Short Communication

HUMAN CYTOCHROME P450 INDUCTION AND INHIBITION POTENTIAL OF CLEVIDIPINE AND ITS PRIMARY METABOLITE H152/81

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ABSTRACT:
Clevidipine is a short-acting dihydropyridine calcium channel antagonist under development for treatment of perioperative hypertension. Patients treated with clevidipine are likely to be co-medicated. Therefore, the potential for clevidipine and its major metabolite H152/81 to elicit drug interactions by induction or inhibition of cytochrome P450 was investigated. Induction of CYP1A2, CYP2C9, and CYP3A4 was examined in primary human hepatocytes treated with clevidipine at 1, 10, and 100 μM. Clevidipine was found to be an inducer of CYP3A4, but not of CYP1A2 or CYP2C9, at the 10 μM and 100 μM concentrations of clevidipine tested. Induction response for CYP3A4 to 100 μM clevidipine was approximately 20% of that of the positive control inducer rifampicin. The response of H152/81 was similar. Using cDNA-expressed enzymes, clevidipine inhibited CYP2C9, CYP2C19, and CYP3A4 activities with IC50 values below 10 μM, whereas CYP1A2, CYP2D6, and CYP2E1 activities were not substantially inhibited (IC50 values >70 μM). The K values for CYP2C9 and CYP2C19 were 1.7 and 3.3 μM, respectively, and those for CYP3A4 were 8.3 and 2.9 μM, using two substrates, testosterone and midazolam, respectively. These values are at least 10 times higher than the highest clevidipine concentration typically seen in the clinic. Little or no inhibition by H152/81 was found for the enzyme activities mentioned above (IC50 values ≥ 69 μM). The present study demonstrates that it is highly unlikely for clevidipine or its major metabolite to cause cytochrome P450-related drug interactions when used in the dose range required to manage hypertension in humans.

Materials and Methods
Hepatocytes, Enzymes, and Chemicals. Primary cultured human hepatocytes were obtained from BD Biosciences Discovery Labware (Woburn, MA) (donors 2, 3, and 4) or CellzDirect Inc. (Tucson, AZ) (donors 1 and 5). Donor number, age, sex, and race were as follows: 1/51/F/His; 2/41/M/Cau; 3/57/F/Cau; 4/6/F/AA; 5/61/F/Cau. Medical history was unremarkable, although two donors (1 and 3) had unspecified medication for hypertension. The cDNA-expressed P450 enzymes (Supersomes or lymphoblast cell-derived), hepatocyte culture medium, NADPH regenerating system (NADP+), glucose-6-phosphate, glucose-6-phosphate dehydrogenase), and epidermal growth factor were from BD Biosciences. Glutamine and Fungizone (amphotericin B) were obtained from Invitrogen (Carlsbad, CA). 4-hydroxy(3S,5S)-Mephenytoin was purchased from Amersham Biosciences (Piscataway, NJ). Other chemicals were of high purity grade and purchased from Sigma-Aldrich (St. Louis, MO) or J.T. Baker (Phillipsburg, NJ).

Hepatocyte Culture, Treatment, and Induction Assays. Human hepatocytes plated in collagen I-coated 24-well plates were maintained in culture at least 48 h before treatment in hepatocyte culture medium supplemented with 10 μg/l epidermal growth factor, 50 μg/ml gentamycin, 2 mM L-glutamine, and 0.75 μg/ml Fungizone. Cells were treated in triplicate with 0.08% dimethyl sulfoxide vehicle, 20 μM BN, 20 μM RIF, CLE, or H152/81 at concentrations of 1, 10, and 100 μM for 72 h, with medium change and replenishment every 24 h. After treatment, hepatocytes were washed with medium and then incubated with P450 isoform-specific probe substrates. Cells were incubated at 37°C for 60 min with 100 μM phenacetin (CYP1A2) or 100 μM diclofenac (CYP2C9), or 30 min with 200 μM testosterone (CYP3A4) in volumes of 200 to 400 μl. The reaction was stopped by mixing a 175-μl aliquot of incubation medium with 21.9 μl of 70% perchloric acid (phenacetin), a 300-μl aliquot of incubation medium with 90 μl of acetonitrile/acetic acid, 94/6 v/v (diclofenac), or a 300-μl aliquot of incubation medium with 150 μl of acetonitrile (testosterone). Catalytic activity was determined by quantifying probe substrate metabolites in cell culture medium using high-perfor-
mance liquid chromatography with absorbance detection. Acetamidophenol, 4’-hydroxyclozofenac, and 6β-hydroxytestosterone metabolites were measured as described previously (Stresser et al., 2004).

**Enzyme Inhibition Studies.** Enzyme inhibition analysis was carried out using cDNA-expressed P450 enzymes as described previously (Stresser et al., 2004), with total protein concentration standardized to 0.4 mg/ml. CLE or H152/81 at 10 concentrations ranging from 0.01 to 300 μM was tested in duplicate. For Ki determination, three substrate concentrations were used and were incubated with or without three linearly spaced concentrations of CLE, chosen based on the results from the IC50 determinations. The apparent K_i was determined by nonlinear curve-fitting using SigmaPlot (v. 8) with Enzyme Kinetics Module 1.1 (SPSS Inc., Chicago, IL). Comparisons among competitive, noncompetitive, and mixed inhibition models and choice of best fit were conducted using Akaike’s information criterion and inspection of the residuals and Dixon plots.

**Statistical Analysis.** Statistically significant differences between groups were determined by analysis of variance (Minitab Statistical software, release 13.31; Minitab Inc., State College, PA). When nonhomogeneity in the within-treatment variances was indicated, data were log-transformed (to stabilize the variances). Significant differences (p < 0.05) between groups treated with test substance and vehicle-only treated groups were determined using Dunnett’s post hoc test.

**Results**

The inhibition potential of CLE and H152/81 on major P450 isoforms is shown in Table 1. CLE inhibited CYP2C9, CYP2C19, and CYP3A4 catalytic activities with IC50 values less than 10 μM, whereas H152/81 was far less inhibitory. Activities for other P450s were essentially unaffected by either compound. The inhibition of CLE against CYP2C9, CYP2C19, and CYP3A4 (with both testosterone and midazolam as substrates) was further evaluated by determining K_i values (Table 1).

The induction potential of CLE and H152/81 on three P450 isoforms was examined in hepatocytes from three donors. Basal (vehicle-treated) CYP1A2 activity in hepatocytes varied from 2.7 to 8.4 pmol/mg/min, whereas treatment of hepatocytes with 20 μM BNF induced activity to 105 to 177 pmol/mg/min (14- to 73-fold induction). Treatment with CLE or H 152/81 exhibited no induction of CYP1A2 up to 100 μM. Basal CYP2C9 activity among the three donors ranged from 23 to 59 pmol/mg/min and inductive response from RIF was 4- to 5.5-fold. CLE at 1 μM caused 4.3-fold induction in the hepatocytes from donor 1 but not donors 2 and 3. H152/81 decreased CYP2C9 activity by 70% in donor 1 and 19% in donors 2 and 3, resulting in a mean decrease for all three donors that was significantly different (p < 0.05) from control activities. A similar trend was observed for CLE at 10 and 100 μM, but this was not statistically significant. Basal catalytic activity of CYP3A4 ranged from 5.0 to 96 pmol/mg/min among three donors. With donors 2 and 5, there was a >90-fold induction response to RIF, but the response was 15-fold with donor 4. Low basal activity and not an unusually large induction response from the treated cells appears to explain the higher -fold induction response in donors 2 and 5. CLE resulted in a statistically significant increase in CYP3A4 activity with a mean of 7.3-fold (range 5.5–10) induction at 100 μM concentration (Table 2).

Similarly, H152/81 also caused CYP3A4 induction in hepatocytes at 100 μM, with a mean of 8.7-fold induction. Both CLE and H152/81, when tested at 1 and 10 μM, had no significant effects on CYP3A4 activity.

**Discussion**

Clevidipine, a new dihydropyridine calcium channel antagonist under development by The Medicines Company, exhibits advantages over other dihydropyridines in the treatment of hypertension (Powroznyk et al., 2003). However, until now, the drug-drug interaction potential of CLE has not been investigated. Drug interactions can cause morbidity and mortality in patients undergoing multiple drug therapy (Lin and Lu, 1998), often attributable to P450 induction or inhibition, and this was the subject of the current study.

Many dihydropyridine calcium antagonists such as nifedipine, nicardipine, or nilvadipine are moderate to potent cytochrome P450 inhibitors, and some can give substrate-dependent responses with CYP3A4 (Katoh et al., 2000; Stresser et al., 2000; Niwa et al., 2004; Nakamura et al., 2005). It has been suggested that an inhibition interaction in vivo would “likely” occur if the ratio of inhibitor Cmax/K_i was greater than 1 (Björnsson et al., 2003), “possible” if the ratio is between 1 and 0.1, and “remote” if below 0.1. A long-term (24-h) intravenous infusion with CLE at 7 nmol/min/kg in healthy volunteers showed that the steady-state blood concentrations were approximately 0.1 μM with a rapid clearance after the infusion was stopped (Ericsson et al., 2000). The [I/K] ratios of CLE CYP2C19 and CYP3A4/testosterone were found to be less than 0.1, whereas for CYP2C9 and CYP3A4/midazolam, the corresponding values were 0.18 and 0.1, respectively. In this simplified analysis, we are using total blood concentrations of 0.3 μM (concentrations expected in patients receiving 3 times the normal dose) for [I], and K_i values are based on total (bound plus unbound) concentrations of CLE added in the assay. This is important to note because binding to plasma and microsomal protein may affect [I] and K_i estimates, respectively [discussed in Bachmann (2006) and references therein]. Because CLE is approximately 99.7% protein-bound (Nordlander et al., 2004) in human plasma, the free fraction presumably available to cross membrane barriers and interact with hepatic P450 is expected to be miniscule (≤1 nm). For H152/81, the maximal concentration in blood was found to be 1.1 μM, with a terminal half-life of approximately 8 h (Ericsson et al., 1999). Although the H152/81 can be maintained at the micromoles per liter range in the blood for a few hours after one therapeutic dose of CLE, it was found to be a weak inhibitor in this study. Given the intended use of CLE as an infused drug in a perioperative setting, its rapid clearance, and the very weak inhibition
Effect of clevidipine, H152/81, and positive control inducers on CYP1A2, CYP2C9, and CYP3A4 activity in primary cultures of human hepatocytes from three donors

Data are the mean ± standard deviation from three donors in each group.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>CYP1A2</th>
<th>CYP2C9</th>
<th>CYP3A4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Activity</td>
<td>-Fold Induction</td>
<td>Activity</td>
</tr>
<tr>
<td>CLE</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 µM</td>
<td>4.7 ± 2.5</td>
<td>32 ± 16</td>
<td>36 ± 46</td>
</tr>
<tr>
<td>1 µM</td>
<td>5.2 ± 2.1</td>
<td>56 ± 39*</td>
<td>36 ± 46</td>
</tr>
<tr>
<td>10 µM</td>
<td>4.4 ± 3.1</td>
<td>56 ± 39*</td>
<td>36 ± 46</td>
</tr>
<tr>
<td>100 µM</td>
<td>4.1 ± 2.6</td>
<td>23 ± 15</td>
<td>73 ± 26</td>
</tr>
<tr>
<td>Positive controls*</td>
<td>153 ± 37*</td>
<td>39 ± 30</td>
<td>1099 ± 414*</td>
</tr>
<tr>
<td>H152/81</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 µM</td>
<td>6.0 ± 3.3</td>
<td>33 ± 23</td>
<td>39 ± 49</td>
</tr>
<tr>
<td>1 µM</td>
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<tr>
<td>10 µM</td>
<td>5.5 ± 2.9</td>
<td>18 ± 13*</td>
<td>50 ± 65</td>
</tr>
<tr>
<td>100 µM</td>
<td>5.0 ± 2.9</td>
<td>23 ± 22*</td>
<td>294 ± 316*</td>
</tr>
<tr>
<td>Positive controls</td>
<td>151 ± 28*</td>
<td>34 ± 23</td>
<td>167 ± 128*</td>
</tr>
</tbody>
</table>

* Significantly different from controls (p < 0.05).
* Positive control inducers: 20 µM BNF for CYP1A2; 20 µM RIF for CYP2C9 and CYP3A4.

References


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