IN VITRO CHARACTERIZATION OF CYTOCHROME P450 2D6 INHIBITION BY CLASSIC HISTAMINE H1 RECEPTOR ANTAGONISTS

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(Received June 27, 1997; accepted February 17, 1998)

This paper is available online at http://www.dmd.org

ABSTRACT:

Classic antihistamines, namely diphenhydramine, chlorpheniramine, clemastine, perphenazine, hydroxyzine, and tripelennamine, share structural features with substrates and inhibitors of the polymorphic cytochrome P450 (CYP) isozyme CYP2D6. Therefore, the current study was undertaken to characterize the in vitro inhibition of CYP2D6 by these commonly used, histamine H1 receptor antagonists. Micromolar incubations were performed using bufuralol as a specific CYP2D6 substrate and microsomes derived from human cells transfected with CYP2D6 cDNA. Reaction velocities were assessed in the absence and presence of antihistamines (20 μM) at 11 substrate concentrations (1, 2.5, 5, 7.5, 10, 15, 20, 25, 50, 75, and 100 μM), as well as at three nonsaturating substrate concentrations (2.5, 5, and 20 μM) and three inhibitor concentrations (5, 20, and 50 μM). In the presence of all antihistamines, the Vmax and KM of bufuralol 1'-hydroxylation were significantly altered, compared with the uninhibited reaction (p < 0.05). Lineweaver-Burke plots suggested competitive inhibition of the reaction by diphenhydramine and mixed inhibition by all other antihistamines tested. Diphenhydramine and chlorpheniramine, with estimated Ki values of ~11 μM, were the weakest inhibitors of CYP2D6 in vitro. Whereas tripelennamine, promethazine, and hydroxyzine were similar in their inhibitory capacities (Ki ~ 4–6 μM), clemastine appeared to be significantly more potent, with a Ki of ~2 μM. These data demonstrate that classic histamine H1 receptor antagonists, available in over-the-counter preparations, inhibit CYP2D6 in vitro. Furthermore, the CYP2D6-inhibitory concentrations of these antihistamines are in the range of their expected hepatic blood concentrations, suggesting that, under specific circumstances, clinically relevant interactions between classic antihistamines and CYP2D6 substrates might occur.

The classic histamine H1 receptor antagonists clemastine, diphenhydramine, chlorpheniramine, tripelennamine, promethazine, and hydroxyzine were introduced into clinical practice >50 years ago and are today among the most commonly used drugs in the world (Simons and Simons, 1994). Because these compounds have excellent overall safety records, they are found in a wide variety of over-the-counter cold and allergy treatments, as well as in sleeping aids, and they are often used in combination with other drugs (Simons and Simons, 1994). Despite their widespread use over an extended period, little is known about their pharmacokinetics, particularly their interactions with specific P4501 isozymes.

Several observations argue for an important role of the polymorphic P450 isoform CYP2D6 in the metabolism and the interaction profiles of classic antihistamines. First, the structural criteria elaborated for the optimal binding of diphenhydramine and its analogues to P450 (Rekkka et al., 1989) are very similar to the structural characteristics of many known CYP2D6 substrates and inhibitors (de Groot et al., 1997). Second, a recent study demonstrated that, in vitro, the residual activity of bufuralol 1-hydroxylation was lowest in the presence of clemastine (5% of control activity at a concentration of 100 μM clemastine) and highest in the presence of diphenhydramine (40% of control activity at a concentration of 100 μM diphenhydramine) (Nakamura et al., 1996). The same investigators demonstrated that promethazine and chlorpheniramine inhibited CYP2D6 activity in vitro, although the type of inhibition was not determined (Nakamura et al., 1996). Third, and consistent with these in vitro data and structural considerations, a case study reported a 2-fold increase in the half-life of diphenhydramine in an elderly woman with impaired metabolism of the CYP2D6 substrate imipramine (Glassman et al., 1985). Lastly, the pharmacokinetics of classic antihistamines are characterized by intersubject variability (Chiou et al., 1979; Huang et al., 1982) similar to that described for CYP2D6 substrates; therefore, this variability might be the result of a genetically determined lack of metabolic capacity (Alvan, 1991).

The isoform CYP2D6 has received considerable attention because of the presence of a genetic polymorphism that divides the population into individuals with high enzyme activity (extensive metabolizers) and individuals (5–10% of the population) with low enzyme activity (poor metabolizers) (Mahgoub et al., 1977). Poor metabolizers are predisposed to the accumulation of CYP2D6 substrates and drug-induced adverse effects (Lennard, 1993). Similarly, when extensive metabolizers are treated simultaneously with a substrate and a potent...
inhibitor of CYP2D6, substrate accumulation occurs (Brosen et al., 1987).

The objectives of the present study were to substantiate and extend previous work by determining the type of inhibition of in vitro CYP2D6 activity produced by the classic histamine H1 receptor antagonists diphenhydramine, chlorpheniramine, promethazine, and clemastine. Furthermore, we intended to determine the type and extent of CYP2D6 inhibition produced by two additional, structurally related, classic antihistamines, namely tripelennamine and hydroxyzine.

Materials and Methods

Chemicals. Clemastine fumarate, chlorpheniramine maleate, promethazine hydrochloride, tripelemamine hydrochloride, diphenhydramine hydrochloride, hydroxyzine dihydrochloride, NADP, glucose-6-phosphate, and glucose-6-phosphate dehydrogenase in 3.0 ml of 100 mM potassium phosphate buffer (pH 7.4). Incubation mixtures containing microsomes, substrate, and buffer were preincubated at 37°C for 10 min before the addition of the NADPH-regenerating system. The resulting mixture was incubated for 30 min at 37°C in a Dubnoff incubator (Precision Scientific, Chicago, IL), and the reaction was stopped by the addition of 25 μl of perchloric acid (69–72%, by volume). Proteins were sedimented by centrifugation. All experiments were performed in duplicate.

Clemastine fumarate, chlorpheniramine maleate, promethazine hydrochloride, tripelemamine hydrochloride, diphenhydramine hydrochloride, and hydroxyzine dihydrochloride, NADP, glucose-6-phosphate, and glucose-6-phosphate dehydrogenase were obtained from Sigma Chemical Co. (St. Louis, MO). Magnesium chloride was purchased from Anachemia Science (Ville-St-Pierre, Montreal, Quebec, Canada), and (-)-bufuralol hydrochloride and 1’-hydroxybufuralol were purchased from Gentest Corp. (Woburn, MA). Other chemicals were obtained from the usual commercial sources and were of analytical grade.

Recombinant Human CYP2D6 Isozymes. Human microsomes expressing CYP2D6 protein was purchased from Gentest. This protein was derived from a human AHH-1-TK+/− cell line transfected with cDNA encoding human CYP2D6-Val394Tyr.

Microsomal Incubations. Incubations (final volume, 250 μl) contained substrate (0–350 μM bufuralol in 100 mM potassium phosphate buffer, pH 7.4), 10 μl of microsomes (0.4 mg/ml), 100 μl of a NADPH-regenerating system (7.75 mg of NADP, 7.75 mg of glucose-6-phosphate, and 36 units of glucose-6-phosphate dehydrogenase in 3.0 ml of 100 mM potassium phosphate buffer, pH 7.4), and potassium phosphate buffer (100 mM, pH 7.4). Incubation mixtures containing microsomes, substrate, and buffer were preincubated at 37°C for 10 min before the addition of the NADPH-regenerating system. The resulting mixture was incubated for 30 min at 37°C in a Dubnoff incubator (Precision Scientific, Chicago, IL), and the reaction was stopped by the addition of 25 μl of perchloric acid (69–72%, by volume). Proteins were sedimented by centrifugation. All experiments were performed in duplicate.

Clemastine fumarate, chlorpheniramine maleate, promethazine hydrochloride, tripelemamine hydrochloride, diphenhydramine hydrochloride, and hydroxyzine dihydrochloride were dissolved in potassium phosphate buffer (100 mM, pH 7.4). To characterize the type and extent of inhibition of CYP2D6 by an antihistamine, substrate accumulation occurs. Data for bufuralol 1’-hydroxylation formed per picomole of CYP2D6 per minute. Velocity values for the inhibition of bufuralol 1’-hydroxylation by classic antihistamines (means ± 95% confidence intervals). Inhibition of bufuralol 1’-hydroxylation by classic antihistamines (means ± 95% confidence intervals).

**TABLE 1**

<table>
<thead>
<tr>
<th>Antihistamine</th>
<th>( V_{\text{max}} ) pmol/pmol CYP2D6/min</th>
<th>( K_M ) μM</th>
<th>( K_i ) Competitive μM</th>
<th>( K_i ) Noncompetitive μM</th>
<th>( K_i ) Mixed μM</th>
</tr>
</thead>
<tbody>
<tr>
<td>None (control)</td>
<td>2.51 ± 0.25</td>
<td>10.0 ± 3.3</td>
<td>11.7 ± 2.8a</td>
<td>49.8 ± 13.1</td>
<td>30.4 ± 6.6</td>
</tr>
<tr>
<td>Diphenhydramine</td>
<td>1.75 ± 0.18</td>
<td>18.6 ± 5.2</td>
<td>5.4 ± 0.4</td>
<td>25.6 ± 6.3</td>
<td>10.8 ± 1.4b</td>
</tr>
<tr>
<td>Chlorpheniramine</td>
<td>1.84 ± 0.10</td>
<td>37.8 ± 4.7</td>
<td>0.8 ± 0.1</td>
<td>4.8 ± 1.1</td>
<td>2.0 ± 0.3c</td>
</tr>
<tr>
<td>Clemastine</td>
<td>0.61 ± 0.03</td>
<td>32.8 ± 8.7</td>
<td>3.1 ± 0.4</td>
<td>15.8 ± 4.1</td>
<td>5.6 ± 0.8b</td>
</tr>
<tr>
<td>Tripelemamine</td>
<td>1.74 ± 0.34</td>
<td>54.5 ± 20.9</td>
<td>1.9 ± 0.2</td>
<td>8.5 ± 2.3</td>
<td>3.8 ± 0.7b</td>
</tr>
<tr>
<td>Promethazine</td>
<td>1.05 ± 0.10</td>
<td>37.1 ± 7.8</td>
<td>1.6 ± 0.4</td>
<td>9.3 ± 2.2</td>
<td>3.9 ± 0.5</td>
</tr>
<tr>
<td>Hydroxyzine</td>
<td>1.01 ± 0.21</td>
<td>36.0 ± 7.4</td>
<td>0.4 ± 0.2</td>
<td>25.6 ± 6.3</td>
<td>13.1 ± 3.1</td>
</tr>
</tbody>
</table>

a Competitive inhibitor, according to Lineweaver-Burke plot. b Mixed inhibitors, according to Lineweaver-Burke plots.
sistent with the fact that these agents are perceived as being relatively safe compounds. In fact, if drug interactions between classic antihistamines and other drugs have occurred, they have not been documented in the scientific literature. What was considered most bothersome about this class of agents was that they are not selective for histamine H1 receptors, thus inducing dopaminergic, serotonergic, and cholinergic responses (Simons and Simons, 1994). This pharmacological nonselectivity, combined with the ability to penetrate the blood-brain barrier, leads to the development of significant adverse effects in the central nervous system. These central nervous system effects occur when the drug is administered alone and appear to be related to plasma concentrations (Carruthers et al., 1978) but not to race (Spector et al., 1980), gender, or age (Berlinger et al., 1982).

On the other hand, after oral dosing, classic antihistamines are characterized not only by rapid and extensive distribution but also by considerable accumulation after multiple doses (Paton and Webster, 1985; Huang et al., 1982). If these agents are metabolized by CYP2D6, as shown directly for promethazine (Nakamura et al., 1996) and indirectly for chlorpheniramine (Yasuda et al., 1995), and they inhibit the same enzyme, one might speculate that autoinhibition could occur. However, multiple-dose data are limited to a few subjects and, despite an estimated accumulation factor of 4–9 for chlorpheniramine (Yasuda et al., 1996), as shown directly for promethazine (Nakamura et al., 1996), they have not been documented.

Interactions of classic antihistamines with cardiovascular, antidepressant, and antipsychotic CYP2D6 substrates. Thus, well-controlled studies of the interactions of classic antihistamines with such substrates must be performed with individuals with high and low CYP2D6 activities.

Acknowledgment. The authors thank Michel Bluin for excellent technical assistance.

References


## Table 2

<table>
<thead>
<tr>
<th>Antihistamine</th>
<th>Plasma Concentration</th>
<th>Plasma Concentration</th>
<th>Tissue Concentration</th>
<th>Expected Liver Concentration</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diphenhydramine HCl</td>
<td>0.06–0.09</td>
<td>0.21–0.55</td>
<td>Liver/blood, 10:1</td>
<td>2–5</td>
<td>Blyden et al., 1986; Jones and Pounder, 1987</td>
</tr>
<tr>
<td>Chlorpheniramine maleate</td>
<td>0.02</td>
<td>0.05</td>
<td>Liver/plasma, 22:1</td>
<td>1.2</td>
<td>Athanikar et al., 1979; Kamet al., 1969</td>
</tr>
<tr>
<td>Tripelennamine HCl</td>
<td>0.11</td>
<td>0.36</td>
<td>Liver/blood, 2–7:1</td>
<td>0.7–2.5</td>
<td>Yeh et al., 1986; Rao et al., 1975</td>
</tr>
<tr>
<td>Clemastine fumarate</td>
<td>0.002–0.01</td>
<td>0.004–0.02</td>
<td>Liver/blood, 10:1</td>
<td>1.7</td>
<td>Tham et al., 1978</td>
</tr>
<tr>
<td>Hydroxyzine HCl</td>
<td>0.073–0.08</td>
<td>0.16–0.17</td>
<td>Liver/blood, 43:1</td>
<td>2.3</td>
<td>Pong and Huang, 1974; Simons et al., 1984</td>
</tr>
<tr>
<td>Promethazine HCl</td>
<td>0.017</td>
<td>0.053</td>
<td>Liver/blood, 10:1</td>
<td>1.7</td>
<td>Taylor and Houston, 1982; Huang et al., 1970</td>
</tr>
</tbody>
</table>

* Plasma concentrations after single doses used clinically. 

$^{a}$ Derived from distribution of 14C-labeled drug in rats for chlorpheniramine, tripelennamine, hydroxyzine, and promethazine and derived from human autopsy specimens for diphenhydramine.


