Hepatic Disposition of Fexofenadine: Influence of the Transport Inhibitors Erythromycin and Dibromosulphothalein

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Received April 19, 2000; accepted August 31, 2000.

Purpose. To examine the disposition of fexofenadine in the isolated perfused rat liver and the influence of erythromycin and dibromosulphthalein (DBSP) on the hepatic uptake and biliary excretion of fexofenadine.

Methods. Livers from four groups of rats were perfused in a recirculatory manner with fexofenadine HCl added as a bolus (125, 250, 500, or 1000 μg) to perfusate. Livers from another three groups of rats were perfused with 250 μg of fexofenadine HCl. With one group as control, erythromycin (4.0 μg/ml) or DBSP (136 μg/ml) was added to the perfusate of the other groups. In all experiments, perfusate and bile were collected for 60 min; in addition, livers from the second experiment were retained for assay. Fexofenadine was determined in perfusate, bile, and homogenized liver by HPLC.

Results. The area under the curve (AUC) of fexofenadine was linearly related to concentration. It was unchanged from control (12,800 ± 200 ng·h/ml) by erythromycin (14,400 ± 2000 ng·h/ml), but was increased 95% by DBSP (25,000 ± 2600 ng·h/ml, $P < 0.001$). The ratios of the concentrations of fexofenadine in liver/perfusate were decreased significantly by DBSP; those for bile/liver were increased by erythromycin.

Conclusions. Erythromycin reduced the canalicular transport of fexofenadine into bile, whereas DBSP reduced uptake across the sinusoidal membrane.

KEY WORDS: fexofenadine; hepatic transport; inhibition; erythromycin; dibromosulphothalein.

INTRODUCTION

Fexofenadine is one of the more recent second-generation H_1-histamine receptor antagonists approved for the relief of allergic rhinitis. It is the carboxylate metabolite of terfenadine. The latter has been withdrawn from sale in many countries because of concerns over its cardiotoxicity when coadministered with drugs known to inhibit the metabolism of terfenadine to fexofenadine, a path catalyzed by CYP3A4 (1). The cardiotoxicity of fexofenadine appears to be minimal (2). Preliminary observations suggest that, following oral administration, fexofenadine is eliminated essentially unchanged, with the majority of the dose appearing in feces (about 80%) and around 12% in urine (1,3).

Our interest in the hepatic disposition of fexofenadine was stimulated by reports of significant increases in the area under the curve (AUC) of terfenadine and also of fexofenadine (4–6) when inhibitors of CYP3A4-catalyzed metabolism, such as erythromycin, ketoconazole, itraconazole, and fluconazole, were coadministered with terfenadine. Some of these inhibitors of CYP3A4, such as erythromycin and ketoconazole, have also been found to inhibit transport catalyzed by P-glycoprotein (7,8). Recent studies have observed a net efflux of fexofenadine from the basolateral to apical side of a monolayer of Caco-2 cells (9); such efflux has been confirmed with layered L-MDR1 cells expressing P-glycoprotein (10). The latter workers also administered fexofenadine intravenously and orally to normal mice and mice lacking the *mdr1a* gene (–/–) and found approximately 5-fold greater concentrations of fexofenadine in plasma and in tissues of the liver, kidney, and brain of the (–/–) mice. In addition, these workers observed saturable cellular uptake of fexofenadine that was mediated by the organic anion transporting protein (OATP). OATP is located in the sinusoidal membrane of hepatocytes, whereas P-glycoprotein is located in the canalicular membrane (11).

Hence, our hypothesis was that fexofenadine may be actively transported across the sinusoidal membrane into hepatocytes via OATP, and secreted into biliary canaliculi via P-glycoprotein. Furthermore, it is possible that substrates for sinusoidal uptake, such as dibromosulphothalein (DBSP) (12), and inhibitors of transport by P-glycoprotein, such as erythromycin (7), may modify the hepatic disposition of fexofenadine. The latter may explain in part the increased AUC of fexofenadine in humans during concurrent administration of terfenadine or fexofenadine with erythromycin (4,13).

Therefore, the aims of our study were to examine the disposition of fexofenadine in the isolated perfused rat liver and investigate the influence of erythromycin and DBSP on the hepatic uptake and biliary excretion of fexofenadine, and on its concentrations within the liver.

MATERIALS AND METHODS

Chemicals

Fexofenadine HCl (lot no. Q00514) was a gift from Hoechst Marion Roussel, Inc. (Bridgewater, NJ). Erythromycin was obtained from Sigma Chemical Co. (St. Louis, MO), and DBSP from S.E.R.B. (Paris, France). Acetonitrile (UV cut-off 190 nm, BDH, Poole, England), potassium dihydrogen orthophosphate (AR grade, Ajax Chemicals, Auburn, Australia), and Milli-Q water were used for HPLC. All other chemicals for the preparation of perfusing media were of analytical grade and used as supplied commercially.

Surgery and Perfusion of Livers

Male Sprague-Dawley rats (250–350 g, Gilles Plains Animal Resource Centre, Adelaide, Australia) were housed in plastic cages in a room with a 12-h light/dark cycle, and allowed free access to water and food (Mouse Cubes, Rigley

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ABBREVIATIONS: CYP, cytochrome P450; AUC, area under the curve; OATP, human organic anion transporting protein; oatp1, isoform of rat organic anion transporting protein; cMOAT, canalicular multispecific organic anion transporter; DBSP, dibromosulphthalein; CL_b, biliary clearance.

0724-8741/00/1200-1511$18.00/0

Agriproducts, Murray Bridge, Australia). Approval for their use in experiments was obtained from the institutional animal ethics committee. Anesthesia was induced by intraperitoneal injection of 60 mg/kg of sodium pentobarbitone (Nembutal 60 mg/ml, Boehringer Ingelheim, North Ryde, Australia) prior to surgery. Livers were prepared for perfusion in-situ, as described previously (14). Freshly prepared and filtered (0.2 μm) erythrocyte- and albumin-free Krebs bicarbonate buffer (pH 7.4), supplemented with glucose (16.5 mM) and sodium taurocholate (8.33 μM), was pumped through the liver at a flow rate of 30 ml/min via a catheter inserted in the portal vein. Perfusion was performed at 37°C in a recirculatory manner, from a reservoir containing 250 ml of medium, through an 8 μm in-line filter, and a bubble trap was placed before the liver.

After allowing time for the liver to stabilize, the compound(s) of interest were added to the reservoir. Samples of perfusate (0.6 ml) were taken from the reservoir immediately and at 1, 3, 5, 10, 15, 20, 25, 30, 35, 40, 50, and 60 min after the addition of fexofenadine HCl; all bile was collected at intervals of 10 min.

Experimental Design

Initial experiments were conducted to examine the influence of the concentration of fexofenadine in the perfusing medium on its hepatic disposition. Rats were divided into four groups and, following stabilization, 125, 250, 500, or 1000 μg of fexofenadine HCl was added to the reservoir, generating nominal concentrations in perfusate of 500, 1000, 2000, and 4000 ng/ml. Five livers were perfused at an initial concentration of 1000 ng/ml and four at the other concentrations.

To examine the influence of erythromycin and DBSP on the hepatic disposition of fexofenadine, 15 rats were divided equally into three groups, and each was perfused with fexofenadine at an initial concentration of 1000 ng/ml. While one group served as the control, to the second and third groups erythromycin or DBSP was added to the reservoir 5 min after the addition of fexofenadine HCl at initial concentrations of 4.0 and 136 μg/ml, respectively.

Viability of the livers was monitored throughout each experiment by assessing their gross appearance (uniformly pink to brown) and consumption of oxygen, the flow of bile (>5 μl/min), and the pH of perfusate (inflow in the range 7.35–7.45, outflow 7.20–7.35).

Concentrations of fexofenadine HCl in bile and perfusate were determined immediately after the experiment. Perfused livers were stored at −20°C.

Measurement of Fexofenadine

Perfusate and bile (diluted 1 in 500 with drug-free perfusate) were injected (WISP 712, Waters Assoc., Lane Cove, Australia) in a volume of 0.1 ml on to a Nova-Pak C_{18} 4 μm Radial-Pak cartridge, 10 cm × 5 mm, pressurized in a RCM 8 × 10 compression module (Waters Assoc.). The analytical column was preceded by a Guard-Pak insert containing μBondapak C_{18} (Waters Assoc.). Fexofenadine was eluted with a mobile phase of acetonitrile and 0.024 M potassium dihydrogen orthophosphate (30:70) pumped at 1 ml/min (LC-10AD, Shimadzu Corp., Kyoto, Japan), and detected by UV absorbance at 225 nm (SPD-10AV, Shimadzu Corp.). Data for peak area were collected and processed on a C-R6A integrator (Shimadzu Corp.). The retention time for fexofenadine was about 12.4 min.

Concentrations of fexofenadine HCl in perfusate and bile were calculated by reference to a set of calibration standards (20–4000 ng/ml of fexofenadine HCl) prepared in perfusate that had been passed through the liver. At least eight concentrations were included in the range, with the maximum concentration determined by the initial concentration perfusing the liver. Standard curves were constructed by linear regression without weighting. The concentrations of fexofenadine HCl were determined in duplicate quality controls prepared separately at 90% and 50% of the highest standard, and at the level of the lowest calibration standard (20 ng/ml). Quality controls and working standards of fexofenadine HCl in water were stored at −20°C.

Results from intra- and inter-day determination of quality controls demonstrated accuracy ranging from 93% to 103% ($n = 6$), and from 88% to 105% ($n = 12$) of the nominal concentrations, respectively; intra- and inter-day reproducibility ranged from 1.39% to 19.4%, and from 6.3% to 15.9%, respectively. The limit of quantification was set at 20 ng/ml.

Livers were thawed, weighed, and homogenized in an equal volume of water. Homogenate (1 ml) was centrifuged and the supernatant mixed with an equal volume of acetonitrile. Following centrifugation and passage through a 0.1 μm filter, 0.05 ml was injected onto the column. Calibration standards were prepared in drug-free homogenate over the range 0.5–8.0 μg/ml; quality controls were similarly prepared at concentrations of 1.0, 2.0, and 6.0 μg/ml.

The stability of fexofenadine HCl in perfusate (at 20, 500, and 900 ng/ml) was established during storage at −20°C. Samples were taken for assay at 0, 7, 14, 21, 43, 62, and 90 days. Mean (±SD) concentrations of fexofenadine HCl in the samples stored for up to 90 days at −20°C were 20.3 ± 4.8, 520 ± 29, and 988 ± 70 ng/ml, with no apparent trends in measured concentration with time.

Pharmacokinetic and Statistical Analysis

From the concentrations of fexofenadine HCl in perfusate-time profiles up to 60 min, an AUC was calculated using the linear trapezoidal method. Biliary clearance was the quotient of the cumulative amount of fexofenadine HCl excreted into bile and the AUC. For the interaction study, concentrations in perfusate, bile, and homogenized liver at 60 min were used to calculate the ratios of the concentrations of fexofenadine in liver to perfusate and bile to liver.

Statistical comparisons of AUC and biliary clearance between groups were performed by single-factor analysis of variance; *a posteriori* comparisons were made using the method of least significant difference. Comparisons between ratios of concentrations were conducted using the Kruskal–Wallis test. Differences between groups were considered significant if $p < 0.05$.

RESULTS

Figure 1 shows the concentrations of fexofenadine HCl determined in perfusate when livers were perfused at four different concentrations. Figure 2 shows the relationship be-

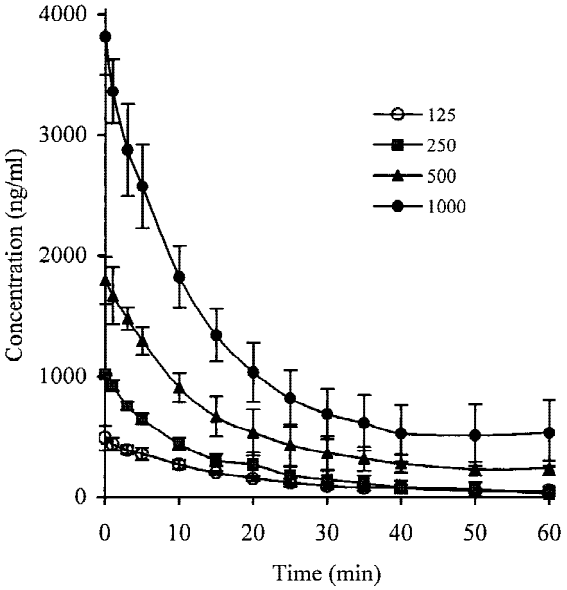

Fig. 1. Concentrations of fexofenadine HCl in perfusate-time profiles following addition of different amounts of fexofenadine HCl (125, 250 500, or 1000 μg) to the perfusing medium.

tween AUC and amount of fexofenadine HCl added to the medium.

Mean (±SD) values for biliary clearance ranged from 3.24 ± 1.21 to 3.95 ± 1.68 ml/min, with no significant differences ($p = 0.96$) in values between the four doses. Across all experiments the mean recovery of fexofenadine HCl in bile up to 60 min was 22.4%; summed recovery of unchanged drug in bile and perfusate at 60 min was 32.5%.

Concentrations of fexofenadine HCl in perfusate during concurrent perfusion with erythromycin and DBSP are shown in Fig. 3. Although the AUC of fexofenadine HCl was unchanged from control by concurrent perfusion with erythromycin, there was a significant increase of 95% in the AUC when DBSP was added to the medium (Table 1). Figure 4

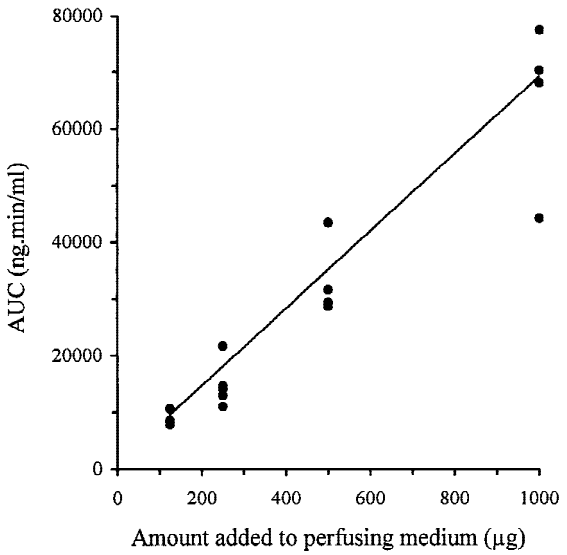

Fig. 2. Relationship between AUC of fexofenadine HCl in perfusate and amount added to medium at time zero. (The variables were related by the equation: $y = 65.3x - 160$; $p < 0.001$).

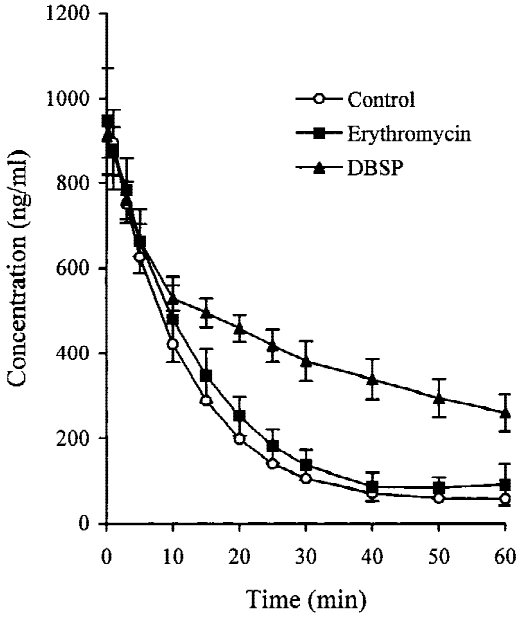

Fig. 3. Influence of erythromycin and DBSP on concentrations of fexofenadine HCl in perfusate following addition of 250 μg of fexofenadine HCl to the perfusing medium.

shows the influence of erythromycin and DBSP on the cumulative amount of fexofenadine HCl excreted in bile. Cumulative excretion to 60 min was decreased significantly ($p < 0.05$) by erythromycin (56%) and DBSP (52%). The biliary clearance of fexofenadine was decreased 46% by erythromycin and 68% by DBSP (Table 1). Of the summed recoveries of fexofenadine in bile, perfusate and liver at 60 min (Table 1), 52 ± 5% (control), 60 ± 8% (erythromycin), and 34 ± 4% (DBSP), was found in the liver.

The ratio of the concentrations of fexofenadine in liver to perfusate in the group coperfused with DBSP was significantly different ($p < 0.05$) from the control group and the group coperfused with erythromycin (Table 1). There was also a significant difference ($p < 0.05$) between the control and the group coperfused with erythromycin in the ratio of fexofenadine in liver to bile (Table 1).

Table 1. Comparison of Parameters Between Control Livers Perfused with Fexofenadine HCl Only and Livers Coperfused with Erythromycin or DBSP

	Treatment group		
Parameter[a]	Control	Erythromycin	DBSP
AUC (ng·min/ml)	12800 ± 200	14400 ± 2000	25000 ± 2600[e]
CL_b (ml/min)	6.87 ± 1.70	3.67 ± 1.89[e]	2.23 ± 0.86[e]
Summed recovery[b]	84 ± 11	73 ± 10	73 ± 6
Liver/perfusate[c]	118 ± 86	120 ± 90	20.6 ± 1.4[f]
Bile/liver[d]	18.4 ± 4.9	10.5 ± 3.1[e]	14.9 ± 2.4

[a] Mean ± SD.
[b] Summed recovery from perfusate, liver and bile as a percentage of the amount added.
[c] Ratio of concentrations of fexofenadine in liver to perfusate.
[d] Ratio of concentrations of fexofenadine in bile to liver.
[e] Significantly different from control, $P < 0.05$.
[f] Significantly different from control livers and livers co-perfused with erythromycin, $P < 0.05$.

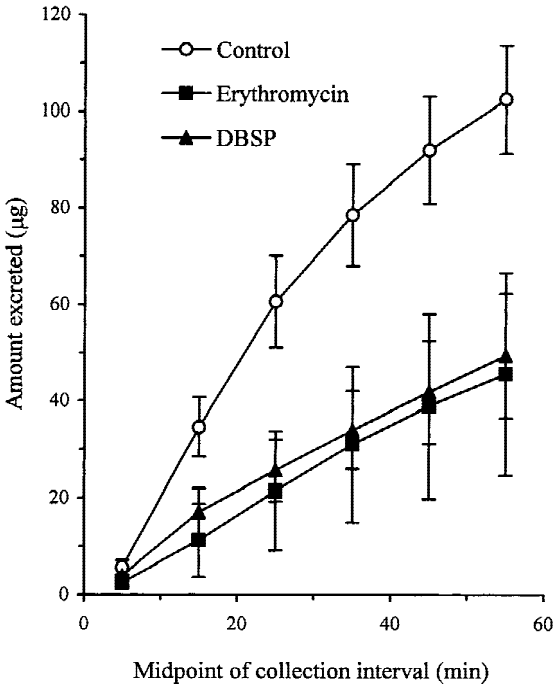

Fig. 4. Cumulative biliary excretion of fexofenadine HCl in controls and livers coperfused with erythromycin and DBSP.

DISCUSSION

Terfenadine, when administered orally to humans, is metabolized almost completely, and an estimated 40% of an oral dose is recovered in urine and feces as fexofenadine (15,16). Previous work found that the AUC of fexofenadine in humans was increased by 170% and 57% when terfenadine was coadministered with erythromycin (17) and ketoconazole (18). Product information from the pharmaceutical manufacturer, Hoechst Marion Roussel, states that a 2- to 3-times increase in the AUC of fexofenadine may occur during concurrent administration of erythromycin with oral fexofenadine (13). On the basis of the recovery of radiolabeled compound from urine and feces following administration of an oral dose of labeled fexofenadine, it was concluded that the metabolism of fexofenadine is minor (3). Hence, it is unlikely that the considerable increase in AUC of fexofenadine is due to a metabolic interaction with erythromycin.

The data from experiments perfusing livers over a wide range of concentrations demonstrates linearity in the disposition of fexofenadine between 500 and 4000 ng/ml (Fig. 2). Following the administration of recommended oral doses of fexofenadine (120–180 mg daily in two divided doses), maximum concentrations achieved in plasma were approximately 500 ng/ml (1). Thus, with reported half-lives in humans of 1 h, concentrations over a dosing interval would be comparable to those observed in perfusate in the present experiments. Furthermore, fexofenadine was taken up avidly by the liver, and after 60 min only 22% was recovered in bile while a further 10% remained in perfusate. Given that the metabolism of fexofenadine in humans is minor (3), and that our preliminary analyses of bile indicated negligible formation of alkali-labile conjugates, it may be concluded that a considerable portion of fexofenadine taken up by the liver is retained within hepatocytes. Indeed, in the control livers of the interaction study, over half of the recovered fexofenadine remained within the liver after 60 min of perfusion.

DBSP is an organic anion which, following administration to rats, is excreted into bile essentially unchanged (19). It is taken up actively by hepatocytes across the sinusoidal membrane (12,20); uptake that appears to be mediated by oatp1 (21). Furthermore, it may be concluded from previous studies (11,22) that transport of DBSP across the canalicular membrane occurs predominantly via the canalicular multispecific organic anion transporter (cMOAT). DBSP increased significantly the AUC of fexofenadine in perfusate and decreased the ratio of the concentrations in liver to those in perfusate (Table 1), suggesting that DBSP reduced its sinusoidal uptake. Concentrations of DBSP in perfusate were not measured. However, starting from an initial value of 74 μM, previous workers (23) observed a rapid decrease in the concentrations of DBSP in medium recirculating through the isolated perfused rat liver. They reported a half-life of approximately 20 min. The initial concentration of DBSP in the present study was 136 μg/ml (200 μM). Concentrations of DBSP in the range of 1–300 μM were found to inhibit the uptake of estradiol 17β-D-glucuronide by oatp-transfected COS-7 cells and cultured rat hepatocytes (21). Given that fexofenadine is also a substrate for expressed forms of oatp from human and rat (10), it is likely that the two substrates compete for sinusoidal uptake via the transporter. Coperfusion with DBSP also reduced the biliary clearance of fexofenadine (Table 1). However, this is likely to have been a consequence of the diminished hepatic uptake and, therefore, reduced intracellular concentrations of fexofenadine. The ratio of concentrations of fexofenadine in bile to liver were not affected significantly by DBSP, suggesting that transport of fexofenadine via cMOAT is minor.

The net transport of fexofenadine from the basal to apical surface of layered LLC-PK1 cells that do not express P-glycoprotein and of Caco-2 cells that express detectable but modest levels of the protein (24) was relatively low (<1%/h) when compared with L-MDR-1 cells (10) expressing larger levels of the transporter (24). A preliminary report (25) indicated that erythromycin and ketoconazole increased the AUC of fexofenadine in medium recirculated through perfused rat liver. Erythromycin is a substrate for transport in the basal to apical direction across layered cells expressing P-glycoprotein (7,26). Erythromycin (at a concentration of 4 μg/ml or 100 μM, approximately four times the initial concentration in perfusate in the present study) and ketoconazole inhibit similar directional transport of rhodamine 123 (7), a known substrate of P-glycoprotein. Concentrations of erythromycin attained maximum values of 1.7 μg/ml in the plasma of humans administered 250 mg orally three times a day (27). Following intravenous and oral administration of radiolabeled erythromycin to wild-type mice and mice nullizygous for *mdr1a,* elimination of erythromycin was significantly reduced in the plasma and tissues of the nullizygous mice (26). In the present study, even though canalicular transport of fexofenadine was reduced by erythromycin, concentrations of fexofenadine in perfusate were not increased significantly (Table 1). P-glycoprotein is found in the canalicular membrane of hepatocytes, in the apical membranes of intestinal epithelia, and in tubular cells of the kidney (28). The transporter in the latter two locations catalyses the transport of substrates from the basolateral surface to the lumen of the

intestine and renal tubule, respectively. Therefore, during oral administration of fexofenadine to humans, it is likely that the stated increase in the concentrations of fexofenadine in plasma during concurrent oral administration of erythromycin is caused by an inhibition of the transport of fexofenadine in the basolateral to apical direction across enterocytes, resulting in an increase in its oral absorption. However, additional inhibition of biliary secretion across the canalicular membrane in vivo cannot be excluded, especially by the higher hepatic concentrations of erythromycin likely to be achieved during the absorptive phase.

ACKNOWLEDGMENT

The authors wish to thank Hoechst Marion Roussel for providing a sample of fexofenadine HCl.

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