1993; Sattari & Jamali 1997; Teng et al 2003; Arellano et al 2004, 2005a, b, 2007). It has an advantage over some other methods (microsomes, Ussing chambers, cell cultures) in that structural integrity of the tissue is maintained. Previously it was demonstrated that DEA could be formed from microsomal protein isolated from the intestinal tract of rat. Our results further demonstrated, here in an intact tissue environment, that amiodarone could be directly metabolized to DEA. This left open the possibility that the intestinal tract plays a role in the first-pass metabolism of amiodarone to DEA. Given previous observations for halofantrine and amiodarone after ingestion of a high-fat meal, we carried forth with the experiments investigating the influence of bile components and lipids on the metabolism of amiodarone to DEA.

Neither drug nor metabolite was detected at quantifiable concentrations in the serosal fluids within, or mucosal fluids outside of, the everted intestinal sacs. This is consistent with the strong affinity of amiodarone and DEA to tissues and their large volumes of distribution in the rat (Shayeganpour et al 2007b). The absence of a sink-effect imparted in-vivo by flowing blood, including the influence of extensive plasma protein binding in the closed system represented by the everted gut sac system, was clearly evident. In a previous study it was similarly shown that in Ussing chambers in which amiodarone was spiked on one side of the membrane the majority of the drug and metabolite was confined to the tissues (Kalitsky-Szirtes et al 2004).

Given the absence of measurable DEA in the buffer solutions on either side of the intestinal tissue, the most relevant comparator for examining metabolite formation in this closed system is the DEA-to-amiodarone concentration ratio within the tissues (Figure 2). Within each segment (Figure 2, left panel), in general the highest level of DEA-forming activity was apparent in the KH+5% SBS treatment group, and lowest in the peanut oil pretreated rats for which gut sacs were incubated in KH+25% SBS. This infers that the prior administration of peanut oil causes some inhibition in the ability of the gastrointestinal tract to metabolize amiodarone to DEA. Further, it appears that lower concentrations of bile cause an increase in metabolizing efficiency compared with no SBS or high concentrations of SBS (Figure 2, left panel). In general, there was little significant difference noted in the intersegment metabolizing efficiency within each treatment group (Figure 2, right panel). It was observed that for most treatments the most distal segment (G5) seemed to have the lowest level of activity, whereas the mid section (G3) had the highest. This is in line with studies showing that in the distal parts of the gastrointestinal tract, concentrations of CYP are thought to be lower than in the more proximal regions (Kaminsky & Zhang 2003).

When examining the effect of the treatments on overall metabolism of amiodarone to DEA by combining all segments,

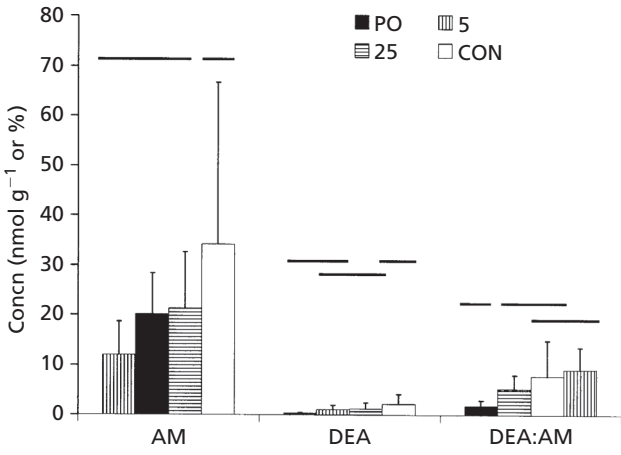

Figure 3 Concentrations of amiodarone (AM), desethylAM (DEA) and metabolite-to-drug ratio when all rat intestinal segments (G1–G5) were combined. Broken lines between groups indicates significant differences. See Figure 1 for abbreviations.

the control KH incubations had the highest levels of amiodarone and DEA (Figure 3). For the important comparator of DEA-to-amiodarone ratio, however, it was found that the lowest metabolizing efficiency for DEA formation was in the peanut oil treated group incubated with KH+25% SBS. The highest level of activity was present in the KH+5% SBS. Both of these findings mirrored those of the segments evaluated separately (Figure 2, left panel). Of the three KH-25% SBS treatments utilized in the experiments, it was found that intestinal sacs from rats pretreated with 1% cholesterol in peanut oil had the lowest level of measurable DEA forming ability (Figure 4). The lower uptake of amiodarone in the everted fasted rat gut sacs exposed to KH+25% SBS+5% Intralipid can perhaps be attributed to sequestration into mixed micelles, which akin to plasma protein binding, limited the ability of the intestinal tissue to readily take up the drug. Despite the lower uptake, the DEA-to-amiodarone ratio was similar to that of KH+25% SBS alone, suggesting that low concentrations of lipid did not alter the metabolizing efficiency of the tissues.

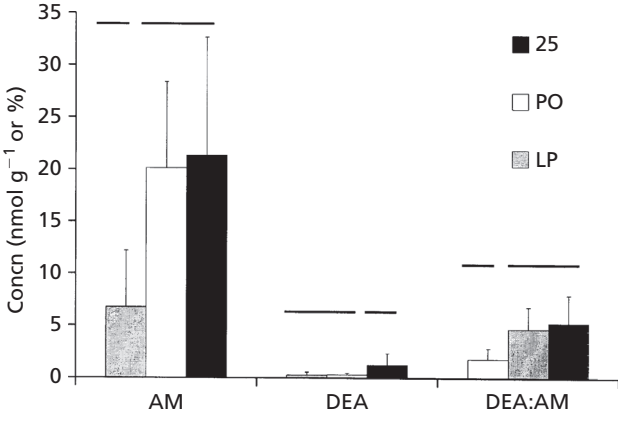

Figure 4 Concentrations of amiodarone (AM), desethylAM (DEA) and metabolite-to-drug ratio when all rat intestinal segments (G1–G5) from incubations involving 25% SBS were combined. Broken lines between groups indicates significant differences. See Figure 1 for abbreviations.

The data raise the question as to why peanut oil pretreatment apparently causes a decrease in metabolizing efficiency within the intestinal tract. It is known that several fatty acids can inhibit CYP enzymes of mammalian species including man and rat. For instance recently it was shown that saturated fatty acids had a very weak inhibitory effect on the CYP3A-mediated metabolism of testosterone 6β-hydroxylation (Hirunpanich et al 2007). In contrast unsaturated fatty acids exhibited stronger inhibitory effects. There was a progression of inhibition in the order of saturated < monounsaturated < polyunsaturated fatty acids. Both the presence of double-bonds and increasing chain length of the fatty acids increased their inhibitory potency (Hirunpanich et al 2007). In a similar fashion, saturated fatty acids had no inhibitory effects on the activity of a number of human CYP isoenzymes (CYP1A2, 2C9, 2C19, 2D6, 2E1 and 3A4) at concentrations up to 200 μM. In contrast the polyunsaturated fatty acids strongly inhibited CYP2C9- and CYP2C19-facilitated reactions, and to a lesser degree competitively inhibited the metabolic reactions catalyzed by CYP1A2, CYP2E1 and CYP3A4 (Yao et al 2006). The main product of linoleic acid metabolism was also observed to have significant inhibitory effects on CYP1A1, 1A2, 2C8 and 2C19 activity in human liver microsomes (Yamazaki & Shimada 1999). Inhibition of CYP through the inhibitory effects of fatty acids was offered as an explanation for the increase in the bioavailability of saquinavir and ciclosporin after ingestion of docosahexaenoic acid by rats in either liver or intestine through inhibition of metabolism but not P-glycoprotein-mediated transport (Hirunpanich et al 2006). It has been observed that high concentrations of plasma lipoproteins can cause a down-regulation of some hepatic CYP isoenzymes. Whether this is also true of intestinal CYP has not yet been investigated.

Peanut oil is composed mostly of monounsaturated fatty acids (81%) (Venkatachalam & Sathe 2006). It also contains other saturated, monounsaturated and polyunsaturated fatty acids in varying amounts, in different varieties of peanut oil (Grosso et al 2000). In rat, amiodarone is known to be metabolized by some of the CYP isoforms present in the intestinal tract (Shayeganpour et al 2006). No significant difference was observed between the intestinal metabolism of amiodarone to DEA in microsomes of control untreated rats and the rats treated with peanut oil. This suggests that peanut oil does not affect the expression of the CYP isoenzymes known to be involved in the metabolism of amiodarone (CYP1A1 and CYP3A1) in the intestinal tract in rat (Shayeganpour et al 2006; Elsherbiny et al 2008). When microsomes are prepared, most of the other cellular constituents are removed, including any free fatty acids, mono-, di- and tri-glycerides that might be present in the cells. For this reason the discrepancy between the decreased DEA formation in everted intestine and unchanged microsomal DEA formation after peanut oil is apparently directly related to the presence of lipids in the enterocytes. Possible explanations for decreased metabolism in everted intestine

Table 1 Pharmacokinetic parameters after a single 10-mg kg^{-1}oral dose of amiodarone with and without oral lipid (1% cholesterol in peanut oil)

	Amiodarone		**Desethylamiodarone**	
	Control	Oral lipid	Control	Oral lipid
AUC_{0-24h} (ng · h mL^{-1})	1967 ± 611*	5431 ± 994	167 ± 56*	681 ± 253
$AUC_{0-\infty}$ (ng · h mL^{-1})	2893 ± 666*	6892 ± 897	322 ± 191*	2481 ± 1579
t½ (h)	16.20 ± 9.98	22.40 ± 4.16	23.8 ± 10.3	50.6 ± 24.6
C_{max} (ng mL^{-1})	234 ± 66*	731 ± 209	16.80 ± 7.76*	102.0 ± 48.7
t_{max} (h)	3.20 ± 0.41*	5.4 ± 2.0	3.5 ± 2.5	5 ± 4

*$P < 0.05$, compared with oral lipid.

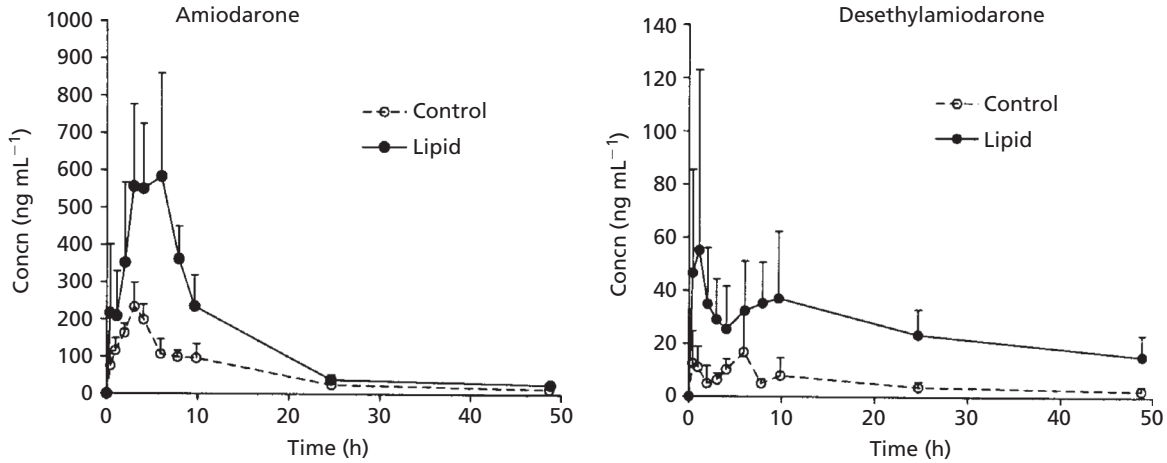

Figure 5 Plasma concentration vs time curves of amiodarone and desethylamiodarone in rat without (Control) and with ingestion of 1% cholesterol in peanut oil (Lipid).

include competitive inhibition for intestinal CYP caused by residual fatty acids remaining within the enterocytes after oral lipid, or depletion of cofactors required for reactions and shunting of cellular resources for preferential metabolism of the residual lipids present in the cells.

The plasma concentrations of both amiodarone and DEA were increased by the ingestion of oral lipid. This was observed previously for amiodarone using an HPLC UV assay, but with the improved sensitivity of the LC-MS assay (Shayeganpour et al 2007a), the AUC of DEA using a smaller dose of amiodarone could now be characterized. The increase in amiodarone concentrations are attributable to a combination of increased bioavailability and decreased hepatic clearance caused by the higher binding of drug to lipoproteins in the post-prandial state (Shayeganpour et al 2005). Although the data showed that pretreatment with oral lipid could decrease the intestinal metabolism of amiodarone to DEA, this did not translate into significant changes in the DEA-to-amiodarone ratio of AUC in the rat. This suggests that in the rat the apparent decrease in intestinal metabolism of amiodarone is outweighed by other factors that increase the plasma concentrations of both amiodarone and DEA. In rat intestine, amiodarone has been suggested to be a substrate for intestinal efflux transport (Kalitsky-Szirtes et al 2004). Hence it is possible that oral lipids decreased intestinal efflux (Custodio & Benet 2005), which in turn could have contributed to the higher amiodarone levels, and subsequently DEA concentrations after its systemic formation by hepatic CYP (Table 1, Figure 5).

In conclusion, it was found that small concentrations of SBS seemed to stimulate the metabolism of amiodarone in the everted gut sacs but at higher concentrations this did not occur. More notably, pretreatment of rats with peanut oil caused a decrease in metabolizing efficiency in the intestinal tissues, but not in microsomal preparations derived from those tissues. Although the results seem to indicate that fatty acids competitively interfere with the local metabolism of amiodarone in intestinal tissues, it did not result in an apparent change in the in-vivo plasma concentrations of metabolite in the rat.

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