IDENTIFICATION OF METABOLIC PATHWAYS INVOLVED IN THE BIOTRANSFORMATION OF TOLPERISONE BY HUMAN MICROsomAL ENZYMES

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(Received October 28, 2002; accepted February 10, 2003)

This article is available online at http://dmd.aspetjournals.org

ABSTRACT:
The in vitro metabolism of tolperisone, 1-(4-methyl-phenyl)-2-methyl-3-{1-(piperidino)}-1-propanone-hydrochloride, a centrally acting muscle relaxant, was examined in human liver microsomes (HLM) and recombinant enzymes. Liquid chromatography-mass spectrometry measurements revealed methyl-hydroxylation (metabolite at m/z 261; M1) as the main metabolic route in HLM, however, metabolites of two mass units greater than the parent compound and the hydroxy-metabolite were also detected (m/z 247 and m/z 263, respectively). The latter was identified as carbonyl-reduced M1, the former was assumed to be the carbonyl-reduced parent compound. Isoform-specific cytochrome P450 (P450) inhibitors, inhibitory antibodies, and experiments with recombinant P450s pointed to CYP2D6 as the prominent enzyme in tolperisone metabolism. CYP2C19, CYP2B6, and CYP1A2 are also involved to a smaller extent. Hydroxymethyl-tolperisone formation was mediated by CYP2D6, CYP2C19, CYP1A2, but not by CYP2B6.

Tolperisone competitively inhibited dextromethorphan O-demethylation and bufuralol hydroxylation (K_i = 17 and 30 μM, respectively). Tolperisone inhibited methyl p-tolyl sulfide oxidation (K_i = 1200 μM) in recombinant flavin-containing monoxygenase 3 (FM03) and resulted in a 3-fold (p < 0.01) higher turnover number using rFMO3 than that of control microsomes. Experiments using nonspecific P450 inhibitors—SKF-525A, 1-aminobenzotriazole, 1-benzylimidazole, and anti-NADPH-P450-reductase antibodies—resulted in 61, 47, 49, and 43% inhibition of intrinsic clearance in HLM, respectively, whereas hydroxymethyl-metabolite formation was inhibited completely by nonspecific chemical inhibitors and by 80% with antibodies. Therefore, it was concluded that tolperisone undergoes P450-dependent and P450-independent microsomal biotransformations to the same extent. On the basis of metabolites formed and indirect evidences of inhibition studies, a considerable involvement of a microsomal reductase is assumed.

Although a few papers are available on the pharmacokinetics of tolperisone, no study has been performed to date on the in vitro metabolism of the compound. In early in vivo studies the drug concentration in the plasma was measured using gas chromatography and by mass fragmentographic techniques (Miyazaki et al., 1972, 1975). Miskolczi and coworkers in 1987 published a new and sensitive gas-liquid chromatographic method for the determination of tolperisone in human plasma and made a comparison of two different brands of tolperisone tablets, Mydeton and Mydocalm. In that study the elimination half-life of tolperisone was 1.55 ± 0.7 h, and the total body clearance was 140.8 ± 33.8 l/h. The oral bioavailability of the two different tolperisone products was 22.3 ± 6% and 16.7 ± 8.9%, respectively.

Metabolic profiling is a useful tool to predict the pharmacokinetic properties of drugs and drug candidates and to assess the possibility of drug-drug interaction risks. The genetic background and actual metabolic state of individuals can vary largely even in the same population group. Therefore, these differences can influence significantly the fate of ingested drugs (i.e., the AUC can differ largely in different individuals). On one hand, the elevated plasma level of a drug can cause toxicity problems, especially those with narrow therapeutic range. On the other hand, high metabolic activity in certain population groups (extensive metabolizers) may prevent therapeutic efficacy of drugs. The goal of the present study was to identify by in vitro reaction phenotyping methods the microsomal enzymes that contribute to the biotransformation of tolperisone and to elucidate the metabolic profile of the compound.

1 Abbreviations used are: AUC, area under the curve; P450, cytochrome P450; FM0, flavin-containing monoxygenase; HPLC, high-performance liquid chromatography; HLM, human liver microsomes; SKF-525A, proadifen-hydrochloride; ABT, 1-aminobenzotriazole; BI, 1-benzylimidazole; MptS, methyl p-tolyl-sulfide; MptSO, methyl p-tolyl-sulfoxide; LC/MS, liquid chromatography-mass spectrometry; V, reaction rate; S, substrate concentration; M1, hydroxymethyl-tolperisone; M2, carbonyl-reduced form of hydroxymethyl-tolperisone; Cl_intrinsic, intrinsic clearance.
Materials and Methods

Chemicals. Tolperisone-hydrochloride and metabolite standards were synthesized in Gedeon Richter Ltd. (Budapest, Hungary). Quinidine was purchased from Sigma-Aldrich (St. Louis, MO). Furafylline and S-mephentoin were from Ultrafine Chemicals Ltd. (Manchester, UK). Testosterone was from Fluka (Buchs, Switzerland); tolbutamide was obtained from Sigma/RBI (Natick, MA). Methyl p-tolyl sulfide and sulfoxide were from Aldrich Chemical Co. (Milwaukee, WI). 1-benzylimidazolide was purchased from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). Na-pyrophosphate, MgCl2, NADPH-sodium salt was obtained from Reanal (Budapest, Hungary).

Incubation Conditions. All incubations were carried out in incubation mixtures containing 6 mM Na-pyrophosphate, 5 mM MgCl2, 5 mM glucose 6-phosphate, 1 U/ml glucose-6-phosphate dehydrogenase, phenacetin, 4-acetamido-phenol and 1-aminobenzotriazole were purchased from Sigma-Aldrich (St. Louis, MO). Furafylline and S-mephentoin were from Ultrafine Chemicals Ltd. (Manchester, UK). Testosterone was from Fluka (Buchs, Switzerland); tolbutamide was obtained from Sigma/RBI (Natick, MA). Methyl p-tolyl sulfide and sulfoxide were from Aldrich Chemical Co. (Milwaukee, WI). 1-benzylimidazolide was purchased from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). Na-pyrophosphate, MgCl2, NADPH-sodium salt was obtained from Reanal (Budapest, Hungary).

(±)-Bufuralol hydrochloride, (±)-1'-hydroxybufuralol maleate, CYP2D6 and CYP3A4-specific monoclonal antibodies, CYP2C8/9/19 and NADPH-P450-reductase specific polyclonal antibodies and baculovirus-insect-cell-expressed human enzymes were purchased from BD Gentest (Woburn, MA). CYP1A2, CYP2A6, CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4) or 1000 pmol/ml enzyme concentration was 1 pmol/ml (CYP2C8/9/19-specific antibodies; 6 and 10 µM of antibodies/100 µg of microsomes, respectively) or for 30 min at room temperature (CYP2C8/9/19-specific and NADPH-P450 reductase-specific antisera; 20 µl and 7.5 µl of antisera/100 µg microsomes, respectively). Anti-reductase inhibition kit contained nonimmune goat serum as control. In experiments with CYP2C8/9/19-specific antisera, normal goat serum from Invitrogen New Zealand Ltd. (Auckland, NZ) was used as control.

High Performance Liquid Chromatography. Analytical measurements were carried out using a Merck-Hitachi LaChrom HPLC system equipped with UV detector. Discovery C18 150 × 4.6 mm (5 µm) column with Supelguard 20 × 4 mm (5 µm) column (Supelco, Bellefonte, PA) was maintained at 40°C for all assays.

HPLC Measurements. Merck-Hitachi LaChrom HPLC system was coupled with mass spectrometer (Merck-Hitachi M-8000) with electrospray ionization interface. The enzyme reaction was stopped with equal volume of 140 ml of methanol and 100 ml of water, supplemented with 100 µl 10% ammonium hydroxide. Eluent B consisted of methanol/0.1 M ammonium-acetate, 27.73% (A) and methanol (B) were used. Gradient (A) was 100 (8 min), 40 (16–17 min), and 100% (20–25 min). Column effluent was monitored at 254 nm (Anderson et al., 1995). Mobile phases for hydroxytolbutamide analysis were methanol/0.1 M ammonium-acetate, 25.75% (A) and methanol (B). Gradient (A) was 100 (4 min), 70 (10–11 min), and 100% (13–20 min). Detection was carried out at 230 nm (Miners et al., 1996). 4-Acetamidophenol, the CYP1A2 metabolite of phenacetin was also examined using gradient method. Mobile phase A was methanol/0.1 M ammonium-acetate, 5.95% and B was methanol. Gradient (A) was 100 (0 min), 50 (12 min), 100% (14–19 min). Column effluent was monitored by UV detector at 249 nm (UV absorption maximum estimated previously). Mobile phase B was methanol/0.1 M ammonium-acetate, 34.66% (A) and methanol (B), Gradient (A) was 100 (5 min), 35 (12 min), and 100% (16–22 min).

LC/MS Measurements. Merck-Hitachi LaChrom HPLC system was coupled with mass spectrometer (Merck-Hitachi M-8000) with electrospray ionization interface. The enzyme reaction was stopped with equal volume of ice-cold methanol. The samples were kept at −20°C for 30 min. After centrifugation, the supernatant was collected, and the pH was set to 9 and extracted with chlorobutane. Organic phase was evaporated to dryness and reextracted in ethanol, and 10 µl was subjected to HPLC. Eluent A consisted of 140 ml of methanol and 100 ml of water, supplemented with 100 µl 10% formic acid and 200 µl 10% ammonium hydroxide. Eluent B consisted of methanol. Gradient: 0. min: 100% A, 8. min: 60% A, 12. min: 100% A. Flow rate was 0.25 ml/min. Samples were monitored at 255 nm with UV detector.
The concentration of each inhibitor was chosen so as to represent the diagnostic value (i.e., the maximal specificity for the given P450). Tolperisone was used at 5 μM.

The results are expressed as the mean of three determinations ± S.D. except for furafylline, where six determinations were done.

100% inhibition using 1000 μM SKF-525A, ABT and BI means that M1 metabolite could not be detected at all.

Inhibitory Antibodies. Human CYP2D6 and human CYP3A4-specific monoclonal and human CYP2C8/9/19-specific polyclonal antibodies were used to test the contribution of respective P450 enzymes to the in vitro metabolism of tolperisone (5 and 50 μM) in pooled human liver microsomes. CYP2D6- and CYP2C-specific antibodies inhibited the clearance of tolperisone and M1 formation (Table 2); antibodies against CYP3A4 had no effect (data not shown). Anti-CYP2C-inhibited tolperisone clearance more effectively when 50 μM substrate was used, whereas the inhibitory effect of anti-CYP2D6 decreased. The same pattern was seen in M1 formation, the 70% inhibition of anti-CYP2D6 at 5 μM decreased to about 50% at 50 μM compound found to substantially inhibit tolperisone consumption and M1 formation was quinidine at a diagnostic 10 μM concentration. Ketoconazole at 10 μM inhibited the loss of parent compound and M1 formation to a lesser extent. S-mephenytoin and sulfaphenazole resulted in slight and concentration independent (data not shown) inhibition both in consumption and M1 formation. Thiourea was also used to assess the contribution of FMO3 to the metabolism of tolperisone. No inhibition was seen at 10 mM (data not shown).

TABLE 2

<table>
<thead>
<tr>
<th>Inhibitor Concentration (μM) a</th>
<th>Inhibition % a</th>
<th>Cl int</th>
<th>M1 Formation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti CYP2C8/9/19 b c</td>
<td>7 ± 1.0</td>
<td>14 ± 2.6</td>
<td>32 ± 5.7</td>
</tr>
<tr>
<td>Anti CYP2D6</td>
<td>18 ± 1.3</td>
<td>11 ± 4.0</td>
<td>70 ± 1.1</td>
</tr>
</tbody>
</table>

*Data are expressed as the mean of three determinations ± S.D.

*In experiments using antisera, goat nonimmunne serum was used as control. Cl int was calculated as described under Materials and Methods.

Prontosil C18 Ace EPS 5 μm, 100–2 mm column (Bischoff, Germany) was used at 35°C.

Data Analysis. Enzyme kinetic data of tolperisone hydroxylation in pooled HLM and recombinant isoforms were fitted to standard Michaelis-Menten plot (reaction rate “V” against substrate concentration “S”) and Eadie-Hofstee plot (“V/S” against “V”). Kinetic parameters (apparent K m and V max) were calculated using nonlinear regression analysis of GraphPad Prism 2.01 (GraphPad Software Inc., San Diego, CA). Standard deviation and p values of Student’s t test were calculated with Microsoft Excel (Redmond, WA). For determination of K m, “K m app” method was used (Kakkar et al., 1999). Cl int in pooled HLM was calculated from initial reaction rate using the following equation:

\[ Cl_{int} = \left( \frac{dc/dt}{c_0} \right) \times 45 \ (\text{ml/min/g liver}) \]

where dc/dt is the initial reaction rate and c0 is the initial concentration of the substrate.

Results

Intrinsic Clearance of Tolperisone. The in vitro Cl int of tolperisone was determined using individual microsomes from nine donors (three female and six male). Tolperisone had a Cl int of 1.27 ± 0.16 ml/min/g of liver, determined as the mean of nine individual clearance values ± S.D. The female and the male pool resulted in 1.39 ± 0.22 and 1.15 ± 0.20 ml/min/g of liver, respectively (mean of three and six individual samples ± S.D., respectively). The sex difference in Cl int of the compound was not statistically significant. The metabolism was NADPH-dependent, since the omission of this coenzyme completely abolished the consumption of tolperisone (data not shown).

Tolperisone is metabolized mainly to hydroxymethyl-tolperisone (hydroxylation on methyl group of 4-methyl-phenyl moiety) in HLM that can be monitored with UV detector. LC/MS measurements revealed three main metabolites represented as molecular ions at m/z 261, 263, and 247. Metabolites m/z 261 (M1) and m/z 263 (M2) were identified as hydroxymethyl-tolperisone and carbonyl-reduced form of M1, respectively. Both metabolites were also found in 24-h rat urine (Miyazaki et al., 1972).

Isoform-Specific Chemical Inhibitors/Index Substrates. P450 isoform-specific chemical inhibitors or index substrates (i.e., competitive inhibitors) were used to inhibit the consumption of tolperisone (5 μM) and the formation of M1 metabolite in pooled HLM. Furafylline (CYP1A2), sulfaphenazole (CYP2C9), S-mephenytoin (CYP2C19), quinidine (CYP2D6), and ketoconazole (CYP3A4) were tested as inhibitors. The summarized results are shown in Table 1. The only
carried out. This reaction is known to be nonspecific for FMO3 (i.e., P450 enzymes might also contribute to the metabolism of tolperisone, metabolite produced by this isozyme still needs to be identified.

also contributes to the consumption of parent compound, however at a much lower rate than CYP2D6 and CYP2C19. CYP2B6 also catalyzed tolperisone hydroxylation at an order of magnitude lower rate than CYP2D6 and CYP2C19.

Recombinant CYP1A2 also catalyzed tolperisone hydroxylation at an order of magnitude lower rate than CYP2D6 and CYP2C19. CYP2B6 also contributed to the consumption of parent compound, however at a much lower rate than CYP2D6 and CYP2C19.

P450s (CYP1A2, CYP2A6, CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4) were used to study the contribution of these enzymes to the metabolism of tolperisone. The reaction mixture contained 20 pmol/ml of each P450 and 1 μM tolperisone. Under these conditions, CYP2D6 and CYP2C19 containing microsomes metabolized tolperisone substantially to the main M1 metabolite (Fig. 1). Recombinant CYP1A2 also catalyzed tolperisone hydroxylation at an order of magnitude lower rate than CYP2D6 and CYP2C19. CYP2B6 also contributes to the consumption of parent compound, however metabolite produced by this isozyme still needs to be identified. Since aforementioned results implied that NADPH-dependent, non-P450 enzymes also contribute to the metabolism of tolperisone, inhibition of MpTS oxidation, as an FMO3-mediated reaction, was carried out. This reaction is known to be nonspecific for FMO3 (i.e., P450s are also involved, Pike et al., 1999), therefore we used recombinant, insect cell-expressed human FMO3 containing microsomes. Analysis of the data revealed a K_{m} of 1197 μM in MpTS oxidase inhibition. The consumption of tolperisone was examined in rFMO3 containing 1 nmol enzyme/ml. In this latter experiment rFMO3 resulted in a turnover number/specific activity of 60 pmol/min/mmol enzyme.

Kinetics of Tolperisone Hydroxylation. Enzyme kinetic analysis of M1 formation was carried out to study the main metabolic route of tolperisone biotransformation. In experiments using HLM tolperisone was used in the concentration range of 1 to 1000 μM. Data of M1 formation were fitted to Michaelis-Menten kinetics (Fig. 2). Calculated apparent kinetic values were summarized in Table 3. Kinetic analysis of M1 formation showed biphasic kinetics, suggesting the contribution of at least two populations of P450s. Eadie-Hofstee plot (Fig. 2B) of data points also revealed that there is a high K_{m} and a low K_{m} component of M1 formation. Fine characterization of M1 formation in expressed human P450s revealed CYP2D6 as the low K_{m} component of M1 formation. Since aforementioned results implied that NADPH-dependent, non-P450 enzymes contribute to the in

### Table 3

<table>
<thead>
<tr>
<th>Component</th>
<th>One K_{m} Model</th>
<th>Two K_{m} Model</th>
</tr>
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<tbody>
<tr>
<td>Low K_{m}</td>
<td>High K_{m}</td>
<td>Low K_{m}</td>
</tr>
<tr>
<td>K_{m} (μM)</td>
<td>V_{max} (pmol/min/mg protein)</td>
<td>V_{max} (pmol/min/mg protein)</td>
</tr>
<tr>
<td>CYP2D6</td>
<td>67 ± 2.8</td>
<td>458 ± 8.2</td>
</tr>
<tr>
<td>CYP2C19</td>
<td>7.9 ± 0.95</td>
<td>172 ± 9.9</td>
</tr>
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</table>

* Apparent kinetic values were calculated using nonlinear regression method. Each parameter represents the mean of three determinations ± S.D.

### Table 4

Kinetic parameters of tolperisone hydroxylation in baculovirus-infected insect cell expressed human P450s

<table>
<thead>
<tr>
<th>Component</th>
<th>One K_{m}</th>
<th>Two K_{m}</th>
</tr>
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<tbody>
<tr>
<td>Low K_{m}</td>
<td>High K_{m}</td>
<td>Low K_{m}</td>
</tr>
<tr>
<td>K_{m} (μM)</td>
<td>V_{max} (μmol/min/pmol)</td>
<td>V_{max} (μmol/min/pmol)</td>
</tr>
<tr>
<td>CYP1A2</td>
<td>160.6 ± 7.1</td>
<td>23.6 ± 5.78</td>
</tr>
<tr>
<td>CYP2C19</td>
<td>24.4 ± 8.6</td>
<td>33.7 ± 9.16</td>
</tr>
<tr>
<td>CYP2D6</td>
<td>1.8 ± 0.29</td>
<td>9.3 ± 2.46</td>
</tr>
</tbody>
</table>

Enzyme kinetic parameters of tolperisone hydroxylation in HLM

<table>
<thead>
<tr>
<th>Component</th>
<th>One K_{m}</th>
<th>Two K_{m}</th>
</tr>
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<tbody>
<tr>
<td>Low K_{m}</td>
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Inhibition Studies for K_{m} Determination. To determine inhibitory potential of tolperisone toward the prominent isozyme, compound was tested to inhibit dextromethorphan O-demethylation and bufuralol hydroxylation—widely used index reactions of CYP2D6. Enzyme kinetic analysis of the data with the “K_{m,app}” method (Kakkar et al., 1999) revealed that tolperisone inhibited dextromethorphan O-demethylation and bufuralol hydroxylation with the K_{m} of 17 and 30 μM, respectively. Tolperisone was also used as inhibitor in testosterone 6β-hydroxylation, phenacetin O-deethylation and tolbutamide-hydroxylation, the index reactions of CYP3A4, CYP1A2, and CYP2C9, respectively. Tolperisone did not inhibit these index reactions in the concentration range used for inhibition of CYP2D6 index reaction (data not shown).

### Contribution of Microsomal Non-P450 Enzymes.

To test whether non-P450, NADPH-dependent enzymes contribute to the in

![A. Michaelis-Menten plot](image1)

![B. Eadie-Hofstee plot](image2)

**Fig. 2.** Kinetic analysis of tolperisone metabolism in HLM.

A. Michaelis-Menten plot of M1 formation reaction. The dotted line represents one-K_{m}, the solid line represents two-K_{m} kinetic calculation. The corresponding r^{2} values are 0.95 and 0.998, respectively. B. Eadie-Hofstee plot of M1 metabolite formation in HLM. V and S stand for the reaction velocity and tolperisone concentration, respectively. Tolperisone was used at 1 to 1000 μM.

These results point toward the decreasing relative contribution of CYP2D6 along with increasing tolperisone concentration. Although CYP2C-specific antibodies resulted in significant inhibition, these results were difficult to interpret since nonimmune goat serum had an intrinsic, yet unidentified metabolizing capacity.

**Recombinant Enzymes.** Insect cell-expressed recombinant human P450s (CYP1A2, CYP2A6, CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4) were used to study the contribution of these enzymes to the metabolism of tolperisone. The reaction mixture contained 20 pmol/ml of each P450 and 1 μM tolperisone. Under these conditions, CYP2D6 and CYP2C19 containing microsomes metabolized tolperisone substantially to the main M1 metabolite (Fig. 1). Recombinant CYP1A2 also catalyzed tolperisone hydroxylation at an order of magnitude lower rate than CYP2D6 and CYP2C19. CYP2B6 also contributes to the consumption of parent compound, however metabolite produced by this isozyme still needs to be identified.
In the present study the in vitro pathways of tolperisone metabolism were examined in pooled human liver microsomes and in recombinant enzymes. In previous pharmacokinetic studies it was found that tolperisone had a low bioavailability (20%, Miskolczi et al., 1987). Here we demonstrated that the rate of NADPH-dependent CLint of tolperisone could account for the low in vivo bioavailability.

Experiments studying metabolic stability of tolperisone also revealed that the compound is metabolized mainly to hydroxymethyltolperisone (M1), in vitro. Stochiometric calculations predicted the formation of other, substantial, non-UV active metabolites besides the main M1. LC/MS measurements showed that as well as M1 (m/z 261), the carbonyl-reduced form of this metabolite (M2, m/z 263), and another metabolite that is two mass units greater than parent compound was produced (m/z 247). Since several carbonyl-reduced metabolites were identified previously in rats (Miyazaki et al., 1972), and one of these was identified in HLM in the present study (M2), it was reasonable to assume that metabolite at m/z 247 was identical with the carbonyl-reduced parent compound. Nonspecific P450 inhibitors (SKF-525A, ABT, BI, and NADPH-P450 reductase-specific antibodies) potently inhibited M1 formation, however parent compound consumption was inhibited to a much lesser extent (40–60% residual microsomal activity at 100% P450 inhibition). This suggested the substantial contribution of P450-independent enzymes. Additionally, anti-reductase antibodies did not inhibit the formation of metabolite M2, whereas the amount of M1 and M2 was decreased. These indirect evidences implied that besides P450s, a significant P450-independent route is involved in tolperisone metabolism. However, further supportive results are needed to identify the reductase and evaluate its role in tolperisone metabolism.

The P450-dependent metabolic processes were characterized in details. In our hands, the most potent chemical inhibitor of tolperisone consumption and M1 metabolite formation (76%). LC/MS measurements revealed that P450-reductase-specific antibodies inhibited the formation of M1 and M2 significantly, but formation of m/z 247 was not affected (data not shown).

Discussion
In the present study the in vitro metabolic pathways of tolperisone was examined in pooled human liver microsomes and in recombinant enzymes. Previous pharmacokinetic studies it was found that tolperisone had a low bioavailability (20%, Miskolczi et al., 1987). Here we demonstrated that the rate of NADPH-dependent CLint of tolperisone could account for the low in vivo bioavailability.

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The P450-dependent metabolic processes were characterized in details. In our hands, the most potent chemical inhibitor of tolperisone consumption and M1 metabolite formation was quinidine that implied the main role of CYP2D6. This result was in concert with results of recombinant P450s, where the most active isoform (i.e., the enzyme with the highest turnover number) was CYP2D6 both in compound loss and M1 formation. More supportive data of the key role of CYP2D6 came from the experiments with specific inhibitory antibodies; 70% inhibition of M1 formation meant that the majority of the P450-dependent biotransformation is mediated by CYP2D6. S-mephenytoin inhibited parent loss about to the same extent as quinidine (22%), however the effect to M1 formation was weak (16%). On the other hand, the activity of recombinant CYP2C19 revealed 60% of rCYP2D6 activity in both compound loss and M1 formation placing recombinant CYP2C19 to the second most active P450 in tolperisone metabolism. Despite this high activity in expressed enzyme system, we concluded that at “physiological” tolperisone concentration the role of CYP2C19 is less essential than that of CYP2D6. The results given by inhibitory antibodies are controversial, since an unidentified interaction of tolperisone with goat serum was seen. However, it was
clearly shown that at a higher concentration, the relative contribution of CYP2D6 decreased. This finding was supported with the results of enzyme kinetic characterization of tolperisone hydroxylation. Reconsti-

bant CYP2D6 revealed the low Km enzyme with low apparent Vmax indicating that at low concentration CYP2D6 plays the prominent role, whereas increasing the concentration of tolperisone increased the role of CYP2C19 as a medium Km-high Vmax enzyme. CYP1A2 turned out to be the highest Km counterpart; however, because of the relative abundance of this isozyme its role is not negligible. Moreover, the role of a nonpolyorphic enzyme is important mainly in a subject relative abundance of this isozyme its role is not negligible. Moreover, the role of a nonpolyorphic enzyme is important mainly in a subject

Acknowledgments. The authors thank Gabriella Pásztor for help in metabolite identification and Teréz Merkl and Marianna Borsos for skilful technical assistance.

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avzation and involvement of CYP2C19 and CYP1A2 were also shown. It was evidenced that P450-independent metabolism was mediated to a small extent by FMO3. Metabolites detected and indirect evidences


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