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Role of unstirred water layer in the exsorption of quinidine

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Abstract—Intestinal exsorption of salicylic acid, thiopentone, theophylline, and quinidine was measured during perfusion of the intestinal lumen with Tyrode solution. The effect of pectin or bovine serum albumin added to the perfusate on intestinal clearance (CL_i) was investigated. Increasing pectin concentration from 0·0 to 0·5, 1·0 and 1·5% gave CL_i values for quinidine of 499 ± 18, 363 ± 35, 237 ± 56, and 300 ± 28 mL h^{-1} kg^{-1}, respectively. One per cent of pectin in the perfusate also decreased the CL_i of thiopentone, but had no effect on the CL_i of salicylic acid or theophylline. Pectin may have increased the thickness of the unstirred water layer on the mucous membrane and the resistance of drug exsorption for some drugs. When bovine serum albumin was added, drug binding in the perfusate increased, and the CL_i of salicylic acid, thiopentone, and theophylline increased; the CL_i of quinidine was unaltered. Co-administration of theophylline with quinidine decreased the CL_i of quinidine without affecting quinidine binding in serum or in the perfusate. The CL_i theophylline was not affected by quinidine. These observations are consistent with the hypothesis that the exsorption of quinidine is rate-limited by diffusion through the unstirred water layer on the mucous membrane. The CL_i of quinidine is affected by the microclimate-pH in the unstirred water layer. An alternative possibility is that quinidine exsorption is mediated by a carrier-transport pathway.

It is widely accepted that there is a relatively unstirred water layer next to biological membranes and that solute molecules move through this layer by simple diffusion. The unstirred 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). Pectin, a polysaccharide, increases the functional thickness of the unstirred water layer and impairs intestinal absorption of many solutes (Flourie et al 1984; Gerencser et al 1984). To investigate the role of the unstirred water layer in the exsorption of quinidine, the effect of pectin on the intestinal clearance of quinidine has been studied and compared with its effect on the clearance of other types of compounds, such as salicylic acid, thiopentone, and theophylline (Huang 1990).

If diffusion of unbound drug through the membrane is the only mechanism involved in the exsorption process, an alteration in drug binding at either the seral or the mucosal side of the membrane should affect intestinal clearance. Increased drug binding in serum has been shown to decrease the intestinal clearance of disopyramide (Huang 1989), and it should therefore result in increased intestinal clearance, unless diffusion in the unstirred water layer is the rate-limiting step. I have therefore also investigated the effect of bovine serum albumin in the perfusate on the intestinal clearance of the test drugs.

Materials and methods

Chemicals. Pectin (from citrus, Lot no. LAN0652) was obtained from Wako Pure Chemical Industries, Ltd (Osaka, Japan). Bovine serum albumin was obtained from Sigma Chemical Co. (St. Louis, MO; Lot 37F-0851). Pectin and bovine serum albumin were dissolved in Tyrode solution at concentrations of 0·5, 1 and 1·5% (w/v). 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 of water.

Animal experiments. The in-situ single-pass perfusion technique was as previously reported (Huang 1990). Male Sprague-Dawley rats, 330–490 g, were used with six animals in each group. The experimental protocol was basically the same as in the previous report with pectin or bovine serum albumin added to the Tyrode solution. In brief, the jugular vein of rats anaesthetized with ethyl carbonate (urethane, 1·0 g kg^{-1} i.p.) was cannulated with a Silastic tube for drug infusion at a rate of 0·2, 1·5, 0·2 and 0·1 mg h^{-1} for salicylic acid, thiopentone, theophylline, and quinidine, respectively. For the purpose of investigating the interaction of theophylline and quinidine, the same doses of quinidine and theophylline were administered together to rats. The carotid artery was cannulated for blood sampling. The bile duct was cannulated with a Tygon tubing to divert the bile flow. The beginning of the duodenum and the end of the ileum were intubated and the intestinal lumen perfused at a rate of approximately 40 mL h^{-1} kg^{-1} with a peristaltic pump. Blood and perfusate samples were taken hourly. The intestinal clearance was calculated as the rate of drug appearance in the intestinal luminal perfusate divided by the drug concentration in serum. The unbound fraction of test compounds in serum and in the perfusate was determined by equilibrium dialysis, using a correction for the volume shift (Huang 1983). In perfusate samples, calculated unbound fractions greater than 1 were taken as unity with no standard error (i.e. no binding) (Table 1). Drug content in the serum sample and the luminal perfusate was assayed by HPLC (Huang 1990).

Results

Effect of pectin concentration on CL_i of quinidine. Adding pectin to the intestinal perfusate decreased the intestinal clearance (CL_i) of quinidine (Table 2); the CL_i of quinidine in the groups of rats (perfused with 0·5, 1 and 1·5% pectin) was significantly decreased compared with that in control rats ($P < 0.05$). However, these reduced CL_i values did not differ significantly from each other ($P > 0.05$). In some individual animals, quinidine binding in the perfusate increased, which may be attributed to

Table 1. Pharmacokinetic parameters in rats perfused with Tyrode solution with 1% pectin or 2% bovine serum albumin (BSA)[a,b].

	Control	1% pectin	2% BSA
Salicylic acid			
CL_i (mL h^{-1} kg^{-1})	1·34 ± 0·12	1·37 ± 0·17	5·63 ± 1·01*
fu (serum)	0·65 ± 0·1	0·65 ± 0·08	0·39 ± 0·09
fu (perfusate)	1·0	1·0	0·068 ± 0·001*
Thiopentone			
CL_i (mL h^{-1} kg^{-1})	8·01 ± 0·58	5·02 ± 0·31*	11·4 ± 1·25*
fu (serum)	0·26 ± 0·03	0·19 ± 0·02	0·21 ± 0·04
fu (perfusate)	0·93 ± 0·03	1·0	0·14 ± 0·01*
Theophylline			
CL_i (mL h^{-1} kg^{-1})	82·9 ± 6·8	73·9 ± 5·7	114·3 ± 7·1*
fu (serum)	0·85 ± 0·02	0·85 ± 0·02	0·92 ± 0·02
fu (perfusate)	1·0	1·0	0·73 ± 0·03*
Quinidine			
CL_i (mL h^{-1} kg^{-1})	499·0 ± 18·0	237·0 ± 56·0*	491·0 ± 71·0
fu (serum)	0·41 ± 0·01	0·40 ± 0·01	0·41 ± 0·01
fu (perfusate)	0·94 ± 0·01	0·90 ± 0·02	0·31 ± 0·01*

[a]: n = 6 for each drug in each group.
[b]: Values are mean ± s.e.m.
* $P < 0.05$ in comparison with the control group.

quinidine binding to pectin or a reduced quinidine diffusion rate in pectin solution.

Effect of 1% pectin on CL_i of drugs. The presence of pectin in the perfusate did not affect the unbound fraction of any of the drugs studied in serum or in the perfusate. The CL_i of quinidine and thiopentone were significantly lower in animals perfused with 1% pectin than those in the control animals ($P < 0.05$). The CL_i of salicylic acid like that of theophylline was not significantly changed after addition of 1% pectin to the intestinal perfusate (Table 1).

Effect of 2% bovine serum albumin on CL_i of drugs. Bovine serum albumin in the intestinal perfusate did not affect the serum protein binding of any of the drugs. However, the unbound fraction of drugs in the perfusate significantly decreased ($P < 0.05$). The CL_i values of salicylic acid, thiopentone, and theophylline were significantly lower than those in control animals when the intestine was perfused with Tyrode solution containing bovine serum albumin (Table 1), whereas the CL_i of quinidine did not differ from control.

Co-administration of theophylline with quinidine. The steady-state concentrations of quinidine and theophylline in rats infused with theophylline and quinidine simultaneously were 0.058 ± 0.004 and 1.81 ± 0.09 $\mu g\ mL^{-1}$, respectively. Neither of these values was significantly different from those obtained from control animals infused at the same dose. The CL_i of quinidine (377 ± 37 mL h^{-1} kg^{-1}) in the presence of theophylline is significantly lower than that in control rats (499 ± 18 mL h^{-1} kg^{-1}, $P < 0.05$). Quinidine binding in serum or in the perfusate was not significantly different between the control and theophylline-treated groups. The CL_i of theophylline (79.8 ± 4.5 mL h^{-1} kg^{-1}) in the presence of quinidine was not significantly different from that of control rats (82.9 ± 6.8 mL h^{-1} kg^{-1}). Theophylline binding in serum or in the perfusate was not affected by quinidine.

Discussion

In previous studies, the intestinal clearance of quinidine was much higher than that of other drugs, such as salicylic acid, thiopentone, and theophylline (Huang 1990). An unstirred water layer with a lower microclimate-pH was postulated to be the rate-limiting step of quinidine exsorption and this may have influenced the high intestinal clearance of quinidine. The observations in this study are consistent with the previous data.

Pectin has been reported to increase the thickness of the unstirred water layer (Flourie et al 1984; Gerencser et al 1984) and hence the resistance of drug diffusion through the layer. By adding pectin to the intestinal perfusate, the intestinal clearance of quinidine and thiopentone was decreased. For salicylic acid and theophylline, the intestinal clearance remained unchanged by pectin possibly due to the fact that diffusion in the unstirred water layer is an important step for exsorption of quinidine and thiopentone, the two lipophilic compounds, but not for the less lipophilic compounds, where membrane permeation may be the rate-limiting step. Interestingly, the two compounds affected by pectin also showed some binding in the perfusate in the control group of rats (Table 1), indicating there may be some binding substances in the unstirred water layer associated with the slow drug diffusion. However, the intestinal clearance due to the exofoliation of drug binding macromolecules is too small to be a major exsorption pathway.

Continuous intestinal perfusion sets up a concentration gradient for drugs subject to exsorption. However, at the distal part of the small intestine, a high concentration may be built up.

Table 2. Pharmacokinetic parameters of quinidine in rats perfused with Tyrode solution with various concentrations of pectin[a,b].

	CL_i (mL h^{-1} kg^{-1})	fu (serum)	fu (perfusate)
Control	499 ± 18	0.41 ± 0.01	0.94 ± 0.01
0.5% pectin	363 ± 35*	0.41 ± 0.01	0.90 ± 0.01*
1% pectin	237 ± 56*	0.40 ± 0.01	0.90 ± 0.02
1.5% pectin	300 ± 28*	0.42 ± 0.01	0.74 ± 0.01*

[a]: n = 6 for each pectin concentration.
[b]: Values are mean ± s.e.m.
* $P < 0.05$ in comparison with the control group.

For high intestinal clearance drugs, such as quinidine and theophylline, the concentration in the perfusate was even higher than that in serum. The addition of bovine serum albumin to the perfusate decreased the unbound fraction of drugs in the perfusate. If only the unbound drug is readily diffusable, bovine serum albumin may decrease the unbound drug concentration in the perfusate and increase the concentration gradient for passive exsorption or decrease the concentration difference for passive absorption. For salicylic acid, thiopentone, and theophylline, the intestinal clearance was indeed increased by bovine serum albumin. For quinidine, the intestinal clearance was unaltered. This observation is consistent with the hypothesis that the diffusion of quinidine in the unstirred water layer is the rate-limiting step of the exsorption. If drugs which bind to bovine serum albumin also diffuse as the unbound drug in the unstirred water layer, the intestinal clearance may be unaltered in the presence of bovine serum albumin. It can also be postulated that quinidine exsorption is mediated by a carrier-mediated transport, which is independent of the drug concentration at the mucosal side of the membrane.

There is an acidic microclimate in the unstirred water layer (Lucas et al 1975). The microclimate-pH was 5.7 in duodenum and higher values were found in the jejunum and ileum (Lucas & Blair 1978). It was also reported that theophylline elevated the microclimate-pH in the rat jejunum (Lucas 1984). Co-administration of theophylline with quinidine decreased the intestinal clearance of quinidine, so the decreased intestinal clearance of quinidine is possibly due to reduced ion-trapping at the higher microclimate-pH. It could also be explained by competition at a carrier-mediated transport site. The possibility that theophylline affects the intestinal clearance of quinidine by a dual action on both carrier competition and elevation of the microclimate-pH remains to be explored.

Based on the observations in this and previous studies, it may be postulated that different compounds have different rate-limiting steps in intestinal exsorption. The exsorption of salicylic acid may be rate-limited by membrane permeation. The exsorption of thiopentone is also controlled by diffusion at the unstirred water layer, and is affected by pectin. Exsorption of theophylline and quinidine seem to have non-diffusional pathways, with exsorbed quinidine being ion-trapped in the unstirred water layer because of the lower microclimate-pH. Diffusion through the acidic water layer is apparently the rate-limiting step in quinidine exsorption.

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