The Digoxin-Propafenone Interaction: Characterization of a Mechanism Using Renal Tubular Cell Monolayers

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ABSTRACT
When propafenone is given with digoxin, digoxin serum concentrations increase. Although the digoxin-propafenone interaction is well known clinically, the mechanism by which propafenone interferes with digoxin elimination is unclear. To test the hypothesis that propafenone or one or both of its two major metabolites, 5-hydroxypropafenone (5-OHP) and N-depropylpropafenone (NDPP), inhibit the P-glycoprotein-mediated net renal tubular secretion of digoxin, we examined the transport of digoxin and the well-studied P-glycoprotein substrate vinblastine across confluent Madin-Darby canine kidney cell monolayers in the absence and presence of propafenone, 5-OHP and NDPP. Propafenone and its two major metabolites significantly inhibit the secretory flux of digoxin and vinblastine (propafenone > 5-OHP >> NDPP). Despite decreases in net transport, cellular digoxin accumulation did not decrease, suggesting that neither propafenone nor its metabolites prohibited digoxin from entering the cells at the basolateral side. NDPP, but not 5-OHP, was detected after 48 hr of incubation of the cells with propafenone alone. When the cells were incubated with propafenone or 5-OHP, apical accumulation of 5-OHP, but not 5-OHP, was observed. These findings are consistent with the hypothesis that the digoxin-propafenone interaction results from the inhibition of the renal tubular transport of digoxin by propafenone and its metabolites. Our data suggest that propafenone is an inhibitor of P-glycoprotein, whereas 5-OHP is a possible substrate.

Propafenone is an antiarrhythmic agent that blocks sodium channels and beta adrenergic receptors (Funck-Brentano et al., 1990). When propafenone is given concurrently with digoxin, steady state serum digoxin concentrations increase as a result of decreased digoxin clearance (Belz et al., 1983; Calvo et al., 1989). The low therapeutic index of digoxin necessitates dose adjustment and careful monitoring for digoxin toxicity. However, although the interaction between digoxin and propafenone is well known clinically, the mechanism by which propafenone interferes with digoxin elimination is unclear. This interaction is especially intriguing because the kidney, a major organ for digoxin elimination, plays virtually no role in the elimination of propafenone.

Digoxin is eliminated mainly by the kidney, with renal tubular secretion accounting for as much as 50% of this elimination (Steiness, 1974). Recent studies have shown that digoxin transport involves an active transport mechanism that is compatible with the MDR protein P-glycoprotein (Taniawara et al., 1992; de Lannoy and Silverman, 1992; Ito et al., 1993b; Schinkel et al., 1995). P-glycoprotein is a drug efflux pump that is expressed in the apical membranes of various cell types, including renal tubular cells (Thiebaut et al., 1987). Most of the drugs known to interact with renal tubular digoxin secretion are substrates of P-glycoprotein; examples include cyclosporine, verapamil and quinidine (Bradley et al., 1988; Ito et al., 1993a).

Propafenone is extensively metabolized by the liver and demonstrates negligible renal elimination with no net renal tubular secretion (Hollmann et al., 1983; Seipel and Breithardt, 1980). The two major metabolites of propafenone, 5-OHP and NDPP, also have sodium channel-blocking activity; however, blockade of beta adrenergic receptors is much weaker than with the parent compound. The hydroxy metabolite is considered to be therapeutically active, whereas NDPP is probably less active; in part because of its lower serum concentrations (Funck-Brentano et al., 1990). Bioconversion of propafenone to 5-OHP is mediated by CYP2D6 (Siddoway et al., 1987). NDPP is reportedly pro-

ABBREVIATIONS: MDR, multidrug resistance; 5-OHP, 5-hydroxypropafenone; NDPP, N-depropylpropafenone; MDCK, Madin-Darby canine kidney; a-MEM/FBS, a-minimum essential medium plus 10% fetal bovine serum; PBS-G, phosphate-buffered saline containing 5 mM glucose and 0.02% albumin; TEA, tetraethylammonium.
duced by CYP3A4 and CYP1A2 (Botsch et al., 1993). Due to a genetic polymorphism, ~1% to 7% of the population lacks the enzyme CYP2D6 (Bertilsson, 1995). The prevalence of this enzyme deficiency is Caucasians > African Americans > Orientals. Recently, existence of the superextensive metabolizer phenotype of CYP2D6-mediated biotransformation resulting from functional gene duplication was identified in 7% of a white population (Agúndez et al., 1995). Although there are substantial interindividual variations in serum concentrations of propafenone and its metabolites due to the wide range of metabolic capacities in the population, serum concentrations of 5-OHP may reach levels comparable to those of the parent compound, especially in extensive metabolizers (Hæfeli et al., 1996). NDPP usually achieves much lower concentrations. If the parent propafenone compound is primarily responsible for the digoxin-propafenone interaction, poor metabolizers of propafenone should have a greater likelihood of these interactions. However, no clinical evidence exists to suggest that these interactions are more pronounced in poor metabolizers than in extensive metabolizers, implying that propafenone metabolites may play a role in digoxin-propafenone interactions. The participation of propafenone metabolites in digoxin-propafenone interactions has not been investigated, nor is it known how these metabolites are handled by the kidney. In fact, the roles of drug metabolites in renal drug-drug interactions have never been thoroughly examined.

Also unknown is the relative contribution of the enantiomers of propafenone to digoxin-propafenone interactions. Propafenone is generally administered as a racemic mixture. The (R)- and (S)-enantiomers do not differ in their abilities to decrease the fast, inward sodium current, although the (S)-isomer is far more potent as a beta antagonist. In addition, the area under the concentration-time curve for (S)-propafenone is almost 2-fold higher than that for the (R)-enantiomer, indicating that (R)-propafenone has higher clearance (Kroemer et al., 1989).

These findings led us to hypothesize that propafenone is an inhibitor of P-glycoprotein-mediated renal tubular digoxin secretion and that propafenone metabolites also contribute to this interaction. Because no enantioselective effects have been reported for P-glycoprotein-mediated drug transport, we also hypothesized that the enantiomers of propafenone are handled similarly and, thus, contribute equally to the digoxin-propafenone renal tubular interaction.

Methods

Cell culture. MDCK cells, originally derived from the kidney of a female cocker spaniel, were obtained from the American Type Culture Collection (Rockville, MD). These cells were seeded onto 25-mm Nunc tissue culture inserts (GIBCO BRL, Oakville, Ontario, Canada) at a density of 2.5 × 10⁶ cells/cm². The inserts were placed in six-well tissue culture plates, and the confluent cell monolayers were bathed on each side with 2 ml of α-MEM/FBS and incubated at 37°C in an atmosphere of 5% CO₂/95% air. Media were changed every 2 to 3 days. Experiments were conducted 10 days after seeding. Cells were used between passages 61 and 142.

Short-term digoxin/vinblastine flux experiments. Cells in the filter preparation were gently rinsed with PBS-G. These cell monolayers were precultivated at 37°C for 30 min with 1 ml of PBS-G in the apical compartment and 1.5 ml of PBS-G in the basal compartment.

The tissue culture inserts containing 1 ml of PBS-G in the apical compartment were placed onto six-well tissue culture plates containing 1.2-ml PBS-G solutions of 2.9 μM [¹⁴C]mannitol and either 0.1 μM [³H]digoxin or 0.025 μM [³H]vinblastine, to which we added various concentrations of one of the following compounds: (R)-propafenone, (S)-propafenone, racemic propafenone, (R)-5-OHP, (S)-5-OHP, racemic 5-OHP or racemic NDPP. Appropriate volumes of solvent were added to the wells to correct for volume differences. The apical media were sampled (25 μl) at 10, 20 and 30 min. The basal media were also sampled (25 μl) at 30 min. The radioactivity of the samples was determined using a scintillation counter (Beckman LS5000CE).

Cellular uptake of digoxin and vinblastine. The cellular accumulation of radiolabeled digoxin and vinblastine in the presence and absence of propafenone, 5-OHP or NDPP was examined after 40 min of incubation. The culture inserts were rinsed on both sides with ice-cold PBS-G (1 ml apical, 1.5 ml basal). The filters were cut out of the tissue culture inserts using a cutting device provided with the inserts. The membranes were placed in tissue culture plates containing 0.6 ml of 0.1% Triton X-100. After solubilization of the cells, 0.3 ml of cell solution was sampled for determination of radioactivity. Disintegration-per-minute (dpm) counts were corrected for contamination by the incubation media. On average, the contamination accounted for <10% of the uptake.

Long-term digoxin flux experiments. Six cell preparations on tissue culture inserts were placed in a six-well tissue culture plate containing 2 ml of α-MEM/FBS with 0.1 μM [³H]digoxin plus or without 20 μM propafenone in each well. The same media (2 ml) were slowly added to the apical compartiments. The cells were incubated at 37°C under an atmosphere of 5% CO₂/95% air. Apical and basal media were sampled (25 μl) at various time periods of incubation over a 24-hr period.

Biotransformation of propafenone. α-MEM/FBS (1.5 ml) containing 5 μM propafenone, 5 μM 5-OHP or solvent alone was added to either side or both sides of the cell monolayer. Cells were incubated at 37°C in 5% CO₂/95% air. After a period of 30 min or 48 hr, 1 ml of apical solution and 1 ml of basolateral solution were sampled. These samples were analyzed by the high-performance liquid chromatographic method described below. The integrity of the cell monolayers was determined after the 48-hr period of incubation by measuring the basolateral-to-apical flux of 5 μM [¹⁴C]mannitol using the procedures described for short-term experiments.

High-performance liquid chromatography assay. Propafenone and its metabolites were quantified as described by Verjee and Giesbrecht (1992). Samples (100 μl) were extracted at alkaline pH with ethyl acetate, and the extract was applied to a C18 Bond Elut Cartridge (1 ml size); interfering polar compounds were washed off with methanol. The drug and metabolites were eluted with 95% methanol/5% of 0.1 N HCl. After drying under nitrogen and reconstitution with 100 μl of 0.1 N HCl, 75 μl was injected into a Whatman Partisil 5 ODS RAC HPLC column with in-line filter. The column was maintained at room temperature and eluted with a mobile phase of 42% acetonitrile in 10 mM phosphate buffer, pH 2.5. Detection was made at 214 nm with a UV spectrophotometer. At a flow rate of 2.3 ml/min, chromatography was complete in 10 to 11 min.

Data analysis. For all experiments, an a priori decision was made to exclude the results obtained from any filters that had apical [¹⁴C]mannitol (a marker of extracellular flux) concentrations of >5% of their respective initial basal concentrations.

Data (in at least triplicates) from different experiments were compared by the two-tailed Student's t test for unpaired data or by analysis of variance for repeated measures. P values of <0.05 were considered statistically significant. Results are expressed as mean ± S.D.

Materials. [³H]Digoxin (16.1 Ci/mmol) and [¹⁴C]mannitol (55.1 mCi/mmol) were purchased from Du Pont Canada (Markham, Ontario, Canada). [³H]Vinblastine (11.2 Ci/mmol) was purchased from Amersham Canada (Oakville, Ontario, Canada). Culture medium was prepared according to the manufacturer's instructions.
was brought from the Ontario Cancer Institute. FBS was obtained from Gibco BRL and added to media in the laboratory. Racemic propafenone was purchased from Sigma Chemical (St. Louis, MO). NDPP and the enantiomers of propafenone and 5-OHP were generously donated by Knoll Pharmaceuticals (Markam, Ontario, Canada).

Results

No results had to be excluded on the basis of the a priori definition of inappropriate cell-filter preparations detected by high mannitol fluxes.

Short-term digoxin flux experiments. Racemic mixtures of propafenone, 5-OHP and NDPP significantly inhibited the total basolateral-to-apical flux of 0.1 µM [3H]digoxin across MDCK cell monolayers over a 30-min time period (fig. 1, top). The hydroxylated metabolite (5-OHP) inhibited digoxin renal tubular secretion to a somewhat lesser extent than the parent compound. NDPP was a much less potent inhibitor, as illustrated in figure 1 (top) by the lack of significant inhibition at 20 µM. The time courses of digoxin secretion were nearly linear in the presence and absence of propafenone (fig. 1, bottom), 5-OHP and NDPP.

The effects of the enantiomers of propafenone (fig. 2) and 5-OHP (data not shown) on 0.1 µM [3H]digoxin basolateral-to-apical flux over a period of 30 min were not significantly different from each other, suggesting that the effects were nonstereospecific (P = .27 and .37 for propafenone and 5-OHP, respectively). Because the inhibitory effects of the enantiomers of propafenone and 5-OHP were comparable to those of the racemic mixtures, the racemates were used in all subsequent transport and metabolism experiments.

Short-term vinblastine flux experiments. As illustrated in figure 3 (top), propafenone, 5-OHP and NDPP also inhibited the basolateral-to-apical flux of 0.025 µM [3H]vinblastine, a prototype P-glycoprotein substrate, across confluent MDCK cell monolayers. Concentrations of 40 µM NDPP did not produce statistically significant differences in vinblastine inhibition, although significance was reached with 100 µM NDPP (61 ± 6% of control at 30 min, P = .02). There were no significant differences in vinblastine basolateral-to-apical flux in the presence of 20 µM propafenone or 5-OHP between the (R)- and (S)-enantiomers. The time courses of vinblastine secretion were nearly linear in the presence and absence of propafenone (fig. 3, bottom), 5-OHP and NDPP.

Cellular uptake of digoxin and vinblastine. Although increasing the concentration of propafenone decreased the net secretory flux of 50 µM [3H]digoxin across confluent MDCK cell monolayers, the cellular uptake of digoxin did not decrease (fig. 4). Similarly, the cellular uptakes of 0.1 µM [3H]digoxin and 0.025 µM [3H]vinblastine were not decreased relative to controls in the presence of propafenone, 5-OHP and NDPP (table 1).

Long-term digoxin flux experiments. Figure 5 shows the inhibition of digoxin secretion by 20 µM propafenone over a 24-hr period. Similar to the short-term time course studies, propafenone inhibited the basolateral-to-apical transport of digoxin against a concentration gradient. In a separate experiment, we demonstrated the ability of cells that were previously exposed to 5 µM propafenone (and then rinsed for 1 hr) to transport 10 nM digoxin to the same extent as cells never exposed to propafenone, indicating that the inhibition of digoxin secretion is reversible and that the cells were still functional after the exposure (data not shown).

Propafenone biotransformation. To identify whether the inhibition of digoxin basolateral-to-apical transport by...
propafenone was due to propafenone itself or instead to its metabolite(s) produced by the cells, the ability of MDCK cells to metabolize propafenone was examined. After a 30-min exposure to propafenone, 5-OHP and NDPP were undetectable. On the other hand, after a 48-hr incubation, NDPP (but not 5-OHP) was detected in approximately equal quantities from media bathing the apical and basolateral sides of cells as well as propafenone itself (fig. 6, top). When cells were incubated with 5-OHP initially placed on both sides of the monolayer, the apical concentrations of 5-OHP were almost double that of the basal compartment (fig. 6, bottom). The integrity of the cell monolayers after a 48-hr exposure to these propafenone compounds was confirmed by the 5 mCi[14C]mannitol basolateral-to-apical flux experiments.

**Discussion**

MDCK cells have properties of distal tubular cells with P-glycoprotein expression on their apical membranes (Horio et al., 1990; Tanigawara et al., 1992). We verified P-glycoprotein expression in our MDCK cell line using monoclonal (C219) and polyclonal (4077) antibodies to P-glycoprotein. In the cell culture system used in this study, these epithelial cells orient themselves in a polarized manner such that the part of the cells that adheres to the inorganic membrane of the tissue culture insert represents the basal or blood side of the renal tubular cell, whereas the opposite end represents the apical or urine side. This orientation facilitates the study of drug transport across renal tubular cells. In addition, MDCK cells have tight junction, which results in relatively high transport-to-diffusion ratios of the P-glycoprotein substrates digoxin and vinblastine across the MDCK cell mono-

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2 S. Ito, C. Woodland, and B. Sarkadi, unpublished observations.
layers. The cell-cell junctions in MDCK cells are much tighter than those found in the proximal tubular cell line LLC-PK1. Hence, the paracellular flux of digoxin and vinblastine is lower in MDCK cells than in LLC-PK1 cells. Moreover, based on functional assays and Western blotting techniques, P-glycoprotein expression is higher in MDCK cells than in LLC-PK1 cells.3 Taken together, these characteristics justify the use of MDCK cells to test our hypotheses.

P-glycoprotein is notable for its broad substrate specificity. Vinblastine is a prototype substrate for this protein, and digoxin was recently identified to also be transported by P-glycoprotein (de Lannoy and Silverman, 1992; Ito et al., 1993b; Schinkel et al., 1995; Tanigawara et al., 1992). Direct contributions of other mechanisms to the renal tubular secretion of digoxin are improbable according to our modeling analysis, which suggests that digoxin renal tubular transport involves a single active transporter (Ito et al., 1997). Based on the inhibitory compounds used in this study (propafenone is a weak organic base), if another mechanism is involved, the most reasonable candidate would be the classic organic cation transport system. This involvement seems unlikely because TEA, a prototype organic cation, lacks effects on digoxin transport in LLC-PK1 renal tubular cells, which express an active transport mechanism for TEA (Inui et al., 1985; Ito et al., 1993a). In addition, we were unable to inhibit the secretory flux of digoxin or vinblastine by MDCK cells with two prototype organic cations, TEA and N-methyl-Nicotinamide.4 In fact, we could not detect carrier-mediated TEA transport in MDCK cells. Horio et al. (1990) also failed to show an effect of TEA on vinblastine transport by this cell line. Therefore, P-glycoprotein appears to be a major apical efflux mechanism for digoxin and vinblastine, although the roles of other transporters remain to be explicitly elucidated.

Inhibition of the unidirectional drug efflux pump P-glycoprotein is often implied when fluxes of substrates across cell monolayers expressing the protein are decreased in the basolateral direction and increased in the apical direction (Horio et al., 1989; Tanigawara et al., 1992). In this study, we assumed that basolateral-to-apical flux represents the P-glycoprotein-mediated component of digoxin transport. This assumption seems valid because we could also demonstrate in a different experimental condition that the net basolateral-to-apical transport of digoxin against a concentration gradient is inhibited by propafenone (fig. 5). In addi-

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3 S. Ito, C. Woodland, and B. Sarkadi, unpublished observations.

4 C. Woodland, unpublished observations.
tion, carrier-mediated transport appears to account for the majority of basolateral-to-apical digoxin/vinblastine flux on the basis of experiments in which unlabeled digoxin/vinblastine significantly inhibited radiolabeled digoxin/vinblastine basolateral-to-apical flux in this experimental system. In our experiments in which unlabeled digoxin/vinblastine significantly inhibited radiolabeled digoxin/vinblastine basolateral-to-apical flux in this experimental system.5 Furthermore, our modeling analysis indicated that in MDCK cells, ~90% of the basolateral-to-apical digoxin flux for the first several hours reflects a function of a single apically located drug efflux pump (Ito et al., 1997).

Our results show that propafenone and its two major metabolites, 5-OHP and NDPP, inhibit digoxin and vinblastine transport across MDCK cell monolayers. The inhibitory effects of these compounds are dose-dependent with an order of potency such that propafenone > 5-OHP > NDPP. In extrapolation of these in vitro data to humans, clinically significant differences in the nature of the digoxin-propafenone interaction are not likely among superextensive, extensive and poor metabolizers of propafenone. To the best of our knowledge, however, digoxin-propafenone interactions have not been analyzed with respect to metabolizer phenotype.

The somewhat increased cellular accumulation of digoxin and vinblastine in the presence of propafenone, 5-OHP and NDPP demonstrates that these compounds do not interfere with the ability of digoxin to enter the cells, implying that the digoxin-propafenone interaction does not take place at the basolateral membrane. These results agree with the general concept that the digoxin-propafenone interaction takes place at P-glycoprotein located in the apical membranes of renal tubular cells.

Although the exact dynamics of the interactions are unclear on the basis of this study, because propafenone and NDPP are not accumulated in the apical compartment (fig. 6), the two compounds may be inhibitors, but not substrates, of P-glycoprotein. The data also indicate that 5-OHP is accumulated on the apical side against a concentration gradient. Although this suggests a competitive nature of the interaction of digoxin and 5-OHP, the identification of 5-OHP as a substrate of the transport system awaits further study.

In the present experiments, digoxin concentrations were nearly 10-fold higher than those observed therapeutically (1–3 nM) due to the constraints induced by the specific activity of the available radiolabeled digoxin. Concentrations of propafenone and 5-OHP used in the experiments (1–40 μM) included relevant therapeutic serum concentrations (propafenone, 1–6 μM; 5-OHP, 0.5–1.5 μM; Siddoway et al., 1987), although protein binding was not taken into account. NDPP concentrations seen in patients receiving therapeutic doses of propafenone are much lower than those used in this study: <1.5 μM in vivo (Kates et al., 1985) vs. 20 to 100 μM in vitro in this study. Taken together, our in vitro data seem valid to infer that propafenone and 5-OHP are responsible for digoxin-propafenone interactions in the kidney in vivo.

The inhibitory effects on digoxin secretion of the enantiomers of both propafenone and 5-OHP are nonstereospecific. As a result, one would not expect to see clinically significant differences in the nature of these digoxin interactions based on serum concentrations of the enantiomers. This nonstereospecific nature of interaction is compatible with substrate/inhibitor polyspecificity of P-glycoprotein-mediated drug transport that was shown for drugs such as verapamil (Ito et al., 1993c) and quinidine (Hedman et al., 1990).

In vivo, propafenone is metabolized in the liver by CYP2D6 to 5-OHP and by CYP3A4 and CYP1A2 to NDPP (Botsch et al., 1993; Siddoway et al., 1987). The absence of production of 5-OHP suggests that MDCK cells lack functional CYP2D6. To the best of our knowledge, CYP2D6 has not been detected in human kidney. Therefore, hepatically produced 5-OHP is likely responsible for the majority of the in vivo interactions between digoxin and metabolites of propafenone.

We found that MDCK cells biotransform propafenone to NDPP. The relevance of the NDPP produced within the kid-

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5 C. Woodland and S. Ito, unpublished observations.

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Fig. 6. Biotransformation of propafenone by MDCK cells. Cell monolayers were incubated with α-MEM containing 5 μM propafenone (top) or 5-OHP (bottom). Apical (solid) and basal (hatched) media were sampled at 48 hr. NDPP was produced by the cells incubated with propafenone. Results are expressed as the mean values of three cell monolayers seeded at the same cell density ± S.D. n.d., not detected.
ney cells is unknown at present; however, because only very high concentrations of NDPP (>40 μM) were able to significantly inhibit the renal tubular digoxin secretion, NDPP produced intrarenally probably does not play an important role in the digoxin-propafenone interaction in vivo.

In summary, our study demonstrates that drug metabolites may contribute significantly to renal tubular drug interactions, although in this case, the parent compound has a greater effect. Our findings suggest that P-glycoprotein is involved in the digoxin-propafenone interaction and that 5-OHP is a possible substrate for P-glycoprotein.

References


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