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

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**Running title:** Induction and Inhibition Potential of P450 by Clevidipine

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*Abbreviations:* CLE, clevidipine; BNF, β-naphthoflavone; RIF, rifampicin; CYP, cytochrome P450; PXR, Pregnane X receptor; CAR, Constitutive androstane receptor; Cau, Caucasian; His, Hispanic; AA, African-American.

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## ABSTRACT

Clevidipine is a short-acting dihydropyridine calcium channel antagonist under development for treatment of peri-operative 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 $IC_{50}$ values below 10 μM, whereas CYP1A2, CYP2D6 and CYP2E1 activities were not substantially inhibited (IC50 values > 70 μM). The Ki values for CYP2C9 and CYP2C19 were 1.7 and 3.3 μM, respectively and that for CYP3A4 was 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 ($IC_{50}$ 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 man.

Clevidipine (CLE), butyroxymethyl methyl 4-(2', 3'-dichlorophenyl)-2, 6-dimethyl-1, 4-dihydropyridine-3, 5-dicarboxylate (Fig. 1), is in Phase III clinical trials as a rapid acting calcium channel antagonist for intravenous control of blood pressure (Nordlander et al., 2004). Clevidipine is rapidly hydrolyzed and inactivated by esterases in blood and extravascular tissues to its primary metabolite H152/81 (Ericsson et al., 1999, 2000). CLE may have advantages over other antihypertensives. For example, CLE effectively controlled blood pressure in hypertensive patients after undergoing elective coronary bypass grafting and hemodynamic changes were less pronounced with CLE, compared to sodium nitroprusside (Powroznyk et al., 2003). CLE successfully decreased systemic vascular resistance and mean arterial pressure without changing heart rate, cardiac index, or cardiac filling pressures (Bailey et al., 2002).

Clevidipine is a member of the dihydropyridine class of compounds which have been shown to be inducers and/or inhibitors of cytochromes P450. For example, nifedipine, nicardipine and isradipine are potent inducers for CYP3A4, CYP2B and CYP2C in human hepatocyte cultures (Drocourt et al., 2001) and nifedipine induces CYP2C in coronary artery segment endothelial cells (Fisslthaler et al., 2000). In addition, nicardipine, benidipine, manifipine and barnidipine are potent inhibitors of many CYP isoforms (Katoh et al., 2000). In this study we have investigated whether CLE and/or H152/81 have the potential to cause CYP induction or inhibition, known causes of drug-drug interactions in humans (Lin and Lu, 1998).

## Materials and Methods

**Hepatocytes, Enzymes and Chemicals.** Primary cultured human hepatocytes were obtained from BD Biosciences Discovery Labware (Woburn, MA) (Donors 2, 3, 4) or CellzDirect Inc (Tucson, Arizona) (Donors 1 and 5). Donor number, age, sex and race were as follows: 1/ 51/F/His; 2/41/M/Cau; 3/57/M/Cau; 4/6/F/AA; 5/61/F/Cau. Medical history was unremarkable

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although two donors (1 and 3) had unspecified medication for hypertension. The cDNA-expressed CYP 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 were from Invitrogen Corp (Carlsbad, CA). 4-[$^{14}$C]-(S)-Mephenytoin was purchased from Amersham Biosciences (Piscataway, NJ). Other chemicals were of high purity grade and purchased from Sigma-Aldrich (St. Louis, MO) or JT 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 prior to 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 % DMSO vehicle, 20 μM BNF, 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 CYP isoform specific probe substrates. Cells were incubated at 37° C for 60 min with 100 μM phenacetin (CYP1A2), 100 μM diclofenac (CYP2C9), or 30 min with 200 μM testosterone (CYP3A4) in volumes of 200-400 μL. The reaction was stopped by mixing a 175 μl aliquot of incubation medium with 21.9 μl 70% perchloric acid (phenacetin), 300 μl aliquot of incubation medium with 90 μl acetonitrile/acetic acid, 94:6 v/v (diclofenac) or 300 μl aliquot of incubation medium with 150 μl acetonitrile (testosterone). Catalytic activity was determined by quantifying probe substrate metabolites in cell culture medium using HPLC with absorbance detection. Acetamidophenol,

4'-hydroxydiclofenac 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 CYP 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 utilized and were incubated with or without three linearly spaced concentrations of CLE, chosen based on the results from the $IC_{50}$ determinations. The apparent $K_i$ was determined by nonlinear curve-fitting using SigmaPlot (v. 8) with Enzyme Kinetics Module 1.1 (SPSS, Chicago). Comparison 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, State College, PA). When non-homogeneity 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 CYP isoforms is shown in Table 1. CLE inhibited CYP2C9, CYP2C19, CYP3A4 catalytic activities with $IC_{50}$ values less than 10 μM whereas H152/81 was far less inhibitory. Activities for other CYPs 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 Ki values (Table 1).

The induction potential of CLE and H152/81 on three CYP 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 – 177 pmol/mg/min (14 – 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 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 to 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 CYP induction or inhibition and this was the subject of the current study.

Many dihydropyridine calcium antagonists such as nifedipine, nicardipine, 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 $C_{MAX}$ /Ki was greater than 1 (Bjornsson 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 ratios of [I]/Ki of CLE CYP2C19 and CYP3A4/testosterone were found to be less than 0.1, where as 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 Ki 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 effect [I] and Ki estimates, respectively [discussed in Bachmann (2006) and references therein]. As 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 μmol/L 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 potential of H152/81, CLE appears to have limited potential to cause drug interactions by inhibiting cytochrome P450 enzymes *in vivo*.

Our finding that CLE and H152/81 induced CYP3A4 is consistent with previous studies with other dihydropyridine compounds. For example, Drocourt et al. (2001) reported that nifedipine, BK8644 and isradipine were potent inducers of CYP3A4 message, protein and catalytic activity in cultured human hepatocytes. The mechanism by which CLE and H152/81 induce CYP3A4 was not investigated in the current study, but it is generally accepted that CYP3A4 inducers operate via interaction with PXR (Goodwin et al., 2002). Consistent with this possibility, dihydropyridine analogs have been shown to transactivate a CYP3A4 promoter construct via activation of PXR (Ekins and Erickson, 2002). Whether CLE exhibits intrinsic induction of CYP3A4 or whether the response is attributable to H152/81 or another metabolite is not known. To our knowledge, the metabolism of CLE has not been investigated in hepatocytes. Esterases are present in liver (Satoh et al., 2002), and this suggests the possibility that CLE can be converted to H152/81 in hepatocyte cultures. Monitoring the metabolism of CLE during the course of the daily incubations would assist assessing the intrinsic induction potential of CLE. Our results show that 100 μM concentrations of CLE and H152/81 caused a moderate induction of CYP3A4, but it is highly unlikely that either compound would ever reach this level in patients. Bjornsson et al (2003) representing the Pharmaceutical Research and Manufacturers of America (PhRMA) suggests that an induction of at least 40% of the positive control induction level would indicate a positive inductive response. As our study showed that corresponding values for CLE or H152/81 at concentrations approximately 1000-fold above therapeutic levels was less than 20 %, coupled with the fact that extended exposure would be low given its intended use, it is

unlikely that CLE would cause CYP3A4 induction nor cause drug interactions via this mechanism. Many CYP3A4 inducers including nifedipine, BK8644 and isradipine also induce CYP2C9 activity in hepatocyte cultures (Drocourt et al., 2001) ostensibly because these enzymes can share elements of induction mechanism (Chen et al, 2004). Thus, it was anticipated that CLE might elevate CYP2C9 activity. Instead, both CLE and H152/81 moderately decreased CYP2C9 activity. Whether this modest effect observed at high concentrations was attributable to inhibition of enzyme activity by residual CLE not removed by the wash steps, or by some other mechanism is presently unknown.

In conclusion, the present study demonstrates that relatively high concentrations of clevidipine and its primary metabolite H152/81 induce CYP3A4, but not CYP1A2 or CYP2C9 activity in hepatocytes in vitro. Clevidipine and H152/81 exhibit P450 inhibition *in vitro* at concentrations which greatly exceed anticipated therapeutic levels. Thus neither CLE nor H152/81 would be likely to cause clinically significant drug-drug interaction via induction and inhibition of cytochrome P450 at therapeutic dose *in vivo*.

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**Legend to Figures**

FIG. 1. Chemical structure of clevidipine ($R=CH_2OCO_2CH_2CH_2CH_3$) and its primary metabolite H152/81 (R=H)

**Table 1. Inhibitory effect of clevidipine and H152/81 on human cytochrome P450 using cDNA-expressed CYP isoforms as an enzyme source.** Incubations were conducted with microsomes containing cDNA-expressed CYP isoforms as outlined in the *Materials and Methods* section. The best fit model for inhibition type for the Ki analysis is shown in parenthesis.

| **Isoform** | **Clevidipine IC50 values (**μM) | **Clevidipine Ki values (**μM) | **H152/81 IC50 values (**μM) |
|---|---|---|---|
| CYP1A2 | > 300 | Not done | >300 |
| CYP2C9 | 4.4 | 1.7 ± 0.12 [a] (competitive) | 92 |
| CYP2C19 | 2.5 | 3.3 ± 0.71 (mixed) | 69 |
| CYP2D6 | 72 | Not done | >300 |
| CYP2E1 | >300 | Not done | >300 |
| CYP3A4 (testosterone) | 8.4 | 8.3 ± 1.9 (mixed) | 198 |
| CYP3A4 (midazolam) | 2.6 | 2.9 ± 1.6 (mixed) | 97 |

[a] – Values represent the fitted value ± the standard error.

**Table 2. Effect of clevidipine, H152/81 and positive control inducers on CYP1A2, CYP2C9 and CYP3A4 activity in primary cultures of human hepatocytes from 3 donors.**

| Treatment | CYP1A2 | | CYP2C9 | | CYP3A4 | |
|---|---|---|---|---|---|---|
| | Activity | Fold induction | Activity | Fold induction | Activity | Fold induction |
| **CLE (μM)** | | | | | | |
| 0 | 4.7 ± 2.5 | - | 32 ± 16 | - | 36 ± 46 | - |
| 1 | 5.2 ± 2.1 | 1.2 ± 0.23 | 56 ± 39* | 2.0 ± 1.9 | 33 ± 41 | 0.95 ± 0.10 |
| 10 | 4.4 ± 3.1 | 0.86 ± 0.18 | 18 ± 10 | 0.57 ± 0.12 | 42 ± 52* | 1.2 ± 0.07 |
| 100 | 4.1 ± 2.6 | 0.82 ± 0.23 | 23 ± 15 | 0.67 ± 0.23 | 225 ± 235* | 7.3 ± 2.6 |
| **Positive controls**[a] | 135 ± 37* | 39 ± 30 | 177 ± 90* | 5.4 ± 0.21 | 1099 ± 414* | 77 ± 56 |
| **H152/81 (μM)** | | | | | | |
| 0 | 6.0 ± 3.3 | - | 33 ± 23 | - | 39 ± 49 | - |
| 1 | 6.1 ± 4.2 | 0.94 ± 0.43 | 31 ± 19 | 0.97 ± 0.11 | 39 ± 51 | 0.96 ± 0.037 |
| 10 | 5.5 ± 2.9 | 0.94 ± 0.042 | 18 ± 13* | 0.54 ± 0.18 | 50 ± 65 | 1.2 ± 0.22 |
| 100 | 5.0 ± 2.9 | 0.81 ± 0.21 | 23 ± 22* | 0.63 ± 0.29 | 294 ± 316* | 8.7 ± 5.0 |
| **Positive controls** | 151 ± 28* | 34 ± 23 | 167 ± 128* | 4.9 ± 0.76 | 1235 ± 322* | 82 ± 60 |

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

[a] Positive control inducers: 20 μM BNF for CYP1A2; 20 μM RIF for CYP2C9 and CYP3A4.

* Significantly different from controls ($p \leq 0.05$)

**Figure 1**