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Active Transport of Cimetidine and Ranitidine Into the Milk of Sprague Dawley Rats

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ABSTRACT

Diffusion determines the extent of accumulation into milk for most xenobiotics. However, cimetidine (CM) and ranitidine (RN) have been reported to accumulate to an extent greater than expected in rat and human milk, suggesting an active transport mechanism. In the present study, lactating Sprague Dawley rats were used in a random crossover design to characterize CM and RN active transport. Rat milk-to-serum ratios (M/S) (29.3 ± 3.2 *vs.* 13.0 ± 6.0; $P < .05$) and systemic clearance, Cl_s (12.9 ± 1.2 *vs.* 4.6 ± 1.0 ml/min, $P < .05$), were significantly reduced when exposed to a higher steady state infusion regimen of CM (0.4 and 30 mg/hr, respectively). By contrast, a infusion regimen of RN (0.4 and 30 mg/hr, respectively) produced modest, but not statistically significant, reductions in M/S (12.7 ± 3.8 *vs.* 9.0 ± 2.6; $P > .05$) and Cl_s (12.2 ± 1.5 *vs.* 9.9 ± 2.7 ml/min; $P > .05$). In a third set of rats, CM M/S (30.4 ± 2.7 *vs.* 27.5 ± 4.6; $P > .05$) and Cl_s (12.5 ± 2.8 *vs.* 10.7 ± 4.8 ml/min; $P > .05$), were marginally reduced by a concomitant RN infusion regimen (30 mg/hr) when compared with CM steady state infusions alone (0.4 mg/hr). By contrast, RN M/S (16.1 ± 2.0 *vs.* 10.5 ± 2.0; $P < .05$) and Cl_s (11.0 ± 1.3 *vs.* 7.1 ± 0.9 ml/min, $P < .05$), were significantly reduced by a concomitant CM infusion regimen (30 mg/hr) when compared with RN steady state infusions alone (0.4 mg/hr). Models for M/S and Cl_s as a function of CM steady state serum concentration were proposed and fitted to the data. Values for the maximum transport velocity of the transport system (Tmax') and the apparent dissociation constant (Km) for the M/S relationship were 326 and 55 μg/ml, respectively. For the Cl_s relationship, estimates of the nonsaturable clearance component (Cl_{ns}), the maximum velocity of the saturable elimination process (Vmax), and Km were 3.6 ml/min, 135 μg/min, and 16 μg/ml, respectively. These observations provide evidence that CM and RN milk transfer can be saturated and inhibited, which would be consistent with the hypothesis that these compounds are actively transported across mammary epithelial cells into rat milk.

Previously, a diffusion model has been developed that predicts the extent of xenobiotic transfer into milk, $M/S_{diffusion}$, from simple *in vitro* experiments (Fleishaker *et al.*, 1987). This model takes into account various factors that influence xenobiotic accumulation into milk; namely, protein binding in serum and skim milk, milk fat partitioning, and pH partitioning between milk and serum. This model has been extensively evaluated in the rabbit using 1) diazepam, phenobarbital, phenytoin, and propranolol (Fleishaker and McNamara, 1988), 2) antipyrine, acetaminophen, and salicylic acid (McNamara *et al.*, 1991), and 3) caffeine and its demethylated metabolites, theophylline, theobromine, and paraxanthine (McNamara *et al.*, 1992a)—and in the rat using caffeine, theophylline, paraxanthine, antipyrine, and diazepam (Meece *et al.*, 1993). In all of these cases, the *in vitro* estimation of M/S ($M/S_{diffusion}$) successfully predicted *in vivo* milk to serum ratios.

Some studies have reported that the H_2 antagonists, RN (Kerns *et al.*, 1985, and Dostal *et al.*, 1990) and CM (Somogyi and Gugler, 1979, and Dostal *et al.*, 1990) can accumulate to a greater extent in milk than predicted by diffusion. One hypothesis for this discrepancy is that these compounds, as in other tissues in the body (*i.e.*, renal tubule), are actively transported into milk of humans and some animals. In a more recent study (McNamara *et al.*, 1992b), rabbit M/S values were well predicted by the diffusional model; however, rat M/S values were six times greater than the predicted transport of cimetidine into milk. The higher accumulation of CM into rat milk was attributed to active transport into milk.

Active transport processes share some common features, such as transport against a concentration gradient, saturation, and competitive inhibition of the transport system. Several of these features may be manipulated *in vivo* to provide evidence of an active transport process. The purpose of the present study is to further characterize CM transfer into rat milk and to establish whether RN exhibits similar properties. To investigate the carrier hypothesis, a crossover design was

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ABBREVIATIONS: M/S, milk-to-serum concentration ratio; RN, ranitidine; CM, cimetidine; Cl_s, systemic clearance; S.D., standard deviation; Vmax, maximum velocity of saturable elimination process; Km, dissociation constant.

used to address the saturation and inhibition characteristics of CM and RN transport into milk.

Material and Methods

Chemicals. Beta-ethylhydroxytheophylline, 4-aminoantipyrine, CM and RN were purchased from Sigma Chemical Co. (St. Louis, MO). CM HCl and RN HCl were purchased from Amerisource (Louisville, KY).

Animals. Lactating female Sprague Dawley rats (250–350 g) with 10- to 12-day-old pups were purchased from Harlan Laboratories (Indianapolis, IN) and maintained in a 12/12-hr light/dark cycle. These rats were housed in standard plastic cages containing wood chips and were allowed free access to food and water. Rats were housed in the same cage as their pups until experimentation. On days 15 to 17, postpartum lactating rats were weighed and anesthetized with a ketamine (85 mg/kg) and acepromazine (1.6 mg/kg) mixture administered i.p. Once anesthesia was ensured by a positive toe pinch, catheters were placed into femoral and jugular veins. The rats were allowed to recover and were returned to their pups.

Cimetidine Infusions

Low and high infusions. Five lactating Sprague Dawley rats (16–20 days postpartum) were administered two i.v. infusion regimens (0.4 and 30 mg/h) of CM in a randomized crossover fashion. A second set of five lactating rats received two i.v. infusion regimens (0.4 and 30 mg/h) of RN in a randomized crossover fashion. Before the infusion, the dams were separated from their pups, and loading doses of 0.4 and 30 mg i.v., respectively, were administered over a 6-min period. CM and RN were administered as the hydrochloride salt. Preliminary experiments determined the maximum dosage range and the time (4 hr) for the steady state serum concentrations to be achieved. The first dose was administered 18 to 24 hours after cannulation. Serum samples were collected 5, 6, 7 and 8 hr after the initiation of the infusion regimen *via* the femoral vein. Because of low milk volume, only one milk sample was obtained at the end of the infusion. After the first infusion, the lactating dams were returned to their pups. After a washout period of 18 to 24 hr, the dams were again separated from the pups, and the second infusion was initiated. All samples were stored at −20°C until analysis.

Cimetidine-ranitidine interaction. To further characterize CM and RN transport into milk, five lactating Sprague Dawley rats (16–20 days postpartum) were administered two 8-hr i.v. infusions of CM (0.4 mg/hr) each in the presence and absence of RN (30 mg/hr) in a random crossover fashion. A second set of five lactating rats recieved two 8-hr i.v. infusions of RN (0.4 mg/hr) each in the presence and absence of CM (30 mg/hr) in a random crossover fashion. Before the infusion, the dams were separated from their pups, and loading doses of 0.4 mg of CM (or RN) with or without 30 mg of RN (or CM) were administered i.v. over a 6-min period. The first infusion was initiated 18 to 24 hr after cannulation with serum samples collected at the fifth, sixth, seventh, and eighth hours of the infusion. Again, because of low milk volume, only one milk sample was collected at the end of the infusion. After terminating the first dose, the lactating dams were returned to their pups. After a washout period of 18 to 24 hr, the dams were again separated from the pups, and the second infusion was administered. As described in the last paragraph, all samples were stored at −20°C until analysis.

Cimetidine dose escalation. Six groups of three lactating Sprague Dawley Rats (16–20 days postpartum) were administered single i.v. infusions of CM (0.095, 0.7, 6.1, 19, 33.7, and 35 mg/hr) over an 8-hr period. Lactating rats were separated from their pups, and loading doses of 0.0095, 0.7, 6.1, 19, 33.7, and 35 mg, respectively, were administered before the infusion over a 6-min period. Serum samples were harvested at the fifth, sixth, seventh, and eighth hours of the infusion. Because of low milk volume, only one milk sample was collected at the end of the infusion. Samples were stored at −20°C until analysis. In addition, data from the previous study (McNamara *et al.*, 1992b) were included in this data set.

Assay methodology. Cimetidine serum and milk samples were analyzed by a modification of a HPLC method originally described by Adeodoyin *et al.* (1985). To a 100 μl sample, 25 μl of internal standard (25 μg/ml beta-ethlyhydroxytheophylline) and 100 μl of 5N NaOH were added. CM was extracted with 3 ml of methylene chloride and vortexed for 30 sec. The sample was then centrifuged for 10 min at 3400 × *g* with the organic layer removed and evaporated with nitrogen gas. The residue was then reconstituted with 100 μl of mobile phase and vortexed for 10 sec. Chromatography was conducted by injecting 30 μl samples onto a HPLC system comprised of a LC-6A liquid chromatograph pump, SDP-6A ultraviolet detector, a CR601 Chromatopac integrator (Shimazdu Scientific Instruments, Columbia, MD), and a WISP autoinjector (Waters Associates, Milford MA). The effluent was monitored at 228 nm with a mobile phase consisting of water:acetonitrile mixture (94:6) with 0.25 μM acetic acid and 0.2 μM triethylamine. Separations were conducted using a Merck Lichro Cart LiChrographer 100 RP-18 5 μm column followed by a Beckman Ultrasphere ODS 3 μm 4.6 × 7.5 mm C-18 column at a flow rate of 1 ml/min.

Samples in the CM/RN interaction study were analyzed by the same method with the exception of a few modifications. Because of a co-elution problem with ranitidine, 4-aminoantipyrine (50 μg/ml) was used as an internal standard instead of beta-ethylhydroxytheophylline. To increase resolution, separations were conducted using a Phenomenex Lichrososphere 3 μm RP-18 4.6 × 30 mm guard column followed by a Phenomenex Lichrososphere 3 μm RP-18 4.0 × 125 mm C-18 column at a flow rate of 1 ml/min.

Analytical procedures for CM and RN in milk and serum were established for both assays with acceptable recoveries (> 70%) and low intra- and inter-day coefficients of variance (< 10%). Peaks for all measured compounds were resolved with no interfering peaks. Retention times for CM and beta-ethylhydroxytheophylline were 12 and 18 min, respectively; for the interaction experiment, CM, RN and 4-amino antipyrine eluted at 12, 16, and 22 min, respectively. CM's major metabolite, cimetidine sulfoxide, was not quantitated.

Data calculations. The observed *in vivo* steady state M/S was calculated from

$$M/S_{obs} = \frac{C_m^{ss}}{C_s^{ss}} \quad (1)$$

where C_m^{ss} is the milk concentration collected at the end of the infusion and $C_s^{ss}$is the average steady state concentration in serum during the infusion. The Cl_s was calculated from

$$Cl_s = \frac{R}{C_s^{ss}} \quad (2)$$

where R is the rate of infusion. The following relationship has been derived (Appendix) to characterize the influence of concentration on M/S:

$$M/S = \left[M/S_{diffusion}\right]\left[1 + \frac{Tmax'}{(Km + C_s^{ss})}\right] \quad (3)$$

where $M/S_{diffusion}$ is the diffusional (*in vitro* prediction) component of the transport, Tmax' is the maximum transport velocity of the transport system, and Km is the apparent dissociation constant for the system. The effect of increased serum concentration on Cl_s was also estimated by

$$Cl_s = Cl_{ns} + \frac{Vmax}{(Km + C_s^{ss})} \quad (4)$$

where Cl_{ns} is the nonsaturable clearance component, Vmax the maximum velocity of the saturable elimination process, and Km is the dissociation constant of CM elimination for the active component of

CM Cl_s. The relationships described by equations 3 and 4 were fitted using a nonlinear fitting program (Scientist, Micro Math Scientific Software, Salt Lake City, UT).

Statistics. A paired Student's *t* test was used to assess differences between parameter estimates with the criterion of significance set at $P < .05$.

Results

Crossover infusions of CM resulted in steady state serum concentrations by 5 hr (Fig. 1, upper panel). Because of limited volume, milk samples were only obtained at the end of the infusion with the 5-, 6-, 7-, and 8-hr serum samples averaged to estimate M/S values. Serum concentrations for CM averaged 0.52 ± 0.05 and 113. ± 26.5 μg/ml for the low and high infusion, respectively. Milk concentrations for CM averaged 15.1 ± 1.56 and 1396.0 ± 439.0 μg/ml for the low and high infusion, respectively. Observed M/S ratios decreased when the rats were infused with the higher rate, 29.3 ± 3.2 *vs.* 13.0 ± 6.0 ($P < .05$; Fig. 2). Cl_s was also significantly decreased when the higher infusion rate was administered, 12.9 ± 1.2 *vs.* 4.6 ± 1.0 ml/min ($P < .05$). The M/S value was seven times the predicted value ($M/S_{diffusion} = 4.2$; McNamara *et al.*, 1992b) for the low infusion, but was only three times the value with the high dose. Crossover infusions of RN also resulted in steady state serum concentrations by 5 hr (Fig. 1, lower panel). Serum concentrations for RN averaged 0.55 ± 0.07 and 54.7 ± 20.6 μg/ml for the low and high infusion, respectively. Milk concentrations for RN averaged 6.87 ± 1.53 and 462.0 ± 101.0 μg/ml for the low and high infusion, respectively. Again, the 5-, 6-, 7-, and 8-hr serum samples were averaged to estimate M/S values. The predicted M/S value for RN was estimated to be 5.6. The higher infusion regimen of RN (0.4 *vs.* 30 mg/hr) produced a modest reduction in M/S (12.7 ± 3.8 *vs.* 9.0 ± 2.6) and Cl_s (12.2 ± 1.5 *vs.* 9.9 ± 2.7 ml/min) that were not statistically significant (Fig. 2).

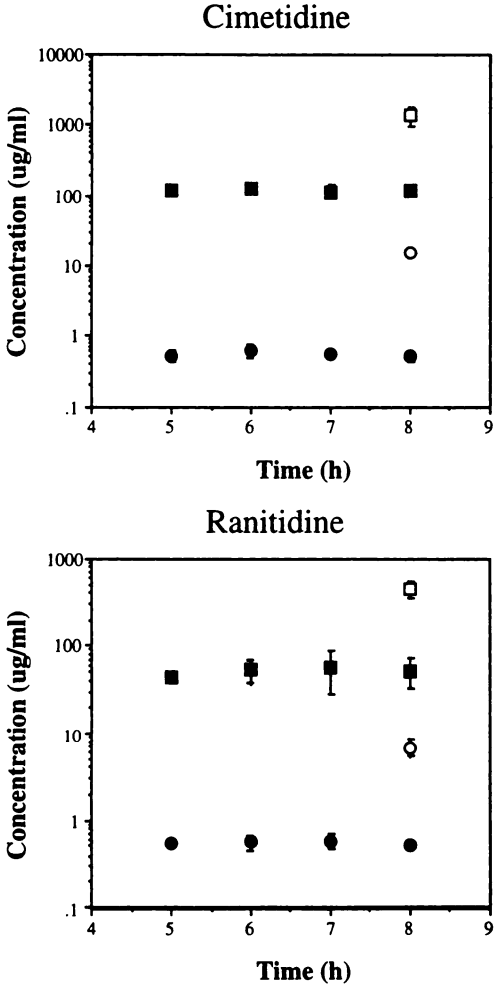

Fig. 1. Mean (± S.D.) serum (closed symbols) and milk (open symbols) concentrations after a low (0.4 mg/hr; circles) and a high (30 mg/hr; squares) infusion of CM (upper panel) or RN (lower panel) in lactating rats ($n = 5$) dosed in a random crossover design.

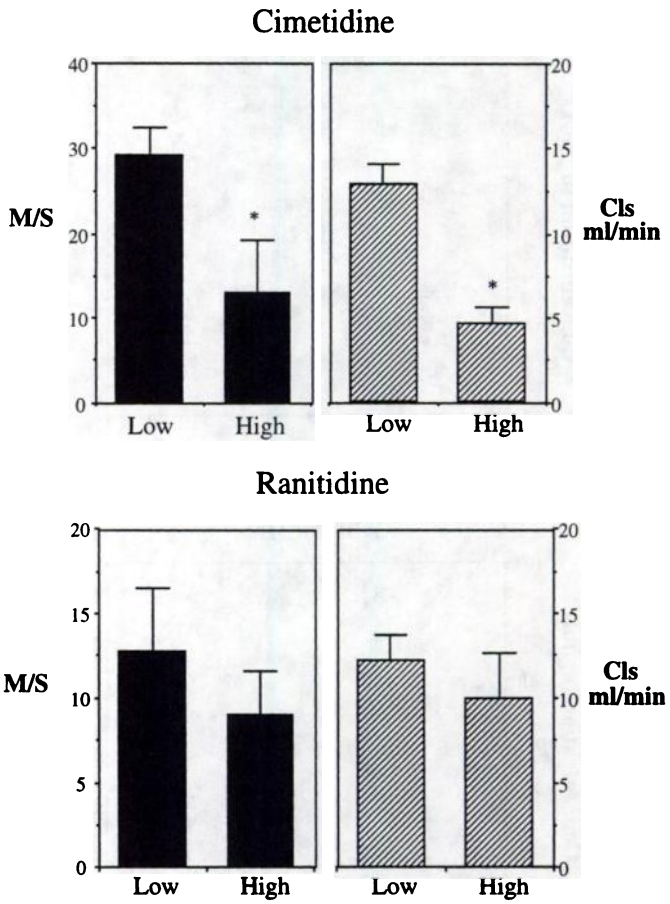

Fig. 2. Mean (± S.D.) M/S (solid bars) and Cl_s (ml/min; hatched bars) of CM (upper panels) or RN (lower panels) after a low (0.4 mg/hr) and high (30 mg/hr) infusion regimens in lactating rats ($n = 5$). *, $P < .05$.

The third set of crossover infusions, CM in the absence and presence of RN, achieved steady state serum levels within 5 hr (Fig. 3, upper panel). Serum concentrations for CM averaged 0.56 (± 0.14) and 0.73 (± 0.31) μg/ml in the absence and presence of RN, respectively. Milk concentrations for CM averaged 16.9 ± 3.74 and 19.2 ± 5.68 μg/ml in the absence and presence of RN, respectively. RN concentrations in serum and milk were 52.2 ± 11.9 and 451 ± 73.9 μg/ml, respectively. Observed M/S, 30.4 ± 2.7 *vs.* 27.5 ± 4.6 ($P < .05$), and Cl_s, 12.5 ± 2.8 *vs.* 10.7 ± 4.8 ml/min, were not significantly different from one another (Fig. 4) in the presence of RN. Serum concentrations for RN averaged 0.61 ± 0.07 and 0.95 ± 0.12 μg/ml in the absence and presence of

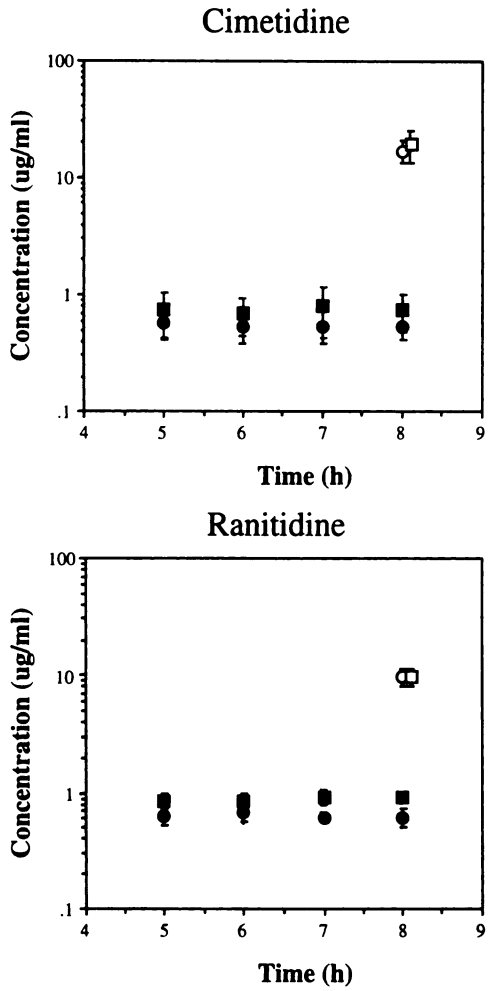

Fig. 3. Mean (± S.D.) serum (closed symbols) and milk (open symbols) concentrations after a low (0.4 mg/hr) infusion of CM (upper panel) or RN (lower panel) in lactating rats (n = 5) dosed in a random crossover design in the absence (circles) and presence (squares) of a high infusion (30 mg/hr) of the opposite H_2 antagonist.

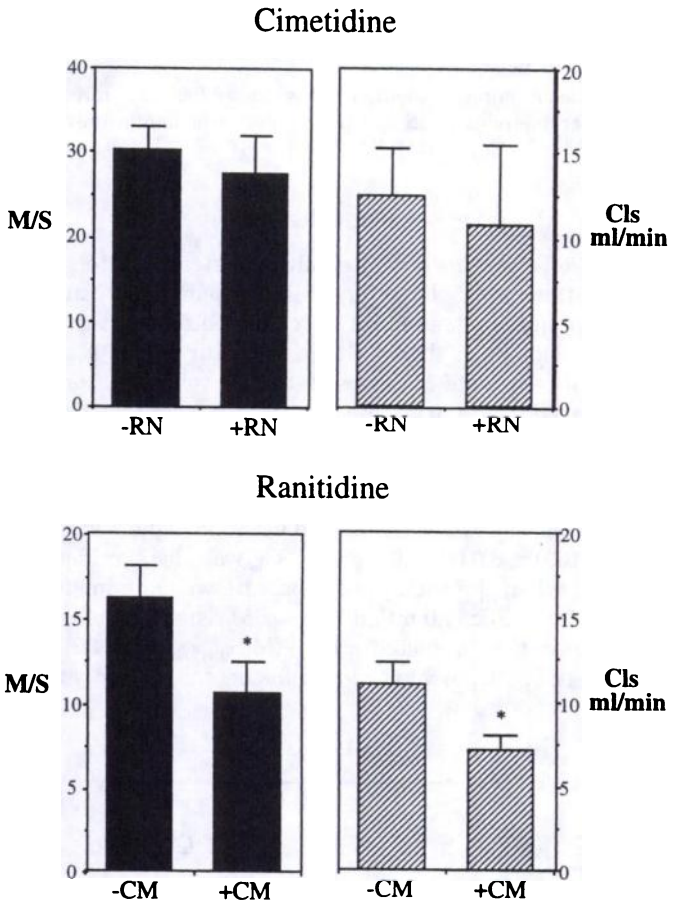

Fig. 4. Mean (± S.D.) M/S (solid bars) and Cl_s (ml/min; hatched bars) of CM (upper panel) or RN (lower panel) after a low (0.4 mg/hr) infusion in lactating rats (n = 5) dosed in a random crossover design in the absence (−) and presence (+) of a high infusion (30 mg/hr) of CM or RN. *, $P < .05$

CM, respectively. Milk concentrations for RN averaged 9.86 ± 1.65 and 9.84 ± 1.51 μg/ml in the absence and presence of CM, respectively. CM concentrations in serum and milk were 95.6 ± 18.6 and 2050.0 ± 290.0 μg/ml, respectively. In contrast to CM, the mean values for RN M/S (16.1 ± 2.0 *vs.* 10.5 ± 2.0) and Cl_s (11.0 ± 1.3 *vs.* 7.1 ± 0.9 ml/min) were significantly reduced from controls (Fig. 4) in the presence of CM ($P < .05$).

M/S and Cl_s values from the final set of infusions and data collected from an earlier study (McNamara *et al.*, 1992b) were modeled by equations 3 and 4. Steady state levels were examined over a wide range of serum concentrations (0.2–200 μg/ml). Higher infusion rates were not possible because of apparent toxicity, and lower infusion rates were limited by assay sensitivity. These two limitations presented some difficulty in fully characterizing the models. To improve the fit in equation 3, the $M/S_{diffusion}$ value (4.2; from McNamara *et al.*, 1992b) was fixed because it represented the diffusional transfer of CM into rat milk. From the fitted relations (Figs. 5 and 6), CM milk transport (M/S) and Cl_s were well described by the model (Table 1).

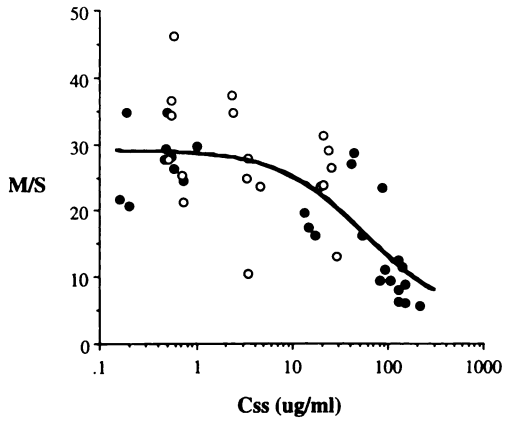

Fig. 5. M/S as a function of CM steady state serum concentrations after infusion regimens in lactating rats. The line represents the fitted relationship defined by equation 3. Values include those from the final set of individual infusions (closed symbols) and data (open symbols) collected from an earlier study (McNamara *et al.*, 1992b). The $M/S_{diffusion}$ value of 4.2 (McNamara *et al.*, 1992b) was fixed.

Discussion

For most xenobiotics, the extent of accumulation in milk is governed by diffusional forces, with most M/S values around unity. However, several studies have reported that certain compounds accumulate in milk to a greater extent than that

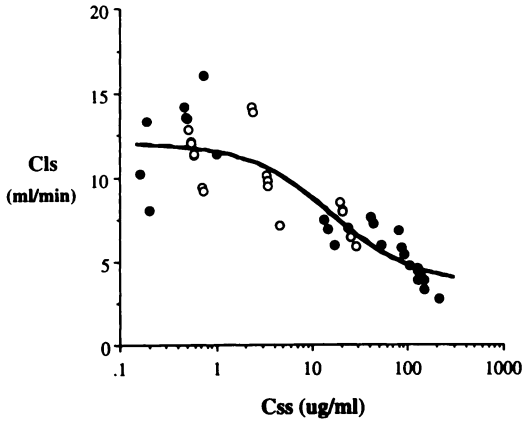

Fig. 6. Cl_s as a function of CM steady state serum concentrations after infusion regimens in lactating rats. The line represents the fitted relationship defined by equation 4. Values include those from the final set of individual infusions (closed symbols) and data (open symbols) collected from an earlier study (McNamara *et al.*, 1992b).

TABLE 1

Parameter estimates for cimetidine M/S and Cl_s as a function of steady state cimetidine serum concentration

Parameter	Estimate (S.D.)[a]
M/S *vs.* C_s	
Tmax' (μg/ml)	326 (91)
Km (μg/ml)	55 (17)
$M/S_{diffusion}$	4.2[b]
Cl_s *vs.* C_s	
Vmax (μg/min)	135 (62)
Km (μg/ml)	16 (6)
Cl_{ns} (ml/min)	3.6 (0.8)

[a] The S.D. is that obtained from the nonlinear regression analysis program (Scientist).

[b] The $M/S_{diffusion}$ value was fixed at 4.2 (McNamara et al., 1992b).

predicted by diffusion, suggesting the involvement of an active transport process (or processes). CM M/S for rats have been shown to be greater than those predicted from diffusion-based models (McNamara *et al.*, 1992b; Dostal *et al.*, 1990). Alternatives to an active transport hypothesis have been pursued (Dostal, 1990), such as pharmacologic effect (effecting milk production) or milk protein binding; however, none of these explanations could account for this deviation from the model. The extent and mechanism of cimetidine transfer into human milk had been largely unknown, except for one case report that suggested that CM may accumulate into human milk (18). Recently, 12 healthy lactating women received single oral doses of 100, 600, and 1200 mg CM in a randomized, crossover design on three different days (Oo *et al.*, 1995). Although there was no influence of dose size on M/S, the overall observed M/S (5.77 ± 1.24) was 5.5 times higher than the M/S predicted by diffusion (1.05 ± 0.18). Peak concentration in milk was delayed (3.3 ± 0.7 hr) when compared with serum peak concentration (1.7 ± 0.6 hr). This study provided the first definitive evidence of an active transport system for drug transfer into human milk.

Based on these previous studies, it was hypothesized that CM is actively transported into milk. In a previous study (McNamara *et al.*, 1992b), Cl_s and M/S decreased with increased serum concentration after CM infusions in the rat. However, because of the large interanimal variation, only the highest infusion rate clearance was found to be significantly different from the other infusion rates. Using a crossover design, the present study found that M/S values declined by 55% at higher CM concentrations. In addition to M/S, Cl_s was decreased significantly (by 64%) at the higher CM infusion regimen. CM is largely eliminated by renal secretion, which is an active process involving the cation transporter and perhaps *p*-glycoprotein (Dutt *et al.*, 1994; Gisclon *et al.*, 1987; McKinney *et al.*, 1981). A reduction of CM clearance and M/S with increased CM concentration suggests that both of these systems may be saturated, a defining characteristic of an active transport process. RN M/S and Cl_s were modestly lower at the higher infusion rate. Attempts to increase RN infusion rates resulted in unacceptable toxicity.

RN did not inhibit CM transport into milk or alter CM clearance, whereas CM was effective in reducing both the mean M/S and Cl_s for RN. Taken together, these observations would suggest that both CM and RN compete for the same transporter, but RN possesses a lower affinity for the transport system in both the mammary epithelial cells and the renal tubules. A similar competition has also been reported in other tissues where CM and RN distribution are thought to be actively mediated, *i.e.*, choroid plexus (Whittico *et al.*, 1990). Reduction in RN M/S was accompanied by a reduction in RN Cl_s in the presence of CM. A reduction in RN Cl_s caused by CM is also consistent with the observations of active transport for CM into renal tubule. Although the mechanism of this inhibition is not fully characterized (*i.e.*, competitive or noncompetitive inhibition of transport), the observations suggest that RN transport into milk may be inhibited by CM, another characteristic of an active transport system.

To more fully characterize the influence of steady state serum concentrations of CM on M/S and Cl_s, a series of infusions were conducted. In addition to these rats, data from previous infusions were included to increase the data base size (McNamara *et al.*, 1992b). As predicted by the models, M/S and Cl_s decreased with increasing steady state serum concentrations. The large interanimal variability in M/S is evident in Figure 5. As predicted by the proposed models (Figs. 5 and 6), M/S and Cl_s values were larger at lower steady state serum concentrations. Thus, the major contributing factor on M/S and Cl_s at these steady state serum levels is the active component of the transport or clearance pathway. As steady state serum levels increase, the active portion of the model contributes less to the overall M/S and Cl_s values, resulting in lower values. With further increased steady state serum levels, CM M/S and Cl_s values approach the $M/S_{diffusion}$ and Cl_{ns} values, suggesting total saturation of the transporter. At higher CM concentrations, most of the milk accumulation and clearance depend upon the nonsaturable, diffusion driven pathways. The saturable clearance pathway is likely to be renal tubule secretion, whereas the milk transporter has yet to be identified.

As stated previously, CM is reported to be actively transported in other tissues in the body (Dutt *et al.*, 1994; Gisclon *et al.*, 1987; McKinney *et al.*, 1981; Whittico *et al.*, 1990), namely in the renal tubule, primarily by the organic cation transporter and *p*-glycoprotein. In this study, evidence suggests that the transporters in the renal tubule and mammary epithelial cell may share some features. Increased CM serum levels reduced M/S and Cl_s in equal proportions (55 and 64% decrease, respectively). Estimates of Km obtained from equations 3 and 4 (Table 1) resulted in values of 16 and 55 μg/ml

(66 and 219 μM), respectively. Similar Km values may suggest that the transporters responsible for CM milk transport and renal elimination may resemble each other. The high M/S value, but low affinity (high Km) further suggests that the transporter(s) may be abundant, but are not selective.

To further study this similarity, other compounds that are actively transported in the renal tubule, both anions and cations, would need to be evaluated. Other agents known to accumulate in milk to a greater extent than predicted by diffusion, *i.e.*, *p*-amino hippuric acid (Rasmussen, 1969a), *n*-acetylated sulphanilamide (Rasmussen, 1969b), 4-amino-antipyrine (Banerjee *et al.*, 1967), benzylpenicillin (Schadewinkel-Scherkl *et al.*, 1993), and nitrofurantoin (Kari, personal communication) are also actively secreted in the renal tubule. It remains to be established whether the link between the active secretion process in the renal tubule and the mammary epithelial cell is more than coincidental.

The present observations provide evidence that CM milk transfer in the rat is saturable and can be inhibited by RN. Such evidence would be consistent with the hypothesis that CM is actively transported across mammary epithelial cells into rat milk. Accumulation in rat milk and, therefore, neonatal dose exposure is greater than predicted based on the physical chemical (*e.g.*, octanol:water partitioning, pH partitioning and protein binding) and pharmacokinetic (*e.g.*, maternal systemic clearance) properties of CM.

Appendix

The transfer of drugs into milk can be governed by passive diffusion or a combination of passive and active transport mechanisms. For a drug which undergoes both mechanisms, the following mass balance relationship can be written:

$$V_m \frac{dC_m}{dt} = Cl_d(C_{s,u\&u} - C_{m,u\&u}) + \frac{Tmax}{(Km + C_s)}C_s \quad (A1)$$

where V_m is the volume of the milk compartment, C_m and C_s are the total concentration of drug in the milk and serum, respectively, Cl_d is the diffusion clearance of drug into and out of the milk, $C_{s,u\&u}$ and $C_{m,u\&u}$ are the concentration of unbound, unionized drug in the serum and milk, respectively, Tmax is the maximum transport velocity of the transport system and Km is the apparent dissociation constant for the system.

Under steady-state conditions [$dC_m/dt = 0$], the relationship rearranges to yield:

$$Cl_d C^{ss}_{s,u\&u} + \frac{Tmax}{(Km + C^{ss}_s)}C^{ss}_s = Cl_d C^{ss}_{m,u\&u} \quad (A2)$$

Substituting unbound for total serum concentration and further rearrangement produces

$$1 + \frac{\left(\frac{Tmax}{(f^u_s f_s Cl_d)}\right)}{(Km + C^{ss}_s)} = \frac{C^{ss}_{m,u\&u}}{C^{ss}_{s,u\&u}} \quad (A3)$$

where f^u_s is the fraction unionized in serum and f_s is the fraction unbound in serum.

Hence the unbound, unionized concentration ratio will always be greater than one. Since total concentrations are more readily available, it is more appropriate to examine the ratio of total concentrations. By taking into account the protein binding and ionization in both serum and milk as well as the partitioning into milk fat (S/W), the following substitution is made.

$$1 + \frac{\left(\frac{Tmax}{(f^u_s f_s Cl_d)}\right)}{(Km + C^{ss}_s)} = \frac{C^{ss}_{m,u\&u}}{C^{ss}_{s,u\&u}} = \frac{f^u_m f_m (S/W) C^{ss}_m}{f^u_s f_s C^{ss}_s} \quad (A4)$$

Rearrangement yields:

$$\frac{C^{ss}_m}{C^{ss}_s} = \left[\frac{f^u_s f_s}{f^u_m f_m (S/W)}\right]\left[1 + \frac{\left(\frac{Tmax}{(f^u_s f_s Cl_d)}\right)}{(Km + C^{ss}_s)}\right] \quad (A5)$$

The initial term in A5 is identical to the M/S term dictated by passive diffusion ($M/S_{diffusion}$, Fleishaker *et al.*, 1987).

$$\frac{M}{S}_{diffusion} = \left[\frac{f^u_s f_s}{f^u_m f_m (S/W)}\right] \quad (A6)$$

By substituting (A6) into (A5) produces:

$$\frac{M}{S} = \left[\frac{M}{S}_{diffusion}\right]\left[1 + \frac{\left(\frac{Tmax}{(f^u_s f_s Cl_d)}\right)}{(Km + C^{ss}_s)}\right] \quad (A7)$$

Since the terms in the numerator of the second factor cannot be isolated from one another, they can be combined to yield the hybrid term Tmax′ which now has concentration units.

$$\frac{M}{S} = \left[\frac{M}{S}_{diffusion}\right]\left[1 + \frac{Tmax'}{(Km + C^{ss}_s)}\right] \quad (A8)$$

Hence, the value of M/S should decrease as a function of increasing serum concentration.

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