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RAPID COMMUNICATION

DRUG-DRUG INTERACTIONS: EFFECT OF QUINIDINE ON NIFEDIPINE BINDING TO HUMAN CYTOCHROME P450 3A4

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ABSTRACT. Quinidine is a known inhibitor of cytochrome P450-mediated nifedipine metabolism. The interactions of nifedipine and quinidine with human cytochrome P450 3A4, which metabolizes these drugs, were examined using the kinetics of CO binding to this P450 as a rapid kinetic probe of protein conformation and dynamics. This approach showed that nifedipine and quinidine bind to different P450 3A4 species, respectively termed species I and II, with distinct conformations. When both drugs were present simultaneously, nifedipine interacted with the quinidine-bound P450 species II, but not species I. These findings indicate that quinidine acts as an allosteric inhibitor by switching nifedipine binding from nifedipine-metabolizing species I to the nonmetabolizing species II.

KEY WORDS. nifedipine; quinidine; drug interactions; cytochrome P450

Increasing attention has been recently directed toward elucidating the various mechanisms of drug-drug interactions [1, 2]. In particular, administration of one drug can alter the metabolism of other drugs via modulation of the cytochrome P450 enzymes. This may occur by two distinct routes. First, a drug may induce a specific P450 that also metabolizes other drugs. Second, direct interaction of a drug with a P450 may reduce its catalytic activity toward other drugs via competitive or noncompetitive inhibition, or enhance its activity through allosteric activation. One drug-drug interaction involves the antiarrhythmic quinidine and the calcium-channel blocker nifedipine [3-6], as quinidine inhibits nifedipine metabolism and thus enhances its pharmacologic response [3, 4]. Both drugs are metabolized by cytochrome P450 3A4, a major human liver P450 that also metabolizes many other important drugs [7, 8], and whose substrates are thus particularly susceptible to drug-drug interactions [9-11].

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We examined the interaction of P450 3A4 with nifedipine and quinidine, both individually and in combination, using the kinetics of CO binding to P450 3A4 as a sensitive probe of P450 conformation and dynamics. This rapid kinetic technique reflects the rate of CO migration from the solvent toward the P450 heme, through an access channel in the protein interior, and contrasts with classical static methodologies that reflect the average properties of a macrosystem [12]. This approach previously revealed subtle and otherwise undetectable mechanistic details for P450s, including the finding that some individual P450 forms, such as P450 3A4, are comprised of multiple species that differ in their conformations and substrate specificities [12]. In this study, we applied this rapid kinetic probe to elucidate P450 3A4-nifedipine-quinidine interactions.

MATERIALS AND METHODS

Human P450 3A4. This P450 was expressed in SF9 insect cells using a recombinant baculovirus as described [13]. Cells were harvested by centrifugation, washed, suspended in phosphate-buffered saline, homogenized, and stored at -80° until used. The total P450 content was determined spectrally [14], and the protein content was measured with bicinchoninic acid [15].

Absorbance Spectra. Substrate-induced spectral changes in the Soret region were recorded at 23° using an Aminco DW2000 spectrophotometer. The sample cuvette contained 1.2 mL of P450 3A4 [2.2 mg/mL cell homogenate protein, suspended in phosphate-buffered saline (pH 7.1) containing 20% glycerol (w/v)]; the contents of the reference cuvette were identical except that extract from wild-type cells without P450 was used. Spectra were recorded after addition of 9 μL of nifedipine (40 mM in methanol) or 18 μL of quinidine (40 mM in methanol) (Sigma, St. Louis, MO) to both cuvettes, and incubation for 10 min. Spectra were also recorded in the presence of both drugs; the order of addition had no effect on these measurements.

CO Flash Photolysis. Reactions were carried out using 0.8 nmol/mL of P450 3A4 (corresponding to 2.2 mg/mL cell protein) and 20 μM CO, at 23° in phosphate-buffered saline (pH 7.1) containing 20% glycerol (w/v). Drugs were added to a final concentration of 300 μM, as initial experiments showed that this concentration produced maximal effects on the CO binding kinetics. The mixture was then incubated for 20 min prior to addition of CO and dithionite. Laser-induced flash photolysis of the P450 heme Fe-CO bond, monitoring of CO recombination kinetics via the absorbance change at 450 nm, and data analysis were performed as described [16]. Neither drug absorbed at 450 nm. A kinetic difference method was used to determine the kinetic parameters of individual P450 species within a sample that contained multiple P450 species. This method evaluates the difference between the kinetic profiles obtained in the presence and absence of a P450 substrate and thus cancels out the contributions from P450s that do not bind the substrate. Using this approach, kinetic parameters for individual substrate specific P450 3A4 species [17] were obtained by least-squares fitting of the kinetic difference curves to equation 1

$$\Delta A_t' - \Delta A_t = \sum_{i=1}^{n} a_i' e^{(-k_i' t)} - \sum_{i=1}^{n} a_i e^{(-k_i t)} \quad (1)$$

where $\Delta A_t'$ and ΔA_t are the absorbance changes observed at time t for the reactions in the presence and absence of substrate; a_i' and a_i are the absorbance changes, and k_i' and k_i are the pseudo-first-order rate constants for the substrate-specific P450 in the presence and absence of substrate, respectively. Thus, when a single P450 species is perturbed ($n = 1$), this equation simply reduces to a difference of two exponential terms.

RESULTS AND DISCUSSION

Absorbance spectra were measured as a probe of the P450 heme region. In particular, the low- and high-spin ferric heme states were reflected by respective absorbance peaks near 417 and 395 nm, and the equilibrium between these was sensitive to substrate binding. The substrate-free enzyme exhibited a band at 417 nm (Fig. 1a), showing that the low-spin state predominated. Addition of nifedipine resulted in the appearance of a 395 nm shoulder, indicating a shift from the low- to the high-spin state (Fig. 1b). In contrast, added quinidine had no effect on the spectrum (not shown). However, addition of both nifedipine and quinidine resulted in a spectrum (Fig. 1c) corresponding to a species that was predominantly low-spin (peak at 417 nm) yet different from that observed in the absence of any substrate (note difference in band shapes). In addition, this spectrum was not intermediate between the spectra obtained in the presence of nifedipine or quinidine alone. These observations indicate that addition of both drugs yields a P450-nifedipine-quinidine ternary complex, rather than a simple mixture of nifedipine-P450 and quinidine-P450 complexes. A rapid kinetic approach was thus applied to provide further details on these ternary interactions.

We measured the kinetics of CO binding to P450 3A4 as a probe that is sensitive to protein conformation and dynamics. Substrate binding can alter P450 conformation to either widen or restrict the CO ligand access channel, which, respectively, accelerates or decreases the CO binding rate. The time courses for CO binding to P450 3A4 in the absence and presence of nifedipine and quinidine are illustrated in Fig. 2. It is evident that nifedipine accelerated (Fig. 2b) CO binding, whereas quinidine decelerated (Fig. 2c) the reaction. In the presence of both drugs (Fig. 2d), the rate was less than that in the absence of substrate (Fig. 2a) but greater than that observed in the presence of quinidine alone (Fig. 2c). Since P450 3A4 is comprised of

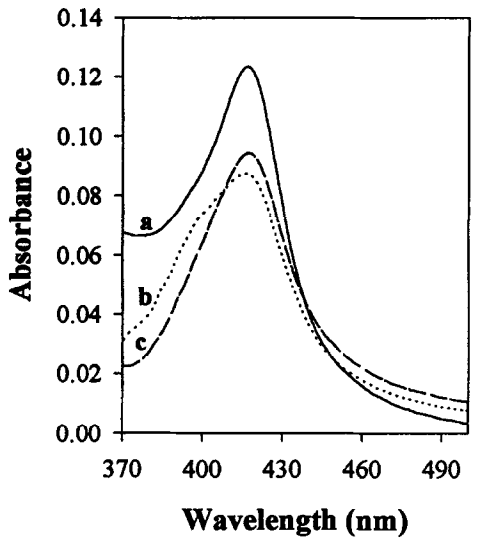

FIG. 1. Effect of nifedipine and quinidine on absorbance spectra of P450 3A4. Spectra of 0.8 nmol/mL P450 3A4 were measured as described in Materials and Methods. Key: (a) P450 3A4 alone, (b) P450 3A4 + 300 μM nifedipine, (c) P450 3A4 + 300 μM nifedipine + 600 μM quinidine (the 417 nm absorbance magnitude was identical using quinidine concentrations of 300, 600, or 900 μM).

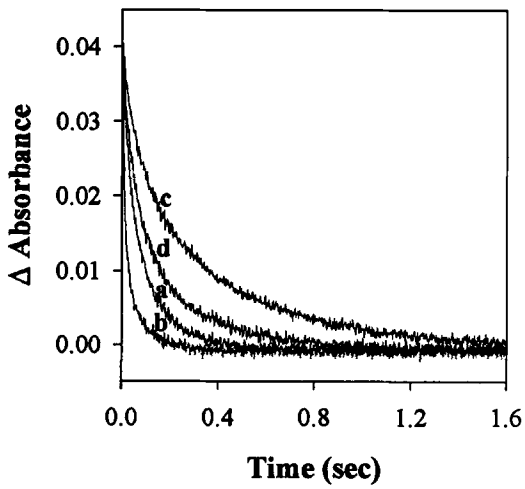

FIG. 2. Effect of nifedipine and quinidine on CO binding kinetics of P450 3A4. Progress curves (a) in the absence, and in the presence of (b) nifedipine, (c) quinidine, and (d) nifedipine + quinidine. P450 concentration was 0.8 nmol/mL; drug concentration was 300 μM.

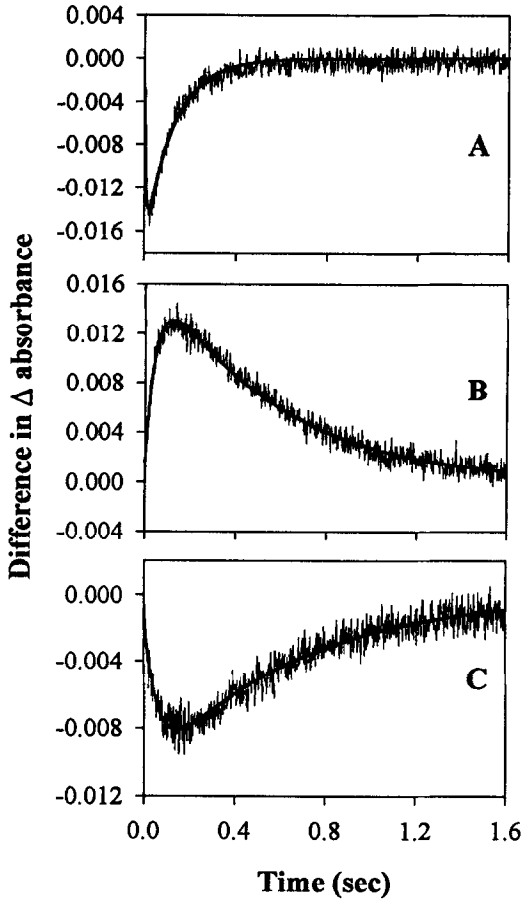

FIG. 3. Difference kinetic analysis for the data in Fig. 2. (A) nifedipine, (B) quinidine, and (C) nifedipine + quinidine, as calculated from the differences between curves (b) and (a), (c) and (a), and (d) and (c) in Fig. 2, respectively. The solid lines represent the best fit according to the kinetic difference equation 1.

multiple, kinetically distinguishable species [17], we applied the kinetic difference method [16, 17] to more precisely define the effect of these drugs on their target species. The basis of this approach is analysis of a difference curve generated by subtracting the kinetic curve obtained in the absence of a drug from that in the presence of the drug. The resulting kinetic difference profile reflects the kinetic properties of the drug-specific P450 species. Figure 3 shows the resultant difference curves for the data in Fig. 2 along with the least-squares curve fits to equation 1. The kinetic difference procedure yielded k_1 and k_1', which represent the CO binding rate constants for a drug-specific P450 3A4 species in the absence and presence of the drug, respectively. This analysis revealed (Table 1) first that nifedipine and quinidine interact with two different P450 3A4 species (termed species I and II), as reflected by the large difference between their corresponding k_1 values (7.7 and 21.0 sec^{-1}). Second, nifedipine accelerated the rate of CO binding to its target species by 18-fold (from 7.7 to 142.1 sec^{-1}), whereas quinidine decreased the rate of its target species by 11-fold (from 21.0 to 1.8 sec^{-1}). These results revealed that nifedipine induces a conformational change in P450 species I which widens the CO access channel, whereas quinidine narrows the CO access channel of species II.

TABLE 1. Kinetic parameters for CO recombination to P450 3A4 in the presence and absence of nifedipine and quinidine, obtained by kinetic difference analysis[a]

P450	Drug	a_1'	k_1' (sec^{-1})	a_1	k_1 (sec^{-1})
3A4	Nifedipine	0.0164 ± 0.0024	142.1 ± 11.0	0.0169 ± 0.0029	7.7 ± 1.3
3A4	Quinidine	0.0192 ± 0.0017	1.8 ± 0.1	0.0158 ± 0.0021	21.0 ± 1.6
3A4 + Quinidine	Nifedipine	0.0120 ± 0.0027	15.5 ± 3.6	0.0135 ± 0.0030	1.7 ± 0.2

[a] Parameters were determined according to the kinetic difference equation 1. k_1 and k_1' are the pseudo-first-order rate constants, and a_1 and a_1' are the corresponding absorbances in the absence and presence of drug, respectively. Means ± SD were derived from at least three experiments.

The next question is: since nifedipine and quinidine interact with different P450 3A4 species, how does quinidine inhibit nifedipine metabolism? If both drugs only interact with their individual target species, their simultaneous addition would result in CO binding curves that are a combination of the curves exhibited by the individual species. Difference kinetic analysis (subtraction of data in Fig. 2a from that in Fig. 2d) would then yield two sets of k and k' values corresponding to P450 species I and II. However, the difference curve did not fit such a two-component model (not shown), eliminating the possibility that in their simultaneous presence, nifedipine and quinidine, respectively, bind only species I and II. Since nifedipine metabolism was inhibited by quinidine, and the spectral results (Fig. 1) indicated a ternary P450-nifedipine-quinidine complex, we next sought to assess the nifedipine interaction with quinidine-bound P450 species II. Kinetic difference analysis was performed by subtracting the CO binding curve in the presence of quinidine alone (Fig. 2c) from that in the presence of both nifedipine and quinidine (Fig. 2d). The resulting difference curve (Fig. 3C) represents nifedipine binding to a single species. On the basis of its CO binding rate (k_1 = 1.7 sec^{-1}, Table 1), this species corresponds to quinidine-bound species II (k_1' = 1.8 sec^{-1}, Table 1). Nifedipine binding increased the CO binding rate of this species by 9-fold (from 1.7 to 15.5 sec^{-1}). But most importantly, the results show that in the presence of quinidine, nifedipine only binds species II and no longer binds species I. This suggests that quinidine inhibits nifedipine metabolism by reducing nifedipine binding to species I, which metabolizes this drug, and enhances binding to quinidine-bound species II, which is presumably less catalytically active for nifedipine metabolism. Since nifedipine preferentially bound species II in the presence of quinidine, these results also imply that the P450 3A4-nifedipine-quinidine complex is more stable than the P450 3A4-nifedipine complex. This interpretation is consistent with the spectral result that the nifedipine-bound high-spin species (Fig. 1b) disappeared upon addition of quinidine and a new low-spin species was formed in the presence of both drugs (Fig. 1c).

These results suggest a mechanism for quinidine inhibition of P450 3A4-mediated nifedipine metabolism. Quinidine acts as a noncompetitive inhibitor, but not in the classical sense. Rather than binding and allosterically modulating all P450 molecules in the system to reduce their catalytic efficiency, quinidine targets a subset of P450 molecules and induces their binding to nifedipine. Nifedipine thus preferentially binds an inactive quinidine-P450 complex rather than an active P450 species. Such a conformational induction mechanism has also been described recently for agonist binding to multiple forms of a receptor [18]. The factors that regulate the distribution of catalytically distinct isoforms of a single enzyme, and how widespread this mechanism is, remain to be elucidated.

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