Effect of Clofibrate on the Chiral Disposition of Ibuprofen in Rats

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ABSTRACT

A potentially clinically important interaction has been described between clofibrate and ibuprofen in vitro. To determine whether this in vitro interaction is paralleled by a change in pharmacokinetics of ibuprofen in vivo two groups of rats were treated orally with clofibrate (n = 8, 280 mg/kg/day) or vehicle (n = 7) for 3 days. On day 3, 2 hr after the last dose of clofibrate, the rats were given an i.v. dose of pseudoracemic ibuprofen (20 mg/kg, 10 mg R-ibuprofen, 10 mg 13C-S-ibuprofen). Plasma concentrations of the enantiomers were monitored by a stereospecific gas chromatography mass spectrometry assay. The clearance of R-ibuprofen more than doubled in the clofibrate-treated group (mean ± S.E.M.; 29.4 ± 4.0 ml/min) as compared to control rats (13.0 ± 1.4 ml/min; P = .003). This increase was similarly reflected in the clearance of inversion (treated, 23.2 ± 3.2 ml/min, untreated, 10.0 ± 1.2 ml/min; P = .003) and there was also an increase in the rate of inversion (treated, t1/2 inv; 8.3 ± 1.6 min; untreated, 13.9 ± 1.4 min; P = .029). By contrast, the estimates of fractional chiral inversion were not affected by clofibrate and were in close agreement whether estimated by the area under the plasma concentration-time curve approach (treated, 0.79 ± 0.02; untreated, 0.72 ± 0.02) or by deconvolution (treated, 0.78 ± 0.02; untreated, 0.73 ± 0.02). There was a significant increase in volume of distribution at steady-state (treated, 4.42 ± 1.12 liter/kg/untreated, 1.03 ± 0.30 liter/kg; P = .017) observed for the R-enantiomer but not the S-enantiomer (treated, 1.04 ± 0.15 liter/kg; untreated, 1.10 ± 0.21 liter/kg). Pretreatment of rats with clofibrate significantly increased the concentrations of ibuprofen in fat, lung, brain and liver tissue. With respect to the protein levels of two key enzymes involved in chiral inversion, clofibrate pretreatment significantly induced expression of long chain acyl-coenzyme A synthetase, although the expression of the epimerase was unaltered. It is concluded, that clofibrate may increase the proportion of R-ibuprofen incorporated into long-lived lipid (“hybrid” lipid) stores.

Ibuprofen is a 2-arylpropionic acid widely used for its antiinflammatory and analgesic activities and is administered almost invariably as its racemate (R,S-ibuprofen). The S-enantiomer is the active agent with respect to inhibition of prostaglandin synthesis (Adams et al., 1967; Geisslinger et al., 1989) as is the case for other members of this group of NSAIDs. However, interest has focused on the stereoselective disposition of the “inactive” R-enantiomers for three reasons. First, in vivo there is stereoselective chiral inversion of some R-2-arylpropionates to their S-enantiomers (Adams et al., 1976; Nakamura et al., 1981; Hutt and Caldwell, 1983; Caldwell et al., 1988). Second, the drugs may perturb lipid biochemistries and be stereoselectively incorporated into long-lived lipid stores (Fears, 1985; Williams et al., 1986; Sallustio et al., 1988; Freneaux et al., 1990; Mayer et al., 1994; Mayer et al., 1995). Third, it has been shown that ibuprofen can activate peroxisome proliferator-activated receptor γ (PPAR γ), a ligand-activated transcription factor known to play a role in adipogenesis (Lehmann et al., 1997). This activation is probably independent of prostaglandin synthesis inhibition (Lehmann et al., 1997). The first metabolic step linking inversion and interactions with lipids is the stereoselective activation of the R-enantiomers to their acyl-CoA thioesters by the long chain acyl-CoA synthetase (Brunner et al., 1996a; Chen et al., 1990; Tracey et al., 1993; Menzel et al., 1994), which is an essential enzyme in the metabolism...

ABBREVIATIONS: AUC, area under the plasma concentration-time curve; AUMC, area under the first moment curve; CL, total body clearance; CLinv, clearance of the R-enantiomer by inversion; CoA, coenzyme A; CYP, cytochrome P450; DECON, deconvolution; Fi, fractional inversion; GCMS, gas chromatography mass spectrometry; MSD, mass selective detector; NSAIDs, nonsteroidal antiinflammatory drugs; t1/2, terminal elimination half-life; VSS, volume of distribution at steady-state; LACS, long chain acyl-CoA synthetase (EC 6.1.2.3).
of fatty acids. In the second step epimerization to S-ibuprofenoyl-CoA is catalyzed by 2-arylpropionyl-CoA epimerase (Reichel et al., 1995, 1997). Finally, the thioesters are hydrolyzed. The rat has become a well accepted model for studying the mechanism of inversion and factors affecting inversion, particularly for ibuprofen.

A mechanistically interesting, and potentially clinically important, interaction has been described between clofibrate and ibuprofen, whereby there is increased inversion of R-ibuprofen in liver homogenate of rats treated with clofibrate. This increased inversion paralleled an increase in hepatic microsomal long-chain CoA synthetase consistent with the central role of the CoA thioester intermediate (Shieh and Chen, 1993; Knights et al., 1991). The main purpose of our study was to determine whether the potentiation of inversion in vitro is also paralleled by a change in the pharmacokinetics of ibuprofen in vivo, and in particular, is reflected by an increase in chiral inversion in rats treated with clofibrate. A technique using pseudoracemic ibuprofen containing 13C-S-ibuprofen with GCMS analysis was developed to achieve this goal. Moreover the influence of clofibrate on the expression of long chain acyl-CoA synthetase and epimerase, two key enzymes in the metabolic chiral inversion was studied.

Methods

Materials

R-ibuprofen (>98.5% optical purity) and 13C-S-ibuprofen (>98% optically pure, >97.8% isotopically pure) were kindly donated by Ethyl Corporation (courtesy of Dr. Denis Bauer, Baton Rouge, LA). The pseudoracemate solution was prepared using an equal proportion of these substances. The internal standard, ring tetradeuterated (D4)-RS-ibuprofen was purchased from Tracer Technologies (Somerville, MA). 14C-RS-ibuprofen (21.6 μCi/mg) was kindly donated by the Boots Company, PLC (Nottingham, UK).

The gas chromatograph used in this study was an HP5890 interfaced to an HP5971A MSD via a capillary splitless injector. The column was a fused silica capillary column (DB-5, 15 m, 0.25-mm inner diameter) faced to an HP5971A MSD via a capillary splitless injector. The mass spectrometric detector was operated under the following conditions: electron impact mode (electron energy, 70 eV), emission temperature, glacial acetic acid (10% v/v); injection port temperature, 270°C, ion source 190°C, emission temperature, 280°C at 10°C/min and held at 280°C for 1 min. The mass spectrometric detector was operated under the following conditions: electron impact mode (electron energy, 70 eV), emission temperature, glacial acetic acid (10% v/v); injection port temperature, 270°C, ion source 190°C, ion source pressure 4 × 10−5 torr. The following mass ions were monitored: m/z 309 (unlabeled ibuprofen), m/z 310 (13C-ibuprofen) and m/z 313 (internal standard, D1-RS-ibuprofen). Data were corrected for natural isotopic abundance of 13C, and for isotopic impurities in the 13C-S-ibuprofen and D1-RS-ibuprofen.

Animal Protocols

Male Wistar rats (200–300 g) were purchased from Charles River (Sulzfeld, Germany). Treatment of animals adhered to the guidelines for research in conscious animals and studies were approved by the local Ethics Committee.

Pharmacokinetic studies. One group of rats (n = 8; 243 ± 3 g) was administered clofibrate (group I, 280 mg/kg/day) by oral gavage as described by Knights et al. (1991) for 3 days. The control group (n = 8; 234 ± 2 g) was similarly treated with vehicle (0.9% NaCl solution) only (group II). On day 3, 2 h after the last dose of clofibrate, the rats were given an i.v. dose of pseudoracemic ibuprofen (20 mg/kg, consisting of 10 mg R-ibuprofen and 10 mg 13C-S-ibuprofen) via the indwelling cannula. The cannula was flushed with saline solution (0.50 ml, 0.9%) to ensure no contamination by administered ibuprofen during blood sampling. Serial blood samples (150 μl) were drawn at the following times: 0, 5, 15, 30, 45, 60, 120, 180, 240, 300, 360 min. After each sampling, blood volume was replaced by injection of an equal volume of saline solution (0.15 ml; 0.9%).

Tissue distribution studies. The design of the study was as for the pharmacokinetic study above. One group of rats (n = 8) was treated with clofibrate and the other group (n = 8) treated with vehicle. Rats then were administered 14C-RS-ibuprofen (20 mg/kg; solution 20 mg/ml = 43.33 μ Ci/ml). 14C-RS-ibuprofen was used because no pure 14C-R-ibuprofen was available. (Administration of radiolabeled racemate is reasonable because it has been shown previously that uptake of ibuprofen into adipose tissue occurs only with the R-enantiomer; Williams et al., 1986). Animals were killed 6 hr after drug administration, and the following tissues collected: liver, kidney, brain, lung and fat. Tissues were washed thoroughly with Ringers solution and frozen for later analysis.

Enzyme Expression Studies

The design of the study was as for the pharmacokinetic study above. One group (n = 3) received clofibrate, the other group (n = 3) received vehicle (0.9% NaCl). On day 3, 2 hr after the last dose of clofibrate, animals were killed. Livers were removed, washed thoroughly with Ringers solution and frozen for later analysis.

Determination of Plasma Ibuprofen Concentrations

The method was based on a modification of previously described GCMS techniques whereby the enantiomers were analysed as their diastereomeric phenylethylamide derivatives (Baillie et al., 1989; Sanins et al., 1991; Rudy et al., 1991). Standards of R-ibuprofen, S-ibuprofen and 13C-S-ibuprofen were prepared at the following concentrations: 0.05, 0.25, 0.5, 2.5, 5.0, 50.0 and 250.0 μg/ml in rat plasma. Replicate (n = 6) samples were assayed at 0.25, 2.5 and 25.0 μg/ml for validation of accuracy and precision of the assay. Plasma samples (50 μl) and standards (100 μl) were extracted with hexane-ether (70:30, v/v, 4 ml) after addition of internal standard (D1-Rs-ibuprofen, 5 μg/ml, 200 μl) and hydrochloric acid solution (200 μl, 2 M). The organic layer was transferred to a tapered tube and was taken to dryness under nitrogen with gentle warming (40°C). The residue was treated with a freshly prepared solution of 1,1-carbonimidazolide in toluene (0.5 ml, 5 mg/ml). After 10 min at room temperature, glacial acetic acid (10 μl) was added followed by R-(+)-α-phenylethylamine (50 μl). The reaction was allowed to proceed at room temperature for 1 hr, and after acidification (0.2 M HCl, 5 ml), the samples were extracted with toluene (0.5 ml). An aliquot of the toluene phase (800 μl) was taken to dryness under nitrogen and the residue resuspended in toluene (20 μl). An aliquot (1 μl) was taken for GCMS analysis.

Determination of Ibuprofen in Tissues

Tissue (about 1 g) was solubilized by vortexing thoroughly with a blend of toluene and dimethyl dialkyl quaternary ammonium hydroxide and methanol (10 ml; Solune-350, Canberra-Packard, Dreieich, Germany) followed by incubation over 60 hr at 60°C. An aliquot (1.0 ml) of the solubilized tissue solution was mixed with scintillant solution (2 ml; Ultima Gold, Canberra-Packard) and the radioactivity was determined as counts/min (Beckman scintillation counter; Beckman, Munich, Germany). These data were then expressed as μg/g RS-ibuprofen “equivalents.”

Sodium dodecylsulphate polyacrylamide gel electrophoresis and Western Blot Analysis

Purification and characterization of long-chain acyl-CoA synthetase (LACS; EC 6.1.2.3) and 2-arylpropionyl-CoA epimerase from rat liver was performed according to previously reported protocols of Brugger et al. (1996a) and Shieh and Chen (1993), respectively.
Deconvolution methods were used to estimate the fraction of R-ibuprofen and 13C-S-ibuprofen (AUCR, AUCS, and AUC13C-S, respectively) and is based on linear systems analysis (Cutler, 1978). Two deconvolution methods were described (McLachlan and Williams, 1995; Karol and Goodrich, 1989), respectively.

The elimination half-life for each compound was calculated from the slope of the terminal portion of the log concentration-time plot.

The Fi was calculated using two approaches, the AUC comparison and the deconvolution method. The calculation of the fractional inversion (FiAUC) using the AUC comparison method (equation 1) is based on the approach summarised by Rowland and Tozer (1989) to describe the pharmacokinetics of drug metabolites.

$$Fi_{AUC} = \frac{AUC_{S} \cdot Fi_{13C-S}}{AUC_{R} \cdot CL_{R}} \tag{1}$$

where AUCS and AUCR are the AUC for S-ibuprofen and R-ibuprofen, respectively, after the administration of R-ibuprofen, and CL13C-S and CLR are the clearance of 13C-S-ibuprofen and R-ibuprofen, respectively.

The deconvolution approach to estimating Fi has been previously described (McLachlan and Williams, 1995; Karol and Goodrich, 1988) and is based on linear systems analysis (Cutler, 1978). Two deconvolution methods were used to estimate the fraction of R-ibuprofen inverted to S-ibuprofen (FiDECON). The staircase approximation deconvolution method (Cutler, 1981; also called the point inversion, was calculated using the AUC and AUMC as described previously (Rowland and Tozer, 1989).

The half-life of chiral inversion, a measure of the rate of inversion, was calculated as the time taken to achieve 50% of the plateau value. Data were analyzed by analysis of variance. Comparisons of data between groups was performed using the Student’s unpaired t test with significance defined as P < .05.

### Results

The stereospecific GCMS assay was shown to be accurate and precise. The within-day coefficients of variation (n = 6) were less than 7.0% for each of R-, S- and 13C-S-ibuprofen at concentrations of 1.0 and 5.0 µg/ml based on the analysis of 50 µl samples of plasma. The day-to-day coefficient of variation was similarly less than 4.7%. The assay could reliably measure down to 0.05 µg/ml (<15% coefficient of variation).

There was no significant difference between the weights of the two groups of rats following the treatment period (control: 253 ± 2 g; treated: 251 ± 2 g).

There were significant increases in the total clearance of R-ibuprofen and the clearance of R-ibuprofen by inversion (CLR1) in rats pretreated with clofibrate (table 1). However, the mean half-life of R-ibuprofen approximately doubled in the pretreated rats despite this increased clearance because of an approximately 4-fold increase in VSS of the R-enantiomer (table 1; fig. 1). Interestingly, the fractional inversion of R-ibuprofen to S-ibuprofen was unaltered. By contrast with the effect of clofibrate on the pharmacokinetics of the R-enantiomer, clofibrate did not alter either the clearance, half-life or, notably, the VSS of S-ibuprofen (table 1; fig. 1). The AUC of the S-enantiomer was also unchanged (control: 2241 ± 508 µg ml⁻¹; treated: 1917 ± 153 µg ml⁻¹; P = .575).

There was excellent agreement between the estimates of the fraction of R-ibuprofen inverted to S-ibuprofen based on both deconvolution analysis and those based on conventional AUC analysis (table 2). Furthermore, there was also good agreement between the staircase approximation approach (data not shown) and the polyexponential deconvolution methods. Deconvolution analysis provided data on the cumulative fraction of R-ibuprofen inverted to S-ibuprofen (table 2; fig. 2) and demonstrated that there was a significant increase in the rate of inversion reflected by the reduction in the half-life of inversion from 13.9 ± 3.7 to 8.3 ± 4.4 min (P = .029) in the clofibrate-treated group.

Pretreatment with clofibrate significantly increased the plasma concentrations of radiolabeled 14C-RS-ibuprofen in the following tissues: fat, lung, brain and liver (table 3). In contrast, the distribution into kidney was unaltered.

The Western blot analysis for LACS and epimerase, respectively, in rat livers are shown in figure 3. Densitometric analysis yielded an approximately 3-fold increase in the protein levels of LACS, due to the 3-day clofibrate pretreatment period (control: 18444 ± 66799 arbitrary units; treated: 54151 ± 37819 arbitrary units; P < .01), whereas no changes were detected for the epimerase (control: 82581 ± 3703 arbitrary units; treated: 90776 ± 13551 arbitrary units; P = .6).

**TABLE 1**

Mean (± S.E.M.) pharmacokinetic parameters of the enantiomers of ibuprofen in clofibrate treated (n = 8) and control (n = 7) rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>13C-S-Ibuprofen</th>
<th></th>
<th></th>
<th>R-Ibuprofen</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td></td>
<td></td>
<td>Treated</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CL (ml/min)</td>
<td>13.0 (± 1.4)</td>
<td>29.4 (± 4.0)</td>
<td>.003</td>
<td>12.0 (± 1.4)</td>
<td>35.8 (± 6.5)</td>
<td>.003</td>
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<tr>
<td>Vss (liter/kg)</td>
<td>1.03 (± 0.30)</td>
<td>4.42 (± 1.12)</td>
<td>.017</td>
<td>1.00 (± 0.30)</td>
<td>4.42 (± 1.12)</td>
<td>.017</td>
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<tr>
<td>t1/2 (min)</td>
<td>98.5 (± 8.1)</td>
<td>89.3 (± 29.9)</td>
<td>.148</td>
<td>98.5 (± 8.1)</td>
<td>89.3 (± 29.9)</td>
<td>.148</td>
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<tr>
<td>CLr (ml/min)</td>
<td>10.0 (± 1.2)</td>
<td>23.2 (± 3.2)</td>
<td>.003</td>
<td>10.0 (± 1.2)</td>
<td>23.2 (± 3.2)</td>
<td>.003</td>
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<table>
<thead>
<tr>
<th>Parameter</th>
<th>13C-S-Ibuprofen</th>
<th></th>
<th></th>
<th>R-Ibuprofen</th>
<th></th>
<th></th>
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<tbody>
<tr>
<td></td>
<td>Control</td>
<td></td>
<td></td>
<td>Treated</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CL (ml/min)</td>
<td>4.2 (± 0.3)</td>
<td>4.5 (± 0.7)</td>
<td>.65</td>
<td>4.2 (± 0.3)</td>
<td>4.5 (± 0.7)</td>
<td>.65</td>
</tr>
<tr>
<td>Vss (liter/kg)</td>
<td>1.10 (± 0.21)</td>
<td>1.04 (± 0.13)</td>
<td>.82</td>
<td>1.10 (± 0.21)</td>
<td>1.04 (± 0.13)</td>
<td>.82</td>
</tr>
<tr>
<td>t1/2 (min)</td>
<td>107.6 (± 23.0)</td>
<td>101.0 (± 16.7)</td>
<td>.81</td>
<td>107.6 (± 23.0)</td>
<td>101.0 (± 16.7)</td>
<td>.81</td>
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</tbody>
</table>
R-ibuprofen, 10 mg 13C-S-ibuprofen). Mean (± S.E.M.) plasma concentration-time data for S-ibuprofen for control rats (•, n = 7), and for rats treated with clofibrate (▲, n = 8) after administration of pseudoracemic-ibuprofen (10 mg R-ibuprofen, 10 mg 13C-S-ibuprofen). B, Mean (± S.E.M.) plasma concentration-time data for S-ibuprofen for control rats (•, n = 7), and for rats treated with clofibrate (▲, n = 8) after administration of pseudoracemic-ibuprofen (10 mg R-ibuprofen, 10 mg 13C-S-ibuprofen). C, Mean (± S.E.M.) plasma concentration-time data for 13C-S-ibuprofen for control rats (•, n = 7), and for rats treated with clofibrate (▲, n = 8) after administration of pseudoracemic-ibuprofen (10 mg R-ibuprofen, 10 mg 13C-S-ibuprofen).

**TABLE 2**
Data for the extent and half-life of inversion calculated by deconvolution or area analysis

<table>
<thead>
<tr>
<th></th>
<th>Control Group</th>
<th>Clofibrate Treated Group</th>
<th>Rat</th>
<th>Fi (Decom)</th>
<th>AUC (A)</th>
<th>t_{1/2} (min)</th>
<th>Rat</th>
<th>Fi (Decom)</th>
<th>AUC (A)</th>
<th>t_{1/2} (min)</th>
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<tr>
<td>1</td>
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<tr>
<td>2</td>
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<td>0.78</td>
<td>5.0</td>
<td>10</td>
<td>0.78</td>
<td>0.75</td>
<td>5.0</td>
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<td>3</td>
<td>0.67</td>
<td>0.69</td>
<td>16.5</td>
<td>11</td>
<td>0.81</td>
<td>6.0</td>
<td>11</td>
<td>0.74</td>
<td>0.75</td>
<td>6.5</td>
</tr>
<tr>
<td>4</td>
<td>0.75</td>
<td>0.78</td>
<td>8.0</td>
<td>12</td>
<td>0.74</td>
<td>6.5</td>
<td>12</td>
<td>0.80</td>
<td>0.82</td>
<td>5.5</td>
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<tr>
<td>5</td>
<td>0.73</td>
<td>0.80</td>
<td>15.0</td>
<td>13</td>
<td>0.80</td>
<td>6.5</td>
<td>13</td>
<td>0.80</td>
<td>0.82</td>
<td>6.5</td>
</tr>
<tr>
<td>6</td>
<td>0.78</td>
<td>0.85</td>
<td>20.0</td>
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<td>0.81</td>
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<td>0.81</td>
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<tr>
<td>7</td>
<td>0.73</td>
<td>0.81</td>
<td>13.0</td>
<td>15</td>
<td>0.75</td>
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<td>0.75</td>
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<td>7.0</td>
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<tr>
<td>8</td>
<td>Mean</td>
<td>0.73</td>
<td>13.9</td>
<td>0.78</td>
<td>0.79</td>
<td>8.3</td>
<td>S.E.M. 0.02</td>
<td>0.02</td>
<td>1.4</td>
<td>0.02</td>
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</tbody>
</table>

Data based on AUC analysis. Data obtained by the polyexponential deconvolution method. Data based on AUC analysis. Rat 8 was excluded because of problems during blood sampling.

**TABLE 3**
Effect of clofibrate pretreatment on the distribution of ibuprofen into various tissues [data are given as ibuprofen/tissue (μg/g); mean ± S.E.M.]

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Concentration of Ibuprofen (μg ibuprofen/g tissue)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>Clofibrate Group</td>
<td></td>
</tr>
<tr>
<td>Fat</td>
<td>5.70 ± 1.19</td>
<td>8.42 ± 1.18</td>
</tr>
<tr>
<td>Lung</td>
<td>5.23 ± 2.18</td>
<td>8.62 ± 3.18</td>
</tr>
<tr>
<td>Brain</td>
<td>0.89 ± 0.42</td>
<td>1.93 ± 1.16</td>
</tr>
<tr>
<td>Kidney</td>
<td>17.32 ± 11.50</td>
<td>24.95 ± 10.51</td>
</tr>
<tr>
<td>Liver</td>
<td>13.32 ± 8.50</td>
<td>34.71 ± 17.73</td>
</tr>
</tbody>
</table>

*p* = .110 .078 .029

Fig. 1. Mean (± S.E.M.) plasma concentration-time data for R-ibuprofen for control rats (•, n = 7), and for rats treated with clofibrate (▲, n = 8) after administration of pseudoracemic-ibuprofen (10 mg R-ibuprofen, 10 mg 13C-S-ibuprofen). B, Mean (± S.E.M.) plasma concentration-time data for S-ibuprofen for control rats (•, n = 7), and for rats treated with clofibrate (▲, n = 8) after administration of pseudoracemic-ibuprofen (10 mg R-ibuprofen, 10 mg 13C-S-ibuprofen). C, Mean (± S.E.M.) plasma concentration-time data for 13C-S-ibuprofen for control rats (•, n = 7), and for rats treated with clofibrate (▲, n = 8) after administration of pseudoracemic-ibuprofen (10 mg R-ibuprofen, 10 mg 13C-S-ibuprofen).

Fig. 2. The mean cumulative fractional inversion (Fi) of R-ibuprofen to S-ibuprofen versus time in control rats (—, n = 7) and in rats pretreated with clofibrate (—, n = 8) as determined by plasma analysis.

**Discussion**

The use of stable isotopes has proved to be a valuable approach for pharmacokinetic studies and has been applied to studies of the kinetics and mechanism of inversion of ibuprofen in rats (Sanins et al., 1991) and man (Baillie et al., 1989; Rudy et al., 1991). Importantly, the use of isotopically labeled S-ibuprofen in combination with the R-enantiomer (pseudoracemate), allows an estimation of the fractional inversion of R-ibuprofen in the form it is usually administered, i.e., the racemate. Furthermore, only a single dose of drug needs to be administered. This is useful because data indicate that there are interactions between the enantiomers of ibuprofen at protein binding sites such that each enantiomer modifies the kinetics of the other (Lee et al., 1985; Evans et al., 1989). In the absence of a pseudoracemic preparation and associated GCMS methodology, estimations of inversion require separate administration of the enantiomers and the assumption that interactions between enantiomers do not occur.

The dosage of clofibrate used to treat the rats is large. However, we followed the same dosage regimen of Knights et al. (1991) to allow a direct comparison between their in vitro and our in vivo data. (In this respect the minimum dose required to induce the changes recorded in our study is not known.) The absolute increase in inversion of R-ibuprofen observed in preparations of rat liver homogenate was not observed in vivo where the Fi was not significantly altered by pretreatment with clofibrate. The degree of inversion in the control group (77%) was similar to previous estimates of inversion in Wistar rats (50–70%; Knihinicki et al., 1990) and to estimates of inversion in humans (57–71%; Lee et al., 1991) where the Fi was not significantly altered by pretreatment with clofibrate. However, we followed the same dosage regimen of Knights et al. (1991) to allow a direct comparison between their in vivo data. (In this respect the minimum dose required to induce the changes recorded in our study is not known.) The absolute increase in inversion of R-ibuprofen observed in preparations of rat liver homogenate was not observed in vivo where the Fi was not significantly altered by pretreatment with clofibrate. The degree of inversion in the control group (77%) was similar to previous estimates of inversion in Wistar rats (50–70%; Knihinicki et al., 1990) and to estimates of inversion in humans (57–71%; Lee et al., 1991).
a half-life of inversion of approximately 32 min was estimated in the earlier investigation (Knihinicki et al., 1990), contrasting with the 14 min in our study.

Although the total fraction of R-ibuprofen inverted was unchanged, there was a significant increase in the total clearance and the clearance of R-ibuprofen by inversion. It is difficult to be certain that noninversion pathways of ibuprofen metabolism were affected by clofibrate. In association with its well-known induction of peroxisomal proliferation, clofibrate induces a range of enzymes including the cytochrome P450 (CYP) 4A (fatty acid ω-hydroxylation) family of enzymes (Sundseth and Waxman, 1992), acyl-CoA synthetases and hydrolases (Knights et al., 1988; Mentlein et al., 1986), and fatty acyl-CoA oxidases (Gronn et al., 1992). Noninversion, oxidative pathways are marginally stereoselective for the S-enantiomer in humans (Rudy et al., 1991), whereas clearly inversion is the dominant pathway of metabolism of R-ibuprofen. Oxidation occurs on the isobutyl side chain of ibuprofen and oxidation is mediated by the CYP2C subfamily (Leemann et al., 1994). A recent study suggested both regioselective and stereooslective metabolism of ibuprofen (Hamman et al., 1997). CYP2C9 favored formation of S-2- and S-3 hydroxyibuprofen whereas CYP2C8 favored R-2 hydroxyibuprofen (Hamman et al., 1997). Thus the expression of these two isozymes may influence the disposition of ibuprofen in vivo. However, there are no reports to this time suggesting that xenobiotics induce CYP 2C8/9. The reason the proportion of ibuprofen inverted to the S-enantiomer was unaffected is that there must be a proportional diversion of drug to other pathways. Taken with our evidence that clofibrate had no effect on the disposition of S-ibuprofen, it can reasonably be assumed that the oxidation of R-ibuprofen was not affected by clofibrate. It was thus an hypothesis generated from these data that the increase in the Vss and the increase in metabolic clearance reflected an increase in the rate of incorporation of drug into what have been termed “hybrid” lipids (Williams et al., 1986) i.e., lipids in which the normal endogenous fatty acid is replaced by a xenobiotic (Fears, 1985). R-ibuprofenoyl-CoA is the precursor both for inversion (Shieh and Chen, 1993), and for incorporation of the drug into hybrid lipids. One would hypothesize on this basis that the overall fraction of the dose ending up in hybrid lipids might increase if the rate of formation of R-ibuprofenoyl-CoA was increased.

The liver is the major site of inversion of ibuprofen and of LACS expression (Brugger et al., 1996b). Consequently, to further investigate our hypothesis of enhanced lipid incorporation the effect of clofibrate on LACS expression was investigated in the liver. The data from the Western blot analysis demonstrated that clofibrate significantly increased the expression of LACS. Furthermore, as the enzyme stereospecifically activates R-ibuprofen to its CoA-thioester (Brugger et al., 1996a) increased formation of R-ibuprofenoyl-CoA may result in increased incorporation into hybrid lipids. Moreover, the fact that expression of the 2-arylpropionyl-CoA epimerase, the key enzyme that epimerizes the chiral center of the ibuprofenoyl-CoA thioester, was unaltered by clofibrate, was also consistent with our additional finding that the Fi remained unchanged. Thus increased formation of R-ibuprofenoyl-CoA thioester via up-regulation of LACS in the absence of a change in the epimerase is the most likely explanation for our findings that the Vss for R-ibuprofen increased although the Fi was unaltered.

This thesis was further supported by our investigation of tissue distribution using radiolabeled (14C) racemic ibuprofen. The magnitude of the increase in distribution of radiolabeled ibuprofen into tissues (1.5–6.0 times) after clofibrate treatment was of the same order as the increase in the Vss (2.5 times). The pharmacological relevance of the clofibrate-induced changes in ibuprofen tissue distribution and on the enzymatic level remains unclear. Although increased incorporation of R-ibuprofen into hybrid lipids may be of toxicological relevance it has also been shown that ibuprofenoyl-CoA thioesters inhibit cyclooxygenase-2 mediated prostaglandin E2 synthesis (Neupert et al., 1997) that might have therapeutic significance. Thus, the finding that clofibrate induces acyl-CoA-synthetase and thus promotes forma-

Fig. 3. Expression of LACS and epimerase in rat liver, using Western blotting. Protein levels of LACS significantly increased after clofibrate pretreatment (n = 3) as compared to controls (n = 3). Expression of the epimerase did not change. The different lanes of Western blots represent different animals.
tion of R-ibuprofenoyl-CoA thioester is an interesting finding deserving further investigation.

Only male animals were investigated in our study. It is reported that male rats are more susceptible to enzyme induction by clofibrate than female rats (Sundseth and Worman, 1992), and thus sex may also be an important determinant of the interaction, and a factor to be considered when investigating inversion in humans. Another potential contribution to the change in kinetics induced by clofibrate is an effect on biliary function. In contrast to humans, rats excrete some unchanged ibuprofen in bile (approximately 12%; Dietzel et al., 1990). However, the overall excretion is not high and in any case clofibrate has been reported to decrease biliary flow (James and Ahokas, 1992) and so this consideration would not appear to be an explanation for our results.

Displacement of R-ibuprofen by clofibrate from plasma protein binding sites might also be invoked to account for the increased clearance of R-ibuprofen. Clofibrate is reported to have a moderately long half-life of between 7 and 24 hr in rats (Brodie et al., 1976), and, therefore, although not measured, was likely to have been present in blood at relatively high concentrations at the time the ibuprofen dose was administered. However, it is to be noted that neither the clearance nor the volume of distribution of the S-enantiomer were affected in the clofibrate-treated group. S-ibuprofen binds less avidly to the common binding site on the albumin molecule than R-ibuprofen, and there is mutual displacement of the enantiomers from this binding site (Evans et al., 1989).

The S-enantiomer is thus more susceptible to protein binding displacement interactions. However, as noted, there was no evidence that the pharmacokinetic parameters of S-ibuprofen were affected by clofibrate. These data suggest that the increase in the clearance and the volume of distribution of R-ibuprofen was not due to a protein binding interaction.

In conclusion, the study confirmed the value of stable isotopes for the study of chiral inversion when the drug is administered in the usual form, i.e., the racemate. It was demonstrated that the previously observed clofibrate induced increased fractional inversion of R-ibuprofen to S-ibuprofen in vitro in rats (Knights et al., 1991) was not paralleled by an increased Fi in vivo, despite the substantial increase in the clearance by inversion of R-ibuprofen. Data on tissue distribution and enzyme expression suggest that more drug is diverted to other pathways, in particular incorporation into hybrid lipids, after exposure to clofibrate. Such an interaction may also occur in humans and the methodology developed in our study is suited to such an investigation.

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