The Meta Isomer of Acetaminophen Is A Time Dependent Inhibitor of Human CYP2E1

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Abstract

*N*-acetyl-*m*-aminophenol (3'-hydroxyacetanilide, AMAP) is the *meta* isomer of acetaminophen (4'-hydroxyacetanilide, APAP), the widely used analgesic that is safe at therapeutic doses but is hepatotoxic at larger doses. Unlike APAP, AMAP does not cause hepatotoxicity in mice even though AMAP and its metabolites covalently bind to hepatic proteins at levels comparable to APAP. Therefore, comparative studies with APAP and AMAP have been used in order to investigate mechanisms of toxicity and structure-toxicity relationships. However, the relationship between AMAP and CYP2E1, the enzyme generally implicated in the amplification of APAP-induced hepatotoxicity after ethanol ingestion, has not been fully elucidated. The microsomal metabolism of AMAP to reactive metabolites has been studied however, the identity of the reactive metabolite(s) of AMAP that bind to CYP2E1 has not been unequivocally determined. Therefore, we hypothesized that AMAP would covalently bind to and inhibit CYP2E1 in a reconstituted system and that mass spectral analysis would provide structural information for the reactive metabolite. Deconvoluted mass spectra indicated that a reactive metabolite of AMAP forms mono- and diadducts with CYP2E1 apoprotein (experimentally measured masses = 54622.4 ± 8.9 Da, 54791.3 ± 6.1 Da, and 54451.7 ± 5.5 Da, respectively) but not to other incubation components (i.e., heme, cytochrome b5, or cytochrome P450 reductase). NADPH was required for adduct formation while glutathione prevented it. The data indicated that reactive metabolite formation probably involves the addition of one oxygen atom to AMAP (MW$_{AMAP}$ = 151.2 Da; MW$_{oxidized \; AMAP}$ = 151.2 + 16.0 = 167.2 Da; experimentally determined mass of the small molecule adducted to CYP2E1 = 167.5 ± 7.1 Da. Therefore, the reactive metabolite of AMAP that covalently binds to CYP2E1 is likely formed from aromatic oxidation (quinone formation).

Disciplines
Pharmacy and Pharmaceutical Sciences

Comments
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Table of detected masses for CYP2E1, cytochrome P450 reductase, cytochrome b5, and heme analyzed under various incubation conditions:

<table>
<thead>
<tr>
<th>Component</th>
<th>MW (Da)</th>
<th>Reaction</th>
<th>% Control Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffered</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ 20 mM AMAP</td>
<td>54627</td>
<td>CYP2E1 + AMAP metabolite</td>
<td>21.2 ± 3.6</td>
</tr>
<tr>
<td>+ 40 mM AMAP</td>
<td>54787</td>
<td>CYP2E1 + 2 AMAP metabolites</td>
<td>19.8 ± 3.2</td>
</tr>
</tbody>
</table>

Research Objective

Characterize CYP2E1-AMAP interactions and assess the utility of AMAP as a probe of human CYP2E1 structure-function by testing for mechanism-based inactivation.

Results

1. Inactivation of human CYP2E1-mediated chlorzoxazone hydroxylase activity following pre-incubation with AMAP and NADPH. Data points are the average of at least three experiments conducted on separate days.

2. Remaining chlorzoxazone hydroxylase activity following 72 minutes preincubation. Values are the average of two experiments conducted in triplicate on separate days.

Introduction

- To reduce the risk of potential drug-drug interactions (DDIs) new chemical entities (NCEs) are commonly assessed for time dependent inhibition of major drug metabolizing enzymes (Grimm SW et al (2009) Drug Metabolism and Disposition 37: 1355-1370).

- Whereas time dependent inhibition (TDI) is typically an undesired characteristic for prospective drugs, a time dependent inhibitor can be a useful tool to generate new information about protein structure-function relationships.

Previous studies suggest N-acetyl-m-amino-phenol may be an effective probe of human CYP2E1.

N-Acetyl-m-amino-phenol (3'-hydroxyacetanilide, AMAP) is the meta isomer of acetaminophen (4'-hydroxyacetanilide, APAP), the widely used analgesic that is hepatotoxic in overdose situations. Unlike APAP, AMAP does not cause hepatotoxicity in mice even though AMAP and its metabolites covalently bind to hepatic proteins at levels comparable to APAP (Nelson EB (1980) Res Commun Chem Pathol Pharmacol 28:447-456).

- p-Nitrophenol hydroxylase activity is decreased in mouse liver microsomes and an anti-arylacetamide antibody identified a 50-kDa protein that comigrated with CYP2E1.

- Mass spectra of human CYP2E1

Mass spectra indicate adduct formation is NADPH dependent and specific for CYP2E1 in the reconstituted system.

Conclusions

1. Inhibition data is consistent with mass spectral data:
   - Mass spectra indicated CYP2E1-AMAP adduct formation is NADPH dependent.
   - Inhibition of chlorzoxazone hydroxylase activity is NADPH dependent.
   - Glutathione protects CYP2E1 from inactivation.

2. Mass spectral data indicated metabolite formation involves the addition of one

$\text{AMAP} \text{+ reconstituted CYP2E1} \rightarrow [\text{AMAP}\text{-CYP2E1}]^* \rightarrow [\text{AMAP}\text{-CYP2E1}]$.

\begin{align*}
\text{MW} & = [\text{MW}_{\text{CYP2E1}}] + [\text{MW}_{\text{AMAP}}] + 200

3. $1/K_{inact}$ versus 1/(AMAP)

Conclusions

1. Inhibition data is consistent with mass spectral data:
   - Mass spectra indicated CYP2E1-AMAP adduct formation is NADPH dependent.
   - Inhibition of chlorzoxazone hydroxylase activity is NADPH dependent.
   - Glutathione protects CYP2E1 from inactivation.

2. Mass spectral data indicated metabolite formation involves the addition of one oxygen atom to AMAP ($\text{MW}_{\text{AMAP}} = 151.2$ Da; $\text{MW}_{\text{oxidized AMAP}} = 151.2 + 16.0 = 167.2$ Da; experimentally determined mass of the small molecule adducted to CYP2E1 was $167.5 \pm 7.1$ Da ($n = 3$).

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