trans-Cinnamic Aldehyde Is A Time-Dependent Inhibitor of Human CYP2A6

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Abstract
Cytochrome P450 2A6 is involved in the metabolism of nicotine and the activation of carcinogenic nitrosamines. It is expressed in the liver and several extrahepatic tissues including lung and nasal mucosa. Genetic variability of CYP2A6 is considerable and has clinical implications. CYP2A6 polymorphisms are suggested to influence smoking behavior and cancer risk (Piazezza et al., 1998 and Kamataki et al., 1999). The clearance of letrozole, an aromatase inhibitor, can be altered by CYP2A6 variants (Desta et al., 2010). While the relationship between CYP2A6 polymorphisms and nicotine metabolism is established, the impact of dietary constituents and other xenobiotics as CYP2A6 modulators is less understood. With this in mind, cinnamic aldehyde and related analogs were assessed for their capacity to modulate CYP2A6 activity in vitro. Cinnamic aldehyde is the major constituent in cinnamon oil and is present in a variety of foods, gums, cosmetic products, and supplements. Of three cinnamic derivatives cinnamic aldehyde displayed the greatest potency in IC$_{50}$ studies (IC$_{50}$ = 7.0 µM; IC$_{50}$ = 354.4 µM and IC$_{50}$ > 2500 µM for cinnamic alcohol and cinnamic acid, respectively). Cinnamic aldehyde also exhibited time and NADPH dependent inhibition of coumarin hydroxylase activity (K$_{i}$ = 21.0 µM and k$_{inact}$ = 0.038 min$^{-1}$). Molecular modeling identified five amino acids interacting with cinnamic aldehyde and threonine 305 was identified as a potential site for covalent modification by a reactive metabolite generated from activation of cinnamic aldehyde. The investigation of this model Michael acceptor molecule will facilitate the understanding of the molecular mechanism of CYP2A6 inactivation by phenylpropanoids.

Disciplines
Pharmacy and Pharmaceutical Sciences

Comments
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**trans-Cinnamic Aldehyde Is A Time-Dependent Inhibitor of Human CYP2A6**

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**Objectives**

1. Assess the potential for cinnamic derivatives to modulate human CYP2A6 activity and to test the most potent inhibitors for mechanism based inactivation.
2. Dock cinnamic derivatives with CYP2A6 to identify prospective protein-substrate interactions.

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**Introduction**

A relationship between CYP2A6 genetic polymorphisms and nicotine metabolism has been described and the influence of social environment (e.g., peer group, family, etc.) on smoking behavior has also been studied1-3. Information describing the impact of dietary constituents as CYP2A6 modulators is less available.

The functional impact of amino acid changes resulting from genetic polymorphisms and the structural basis for CYP2A6 substrate/product selectivity has yet to be elucidated. In an effort to shed light on these topics, cinnamic aldehyde and related analogs were assessed for their potential to probe and modulate CYP2A6 activity.

CYP2A6 is the major human nicotine metabolizing enzyme: CYP2A6 is the major human enzyme catalyzing the initial oxidation of nicotine, which is a primary route of clearance. There is evidence CYP2A6 genetic polymorphisms influence smoking behaviors (e.g., slow metabolizers exhibit decreased smoking intensity)2. CYP2A6 is also involved in the activation of carcinogenic nitrosamines and the N-dealkylation of N-(4-hydroxyphenyl)acetamide (Femara®), an aromatase inhibitor used in the treatment of breast cancer in postmenopausal women4-6.

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**Results**

1. Inactivation of human CYP2A6 mediated coumarin hydroxylase activity following pre-incubation with trans-cinnamic aldehyde and NADPH. Data points are the average of at least three trials conducted on separate days.

2. Remaining coumarin hydroxylase activity following 18 minute preincubation. Values are the average of three trials conducted on separate days.

3. Cinnamic aldehyde is the most potent inhibitor of the cinnamic derivatives tested.

4. NADPH is required for time-dependent inhibition of CYP2A6 by cinnamic aldehyde.

5. Molecular docking of cinnamic aldehyde (A), cinnamic alcohol (B), and the proposed 2,3-epoxy cinnamic aldehyde (C) into the CYP2A6 crystal structure (1Z10). The docked molecule is displayed in green, the macrocycle in cyan, the heme in red, and interacting amino acids in yellow (AutoDock 4.2, Release 4.2.2.1, The Scripps Research Institute, La Jolla, CA).

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**Discussion**

1. trans-Cinnamic aldehyde is a time-dependent inhibitor of CYP2A6 (K1 = 17.2 µM and k1 inact = 0.93 min⁻¹).

2. The NADPH requirement for time-dependent inhibition of CYP2A6 by cinnamic aldehyde and trapping of a presumptive metabolite by GSH indicates that a metabolite of cinnamic aldehyde, rather than the parent molecule, is the inactivating agent.

3. 2,3-epoxy cinnamic aldehyde is proposed as a reactive metabolite that results in CYP2A6 inactivation.

4. Docking of cinnamic aldehydes and 2,3-epoxy cinnamic aldehyde identified F118, G301, and T305 as residues interacting with both probe molecules, with T305 serving as a potential reactive site for covalent modification by 2,3-epoxy cinnamic aldehyde.

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**Cinnamic aldehyde and related analogs as probes of CYP2A6:****

Cinnamic aldehyde is the major constituent in cinnamon oil and is present in a variety supplements and herbal medicines as well as foods, gums, and cosmetic products.

Crystal structures of CYP2A6 indicate the active site is relatively small compared to other drug metabolizing CYPs and this is often reflected in CYP2A6 substrate selectivity. That is, CYP2A6 substrates are generally low molecular weight molecules. We hypothesized that dietary phenylpropanoids such as cinnamic aldehyde may serve as probes of CYP2A6 function-reactor relationships.

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**Log IC50:**

<table>
<thead>
<tr>
<th>Cinnamic Derivative</th>
<th>IC50 (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aldehyde</td>
<td>6.9 ± 1.2</td>
</tr>
<tr>
<td>Alcohol</td>
<td>3.4 ± 1.4</td>
</tr>
<tr>
<td>Acid</td>
<td>&gt; 2500</td>
</tr>
</tbody>
</table>

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**References**


