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Gender-Related Differences in Mycophenolate Mofetil-Induced Gastrointestinal Toxicity in Rats

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Abbreviations: AUC, area under the concentration versus time curve; GI, gastrointestinal; HPLC, high performance liquid chromatography; MMF, mycophenolate mofetil; MPA, mycophenolic acid; MPAG, mycophenolic acid phenolic glucuronide; SD, Sprague-Dawley rats; UGT, uridine diphosphate glucuronosyltransferase

ABSTRACT

Mycophenolate mofetil (MMF), the prodrug of mycophenolic acid (MPA), is included in current combination immunosuppressive regimens following organ transplant. Treatment with MMF often results in dose-limiting gastrointestinal (GI) side effects. The underlying mechanisms responsible for these side effects are not fully understood, but exposure of the intestinal epithelia to MPA during enterohepatic recycling may be involved. The present study demonstrated that female rats are more susceptible to MMF-induced GI toxicity than male rats. Female SD rats treated chronically with an oral dose of 50 mg MPA equivalents/kg/day experienced greater GI toxicity than male rats, as measured by diarrhea grade and weight loss. Intestinal microsomes harvested from the upper jejunum of female rats had approximately three-fold lower MPA glucuronidation rates in comparison to male rats. In the remaining areas of the small and large intestine, there was also a trend toward decreased glucuronidation in the female rats. The area under the plasma concentration-time curve (AUC) for MPA following an oral 50 mg MPA equivalents/kg dose was roughly similar between genders, while the AUC for mycophenolic acid phenolic glucuronide (MPAG) was significantly lower in female rats. Female rats also excreted half of the biliary MPAG as male rats. The greater susceptibility of female rats to MMF-induced gastrointestinal toxicity, despite diminished intestinal MPA exposure via reduced biliary excretion of MPAG, may result from reduced protection of enterocytes by *in situ* glucuronidation. Likewise, susceptibility to MMF-induced GI toxicity in humans may also result from variable intestinal glucuronidation due to UGT polymorphisms or differential expression.

Mycophenolate mofetil (MMF, CellCept®, Roche Pharmaceuticals, Nutley, NJ), the prodrug of mycophenolic acid (MPA), is an important transplant medication (Sollinger, 2004). This agent is commonly used in combination with calcineurin inhibitors, such as tacrolimus or cyclosporine, as part of a combination immunosuppressive regimen to prevent allograft rejection following organ transplant (Takatsuka et al., 2003). MPA exerts its pharmacological effect via inhibition of inosine monophosphate dehydrogenase, the rate-limiting enzyme in *de novo* purine synthesis. This ultimately leads to the selective inhibition of lymphocyte cell division (Ransom, 1995). MMF is efficiently cleaved to MPA after oral administration, with very little intraduodenal hydrolysis observed (Sugioka et al., 1995; Sugioka et al., 1996). The primary metabolic pathway for MPA in both rats and humans is glucuronidation to form a phenolic glucuronide (MPAG) (Bullingham et al., 1998; Sugioka et al., 1996). MPAG can be excreted into the bile by the transporter, MRP2/ABCC2, and subsequently cleaved by β-glucuronidase to regenerate the parent, MPA (Kobayashi et al., 2004). MPA can then be reabsorbed, completing a cycle of enterohepatic circulation. Extensive enterohepatic recycling of MPA prolongs its half-life and increases intestinal exposure. Enhanced exposure of the enterocytes to MPA, via enterohepatic recycling, is thought to play a role in one of MMFs major side effects, gastrointestinal (GI) toxicity, which manifests as varying degrees of diarrhea (Behrend, 2001). Another factor that may play a role in modulating MPA GI toxicity is *in situ* metabolism of MPA. Several MPA-metabolizing uridine diphosphate glucuronosyltransferase (UGT) isoforms found within the GI tract are known to be polymorphically expressed, including UGTs 1A7, 1A8, and 1A9 (Huang et al., 2002; Vogel et al., 2002; Paoluzzi et al., 2004). Differential expression of intestinal UGTs may account for the variability in MPA GI toxicity seen in the patient population. The rat has been used previously as an animal model of MPA-induced GI

toxicity and, like humans, also develops diarrhea and anorexia (Matsumoto et al., 2002). The present study demonstrates that female rats are more sensitive to mycophenolate GI toxicity than male rats, despite having decreased gastrointestinal exposure via biliary excretion of MPAG. Metabolism studies support the hypothesis that diminished *in situ* glucuronidation within the intestine of female rats predispose them to mycophenolate GI toxicity.

MATERIALS AND METHODS

Materials. MPA (≥ 98% pure), *m*-hydroxybenzoic acid, and suprofen were purchased from Sigma-Aldrich Co. (St. Louis, MO). MPAG reference standard was prepared and characterized as described previously (Wiwattanawongsa et al., 2001). Solvents used for sample preparation and high performance liquid chromatography (HPLC) were obtained from Fisher Scientific Co. (Pittsburgh, PA). All other chemicals for this study were of the highest purity possible purchased from Sigma-Aldrich.

Animals and treatments. Three- to five-month-old male and female Sprague-Dawley (SD) rats were purchased from Charles River Laboratories (Wilmington, MA) and housed in a temperature- and humidity-controlled facility with 12 hr light-dark cycles. Animals were held in plastic cages with hardwood chips (Beta Chips, Northeastern Products Corp., Warrensburg, WI). They were provided with food (Prolab RMH #3000, PMI Nutritional International, Bretwood, MO) and water *ad libitum*. Animals were allowed to acclimate to housing conditions for at least one week prior to initiation of experiments. All animal studies were conducted after approval of protocols by the University Institutional Animal Care and Use Committee in approved facilities.

For the toxicology studies, male and female rats, five per group, were treated daily by oral gavage with a suspension of 70 or 105 mg MMF/kg (50 or 75 mg/kg in MPA equivalents, respectively) or aqueous vehicle for six days. Body weight and stools were monitored daily. Stools were graded for degree of diarrhea by the following scale: 0- firm stool, 1- malformed stool, 2- watery stool with perianal staining, 3- severe perianal staining (Takasuna et al., 1996). At the termination of the study, rats were killed by carbon dioxide asphyxiation.

For the pharmacokinetic study, male and female rats, five per group, were treated with oral doses of MMF, 50 mg MPA equivalents/kg/day, for two days. On the second day, following

the daily dose of MMF, blood (50 μL) was collected by tail nick for determination of MPA and MPAG pharmacokinetic profiles. Blood collection times were 15, 30, 60, 120, 180, 240, 360 and 540 minutes.

For the bile cannulation study, male and female rats, three per group, were purchased from Charles River Laboratories with in-dwelling recirculating bile catheters and externalized loops to access bile. Rats were treated with a suspension of 70 mg MMF/kg (50 mg/kg in MPA equivalents) by oral gavage. Bile collection intervals were concluded at 15, 30, 60, 120, 180, 240, 360, 480 minutes. Bile samples were diluted 1:20 with 100 mM sodium acetate (pH 5) in duplicate. Diluted samples were treated with β-glucuronidase (1250 u/mL) and incubated for 2 h at 37°C. Reactions were terminated by the addition four volumes of ice-cold acetonitrile. MPA concentration in the incubates was determined by HPLC. The increase in MPA area after β-glucuronidase treatment was attributed to MPAG.

Microsome Preparation. Rat hepatic, renal, and intestinal microsomes were prepared from male and female rats. Intestinal segments were defined as follows: duodenum - pyloric sphincter to ligament of Trietz; upper jejunum - upper half of remaining small intestine; lower jejunum-ileum - lower half of small intestine; colon - cecum to rectum (Kararli, 1995). Intestinal scrapings and portions of excised kidneys and livers were homogenized, and the microsomes isolated through differential ultracentrifugation (Tallman et al., 2005). Protein concentrations were determined using the Bradford assay with bovine serum albumin as the standard.

In Vitro MPA Glucuronidation Assay. The microsomal incubation conditions for determining the rate of MPAG formation were: Brij 35/mg (0.05%/mg protein), D-saccharic acid-1,4-lactone (10 mM), UDP-glucuronic acid (2 mM), magnesium chloride (10 mM), MPA (2 mM), and microsomal protein (0.25 mg/mL) in a total reaction volume of 1 ml Tris buffer (pH

7.0, 37°C). The MPA concentration employed (2mm) was several-fold greater than the Km in pooled rat hepatic, renal and intestinal microsomes (0.32, 0.44 and 0.48 mM, respectively), which was determined in a pilot study. By using this concentration, the reaction would proceed at Vmax and the resulting MPA glucuronidation rate could be used as a surrogate measure for UGT expression. Reactions were terminated at 10-30 min by the addition of four volumes of ice-cold acetonitrile. The reaction was found to be linear with respect to time and quantity of microsomal protein. The amount of MPAG formed in the incubations was determined by HPLC.

MPA and MPAG Analysis. Bile cannulation and microsomal incubation samples were precipitated with acetonitrile, spiked with suprofen internal standard (10 ug/mL in sample) and assayed for MPA and MPAG by HPLC with UV detection (Wiwattanawongsa et al., 2001). The HPLC conditions included a C-18 column (150 X 4.6 mm, Axxiom, Moorpark, CA), isocratic mobile phase (55% methanol: 45% aqueous trifluoroacetic acid [0.1%, pH 2.5]), flow rate of 1.5 mL/min, and UV detection at 250 nm.

Plasma samples were precipitated with acetonitrile, spiked with *m*-hydroxybenzoic acid as an internal standard (10 ug/mL in sample), and assayed for MPA and MPAG by liquid chromatography coupled with mass spectrometry. Samples were injected onto a Zorbax RX-C8 column (150 x 2.1 mm, 5 µM) (Agilent, Palo Alto, CA) at a flow rate of 0.3 mL/min under isocratic conditions. The mobile phase consisted of 55% methanol: 45% acetic acid (1%, pH 2.5). Compounds were detected using a single quadrupole mass spectrometer (Sciex API 100; MDS Sciex, Concord, CA), coupled with a TurboIonSpray (PE Sciex) electrospray interface in negative ion mode. Selected ion monitoring was used to detect hydroxybenzoic acid (m/z 137), MPAG (m/z 495), and MPA (m/z 319), at 2.0, 3.8, and 13.8 minutes, respectively. Analyte concentrations in samples were determined by comparison to the appropriate MPA and MPAG

standard curves prepared in blank rat plasma. The standard curve ranges for both MPA and MPAG were 0.2 -20 μg/mL. Intra- and inter-day variability was less than 5%.

Statistical Analyses. Non-compartmental analysis of plasma MPA and MPAG concentrations provided pharmacokinetic parameter estimates (WinNonlin). AUC values were calculated from 0-540 min since the dosing was to apparent steady-state and estimates of the extrapolated AUC were complicated by unreliable terminal half-life estimates, likely due to enterohepatic recycling. Statistical differences ($n \geq 3$, $p \leq 0.05$) were determined by Student's *t*-test. Finite diarrhea scores were analyzed using the Kruskal-Wallis nonparametric ANOVA and the rank sum test.

RESULTS

Effect of MMF on GI toxicity in male and female rats. Treatment of male and female rats with 50 mg MPA equivalents/kg/day for six days resulted in significant differences in percent loss of body weight (Figure 1a) and diarrhea score between genders (Figure 2a). At this lower dose, female rats had a significantly greater percent loss of body weight and diarrhea score than males. Female rats lost weight from treatment day 4 on, with a maximum loss of 25% of their initial body weight, while the body weight of male rats did not drop below their pre-treatment baseline weight (Figure 1a). Diarrhea in male rats was delayed two days relative to female rats, reaching an average score of 0.8 compared to 2.2 for females at the termination of the study on day 7 (Figure 2a). The higher dose of MPA employed was not discriminating between males and females. Treatment of rats at the 75 mg MPA equivalents/kg/day dose level resulted in a trend toward greater loss of body weight and diarrhea score in females compared to males that failed to reach significance (Figures 1b and 2b). Control animals in both the 50 and 75 mg/kg treatment groups gained weight over the study period and maintained a diarrhea score of zero (data not shown).

Pharmacokinetic study. The pharmacokinetic profiles of MPA and MPAG were similar for male and female rats following oral administration of 50 mg MPA equivalents/kg/day for two days (Figure 3). The MPA and MPAG profiles show a secondary peak at 3 hr for both genders, indicative of enterohepatic recycling. The pharmacokinetic parameters estimated from the plasma MPA and MPAG profiles are displayed in Table 1. Male rats had significantly greater area under the plasma time-concentration curve values (AUC) for MPAG than females (322 ± 42 compared to 248 ± 29 μg*min/mL in MPA equivalents), and there was a trend toward greater MPA AUC in females that was not significant (5530 ± 692 versus 3940 ± 787 ug*min/mL). The

plasma concentration maximum (C_{max}) and the time to maximum concentration (t_{max}) were similar in males and females for both MPA and MPAG. The plasma half-life ($t_{1/2}$) was not reported, as it was quite variable, possibly due to enterohepatic recycling.

Microsomal glucuronidation of MPA *in vitro*. The rate of renal microsomal glucuronidation of MPA was half the rate of hepatic glucuronidation, and did not differ significantly between males and females (Figure 4). The rate of renal glucuronidation for male and female rats was 1.6 ± 0.14 and 1.3 ± 2.2 nmoles/min/mg microsomal protein, respectively. The rate of hepatic glucuronidation for male and female rats was 2.2 ± 0.16 and 2.2 ± 0.17 nmoles/min/mg microsomal protein, respectively.

Segmental analysis of MPA glucuronidation in the intestine revealed that the upper jejunum had the highest activity, followed in descending order by the lower jejunum-ileum, duodenum, and colon. The microsomal MPA glucuronidation rate in the upper jejunum of male rats was more than twice as high as female rats, 1.65 ± 0.28 vs. 0.67 ± 0.19 nmoles/min/mg microsomal protein, respectively (Figure 5). In the duodenum, lower jejunum-ileum, and colon, there was a trend toward greater MPA glucuronidation in the males that did not reach significance (Figure 5).

Bile Cannulation Study. Male and female rats excreted significantly different amounts of MPAG into the bile after an oral dose of MMF (Figure 6). The amount of unconjugated MPA excreted into the bile by either gender was negligible (data not shown). Male rats excreted more than twice as much biliary MPAG as females in the eight hour collection period, 20 ± 7% versus 50 ± 11% of administered drug (mean ± SE), respectively.

DISCUSSION

Female rats, in comparison to males, had significantly lower plasma MPAG levels and a trend towards higher plasma MPA levels (Table 1). The ratio of the plasma concentration of MPAG to MPA, at MPAG t_{max}, was more than twice as high for males as females (0.17 ± 0.05 versus 0.060 ± 0.006, $p \leq 0.05$). Interestingly, a clinical study has also observed higher average MPAG/MPA plasma concentration ratios in the male subjects than females (Morissette et al., 2001), indicating that male and female rats may be a good model for MPA/MPAG disposition for their respective sex in the human population.

The bioavailability of MPA following intraduodenal MMF in the male rat has previously been determined to range from 64-84%, with the lower values corresponding to the higher 33 mg MMF/kg doses utilized (Sugioka et al., 1996). If one assumes that bioavailability is approximately 50% for the 70 mg MMF/kg dose administered in the present biliary excretion study, then the estimates of excreted dose would be 40% and 100% of the bioavailable drug for female and male rats, respectively. The increased excretion of biliary MPAG by male rats (Figure 6) may result from elevated transport or enhanced generation of MPAG in the male rats. While evidence that MPAG is excreted into the bile by MRP2 (Kobayashi et al., 2004) was recently confirmed using TR- rats (Westley et al., 2006), studies have not observed differences in hepatic MRP2 mRNA levels between male and female rats at three months of age on (Johnson et al., 2002). Thus, it would appear that the increased excretion of MPAG into the bile of male rats is the direct result of increased formation of the metabolite rather than increased transport in the male rats. The greater MPA glucuronidation activity observed in the intestine of male rats may be responsible for this increased rate of biliary excretion, via first-pass metabolism during absorption of MPA (Figure 5), since there is evidence that intestinal metabolism may influence

systemic MPA exposure in rats (Sugioka et al., 1996). By comparing plasma MPA profiles following intraduodenal administration or portal infusion of MPA, Sugioka *et al.* estimated that poor absorption or intestinal metabolism accounts for a 10-30% decrease in MPA bioavailability in the rat. Portal and systemic circulation of intestinally generated MPAG may account for the observed gender differences in plasma and biliary MPAG, respectively, in the absence of differences in hepatic MPAG formation rates (Figure 4). Reports indicate that intestinal glucuronidation was found to be an important source of biliary glucuronide, such as for the 7-O-β-glucuronide of genestien in Sprague-Dawley rats (Sfakianos et al., 1997).

The acyl glucuronide of MPA is a minor metabolite, with plasma concentrations less than 1% of MPAG in patients (Tedesco-Silva et al., 2005). In our rat studies, the acyl glucuronide also represented a minor metabolite, accounting for less then approximately 5% of total glucuronides (data not shown), in agreement with formation rates in perfused rat liver (Westley et al., 2006). Some researchers, however, have proposed that even though the acyl glucuronide is a minor metabolite, it could play a role in MMF GI side-effects and therapeutic efficacy (Shipkova et al., 2002). Acyl glucuronides are known to be reactive and the acyl glucuronide of MPA has been shown to covalently bind proteins *in vivo* (Shipkova et al., 2002; Shipkova et al., 2004). Additionally, the acyl glucuronide of MPA has been shown to cause release of inflammatory cytokines from leukocytes *in vitro*, which could contribute to MMF-induced GI inflammation (Wieland et al., 2000). The acyl glucuronide of MPA may also possess immunosuppressive activity and, thus, contribute to therapeutic efficacy (Shipkova et al., 2001). However, the *in vitro* leukocyte proliferation data supporting pharmacological activity of the acyl glucuronide did not control for ester hydrolysis and could have been due to contamination with MPA itself. Since this is such a minor metabolite and difficult to stabilize, plasma or

intestinal acyl glucuronide concentrations were not measured in the present study. Nevertheless, in rats treated chronically with MMF, one colonic protein was reported to be a putative target of acyl MPAG, however, the putative MPA adduct was not confirmed beyond the presence of a reactive spot on a 2D gel with MPA-derived antibody (Shipkova et al., 2004).

Enterohepatic recycling of MPA increases systemic and intestinal exposure in man and rats (Behrend, 2001), and would be expected to contribute to intestinal toxicity. Contrary to this expectation, female rats experienced greater GI toxicity than male rats despite excreting less biliary MPAG (Figures 1, 2, and 6), a process integral to enterohepatic recycling. MPAG excreted into rat bile would be expected to be efficiently cleaved to MPA via intestinal β-glucuronidase, an enzyme produced by intestinal bacteria. Studies have shown that intestinal β-glucuronidase in the rat is extremely high in comparison to other species (Hawksworth *et al.*, 1971), and due to this excess capacity it is highly unlikely that gender related difference in bacterial B-glucuronidase would account for the observed differences in toxicity. The increased sensitivity of female rats to MMF-related GI side effects may have resulted from increased systemic exposure to MPA (Table 1), since there was a trend toward greater plasma MPA AUC and C_{max} in the female rats. However, this trend did not reach significance, and to date there is limited evidence for systemic exposure contributing to intestinal toxicity. In support of a role for systemic exposure in MPA intestinal toxicity, adverse GI events have been shown to correlate with the dose of MMF administered and MPA AUC in clinical trials (Sollinger et al., 1992; Van Gelder et al., 1999). Since MMF dose and MPA AUC would also correlate with intestinal exposure via enterohepatic recycling, these data support the involvement of intestinal exposure as well.

The increased sensitivity of female rats to mycophenolate-induced GI toxicity may be explained by the fact that female rats have decreased intestinal MPA glucuronidation activity in the upper jejunum and a trend toward decreased activity in other regions of the intestine as well. This pattern of intestinal glucuronidation activity, excluding the low duodenal activity, is similar to the pattern observed for p-nitrophenol, which is also a substrate for UGT 1A6 (Koster et al., 1985; Miles et al., 2006). The upper jejunal segment analyzed comprises approximately half of the rat small intestine, thus decreased MPA glucuronidation ability in this region may predispose the female rat to greater MMF-induced GI toxicity. This mechanism could also explain the fact that the higher 75 mg MPA/kg dose was not discriminating between males and females, as saturation of glucuronidation could conceivably have occurred at this higher dose (Figures 1 and 2).

Gender-related differences in glucuronidation and UGT expression patterns have been observed previously in the rat. For example, male rat liver microsomes were reported to have 47% greater glucuronidation activity for p-nitrophenol than females (Catania et al., 1995) and female rat livers express three times as much UGT1A8 mRNA than males (Shelby et al., 2003). However, the pattern of intestinal MPA glucuronidation activity in the present study does not agree with the mRNA expression patterns of the rat intestinal UGTs that conjugate MPA, UGTs 1A1, 1A6, and 1A7 (Shelby et al., 2003; Miles et al., 2005). The mRNA expression of rat UGT 1A1, 1A6, and 1A7 were reported to be similar throughout the small intestine, with greater expression of 1A1 and 1A6 in the large intestine, and no gender-dependent expression patterns (Shelby et al., 2003). This discrepancy may be explained by the fact that the relationship between amount of mRNA and "signal" in the branched chain assay (method used by Shelby et al.) is not necessarily constant, due to the sequences of the oligonucleotide probes and their

specific characteristics. Alternatively, these inconsistencies may be explained by post-translational changes in UGT activity resulting from phosphorylation or glycosylation, or due to inconsistent relationships between mRNA and protein expression when different proteins are compared (Barbier et al., 2000; Basu et al., 2003).

The hypothesis that diminished intestinal glucuronidation of mycophenolic acid is responsible for the greater susceptibility of the female rats is intriguing, since several human MPA metabolizing UGT isoforms found within the GI tract are known to be polymorphically expressed, including UGT 1A7, 1A8, 1A9 (Huang et al., 2002; Vogel et al., 2002; Paoluzzi et al., 2004). Recently, an association between UGT1A9 polymorphism and MPA systemic exposure has been demonstrated in renal allograft patients receiving MMF (Kuypers et al., 2005). The influence of the UGT1A9 polymorphism on MPA systemic exposure is thought to be at least partially the result of altered enterohepatic recycling, possible due to variable intestinal glucuronidation of MPA (Hesselink and Gelder, 2005). The 1A9 polymorphism and MPA systemic exposure in this clinical study also correlated with GI side effects, though this correlation did not reach significance (Kuypers et al., 2005). In addition to genetic polymorphisms, variable expression of intestinal UGTs, resulting from other genetic or environmental factors, may also be responsible for mitigating MPA-induced GI toxicity in man. Cumulatively, the resulting inter-individual variability in intestinal MPA metabolism could account for the susceptibility to GI side-effects observed in mycophenolate patients.

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FOOTNOTES:

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LEGENDS FOR FIGURES:

Figure 1. Toxicology data for SD rats treated with MMF. Body weight measurements (mean ± SE, *n* = 5) for male (-■-) and female (-□-) rats treated with **(a)** 50 or **(b)** 75 mg MPA equivalents /kg/day for 6 days. * indicates $p < 0.05$ as determined by Student's *t* test.

Figure 2. Toxicology data for SD rats treated with MMF. Diarrhea score measurements (mean ± SE, *n* = 5) for male (-■-) and female (-□-) rats treated with **(a)** 50 or **(b)** 75 mg MPA equivalents/kg/day for 6 days). Stools were graded for degree of diarrhea by the following scale: 0- firm stool, 1- malformed stool, 2-watery stool with perianal staining, 3-severe perianal staining. * indicates $p<0.05$ as determined by a Kruskal-Wallis nonparametric ANOVA and rank sum test.

Figure 3. MPA (a) and MPAG (b) plasma pharmacokinetic profiles for male (-▲-) and female (-■-) rats treated with 50 mg MMF/kg/day given via oral gavage for two days. Each data point with associated SE bars represents the average concentration from n = 5 rats per gender group.

Figure 4. MPA glucuronidation activity in renal and hepatic microsomes from male (■) and female (□) rats. In vitro reactions contained 2 mM MPA. Bars are mean glucuronidation rate per gender group ± SE (*n*=5).

Figure 5. MPA glucuronidation activity in intestinal microsomes from male (■) and female (□) rats. In vitro reactions contained 2 mM MPA. Bars are mean glucuronidation rate per gender group ± SE (*n*=5). * indicates $p<0.05$ as determined by Student's *t* test.

Figure 6. Biliary MPAG excretion in male (■) and female (□) rats treated with 50 mg MPA equivalents/kg. Bars are mean glucuronidation rate per gender group ± SE (*n*=5). * indicates $p<0.05$ as determined by Student's *t* test.

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Table 1

Non-compartmental plasma MPA and MPAG pharmacokinetic parameters for male and female SD rats treated with 50 mg MPA equiv./kg/day for two days. Data are mean ± SE from n = 5 rats. Values for MPAG AUC and C_{max} are expressed in MPA equivalents.

	MPA		**MPAG**	
	Male	Female	Male	Female
AUC[a] (μg*min/mL)	3940 ± 787	5530 ± 692	**322 ± 42**[b]	**252 ± 29**
C_{max} (μg/mL)	21 ± 5	26 ± 3	0.7 ± 0.3	0.5 ± 0.1
t_{max} (min)	30 ± 0	30 ± 0	121 ± 81	59 ± 39

[a] AUC from 0 to the last sampling time, 540 min
[b] *p<0.05*

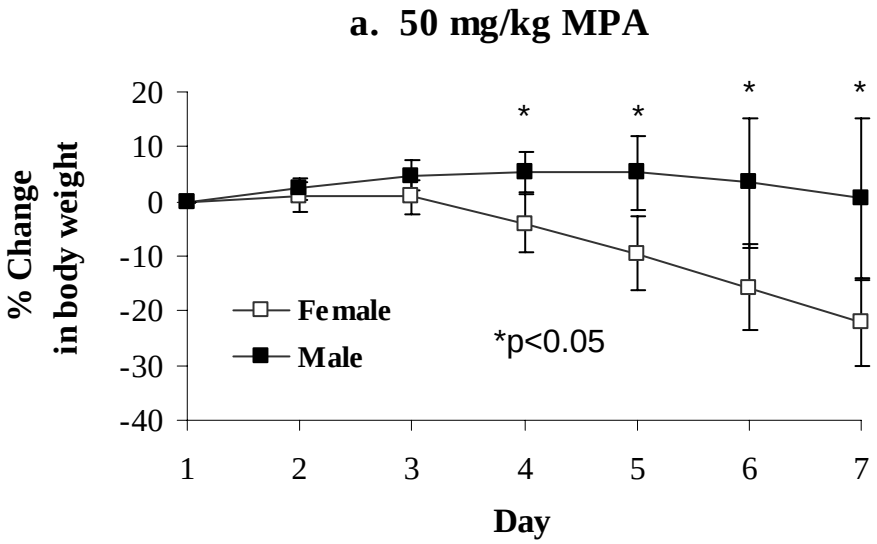
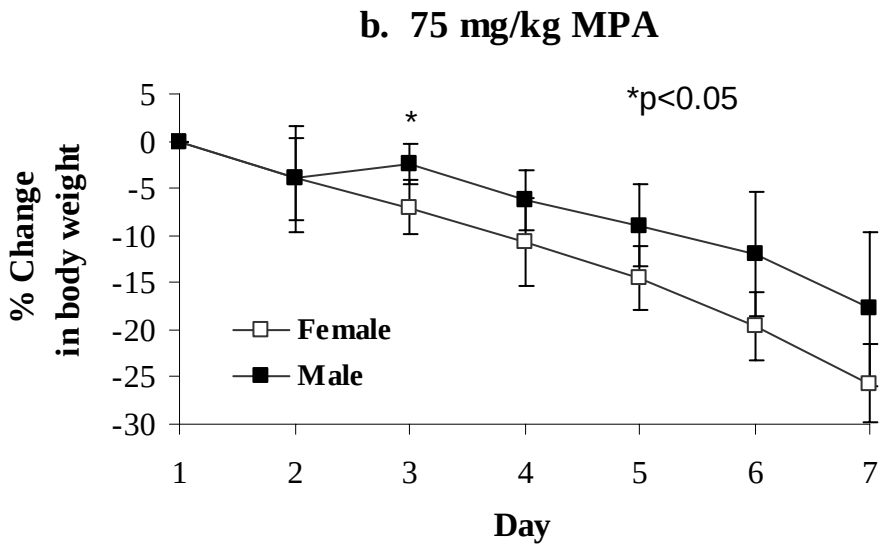


Figure 1a



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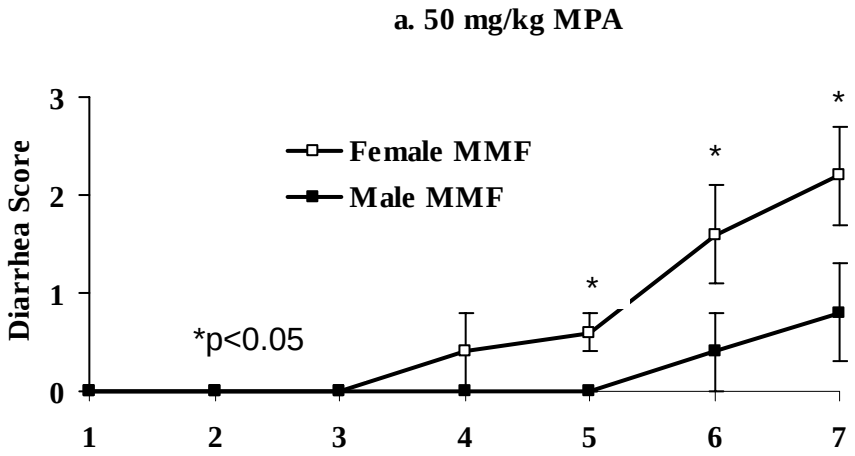

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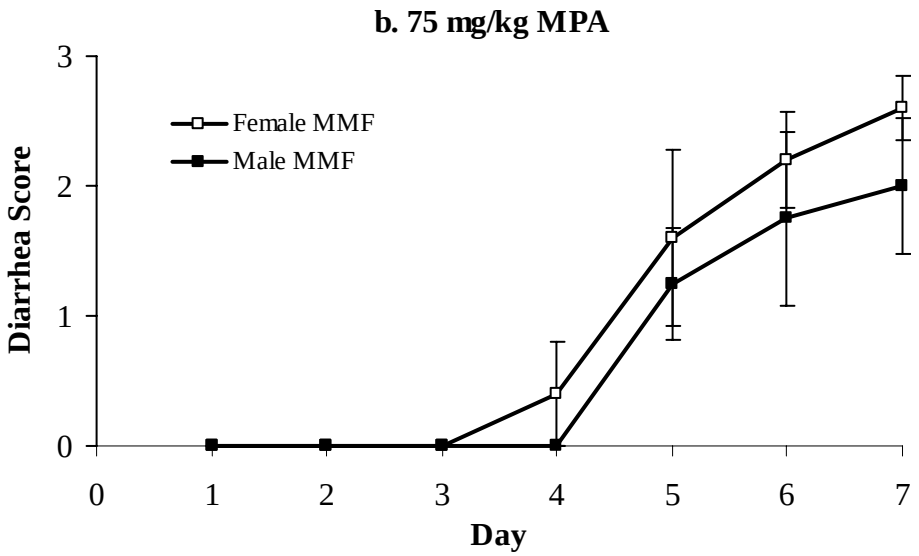

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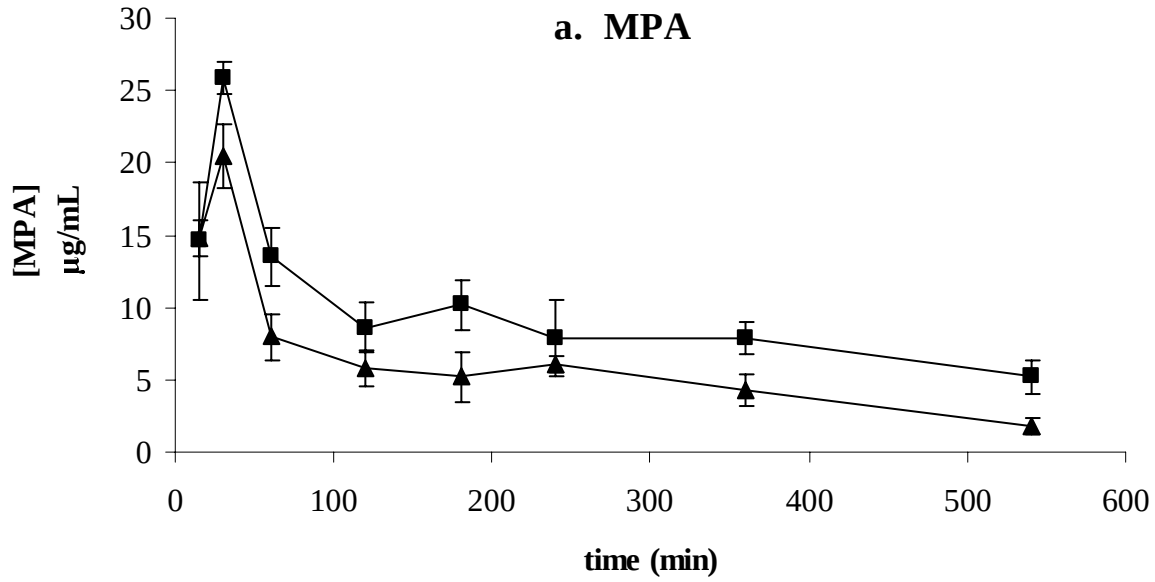
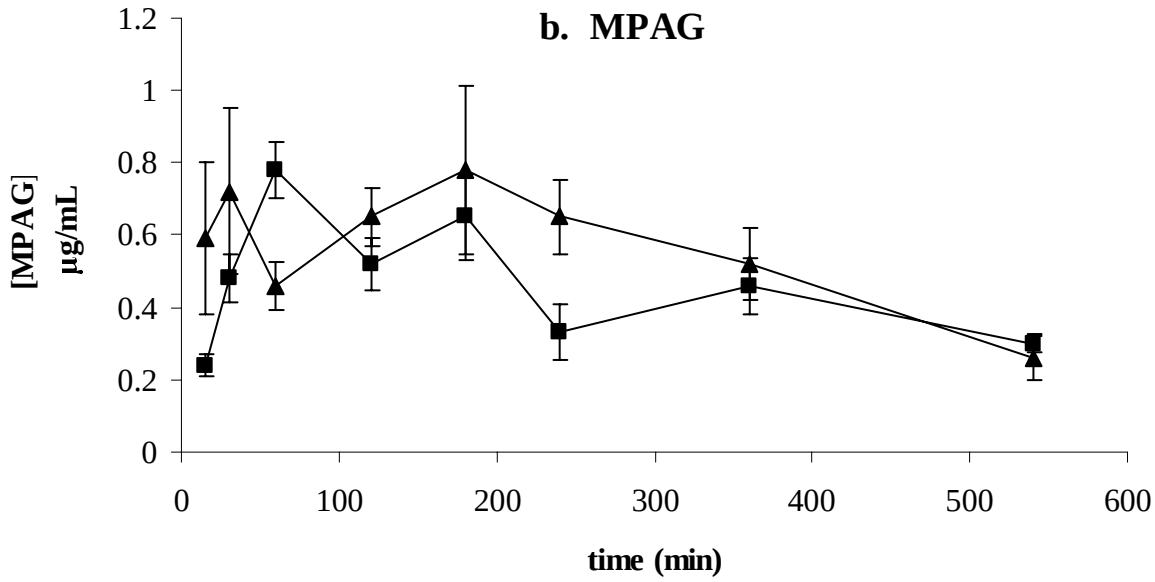


Figure 3a.



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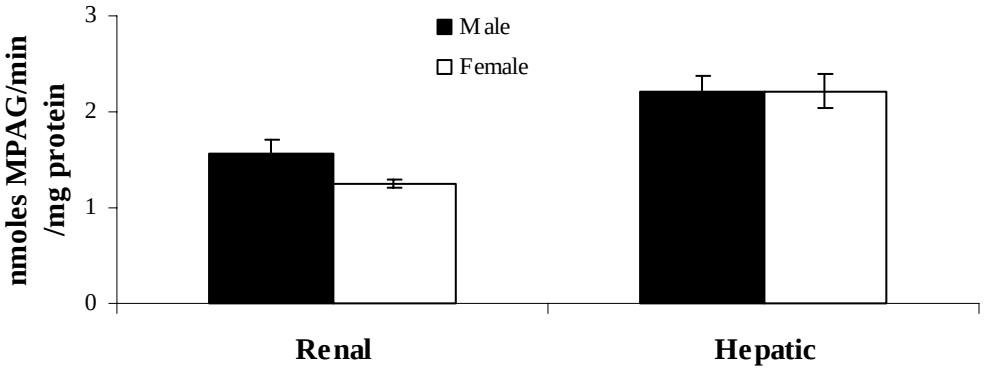

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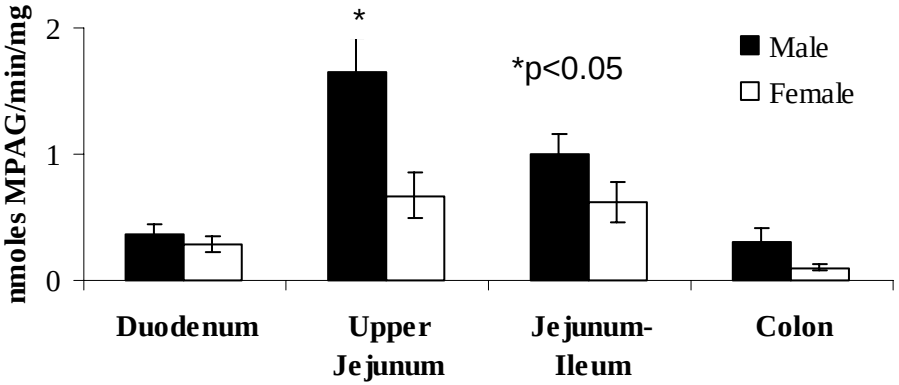

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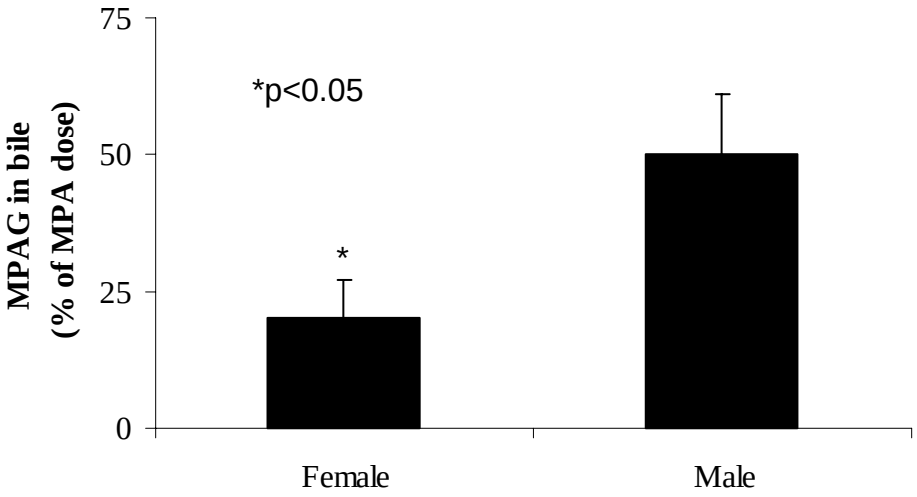

Figure 6.