Short Communication

THE MAIN ROLE OF UGT1A9 IN THE HEPATIC METABOLISM OF MYCOPHENOLIC ACID AND THE EFFECTS OF NATURALLY OCCURRING VARIANTS

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ABSTRACT:

Mycophenolic acid (MPA), a standard immunosuppressive drug, is characterized by unexplained highly variable pharmacokinetics in transplant recipients. The primary metabolic pathway of MPA is glucuronidation; however, literature reports are inconsistent and the contribution of all human UDP-glucuronosyltransferases (UGTs) has never been systematically assessed. Sixteen heterologously expressed UG Ts were studied for MPA 7-O-glucuronidation and compared with liver, kidney, and intestine microsomes. For direct measurement of MPA and mycophenolic acid 7-glucuronide (MPAG), we used a liquid chromatography-mass spectrometry protocol. Metabolic studies demonstrated that the glucuronidation of MPA in humans occurs mainly in the liver at variable rates, as well as in the kidney and the intestine. Kinetic data normalized for UGT expression levels point to 1A8 and 1A9 as the main UGTs, as well as to low-activity enzymes, 1A1, 1A7, and 1A10. According to their expression profiles, 1A8 and 1A9 are proposed, respectively, as the main extrahepatic and the only hepatic enzymes involved. The effects of naturally occurring 1A6 (*2 and *3) and 1A9 (*2 and *3) variants on MPA 7-O-glucuronidation were studied and data suggest that the C277Y of 1A8 and the M35T of 1A9 may influence MPAG levels. A number of low-activity alleles were also identified in 1A1 (*6 (G71R), *7 (Y486D), *27 (P229Q), and *35 (L233R)), 1A7 (*3 (K129K131D139R208), *4 (R208), *5 (S115), *8 (K128K131D139R208), and *9 (S115K129K131)), and 1A10 (*2 (E139K)). Our study provides preliminary evidences that genetic factors, especially in the hepatic UGT1A9, may contribute to the variability of MPA pharmacokinetics observed in transplant patients.

Mythophenolic acid (MPA), the active metabolite of the produg mycophenolate mofetil (MMF), is a standard immunosuppressive drug used after solid organ transplant and in several autoimmune diseases. MMF is currently being used for the prophylaxis of acute rejection in liver, kidney, heart, and lung transplantation (Shaw et al., 1998). Its mechanism of action involves the direct inhibition of inosine monophosphate dehydrogenase, thus preventing the proliferation of B and T lymphocytes (Lipsky, 1996). Several studies in various transplant subpopulations have recognized the large variability in the pharmacokinetic parameters of the drug, thus emphasizing the need to individualize MMF dosing (Bullingham et al., 1998; Ensom et al., 2002). Potential causes of such variability include genetic variations in the metabolic pathways of the drug. To explore this hypothesis, we need to accumulate data on the enzymes involved in the metabolism of MPA. Its metabolism of MPA is for the most part mediated by UDP-glucuronosyltransferases (UGTs) (Bullingham et al., 1998).

In vitro metabolic studies with microsomes prepared from human tissues predict that the formation of MPAG occurs in the kidney and the intestine but predominantly in the liver (Bowlagha and Miners, 2001; Shipkova et al., 2001). However, the identity of specific UGT enzymes involved in the formation of MPA glucuronide derivatives is still not well defined since literature reports are inconsistent, and there have been no systematic studies of all known functional UGTs. To date, 16 functional proteins have been identified in humans and are classified in distinct families and subfamilies based on their sequence homology. The UGT1A subfamily includes nine functional isoforms (UGT1A1 and 1A3–10), while the UGT2B subfamily comprises seven isoforms (UGT2B4, 2B7, 2B10, 2B11, 2B15, 2B17, and 2B28) (Guillemette, 2003). A first study, using thin-layer chromatography to detect the formation of MPA glucuronide metabolites in enzymatic assays with 14C-UDP-glucuronic acid, indicated UGT1A8, 1A9, and 1A10 as potent isoforms toward MPA, whereas other isoenzymes, namely UGT1A1, 1A3, 1A4, 1A6, 2B7, and 2B10, were found to be totally inactive (Mackenzie, 2000). In contrast, a second study, supported by a high-performance liquid chromatography protocol with UV detection, found that UGT1A1, 1A3, 1A4, 1A6, 1A7, 1A9, 1A10, 2B4, 2B7, and 2B15 had similar capacities for the formation of MPAG, 1A7 and 2B4 being most efficient (Shipkova et al., 2001). Not all UGTs were tested in the same experimental conditions in both of these studies, and a systematic approach to identify the UGT(s) responsible for the formation of the main metabolite MPAG was still missing.

The main objective of this study was to test all 16 UGT1A and UGT2B human recombinant UGT proteins known to date for their capacity to form MPAG in the same experimental conditions. For the determination of MPA and MPAG, we have first developed a simple, rapid, and sensitive high-performance liquid chromatography coupled

ABBREVIATIONS: MPA, mycophenolic acid; UGT, UDP-glucuronosyltransferase; MPAG, mycophenolic acid 7-O-glucuronide; MMF, mycophenolate mofetil; LC/MS, liquid chromatography coupled with mass spectrometry.
with mass spectrometry (LC/MS) method. Experiments were performed in parallel to assess the conjugation activities of liver, kidney, and intestine microsomes, and those were compared with recombinant UGTs. Finally, the effects of common polymorphism of UGT allozymes involved in the metabolism of MPA were investigated to gain information on the potential role of genetic factors to the variability in pharmacokinetic parameters of MMF therapy.

Materials and Methods

Chemicals and Reagents. UDP-glucuronic acid and MPA were obtained from Sigma Diagnostics Canada (Mississauga, ON, Canada). MPAG was a generous gift from Hoffman-La Roche (Mississauga, ON, CA). All other chemicals and reagents were of the highest grade and commercially available.

Human UGT1A and UGT2B Alleles and Tissues. UGT-HEK293 cells including 1A1, 1A7, and 1A9 polymorphic allozymes have been described previously (Gagne et al., 2002; Villeneuve et al., 2003). Before enzymic assays, Western blot analyses and catalytic activities of known substrates were performed for each preparation. In addition, preparations of UGT1A1, 1A9 (BD Gentest, Woburn, MA), 1A7, and 1A0 (PanVera Corp., Madison, WI) were obtained from commercial sources to validate results obtained with UGT-HEK293 cells. Two pools of human liver (n = 5 subjects), two human kidney samples (white male/head trauma and white female/stroke) and two pools of human intestine (jejunum, n = 10; ileum, n = 5) were obtained from Tissue Transformation Technologies (Edison, NJ). Four additional human liver samples obtained from Human Cell Culture Center Inc. (Laurel, MD) were also studied. UGT1A8 and 1A9 (BD Gentest, Woburn, MA), 1A7, and 1A10 (PanVera Corp., Madison, WI) were obtained from commercial sources including public and proprietary sources.

Enzymatic Assays. To determine which UGTs might be involved in the formation of MPAG, initial experiments with UGT1A and UGT2B microsomes including commercial preparations consisted of 4- and 16-h incubations at 37°C with 0.2 mM MPA, 10 to 50 μg of UGT membrane protein, 50 mM Tris-HCl, 10 mM MgCl₂, 1 mM UDP-glucuronic acid, pepstatin, and phosphatidylcholine, yielding a pH of 6.8, in a standard procedure performed in our laboratory (Gagne et al., 2002). Detergent was omitted in the enzymic assays since a previous study demonstrated no beneficial activation with common detergents (Shipkova et al., 2001). Determination of Vₘₐₓ and Kₘ was then performed for UGTs with a significant glucuronidation activity for MPA (>5% glucuronide formation). UGTs and human liver, kidney, and intestine microsomes were incubated for 1 h with MPA (1–1000 μM). Kinetic parameters were also determined for allelic variants UGT1A8*1, *2 (A 173 G), and *3 (C⁷⁷⁸Y), and for UGT1A9*1, *2 (C⁶⁷⁷G), and *3 (M⁸⁸⁸F). For the low-activity UGTs, namely 1A1, 1A7, and 1A10, activities were determined using 1 mM MPA. Visual inspection of fitted functions (V as a function of [S]) and Eadie-Hofstee plots (V as a function of [S][S]) was used to select the best-fit enzyme kinetic model. Kinetic parameter calculations were performed with SigmaPlot 8.0 software assisted by Enzyme Kinetics 1.1 software (SPSS Inc., Chicago, IL). Values were expressed as the mean of at least two experiments performed in duplicate. p value calculations were performed with JMP 4.0.2 software (SAS Institute, Cary, NC) using Student’s t test.

Results and Discussion

The present in vitro incubation studies using human liver, intestine, and kidney microsomes and cDNA-expressed human UGTs clearly demonstrate that the metabolism of MPA occurs at multiple sites and is mediated primarily by two UGT isoforms, the extrahepatic UGT1A8 and the hepatic UGT1A9. Specific coding region polymorphic alleles of the high MPA-glucuronidating activity UGT1A8 and UGT1A9, as well as in the low-activity UGTs, namely UGT1A1, 1A7, and 1A10, are predicted to have an impact on the metabolism of MPA.

MPAG Formation by Liver, Kidney, and Intestine Microsomes

Previous studies proposed the liver to be the main organ involved in the metabolism of MPA, whereas the kidney demonstrated one of the highest efficiencies to generate MPAG of all tissues tested (Bovalgaha and Miners, 2001; Shipkova et al., 2001). In this study, the two pools of human liver microsomes and individual samples of human kidney displayed similar capacities to generate MPAG (Clₘₐₓ from 42 to 102 μl/min/mg protein) (Table 1). A considerable variability in kinetic parameters was observed between individual liver samples as well as between pools, especially in the values of velocity, ranging

![Table 1](https://example.com/table1.png)

**Table 1**

Kinetic parameter estimates for MPAG formation by human liver, kidney, and jejunum microsomes

The values of apparent Kₘ and Vₘₐₓ for the formation of MPAG were determined using micromolar preparations from two pools of human liver (HL-P1/2), four human liver samples (HL-S1, -2, -3, and -4), two individual samples of human kidney (HK-S1/2), a pool of human jejunum (HI-P), and a pool of human ileum (HI-P), all obtained from commercial sources.
from 4822 to 13,790 pmol/min/mg protein. The highest affinity was observed using pooled liver samples with $K_m$ values of 95 to 135 $\mu$M, whereas the highest rates were observed with kidney microsomes, with velocities ranging from 23 to 36 nmol/min/mg, 3-fold higher in mean than liver microsomes. On the other hand, pooled kidney microsomes demonstrated the lowest affinity, with $K_m$ values ranging from 553 to 887 $\mu$M. Interestingly, HL-P2, which demonstrated a superior catalytic efficiency for MPA glucuronidation, was also found to have the highest UGT1A9 protein expression compared with other liver samples (data not shown). Additional liver samples from four unrelated subjects were also investigated, and the affinity for the formation of MPAG was in the same range as the $K_m$ observed for pooled livers, ranging from 151 to 272 $\mu$M (Table 1). Three liver samples, HL-S1, HL-S2, and HL-S3, were found to have similar capacities to generate MPAG with $Cl_{\text{int}}$ ranging from 12 to 18 $\mu$l/min/mg protein. The fourth sample, HL-S4, had a 2-fold higher catalytic efficiency for MPAG formation compared with the other samples. As for the human liver pools, catalytic efficiencies of all four liver samples were found to be associated with the level of UGT protein content assessed by Western blot analysis (data not shown).

The two pools of jejunum and ileum microsomes had a significantly lower activity toward MPA, with 2- to 7-fold reduced catalytic efficiencies compared with the other tissues tested. In a previous study, it was reported that MPA glucuronidation activity is similar in jejunum and ileum (Shipkova et al., 2001), consistent with a contribution of the gastrointestinal tract to the first-pass metabolism of MPA. The present study has found ileum microsomes to have a lower capacity to generate MPAG compared with jejunum. This observation is in agreement with previous studies reporting that UGT activity is the highest in the jejunum within the small intestine (Tukey and Strassburg, 2000).

**Identification of Human UGTs Involved in the Formation of MPAG.** For the first time, all 16 known human UGTs were tested in the same experimental conditions. A sensitive LC/MS protocol was used for the detection of MPA and MPAG with authentic standards, the same experimental conditions. A sensitive LC/MS protocol was performed for all UGTs for which MPAG formation was significant, namely, UGT1A1, UGT1A7, UGT1A8, UGT1A9, and UGT1A10, as well as for variant allozymes of UGT1A8 and UGT1A9. Sites of expression are: L, liver; K, kidney; GI, gastrointestinal tract; --, not expressed in L, K, or GI. Kinetic profiles are: H, hyperbolic; S, sigmoid.

**TABLE 2**

**Kinetic parameter estimates for MPAG formation by all reactive UGTs and their variant allozymes**

Determination of apparent $V_{\text{max}}$ and $K_m$ was performed for all UGTs for which MPAG formation was significant, namely, UGT1A1, UGT1A7, UGT1A8, UGT1A9, and UGT1A10, as well as for variant allozymes of UGT1A8 and UGT1A9. Sites of expression are: L, liver; K, kidney; GI, gastrointestinal tract; --, not expressed in L, K, or GI. Kinetic profiles are: H, hyperbolic; S, sigmoid.

<table>
<thead>
<tr>
<th>UGT Source</th>
<th>Sites of Expression</th>
<th>$n$</th>
<th>$K_m$ $\mu$M</th>
<th>$V_{\text{max}}$ pmol/min/mg</th>
<th>$V_{\text{max}}$ pmol/min/mg</th>
<th>$Cl_{\text{int}}$ $\mu$l/min/mg</th>
<th>Kinetic Profile</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A1*</td>
<td>L, GI</td>
<td>--</td>
<td>185 ± 86</td>
<td>336 ± 37</td>
<td>24 ± 3</td>
<td>0.1</td>
<td>H</td>
</tr>
<tr>
<td>1A7*</td>
<td>--</td>
<td>--</td>
<td>30 ± 1</td>
<td>370 ± 7</td>
<td>23 ± 0.4</td>
<td>0.8</td>
<td>H</td>
</tr>
<tr>
<td>1A8</td>
<td>GI</td>
<td>1.7</td>
<td>298 ± 82</td>
<td>32,570 ± 5742</td>
<td>25,252 ± 4452</td>
<td>43</td>
<td>S</td>
</tr>
<tr>
<td>*1</td>
<td>(A$^{177}$G)</td>
<td>1.5</td>
<td>317 ± 69</td>
<td>26,858 ± 4760</td>
<td>24,461 ± 4335</td>
<td>41</td>
<td></td>
</tr>
<tr>
<td>*3 (C$^{177}$Y)</td>
<td>1.3</td>
<td>118 ± 56</td>
<td>1864 ± 614</td>
<td>5371 ± 1711</td>
<td>28</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1A9</td>
<td>L, K, GI</td>
<td>1.7</td>
<td>291 ± 31</td>
<td>8380 ± 1506</td>
<td>8380 ± 1506</td>
<td>29</td>
<td>H</td>
</tr>
<tr>
<td>*2 (C$^{174}$Y)</td>
<td>2.2</td>
<td>224 ± 32</td>
<td>9873 ± 2865</td>
<td>10,703 ± 3105</td>
<td>48</td>
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<td></td>
</tr>
<tr>
<td>*3 (M$^{179}$T)</td>
<td>1.7</td>
<td>720 ± 250$^{a}$</td>
<td>4416 ± 500</td>
<td>11,888 ± 1346</td>
<td>17</td>
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<td></td>
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<tr>
<td>1A10*</td>
<td>GI</td>
<td>--</td>
<td>119 ± 12</td>
<td>434 ± 37</td>
<td>10 ± 0.8</td>
<td>0.1</td>
<td>H</td>
</tr>
</tbody>
</table>

$^{a}$ For relative velocities, absolute rates were divided by UGT protein expression values assessed by Western blot using a polyclonal anti-UGT1A antibody as described under Materials and Methods.

$^{b}$ Microsomes obtained from commercial sources.

$^{p} < 0.05$ vs. *1; $^{p} < 0.05$ vs. *2.
more, only UGT1A9 was reported to be expressed in the kidney, a tissue that exhibits a significant glucuronidation activity for MPA (Bowalgaha and Miners, 2001; Shipkova et al., 2001).

**MPAG Formation by UGT Variants.** Genetic polymorphisms that affect the metabolic activity or expression of UGT biotransformation enzymes, especially UGT1A8 and UGT1A9, may be important contributors to interindividual differences in MPA pharmacokinetics. In line with this hypothesis, we have investigated several known polymorphisms of the UGT1A8 and UGT1A9 genes (Table 2). UGT1A8*1 and *2 (A173G) allelic variants possess similar kinetic values, 298 ± 82 and 317 ± 69 μM, respectively. In turn, the Km of the UGT1A8*3 (C277Y) allele was not significantly lower (118 ± 56 μM; p = 0.12). After normalization for the level of UGT-expressed proteins, the relative velocity of the UGT1A8*3 allozyme (C277Y) (5371 pmol/min/mg) was drastically decreased by 5-fold, compared with the UGT1A8*1 protein (25,252 pmol/min/mg) (p = 0.03). Conversely, compared with the UGT1A9*1 protein, the velocities of the *2 and *3 alleles remain unchanged. In turn, compared with the UGT1A9*1 (Km = 291 ± 31 μM) and UGT1A9*2 (Km = 224 ± 32 μM) proteins, the affinity of the UGT1A9*3 (M33T) allozyme was significantly decreased (Km = 720 ± 250 μM; p = 0.05) (Table 2). Clint of the UGT1A9*3 allele was 1.7-fold lower than that of the UGT1A9*1 protein. In previous studies, several mutations in the UGT1A1 gene were identified that induced nonsynonymous amino acid alterations, including G129R (*6), Y146D (*7), P229Q (*27), and L233R (*35) (Guillemette, 2003). All variant UGT1A1 proteins were shown to have a substantial decrease (reduced by 86–100%) toward the formation of MPAG compared with the 1A1*1 protein. Since both UGT1A7 and UGT1A10 demonstrated detectable MPA glucuronidation activity, all nine 1A7 and the two 1A10 variant proteins described previously were also tested (Elahi et al., 2003; Villeneuve et al., 2003). In the analysis of UGT1A7 allozymes, the highest MPA glucuronidation activity was observed for UGT1A7*1, *2 (K129K131), *6 (D139), and *7 (K129K131D139). The previously described low-activity variant proteins, namely *3 (K129R131R208), *4 (R208), *5 (S115), *8 (K129K131D139R208), and *9 (S115K129K131), presented lower rates of MPAG formation compared with *1 (G129K129R131E139W208), similar to what was observed for every other substrate tested so far (Gagne et al., 2002; Villeneuve and Guillemette, 2003). Furthermore, a 1.5-fold reduced MPA glucuronidation activity was also observed for the UGT1A10*2 (E139K) protein compared with the *1 protein (data not shown).

In conclusion, the UGT1A8*3 (C277Y) allozyme demonstrated a significantly altered catalytic efficiency toward MPA. A similar impact of these variations was reported with other substrates of the UGT1A8 protein (Huang et al., 2002). Since UGT1A8 is not present in the liver, the effect of the *3 allele may be limited to the metabolism of MPA in the gastrointestinal tract and could have a potential role in the toxicities associated with MMF in this tissue (Shaw et al., 1998). Similarly, the UGT1A9*3 (M33T) protein demonstrated a lower Clint compared with the *1 and suggests a potential impact of this variation to the in vivo metabolism of the drug, particularly in the liver, and to the interindividual variability in the pharmacokinetics of MMF. However, the low frequency of the UGT1A8*3 and UGT1A9*3 alleles, present in less than 5% of the population, would indicate a limited impact overall (Huang et al., 2002; Villeneuve et al., 2003). Based on the results of our studies, a unique involvement of the hepatic UGT1A9 enzyme in MPAG formation in the liver is shown and is predicted to impact the in vivo variability of the drug in this tissue. Further studies on genetic factors, mainly of the UGT1A9 gene, associated with altered glucuronidation profiles of MPA are needed.

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**References**


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