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Effect of Theophylline on the Intestinal Clearance of Drugs in Rats

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Abstract—Intestinal exsorption of salicylic acid, urea and quinidine was measured during the perfusion of the rat intestinal lumen with Tyrode solution. The intestinal clearance (CL_i) of the three compounds was measured by dividing the rate of appearance in the intestinal luminal perfusate by the plasma concentration of the compound. Co-administration of theophylline (0·2 mg h^{-1}) with the test agents increased the CL_i of salicylic acid, did not alter the CL_i of urea, but decreased the CL_i of quinidine. The effect of theophylline on the CL_i of quinidine was enhanced with increasing dose. Theophylline was found to increase microclimate-pH at the intestinal surface, but the magnitude of ΔpH alone could not explain the effect of theophylline on the CL_i of quinidine. The data, together with previous observations, suggest that the intestinal exsorption of drugs was affected by the microclimate pH and by the unstirred water layer. Theophylline affects CL_i of salicylic acid and quinidine partly by increasing the microclimate pH of the intestine. Theophylline may also affect quinidine CL_i by inhibiting the carrier-mediated pathway.

We have reported that the rate of exsorption of solute decreases with increased molecular weight. Among the small molecules, basic compounds tend to have a high intestinal clearance and acidic compounds have a low intestinal clearance (Huang 1990a). For some basic compounds, the unbound, un-ionized drug concentration in the bulk phase of intestinal luminal perfusate is higher than that in plasma. In order to delineate the possible mechanisms, we investigated the role of the unstirred water layer in the exsorption of quinidine and showed that the exsorption of quinidine is affected by the microclimate-pH in the unstirred water layer (Huang 1990b). The possibility of carrier-mediated transport was also considered. The binding to serum protein was found to affect the intestinal clearance, but altering the rate of luminal perfusion showed no effect (Huang 1989).

An acidic microclimate-pH has been measured in the unstirred water layer (Lucas et al 1975). The microclimate-pH was estimated at 5·7 in duodenum and higher values were found in the jejunum and ileum (Lucas & Blair 1978). It has also been reported that theophylline elevated the microclimate pH in the rat jejunum (Lucas 1984; McKie et al 1988). Our hypothesis is that if the exsorption of a drug is rate-limited by diffusion in the unstirred water layer, an increase in the microclimate-pH will result in an increase in the intestinal clearance (CL_i) for acidic compounds, an unaltered CL_i for neutral compounds, and a decrease in CL_i for basic compounds. In this study, we have investigated the effect of theophylline-increased microclimate pH on the rat intestinal clearance of salicylic acid, urea, and quinidine, model compounds for acidic, neutral, and basic compounds, respectively.

Materials and Methods

Chemicals

Quinidine sulphate, sodium salicylate, and theophylline were obtained from Sigma Chemical Co. (St Louis, MO, USA). [^{14}C]Urea (Lot no. 2477–100, 5 mCi $mmol^{-1}$) was obtained from New England Nuclear (Boston, MA, USA). 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

The in-situ single-pass perfusion technique was as previously reported (Huang 1990a). Male Sprague-Dawley rats, 330–400 g, bred and housed in the animal center of National Cheng Kung University, Medical College, were used. Food was withheld one day before the experiment but water was freely available. Urethane was injected intraperitoneally at a dose of 1 g kg^{-1}. Before the end of the experiment, 25–50 mg of urethane was added to maintain anaesthesia when necessary. The jugular vein of anaesthetized rats was cannulated with silastic tubing (0·5 mm i.d., 0·95 mm o.d.; Dow Corning Co., Midland, MI, USA) for drug infusion. Solutions containing test compounds were infused at 1·02 mL h^{-1} with a syringe pump. The carotid artery was also cannulated for blood sampling. The bile duct was cannulated with Tygon tubing (0·25 mm i.d., 0·76 mm o.d.; Norton/Chemplast, Wayne, NJ, USA) to divert the bile flow. The beginning of the duodenum and the end of ileum were intubated with Teflon tubing (3 mm i.d., 4 mm o.d.) and perfused with Tyrode solution at a rate of approximately 50 mL h^{-1} kg^{-1} using a peristaltic pump. The Tyrode solution was maintained at 37°C. 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.

Equilibrium dialysis

The unbound fraction of test compounds, salicylic acid or

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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.

Assay of drugs

Concentrations of salicylic acid, quinidine, and theophylline in plasma, or in the luminal perfusate, were determined by HPLC (Huang 1990a). [^{14}C]Urea content in samples was determined by radioactivity counting.

Determination of the microclimate pH

Determination of the microclimate pH was based on a method described by Iwatsubo et al (1986) using a miniature glass electrode (MI506, Microelectrodes, Inc., Londonderry, NH, USA) and a disposable skin reference electrode (A-35, Microelectrodes, Inc.). The anaesthetized rats were infused with theophylline (0·5 mg h^{-1}) followed by a loading bolus dose of 1 mg. For control rats, saline was infused. An acrylate chamber to clamp the intestine wall was prepared (Iwatsubo et al 1986). The chamber was perfused with Tyrode solution gassed with 5% CO_2–95% O_2 at a flow rate of 20 mL h^{-1}. The microelectrode was attached to a micromanipulator (MM133, minimum working scale 10 μm; Narishige Scientific Instrument Lab., Tokyo, Japan) and pushed toward the mucosa. When the microelectrode was pushed to the end and the pH value approached a constant, the value was taken as the microclimate-pH of the intestinal mucosa. At the end of the study, blood was sampled from the femoral vein to assay for theophylline concentration.

Model and simulation

Based on a model similar to that previously described by Hogben et al (1959; Fig. 1), an equation has been derived (see Appendix):

$$\frac{f_{uip}}{CL_i}=\frac{1}{CL_m}+\frac{f_{uim}}{CL_{aq}}+\frac{f_{uim}}{Q_i} \quad (1)$$

where CL_i is the intestinal clearance, CL_m the diffusional clearance of un-ionized solute through the membrane, CL_{aq} the diffusional clearance of ionized and un-ionized solute through the aqueous layer, Q_i the rate of luminal perfusion, f_{uip} and f_{uim} the unbound and un-ionized fraction of the solute in plasma and at the luminal side of the membrane, respectively. When there is an alteration in microclimate-pH, the un-ionized fraction of weak electrolytes was calculated based on the Henderson–Hasselbach equation. Assuming that there is no alteration in binding to macromolecules, the effect of altered microclimate-pH on the intestinal clearance can be simulated by equation 1.

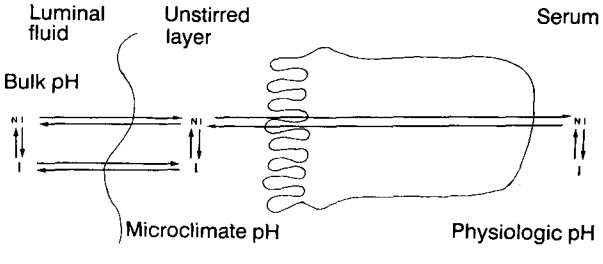

FIG. 1. A physiological model of the intestinal transport of an ionized compound. It is assumed that only un-ionized drug can diffuse through the mucous cells and both ionized and un-ionized drug will diffuse through the unstirred water layer.

Results

Effect of theophylline on CL_i of salicylic acid

When salicylic acid alone was infused at 0·2 mg h^{-1}, the CL_i of salicylic acid was 1·34 ± 0·12 mL h^{-1} kg^{-1} (n = 6, mean ± s.e.m.). Adding theophylline (0·2 mg h^{-1}) in the infusing solution decreased the CL_i of salicylic acid, but a higher dose (0·4 mg h^{-1}) of theophylline did not alter it (Table 1).

Effect of theophylline on CL_i of urea

When tracer dose (0·038 mg h^{-1}) of [^{14}C]urea was infused alone, the CL_i of urea was 17·1 ± 2·1 mL h^{-1} kg^{-1}. Theophylline infusion did not affect the CL_i of urea, the model neutral compound. The CL_i of urea was 18·0 ± 0·8 and 16·1 ± 2·7 mL h^{-1} kg^{-1} at infusion rates of 0·2 and 0·4 mg h^{-1} theophylline, respectively.

Effect of theophylline on CL_i of quinidine

When quinidine alone was infused at 0·1 mg h^{-1}, the CL_i of quinidine was 499 ± 19 mL h^{-1} kg^{-1} (n = 6, mean ± s.e.m.). Adding theophylline (0·2–0·4 mg h^{-1}) in the infusing solution significantly decreased the CL_i of quinidine (Table 2). The effect was dose-dependent; at 0·4 mg h^{-1} of theophylline infusion (steady-state plasma concentration of 4 μg mL^{-1}), 90% of quinidine exsorption was blocked.

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

Theophylline infusion at 0·5 mg h^{-1} resulted in a plasma concentration of 9·7 ± 1·0 μg mL^{-1} (n = 6). Theophylline infusion apparently increased the microclimate-pH of the jejunum and ileum (Fig. 2). Among the test sites, the increase is the largest (0·4 pH unit) at the middle of the jejunum. The difference between the control and theophylline-treated rats is significant at the middle jejunum (sites B and C) and the end of the ileum (site F).

Discussion

It is now widely accepted that there is 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). It has been postulated that the hydrogen ion movement is restricted in the unstirred water layer and the acidic microclimate is therefore maintained (Shiau et al 1985; Shimada 1987).

Hogben et al (1959) first proposed that the observed intestinal absorption rates of weak electrolytes differed quantitatively from values predicted by the pH-partition hypothesis based on the bulk phase pH. To explain this phenomenon, they suggested that the pH was lower at the mucosal surface of the small intestine than in the bulk phase.

Table 1. Effect of theophylline on the intestinal clearance of salicylic acid (n = 6 in each group, mean ± s.e.m.).

Theophylline infusion rate (mg h^{-1})	Salicylate CL_i (mL h^{-1} kg^{-1})	Theophylline concn (mg L^{-1})	Salicylate unbound fraction
0	1·34 ± 0·12	0	0·15 ± 0·01†
0·2	2·00 ± 0·23*	1·51 ± 0·21	0·16 ± 0·01†
0·4	1·43 ± 0·16	4·77 ± 0·29	0·14 ± 0·01†

*$P < 0·05$ compared with control. † Unbound fraction of salicylic acid (10 μg mL^{-1}) in plasma (n = 5) to which had been added theophylline at concentrations of 2 and 5 μg mL^{-1}. After infusion of salicylic acid or theophylline, the unbound fraction of salicylic acid was 1·0 in plasma collected at the end of the study.

Table 2. Effect of theophylline on the intestinal clearance of quinidine (n = 6 in each group, mean ± s.e.m.).

Theophylline infusion rate (mg h^{-1})	Quinidine CL_i (mL h^{-1} kg^{-1})	Theophylline concn (mg L^{-1})	Quinidine unbound fraction
0	499 ± 18	0	0·41 ± 0·01
0·2	377 ± 37*	1·81 ± 0·09	0·41 ± 0·01
0·3	191 ± 26*	3·57 ± 0·16	0·44 ± 0·02
0·4	35·4 ± 3·8*	4·26 ± 0·48	0·52 ± 0·03*

*$P < 0·05$ compared with control.

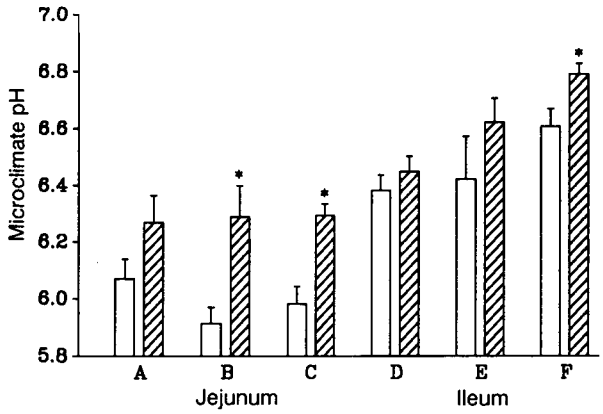

FIG. 2. Effect of theophylline infusion (0·5 mg h^{-1}) on the microclimate pH of the intestine. A, B, C are jejunum sites 5, 15, and 25 cm from the ligament of Treitz, respectively. D, E, F are ileum sites 35, 25, and 15 cm from the caecum, respectively. Theophylline in general increased the microclimate pH. *Indicates a significant difference ($P < 0·05$) between the control and theophylline-treated rats. Control, □; theophylline, ■.

Studies using pH electrodes indicate that rat proximal jejunum maintains a mucosal surface more acidic than neutral bathing medium in-vitro and in-vivo (Lucas et al 1975; Shiau et al 1985; Shimada 1987; McKie et al 1988). The source of mucosal acidity is uncertain, but either hydrogen ion secretion or bicarbonate absorption may be involved. Theophylline (10 mM) was found to increase microclimate pH by increasing the cGMP level, which regulates the rate of Na^+/H^+ exchange (McKie et al 1988). In this study, we have confirmed that there is an acidic microclimate pH and that theophylline increases the pH at a plasma concentration below the therapeutic range (10–20 μg mL^{-1}).

Such an acidic layer may cause ion-trapping of electrolytes and affect the drug transport between the serosal and luminal sides. Based on the proposed model (Fig. 1) and equation 1, it is clear that the microclimate pH, or the un-ionized fraction of electrolytes, should affect the CL_i when membrane permeability (CL_m) is sufficiently large. Theophylline, which increases the microclimate pH, will decrease the unbound, un-ionized fraction of salicylic acid at the surface of mucous (f_{uim}), therefore increases the CL_i of salicylic acid. Similarly, the elevated microclimate pH will increase the f_{uim} of quinidine and decrease the CL_i of quinidine. Urea, which is a neutral compound, should not be affected. Our results are mostly consistent with the trend. Furthermore, increasing the dose and plasma concentration of theophylline did increase the CL_i of quinidine.

The effect of theophylline in decreasing the CL_i of quinidine was, however, too much to be accounted for by the effect on the microclimate pH alone. At an infusion rate of theophylline of 0·5 mg h^{-1}, microclimate pH increased by 0·4 pH units. Assuming a very large CL_m of quinidine, the CL_i should not increase more than 2·5-fold. The fact that there was a 10-fold change in the CL_i of quinidine by theophylline suggests that there are other mechanisms for the inhibition of quinidine CL_i by theophylline. These data together with other experimental data of quinidine exsorption (Bair et al 1992) suggest that quinidine is also exsorbed by a carrier-mediated pathway. In addition to the effect on the microclimate pH, theophylline may also inhibit the carrier-mediated transport of quinidine. In terms of equation 1, not only was the f_{uim} of quinidine increased, CL_m was also decreased by theophylline.

Increasing the theophylline dose from 0·2 to 0·4 mg h^{-1}, however, did not further increase the CL_i of salicylic acid as expected. At 0·4 mg h^{-1}, the CL_i of salicylic acid was not much different from the control. The phenomenon cannot be readily explained. It is possible that the carrier-mediated pathway is also involved in the exsorption of salicylic acid. As for the exsorption of quinidine, a higher dose of

theophylline not only affected the microclimate pH, but also blocked the carrier-mediated pathway. For quinidine, these effects on the CL_i were additive; for salicylic acid, these effects on the CL_i cancelled each other out, resulting in no effect of theophylline at 0·4 mg h^{-1}. Urea, which appeared not to be transported by a carrier system, was not affected by either dose of theophylline.

Theophylline may also affect absorption of weak electrolytes by increasing the microclimate pH. In theory, the effect becomes important for compounds with low membrane permeability (CL_m). The effect has not been reported. The clinical significance of theophylline affecting the microclimate pH and drug absorption in the intestine is to be further explored.

Appendix

Under the experimental condition, a steady-state can be assumed. The rate of solute transfer through the membrane is therefore the same as the rate of transfer through the aqueous layer:

$$CL_m(f_{uip}\,C_p - f_{uim}\,C_m) = CL_{aq}(C_m - C_i) \qquad (A1)$$

where C_p, C_m, and C_i are concentrations of the solute in the plasma, at the membrane surface, and in the intestinal bulk fluid, respectively. The equation can be rearranged as:

$$C_m = \frac{CL_{aq}\,C_i + CL_m\,f_{uip}\,C_p}{CL_{aq} + f_{uim}\,CL_m} \qquad (A2)$$

The rate of solute transfer is also the same as the rate of disappearance from plasma:

$$CL_i\,C_p = CL_{aq}\,(C_m - C_i) \qquad (A3)$$

Substituting equation A2 into A3 gives:

$$CL_i\,C_p = CL_{aq}\left(\frac{CL_{aq}\,C_i + CL_m\,f_{uip}\,C_p}{CL_{aq} + f_{uim}\,CL_m} - C_i\right) \qquad (A4)$$

or

$$CL_i\,C_p = CL_{aq}\,CL_m\frac{f_{uip}\,C_p - f_{uim}\,C_i}{CL_{aq} + f_{uim}\,CL_m} \qquad (A5)$$

Dividing each term of equation A5 by C_p, the equation becomes:

$$CL_i = CL_{aq}\,CL_m\frac{f_{uip} - C_i/C_p\,f_{uim}}{CL_{aq} + f_{uim}\,CL_m} \qquad (A6)$$

At steady state, the rate of removal by perfusion is also equal to the rate of disappearance:

$$CL_i\,C_p = Q_i\,C_i \qquad (A7)$$

So the ratio C_i/C_p is equal to CL_i/Q_i. Substituting the term into equation A6, the equation becomes:

$$CL_i = \frac{f_{uip} - CL_i/Q_i\,f_{uim}}{1/CL_m + f_{uim}/CL_{aq}} \qquad (A8)$$

Rearrangement of equation A8 gives:

$$CL_i = \frac{f_{uip}}{1/CL_m + f_{uim}/CL_{aq} + f_{uim}/Q_i} \qquad (A9)$$

or

$$\frac{f_{uip}}{CL_i} = \frac{1}{CL_m} + \frac{f_{uim}}{CL_{aq}} + \frac{f_{uim}}{Q_i} \qquad (A10)$$

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