Plasma stability-dependent circulation of acyl glucuronide metabolites in humans. How circulating metabolite profiles of muraglitazar and peliglitazar can lead to misleading risk assessment
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Pharmaceutical Candidate Optimization (DZ, NR, LW, YX, MO, WL, RR, ZY, WGH), Discovery Chemistry (SC, ST, HZ, PTC). Bristol-Myers Squibb Research and Development, Princeton, NJ 08543

Supplemental data: Oxidation of glucuronide metabolites of muraglitazar and peliglitazar in human liver microsomes

Muraglitazar AG or peliglitazar AG at 20 μM was separately incubated for 15 min with human liver microsomes (2 mg/mL protein) in 1 mL of 50 mM sodium phosphate buffer with and without 1 mM NADPH. The reaction was quenched by adding one volume of ice-cold acetonitrile containing 2% acetic acid following by centrifugation at 2000xg for 10 min. An aliquot of 100 μL was injected to LC/UV/MS/MS analysis. The samples were analyzed by HPLC system II using a Shimadzu LC-10AT system (Shimadzu Scientific Instruments, Kyoto, Japan) and LC/MS analyses were performed using the analytical column used for the microsomal incubation samples. The mobile phase consisted of two solvents: A) 0.06% TFA in water and B) 0.06% TFA in acetonitrile. The gradient consisted of the following steps: Solvent B started at 5%, then linearly increased to 25% at 5 min, to 40% at 20 min, to 53% at 60 min, to 60% at 63 min, to 90% at 65 min, held at 90% for 7 min, and then decreased to 5% at 75 min. HPLC effluent (1 mL/min) was monitored at 278 nm. The HPLC eluent was partially diverted to a LTQ mass spectrometer (ThermoFisher, San Jose, CA). Full LC/MS (Scan range of 200-1000 Da) and MS/MS spectra were collected.
Figures 1S and 2S show oxidative metabolite profiles of incubations of muraglitazar AG and peliglitazar AG in human liver microsomes in the presence of NADPH. Multiple hydroxylated metabolites and O-demethylated metabolite of muraglitazar and peliglitazar AGs were observed in the incubation starting with muraglitazar AG or peliglitazar AG. After incubations in human liver microsomes, the major components were still the starting materials (muraglitazar and peliglitazar AGs) and the incubations also led to hydrolysis of muraglitazar and peliglitazar AGs (Figures 1S and 2S). Table 1S shows mass spectrometric characterization of these oxidative metabolites. Metabolites M1, M2, and M3 had a molecular ion of m/z 709 from muraglitazar AG and m/z 723 from peliglitazar AG, and respective fragmentation ions at m/z 533 and 547 (loss of 176), which are consistent with hydroxylation products of muraglitazar AG and peliglitazar AG. Although these metabolites could be acyl migration isomers of one metabolite, more likely they are different metabolites with the hydroxyl group at different sites based on retention times. Metabolite M4 had a molecular ion of m/z 679 from muraglitazar AG and m/z 693 from peliglitazar AG, and respective fragmentation ions at m/z 503 and 517 (loss of 176), which are consistent with demethylation products of muraglitazar AG and peliglitazar AG. Metabolites M5, M6, and M7 had a molecular ion of m/z 533 from muraglitazar AG and m/z 547 from peliglitazar AG, which are consistent with hydroxylated products of muraglitazar and peliglitazar. Metabolite M8 had a molecular ion of m/z 503 from muraglitazar AG and m/z 517 from peliglitazar AG, which are consistent with demethylated products of muraglitazar and peliglitazar. Therefore, in addition to these oxidative metabolites of glucuronide, multiple hydroxylated metabolites and an O-demethylated metabolite of muraglitazar were also observed in the incubation.
Without NADPH, no oxidative metabolites of muraglitazar, peliglitazar, muraglitazar AG, or peliglitazar AG were observed. In the incubations without NADPH, glucuronide isomers of muraglitazar or peliglitazar (e.g. the peak after the starting material peaks in Figures 1S and 2S) were observed. The muraglitazar or peliglitazar oxidative metabolites could be formed from muraglitazar or peliglitazar that resulted from hydrolysis of muraglitazar AG or peliglitazar AG or from hydrolysis of oxidized muraglitazar AG or peliglitazar AG. Very similar degradation profiles were observed for the incubation of muraglitazar AG or peliglitazar AG in the human liver microsomes (Figures 1S and 2S).

Figure 3S shows oxidation pathways as well as hydrolysis and isomerization of muraglitazar AG or peliglitazar AG in the in vitro incubations. In the incubations in the presence of NADPH, the oxidized glucuronides could have only been formed by oxidation of the acyl glucuronides since UDPGA was not present in the incubations. There are several reports in the literature on the oxidative metabolism of AGs including diclofenac (Kumar et al., 2002), MRL-C (Kochansky et al., 2007), bilirubin (Crawford et al., 1992), valproic acid (Tang et al., 1996), and gemfibrozil (Ogilvie et al., 2006). In humans, in addition to muraglitazar AG and peliglitazar AG, oxidized (hydroxylated and O-demethylated) acyl glucuronides were also metabolites of muraglitazar and peliglitazar following oral administration (Wang et al., 2006; Wang et al., 2010).

References:


**Supplement Figures**

**Figure 1S.** UV and ion chromatograms of oxidative metabolites of muraglitazar AG formed in HLM incubations in the presence of NADPH.

**Figure 2S.** UV and ion chromatograms of oxidative metabolites of peliglitazar AG formed in HLM incubations in the presence of NADPH.

**Figure 3S.** Hydrolysis, isomerization and metabolic pathways of muraglitazar AG and peliglitazar AG.
Table 1S. LC/MS/MS characterization of oxidative metabolites of muraglitazar AG and peliglitazar AG in human liver microsomes in the presence of NADPH

<table>
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<tr>
<th></th>
<th><strong>Muraglitazar AG</strong></th>
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<th></th>
<th><strong>Peliglitazar AG</strong></th>
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<td></td>
<td><strong>T&lt;sub&gt;R&lt;/sub&gt;</strong> (min)</td>
<td><strong>MH&lt;sup&gt;+&lt;/sup&gt; m/z</strong></td>
<td><strong>Major Fragments m/z</strong></td>
<td><strong>T&lt;sub&gt;R&lt;/sub&gt;</strong> (min)</td>
<td><strong>MH&lt;sup&gt;+&lt;/sup&gt; m/z</strong></td>
<td><strong>Major Fragments m/z</strong></td>
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DMD35048
Fig 1S

Muraglitazar glucuronide

Mura_glu_HLM_NADPH

Channel A
UV:278

Hydroxylated muraglitazar glucuronides

O-Demethylated muraglitazar glucuronide

NL: 7.96E2
m/z = 532.50-533.50
ITMS + c ESI Full ms2
709.00@20.00
Mura_glu_HLM_NADPH

NL: 4.24E3
m/z = 502.50-503.50
ITMS + c ESI Full ms2
679.00@20.00
Mura_glu_HLM_NADPH
Fig 3S

2,3,4-O-glucuronide

Acyl migration

1-O-β-glucuronide

UGT/UDPGA

Hydrolysis

muraglitazar or peliglitazar

P450

Hydrolysis

M1, M2, M3 (hydroxy glucuronide)

M5, M6, M7 (hydroxy metabolite)

M4 (O-demethyl glucuronide)

M8 (O-demethyl metabolite)

R = H for muraglitazar and R = CH₃ for peliglitazar