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Concentration-dependent Exsorption of Quinidine in the Rat Intestine

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Abstract—During intravenous infusion, the luminal concentration of quinidine was higher than the plasma concentration. The intestinal clearance (CL_i) of the drug was measured by dividing the rate of appearance of the drug in the intestinal luminal perfusate by the plasma concentration. The CL_i of quinidine was therefore much higher than the rate of luminal perfusion. Over the infusion dose range of 0·1–2 mg h^{-1}, the CL_i of quinidine decreased with increasing plasma concentration of quinidine. Adding quinidine into the luminal perfusate had little effect on the CL_i of quinidine. Co-administration of quinidine with other agents intravenously did not alter the CL_i of salicylic acid and urea, while the same treatment decreased the CL_i of theophylline and *S*-disopyramide. In-vitro experiments on brush-border membrane vesicles showed that quinidine decreased the rate of Na^+ uptake and H^+ efflux. The inhibition was significant at quinidine concentrations above 20 μM. Quinidine was a more potent inhibitor than amiloride. At quinidine infusion rates less than 2 mg h^{-1}, quinidine concentration in plasma or in the luminal perfusate was at the lower limit of the inhibitory concentration. Microclimate pH at the intestinal surface was also measured. At mid-jejunum, the microclimate pH increased 0·3 pH units by infusing 2 mg h^{-1} of quinidine, while the microclimate pH at most other measuring sites was not significantly altered by quinidine infusion. It was concluded that quinidine is exsorbed from blood into the intestinal lumen by a carrier-mediated pathway in addition to the passive diffusion. At high plasma concentration, quinidine exsorption becomes saturated. Quinidine inhibited the intestinal exsorption of theophylline and *S*-disopyramide possibly by competition on the carrier.

Passive diffusion has been postulated as the most likely mechanism of gastrointestinal excretion of drugs (Levy 1982; Dayton et al 1983; Israili & Dayton 1984). Previously we observed a high intestinal clearance (CL_i) of quindine, *S*-disopyramide, and theophylline in the rat intestine (Huang 1990a). For these compounds, the unbound, un-ionized drug concentration in the bulk phase of intestinal luminal perfusate is higher than that in plasma. Carrier-mediated exsorption has been proposed (Huang 1989, 1990a, b).

It is now widely accepted that there exists a relatively unstirred water layer next to all biological membranes through which solute molecules must move by simple diffusion. Such a layer near the brush-border of the small intestine has been shown to be the major barrier to solute absorption from the intestinal lumen (Wilson & Dietschy 1972; Westergaard & Dietschy 1974). An acidic microclimate pH has been demonstrated in the unstirred water layer (Lucas et al 1975; Bair & Huang 1992). The microclimate pH was estimated to be 5·7 in duodenum and higher values were found in the jejunum and ileum (Lucas & Blair 1978). Quinidine CL_i was affected by adding pectin to the luminal perfusate, but not affected by adding bovine serum albumin (Huang 1990b). The unstirred water layer with a lower microclimate pH was postulated to be responsible for the high intestinal clearance of quinidine.

In this study, we were aiming to explore the mechanism of quinidine exsorption. The high CL_i of quinidine may be due to the carrier-mediated active transport or the passive diffusion enhanced by the acidic microclimate pH, but the effects are difficult to discriminate as factors affecting carrier-mediated exsorption of quinidine may also affect the microclimate pH in the intestine, and vice versa. For example, quinidine was found to increase the microclimate pH in the rat jejunum (Iwatsubo et al 1988); increasing quinidine concentration may not only saturate the carrier, but also increase the microclimate-pH. Either mechanism will decrease the CL_i of quinidine. We have studied the concentration-dependence of the CL_i of quinidine and its effect on the CL_i of other drugs. In order to explain the observed data, we have also investigated the effect of quinidine on the microclimate pH at the intestinal surface and its inhibition of Na^+ uptake and H^+ efflux in brush-border membrane vesicles.

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Materials and Methods

Chemicals and animal preparations

Disopyramide base was extracted from Rythmodan capsules (Roussel Laboratories Ltd, UK). *S*-Disopyramide was separated by fractional crystallization of the disopyramide bitartrate salts (Burke et al 1980; Huang 1988). Quinidine sulphate, sodium salicylate, amiloride hydrochloride, and theophylline were obtained from Sigma Chemical Co. (St Louis, MO, USA). Acridine orange was from E. Merck (Darmstadt, Germany). [^{14}C]Urea (Lot no. 2477-100, 5 mCi $mmol^{-1}$) was obtained from New England Nuclear (Boston, MA, USA). [^{22}Na]NaCl (Batch no. 294, 2 Ci per 2.2 mg Na) was from Amersham International plc (Bucks, UK). The test compounds were dissolved in sterile 0·9% NaCl (saline) before use. The solution was filtered through a Cathivex 0·45 μm sterile filter (Millipore Co., Bedford, MA, USA) during the infusion. Tyrode solution was prepared by dissolving 24 g NaCl, 3 g dextrose, 3 g $NaHCO_3$, 6 mL 10%

KCl, 7·8 mL 10% $MgSO_4 \cdot 7H_2O$, 3·9 mL 5% $NaH_2PO_4 \cdot 2H_2O$, and 5·4 mL 1 M $CaCl_2$ in 3 L water.

Animal preparations were as described by Bair & Huang (1992). The in-situ single-pass perfusion technique was as previously reported (Huang 1990a, b). The intestinal clearance was calculated as the rate of drug appearance in the intestinal luminal perfusate divided by the drug concentration in serum. Concentrations of salicylic acid, quinidine, theophylline, and *S*-disopyramide in plasma, or in the luminal perfusate, were determined by HPLC (Huang 1990a). [^{14}C]Urea or ^{22}Na content in samples were determined by radioactivity counting. The unbound fraction of test compounds, salicylic acid or quinidine, in plasma and in the perfusate was measured by equilibrium dialysis (Huang 1989), and was corrected for volume shift (Huang 1983). Dialyses were in duplicate. The microclimate pH was measured as described by Bair & Huang (1992) in control rats and rats infused with 2 mg h^{-1} of quinidine.

Inhibition of Na^+ uptake and H^+ efflux in BBMV

Brush-border membrane vesicles (BBMV) were prepared using a magnesium precipitation method (Sheikh & Moller 1987). Alkaline phosphatase and aminopeptidase M, the marker enzymes for the brush-border membranes, were enriched 9- and 22-fold in BBMV, respectively. The activity of Na^+, K^+-ATPase, the marker enzyme of basolateral membranes, was very small.

The rate of Na^+ uptake was measured using ^{22}Na (Knickelbein et al 1983). BBMV was preloaded in a buffer of 300 mM mannitol, 25 mM Mes/Tris, pH 6·1 at 4°C for 2–3 h with a dilution of 20 mg protein mL^{-1}. A sample of BBMV (10 μL) was added to 40 μL of reaction mixture (0·25 mM NaCl, 150 mM mannitol, 25 mM HEPES/Tris, pH 7·4, 0·2 μCi of ^{22}Na, and various concentrations of quinidine or amiloride). After 30 s, 1·5 mL of stop solution (150 mM NaCl, 25 mM HEPES/Tris, pH 7·4, 4°C) was added and the mixture rapidly filtered through a 0·45 μm membrane (Whatman WCN). After being washed twice with the stop solution, the ^{22}Na content in BBMV on the membrane was dissolved in 4 mL of scintillation solution and counted.

The pH change in BBMV was measured by fluorescence quenching of acridine organge (Hsyu & Giacomini 1987; Warnock et al 1982). BBMV was preloaded in a buffer of 150 mM NaCl, 1 mM HEPES/Tris, pH 7·4 with a dilution of 15 mg protein mL^{-1}. BBMV solution (20 μL) was added to 3 mL of external solution (150 mM choline chloride, 1 mM HEPES/Tris, 6 μM acridine orange, and various concentrations of quinidine or amiloride) in a cuvette. After the addition of BBMV, fluorescence (Ex = 493 nm, Em = 530 nm) gradually reduced. When a steady state was reached, 20 mM of NaCl was added. The fluorescence then increased to a new steady state. The initial rate of quenching and recovery was measured to indicate the rate of H^+ efflux.

Results

Dose-dependence of quinidine CL_i

Quinidine was infused at a rate of 0·1, 0·2, 0·5, 1·0, 1·5 or 2·0 mg h^{-1} in 36 rats (n = 6 for each dose, data pooled). The CL_i of quinidine decreased with increasing dose or plasma concentration (Fig. 1). The relationship between CL_i and plasma concentration of quinidine can be fitted with an equation assuming a constant component and a saturable component;

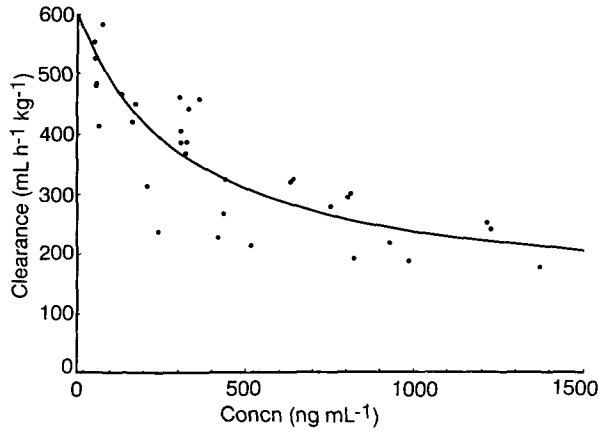

FIG. 1. Concentration dependence of the intestinal clearance of quinidine. Each point represents the value for rat. The data can be fitted by assuming a constant component and a saturable component.

$$CL_i = CL_{max}/(K_m + C_p) + CL_c \quad (1)$$

The constant component (CL_c) was 117 mL h^{-1} kg^{-1}, and the saturable component had a 50%-saturation constant (K_m) of 820 nM and maximum capacity (CL_{max}) of 398 nmol h^{-1} kg^{-1}.

Effects of intraluminal quinidine on CL_i of quinidine

At 0·1 mg h^{-1} of quinidine infusion the plasma concentration and the perfusate concentration were approximately 60 and 800 ng mL^{-1}, respectively. When 1 μg mL^{-1} of quinidine was added into the perfusate and quinidine was infused at the same rate, the quinidine concentration leaving the intestine was approximately 1·8 μg mL^{-1} and the CL_i of quinidine slightly decreased. Adding 4 μg mL^{-1} of quinidine into the perfusate also decreased the CL_i, but the difference did not reach statistical significance ($P > 0.05$).

Effect of quinidine on CL_i of salicylic acid, urea, theophylline, and S-disopyramide

Infusion of quinidine at 1·0 mg h^{-1} resulted in a steady-state plasma concentration of approximately 0·45 μg mL^{-1}. Quinidine decreased the CL_i of theophylline and *S*-disopyramide, but did not affect the CL_i of salicylic acid or urea significantly. The data are summarized in Table 1.

Effect of quinidine on the rate of Na^+ uptake and H^+ efflux in BBMV

Quinidine inhibited the rate of Na^+ uptake and H^+ efflux in BBMV preparations as indicated by the inhibition of Na^+ uptake (Fig. 2) and the rate of fluorescence quenching (Fig. 3). The inhibition was dose-dependent. Quinidine was a more potent inhibitor than amiloride. Quinidine showed significant inhibition of Na^+ uptake or H^+ efflux at a concentration above 20 μM.

Effect of quinidine on the microclimate-pH of the jejunum and ileum

Quinidine infused at 2·0 mg h^{-1} resulted in a plasma

Table 1. Effect of quinidine (1 mg h^{-1}) on the intestinal clearance of salicylic acid, urea, theophylline, and *S*-disopyramide (n = 6 in each group, mean ± s.e.m.).

	CL_i (mL h^{-1} kg^{-1})	Quinidine concn (mg L^{-1})	Unbound fraction
Salicylic acid			
Control	1·34 ± 0·12	0	0·15 ± 0·01†
Treated	1·84 ± 0·23	nd	0·14 ± 0·01†
Urea			
Control	17·1 ± 2·1	0	1
Treated	17·4 ± 1·4	nd	nd
Theophylline			
Control	83 ± 7	0	0·85 ± 0·02
Treated	56 ± 5*	0·43 ± 0·02	0·96 ± 0·01
S-Disopyramide			
Control	219 ± 21	0	0·82 ± 0·05
Treated	117 ± 18*	0·50 ± 0·06	0·84 ± 0·03

*Significantly different from the control group, $P < 0·05$. nd = not determined. †Unbound fraction of salicylic acid (10 μg mL^{-1}) added to blank plasma (n = 5). Quinidine was added at a concentration of 0·5 μg mL^{-1}. After infusion of salicylic acid or quinidine, the unbound fraction of salicylic acid was 1·0 in plasma collected at the end of the study.

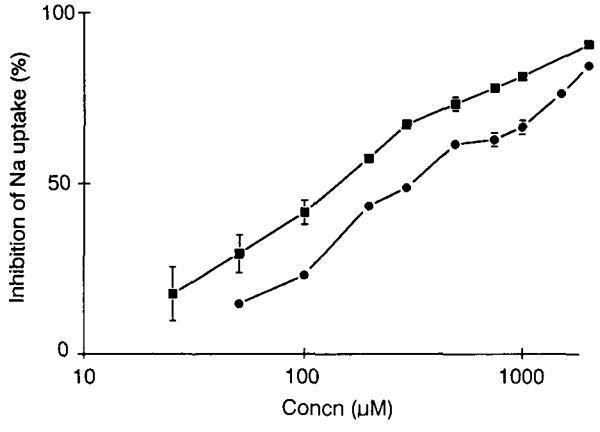

FIG. 2. Dose-response of the inhibition of the Na^+ uptake in BBMV by quinidine (■) and amiloride (●). Uptake of ^{22}Na into BBMV was measured in the presence of an inward pH gradient.

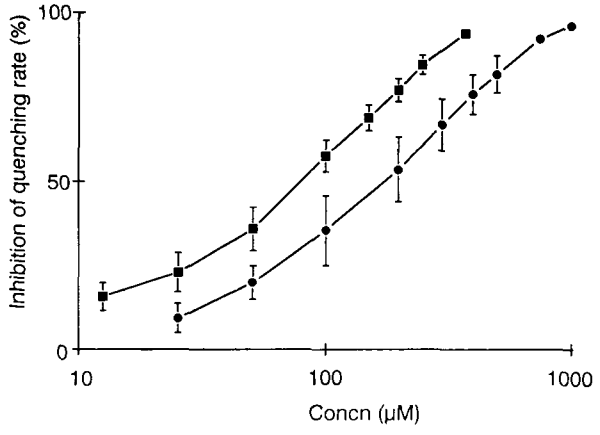

FIG. 3. Dose-response of the inhibition of the initial rate of quenching of the fluorescence of acridine orange in BBMV by quinidine (■) and amiloride (●).

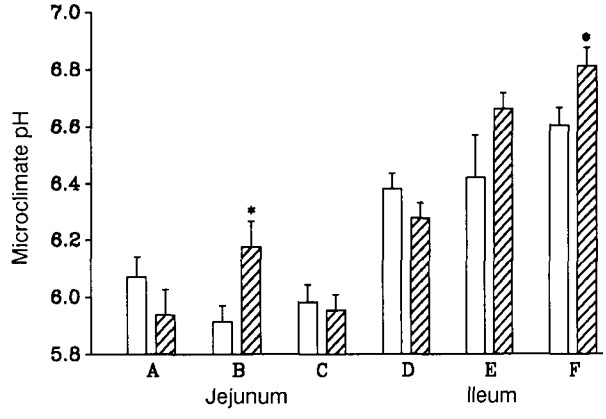

FIG. 4. The effect of quinidine (2 mg h^{-1}) on the microclimate pH of the intestine. A, B, C are jejunum sites 5, 15, and 25 cm from the ligament of Treitz, respectively. D, E, F are ileum sites 35, 25, and 15 cm from the caecum, respectively. Quinidine increased the microclimate pH at site B and F. The effect was not consistent in the whole intestine. *Indicates a significant difference ($P < 0·05$) between the control (□) and quinidine-treated (▨) rats.

concentration of 2·3 ± 0·2 μg mL^{-1} (n = 4). Quinidine did not show a consistent effect on the microclimate pH of the jejunum and ileum (Fig. 4). It decreased the microclimate pH at some measuring sites (A, C, D), but increased the microclimate pH at other sites (B, E, F). At the mid-jejunum (site B), the increment is significant, but the difference is less than 0·3 pH units.

Discussion

The CL_i of quinidine was dependent on the plasma concentration (Fig. 1) and independent of the luminal concentration. Quinidine decreased the CL_i of theophylline and *S*-disopyramide, and theophylline also decreased the CL_i of quinidine (Bair & Huang 1992). Other than assuming a carrier-mediated active transport, the observations can be explained by a physiological model assuming an acidic

microclimate pH (Bair & Huang 1992) and that quinidine increases the microclimate pH. Studies on the effect of quinidine on the microclimate pH (Fig. 4) and on the inhibition of the Na^+ and H^+ transport (Figs 2, 3), however, caused us to reject this hypothesis.

Microclimate pH of the intestine is maintained by the proton secretion and the resistance of proton diffusion (Shiau et al 1985). Inhibition of the Na^+/H^+ exchanger at the brush-border membrane will increase the microclimate pH. Quinidine is known to inhibit the Na^+/H^+ exchanger in the renal tubule (Mahnensmith & Aronson 1985). In our study with the BBMV preparation, we confirmed that quinidine inhibited the rate of Na^+ and H^+ transport. However, the inhibition was minimal at quinidine concentrations below 20 μM. At 2 mg h^{-1} of quinidine infusion, the plasma concentration was approximately 1–2 $\mu g\ mL^{-1}$ whereas the perfusate concentration was $\sim 5\ \mu g\ mL^{-1}$ (15 μM). When the microclimate pH was measured, we found that the effect of quinidine varied at different measuring sites (Fig. 4), which was different from our findings with theophylline (Bair & Huang 1992). Quinidine increased the microclimate pH at a concentration of 1000 μM (Iwatsubo et al 1988). At concentrations of 10 and 100 μM, the effect was not significant. Because the dose range of quinidine was insufficient to result in a concentration which would increase the microclimate pH substantially, the concentration-dependence of quinidine CL_i cannot be due to its pharmacodynamic effect on the intestine and it is probably due to a saturable carrier-mediated exsorption. The inhibition of quinidine on the CL_i of theophylline and *S*-disopyramide is also probably due to its effect on the carrier.

The CL_i of quinidine decreased with increased plasma concentration. The relationship between the quinidine CL_i and the plasma concentration suggested that there is a saturable carrier-mediated transport and another passive component (Fig. 1). The passive component (117 mL h^{-1} kg^{-1}) was higher than the perfusion flow (~ 50 mL h^{-1} kg^{-1}). Due to the acidic microclimate pH, a quinidine concentration in the perfusate higher than that in plasma is favoured. The high passive component of the CL_i of quinidine can be adequately explained by the physiological model and an acidic microclimate pH (Bair & Huang 1992).

The saturable component may be due to an active transport process or a carrier-mediated facilitated diffusion. The carrier may be shared by *S*-disopyramide and theophylline. Quinidine, *S*-disopyramide, and theophylline appear to inhibit each other in the exsorption. Such a carrier-mediated transport will also be affected by the acidic microclimate pH and the unstirred water layer (Wilson & Dietschy 1974).

The hypothesis of microclimate pH was postulated to explain the difference in the CL_i among drugs (Huang 1990a). We have demonstrated that the microclimate pH is indeed an important factor of drug exsorption (Bair & Huang 1992). In this study, the data further indicate that a carrier-mediated pathway for the exsorption of quinidine, theophylline and *S*-disopyramide is also present, but the nature of this carrier is not clear.

Acknowledgements

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