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

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 b_5, 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

Poster presented at the International Society for the Study of Xenobiotics (ISSX) Workshop on Genetic Polymorphisms in Drug Disposition, Indianapolis, IN 2010

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

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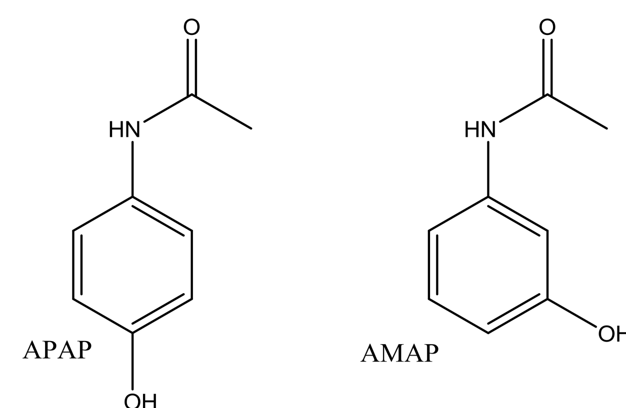
Introduction

- 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.

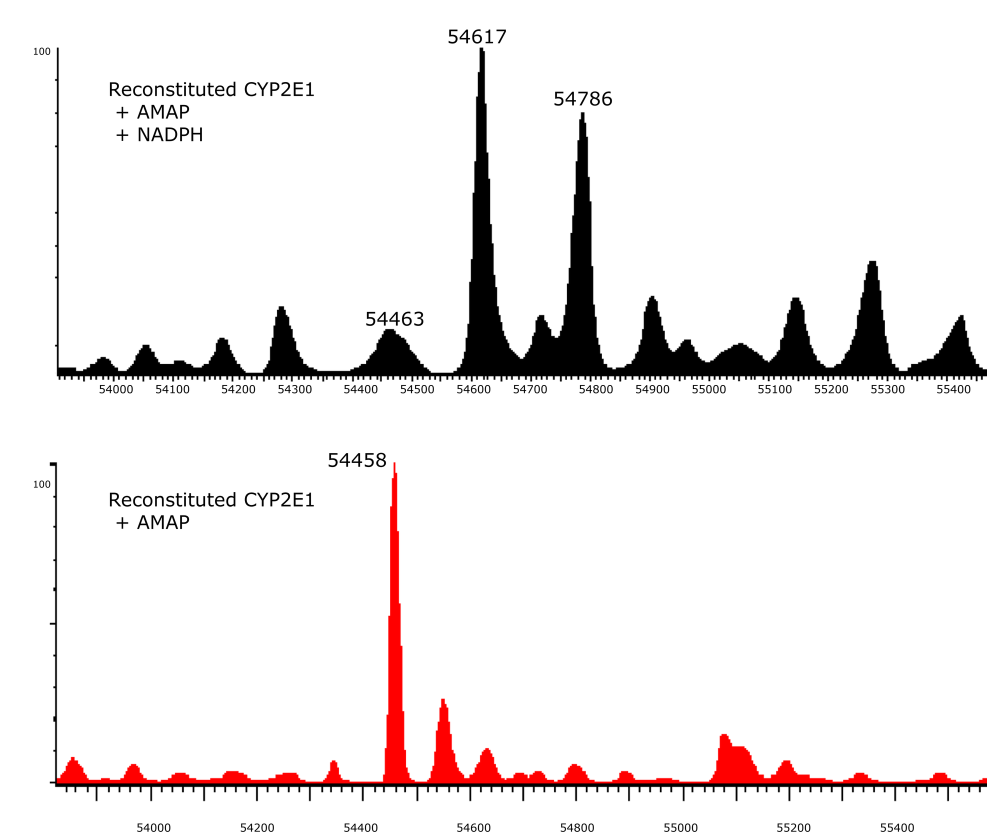
Previous studies suggest *N*-acetyl-*m*-aminophenol may be an effective probe of human CYP2E1.

- *N*-Acetyl-*m*-aminophenol (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 from AMAP-treated mice (Halmes NC, Samokyszyn VM, Hinton TW, Hinson JA, and Pumford NR (1998) *Toxicol Letters* 94:65-71.

- Mass spectra of human CYP2E1-AMAP adducts generated from reconstituted CYP2E1 indicate a reactive metabolite of AMAP forms mono- and diadducts with CYP2E1.



Introduction

- Table of detected masses for CYP2E1, cytochrome P450 reductase, cytochrome b_5, and heme analyzed under various incubation conditions:

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

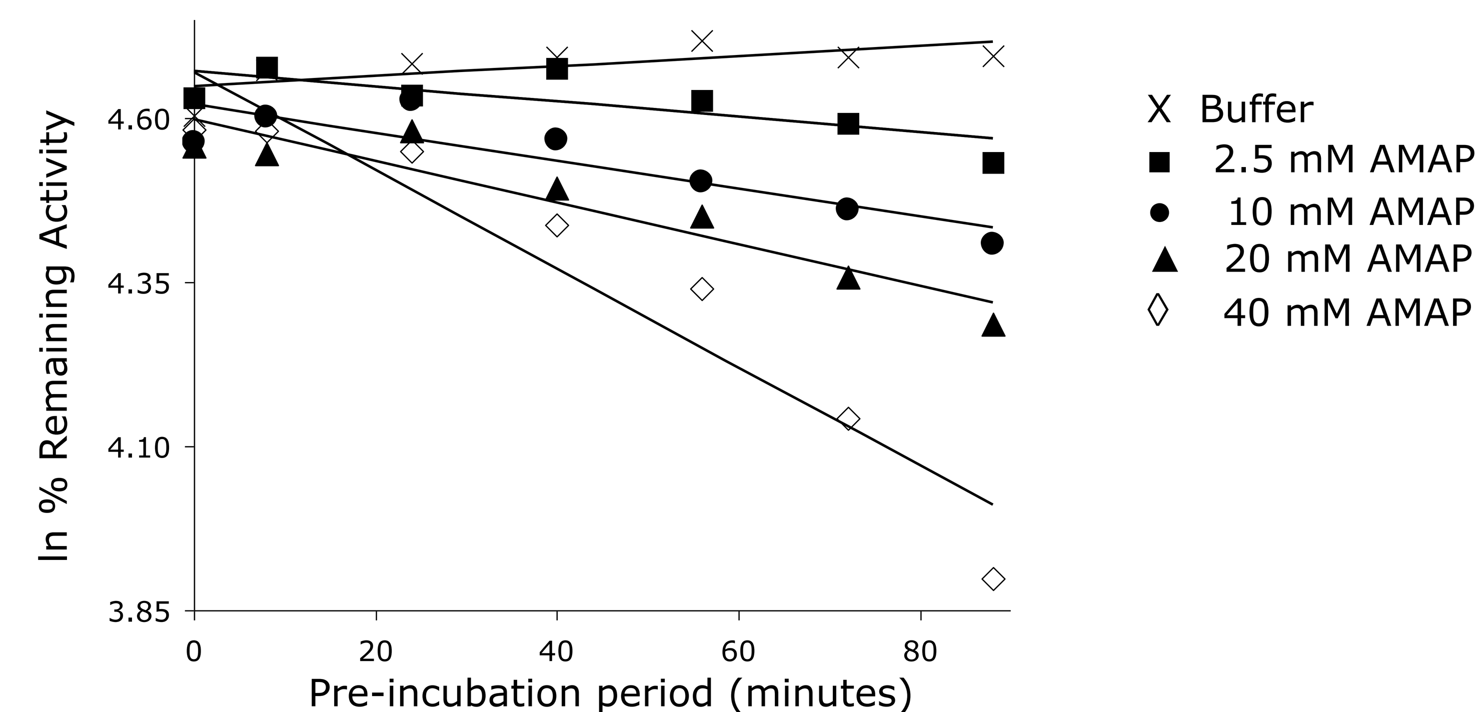
	Detected Masses				
Component	CYP2E1	Reductase	Cytochrome b_5	K^+Heme	n
Reconstituted system	54622.4 ± 8.9	77712.2 ± 2.4	15949.3 ± 0.6	657.3 ± 0.1	6
+ NADPH, + AMAP	54791.3 ± 6.1				
Reconstituted system	54458.7 ± 3.3	77719.2 ± 5.1	15949.0 ± 1.4	657.3 ± 0.0	5
+ AMAP					
Reconstituted system	54465.5 ± 2.0	77721.3 ± 1.3	15949.0 ± 1.2	657.3 ± 0.2	2
+ NADPH					
CYP2E1 standard	54451.7 ± 5.5	NA	NA	657 ± 0.3	3
P450 reductase standard	NA	77714.5 ± 0.7	NA	NA	2
Cytochrome b_5 standard	NA	NA	15948.8 ± 0.4	657.3 ± 0.0	2

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.

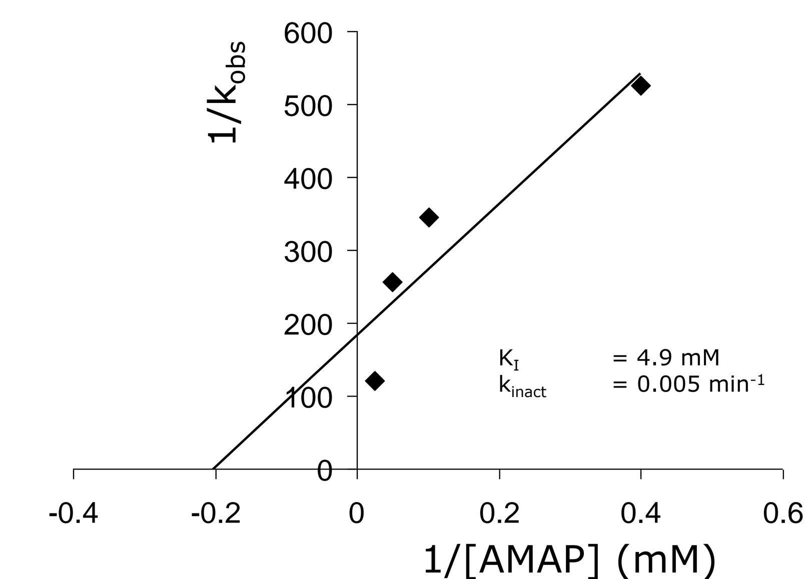


2. Remaining chlorzoxazone hydroxylase activity following 72 minutes preincubation. Values are the average of two experiments conducted in triplicate on separate days.

Pre-Incubation Components	% Control Activity
2E1 Supersomes	100 (12.2 ± 0.5 pmol/min/pmol)
+ NADPH	88.5 ± 2.6
+ 2.5 mM AMAP	98.6 ± 2.5
+ 20 mM AMAP	89.2 ± 2.8
+ 40 mM AMAP	65.6 ± 2.6
+ 20 mM AMAP, + NADPH	64.7 ± 6.2
+ 20 mM AMAP, + NADPH, + 5 mM GSH	91.2 ± 2.9
+ 40 mM AMAP, + NADPH	43.4 ± 3.4
+ ABT, + NADPH	< 1

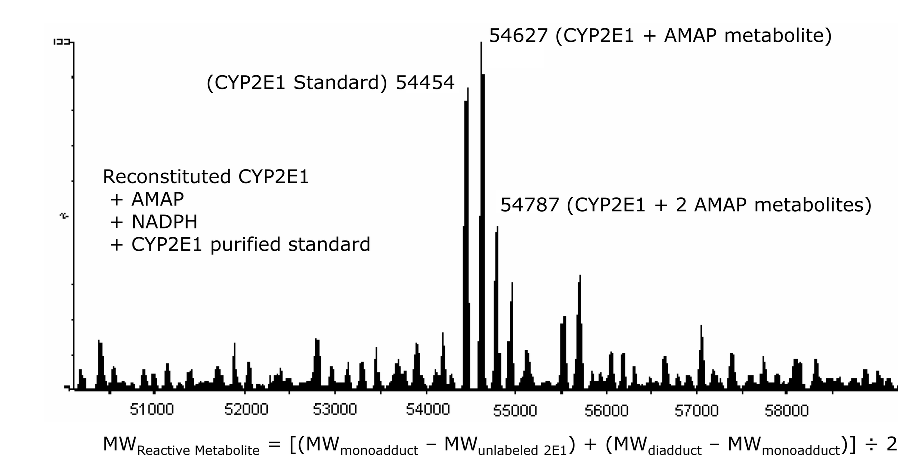
Results

3. $1/k_{obs}$ 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.
   - Mass spectral data indicated metabolite formation 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 (n = 3).



2. Mass spectral data, K_I, and lag period prior to inactivation all support a multi-step activation process involving aromatic hydroxylation followed by a second oxidative event to form a reactive quinone and is consistent with evidence from metabolite identification studies of AMAP glutathione conjugates (Streeter AJ, Bjorge SM, Axworthy DB, Nelson SD, and Baillie TA (1984) *Drug Metab Dispos* 12:565-576.