J. Pharm. Pharmacol. 1990, 42: 167–170
Received September 6, 1989

Comparative Drug Exsorption in the Perfused Rat Intestine

JIN-DING HUANG

Department of Pharmacology, National Cheng Kung University, Medical College, Tainan 70101, Taiwan, Republic of China

Abstract—The factors affecting drug exsorption into the gastrointestinal tract are uncertain. In this study, the intestinal clearance (CL_i) of compounds which vary in their lipophilicity, serum protein binding, molecular weight and ionic charge at physiological pH, has been measured. Male Sprague-Dawley rats with ligated bile ducts were infused with the test compounds through the jugular vein. The small intestine was intubated and perfused with Tyrode solution at 20 mL h^{-1}. The CL_i of the compounds investigated (urea, polyethylene glycol, inulin, albumin, dextran, barbituric acid, salicylic acid, thiobarbital, thiopental, thioseconal, theophylline, *S*-disopyramide and quinidine) was determined under anaesthesia by dividing the rate of a component's appearance rate in the perfusate by its carotid arterial concentration. Serum protein binding of the compounds was determined by equilibrium dialysis. The *n*-octanol–water partition coefficients of the compounds were measured as indices of lipophilicity. The CL_i values of dextran, albumin, inulin, polyethylene glycol and urea were 0·56, 1·03, 4·5, 4·8 and 12·0 mL h^{-1}, respectively. The larger the molecular weight of a compound, the smaller its CL_i. The molecular weight is apparently one of the major determinants of CL_i. Thiobarbital, thiopental and thioseconal are compounds of similar structure with increasing lipophilicity and serum protein binding. The CL_i of thiobarbital, thiopental and thioseconal was proportional to the unbound fraction in serum. The unbound clearance (CL_{ui}) of three thiobarbiturates were similar ($\sim$ 11 mL h^{-1}). The unbound fraction of drug in serum appears to be a factor determining their CL_i. Barbituric acid and salicylic acid, two acidic compounds, showed a low CL_i ($<$ 1 mL h^{-1}). In contrast with barbituric acid and salicylic acid, the CL_i of theophylline, *S*-disopyramide and quinidine was much higher ($>$ 30 mL h^{-1}). The CL_i of dextran, thiopental or *S*-disopyramide was not affected by altered perfusion rate. Other than molecular weight and serum protein binding, the ionic charge of compounds at physiological pH was the most significant factor affecting CL_i. The lipophilicity of compounds and the perfusion rate did not seem to be important factors.

With the exception of biliary excretion, little attention has been paid to the role of the gastrointestinal tract in drug elimination. Biliary excreted drug can be reabsorbed from the intestine unless non-absorbable adsorbents, such as activated charcoal, adsorb the biliary excreted compound thereby interrupting enterohepatic recycling, and enhancing elimination.

Drug elimination from the general circulation, via non-biliary pathways, into the gastrointestinal lumen has seldom been considered to be important. The use of activated charcoal has been shown to enhance the systemic elimination of some drugs (Neuvonen & Elonen 1980; Berg et al 1982; Arimori & Nakano 1986, 1988; Huang 1987, 1988), although none of the drugs studied showed significant biliary excretion. The term "gastrointestinal dialysis" has been coined to describe this drug detoxification process. The rate of drug elimination during the dialysis was described as "gastrointestinal clearance" by Levy (1982).

However, for reasons not understood, gastrointestinal dialysis is not successful for all drugs (Adler et al 1986; Allen et al 1987). Of the several possible mechanisms for non-biliary intestinal drug excretion (Dayton et al 1983; Israili & Dayton 1984), passive diffusion is the most frequently postulated (Levy 1982; Rozman 1986). In addition, exfoliation of intestinal cells and lipid exudation across mucosa have been considered (Rozman 1986). However, none of these possibilities has been documented for their validity.

In the present study, we have compared the intestinal clearance of compounds with a variety of physical properties, in rat perfused intestine. Dextran (mol. wt $\sim$ 70 000), albumin (mol. wt 66 000), inulin (mol. wt $\sim$ 5000), polyethylene glycol (PEG, mol. wt $\sim$ 900) and urea (mol. wt 60) were markers. To represent the normal molecular weight range of drugs, the CL_i of acidic (barbituric acid, salicylic acid), neutral (three thiobarbiturates, theophylline) and basic (*S*-disopyramide, quinidine) compounds was assessed. We set out to relate the drug characteristics with intestinal clearance and to explore their possible influence on intestinal drug exsorption.

Materials and Methods

Chemicals

[^{14}C]Urea (Lot no. 2477-100, 5 mCi $mmol^{-1}$), [1,2-^{3}H]PEG (mol. wt 800–1000; Lot no. 2413-077, 6·75 mCi g^{-1}) and [^{3}H(G)]inulin (mol. wt 5000–5500; Lot no. 2063-194, 295·3 mCi g^{-1}) were obtained from New England Nuclear (Boston, MA, USA). Inulin was purified using Sephadex LH-20 before use as described in the specification sheet. [^{3}H]Dextran (mol. wt 70 000; batch 50, 386 mCi g^{-1}) was from Amersham International plc (Bucks, UK). ^{125}I-Labelled human serum albumin was prepared by a chloramine-T method, and purified with a Sephadex G-25 (medium) column and dialysed before use. 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). 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 drug 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

An in-situ single-pass perfusion technique was used, similar to that of Arimori & Nakano (1985). Male Sprague-Dawley rats (270–470 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. Water was freely available. The jugular vein and the carotid artery were cannulated with silastic tubing (0·5 mm i.d., 0·95 mm o.d.; Dow Corning Co., Midland, MI, USA). The bile duct was ligated and bile was collected through Tygon tubing (0·25 mm i.d., 0·76 mm o.d.; Norton/Chemplast, Wayne, NJ, USA). The beginning of the duodenum and the end of the ileum were intubated with Teflon tubing (3 mm i.d.; 4 mm o.d.) connected to a peristaltic pump, and perfused with Tyrode solution at 37°C. Blood samples (0·5 mL each) were taken via the carotid artery cannula. Blood was allowed to clot and serum separated. Intestinal perfusate was collected for the last 50 min of the 1 h collection. Perfusate pH was measured immediately after sampling. The perfusate was centrifuged to remove exfoliated cells and other solids.

Determination of intestinal clearance

Rats were prepared as described above. Test compounds were infused through the jugular vein at a constant rate. The rate of perfusion (Q_i) measured at the outlet varied slightly in different preparations (Table 1). Dextran, thiopental and *S*-disopyramide were studied at two perfusion rates. Blood samples were taken at 0, 1, 2, 3, 4, 5, 6 and 7 h. Intestinal perfusate was collected for the 1–2, 2–3, 3–4, 4–5, 5–6, and 6–7 h periods. The rate of drug excretion was obtained by multiplying the drug concentration in the intestinal perfusate by Q_i. The intestinal clearance (CL_i) of a compound was calculated by dividing the rate of excretion by the mean drug concentration in serum during the same period. Six measurements were made and averaged in each animal. After the 7 h blood sample, all remaining blood was withdrawn from the animals for serum protein binding determination. The unbound fraction (f_u) of test compounds in serum, and in the perfusate, was determined by equilibrium dialysis. The drug content of the serum sample and of the luminal perfusate was assayed either by HPLC or radioactivity with β- or γ-counters, as appropriate. The unbound clearance (CL_{ui}) was determined by dividing CL_i by the unbound fraction f_u.

Protein binding determinations

The unbound fraction of drugs in serum and perfusate, was measured by equilibrium dialysis (Huang & Øie 1983), and was corrected for volume shift by Huang's method (1983). Dialyses were in duplicate.

Partition coefficient

The partition coefficients of barbituric acid, salicylic acid, thiobarbiturates, theophylline, *S*-disopyramide and quinidine were measured between n-octanol and aqueous buffer, pH 7·4. Drugs were dissolved in either phase, then vortexed with an equal volume of the other phase for 30 s. The drug concentration in both layers was determined by UV-spectroscopy.

HPLC assays

Concentrations of *S*-disopyramide, theophylline, quinidine, thiobarbiturates, salicylic acid and barbituric acid in serum, or in the luminal perfusate, were determined by HPLC. Chromatographic conditions and retention times are shown in Table 2. Previously reported sample extraction methods were used for disopyramide (Huang 1988) and theophylline assay (Huang 1987). Samples of quinidine, thiobarbiturates, barbituric acid and salicylic acid were deproteinated with perchloric acid without further extraction. In brief, the serum or perfusate samples containing drugs were mixed with equal volumes of 20% perchloric acid. After centrifugation for 5 min, a 50 μL sample was injected into the HPLC. Peak

Table 1. Intestinal clearance (CL_i) of compounds with different molecular weight, pK_a, partition coefficient (P), and unbound fraction (f_u) in serum[1].

Chemical	Charge	pK_a	n[2]	Mol. wt	Dose (mg h^{-1})	Perfusion rate (mL h^{-1})	f_u	P	CL_i (mL h^{-1})
Urea			8	60·1	0·038	21·4±0·7		0·025	12·0±0·95
PEG			6	800–1000	0·15	19·6±0·6		0·005	4·84±0·36
Inulin			8	5000–5500	0·0054	21·2±0·6		0·0085	4·50±0·74
Human serum albumin			7	66000	160000[3]	15·2±0·3			1·03±0·19
Dextran			6	50000–	0·0021	17·9±0·8		0·0021	0·56±0·08
			6	125000	0·0021	34·9±0·6			0·89±0·11
Barbituric acid	−1	4·0	6	128·1	7·1	18·1±0·4	0·94	0·005	0·71±0·11
Salicylic acid	−1	3·0	6	138·1	0·2	15·4±0·4	0·4	1·2	0·50±0·05
Thiobarbital	0	7·4	6[4]	200·3	0·3	21·6±0·9	0·61	1·8	6·3±0·7
Thiopental	0	7·45	6[4]	242·3	1·5	21·6±0·9	0·2	3·1	2·4±0·2
			6		1·5	34·1±0·5	0·21		2·2±0·4
Thioseconal	0	7·48	6[4]	254·4	1·5	21·6±0·9	0·14	5·9	1·4±0·02
Theophylline	0	8·6	6	180·2	0·2	14·7±0·6	0·85	1·1	36·7±2·7
S-Disopyramide	+1	10·2	6	339·5	1·0	15·9±1·1	0·7	2·4	79·8±13·3
			6		1·0	30·4±0·9	0·7		83·6±22·9
Quinidine	+1	8·34	6	324·4	0·1	14·9±0·4	0·32	3·0	180·0±21·6

[1] Mean ±s.e.m.
[2] Number of animals studied for each compound.
[3] Unit in counts min^{-1}.
[4] The same 6 animals were used to study 3 drugs simultaneously.

Table 2. The column, mobile phase, and detector used in HPLC analysis of drug concentrations.

Chemical	Column	Mobile phase	Flow rate (mL min^{-1})	Detector	Wavelength (nm)	Retention time (min)
Barbituric acid	μ-Bondapack C18	10 mM acetic acid	1·0	UV	254	3·6
Salicylic acid	Nova-pak C18	30% CH_3CN 0·05% H_3PO_4	1·0	fluorescence	ex. 237 em. 418	3·3
Thiobarbiturates	μ-Bondapack C18	50% CH_3OH 0·1% acetic acid	1·5	UV	287	3·2–8·3
Theophylline	LiChrosorb RP-18	10% CH_3CN 15% CH_3OH 0·1% acetic acid	0·8	UV	270	5·5
S-Disopyramide	LiChrosorb RP-8	45% CH_3CN 0·1% H_3PO_4/NaOH	1·3	UV	260	5·6
Quinidine	LiChrosorb RP-8	5% tetrahydrofuran 0·03% H_3PO_4 0·03% triethylamine	1·2	fluorescence	ex. 245 em. 418	9·5

heights from samples were compared with those of standard solutions for quantification. For thiobarbiturates, 10 μg of phenylethylthiobarbital was added as the internal standard, and the peak-height ratio was used for quantification.

Results

Intestinal clearances of urea, PEG, inulin, albumin and dextran

The CL_i values of compounds are shown in Table 1. Clearance decreased linearly with log (mol. wt). The correlation coefficient was −0·9681 ($P < 0·001$).

Intestinal clearances of thiobarbiturates

Thiobarbital, thiopental, and thioseconal are drugs of similar structure but show increasing serum protein binding and lipophilicity. The intestinal clearance and the unbound fraction (f_u) of the three thiobarbiturates are significantly different from each other ($P < 0·05$). When the clearance calculation was based on the unbound drug concentration in serum (CL_{ui}), no difference was observed (Fig. 1). These results indicated that serum protein binding is a factor determining CL_i in these drugs, whereas the lipophilicity is not.

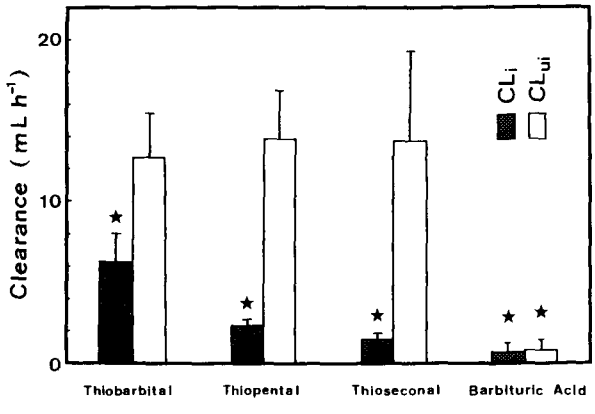

FIG. 1. Comparison of the intestinal clearance (CL_i, hatched columns) and unbound clearance (CL_{ui}, open columns) of thiobarbital, thiopental, thioseconal, and barbituric acid in rats. *, CL_i or CL_{ui} of the compound is significantly different from those of the other compounds ($P < 0·05$).

Intestinal clearances of barbituric acid, salicylic acid, thiobarbiturates, theophylline, S-disopyramide and quinidine

The intestinal clearance, the perfusion rate, the partition coefficient (P), and unbound fractions of each compound were compared (Table 1). The two acidic compounds, barbituric acid and salicylic acid, showed a low CL_i compared with the other compounds, whereas theophylline and two basic compounds, *S*-disopyramide, and quinidine, showed a high CL_i. The high intestinal clearance was reflected by the fact that, in steady state conditions, the drug concentration in the perfusate was higher than that in serum. The intestinal clearance of barbituric acid is much less than that of the thiobarbiturates (Fig. 1).

Intestinal clearance was the dependent variable in a multiple linear regression analysis where the charge, pK_a, molecular weight, perfusion rate, unbound fraction and partition coefficient were the independent variables. For each independent variable, the respective F values were 22·79, 9·72, 4·34, 1·08, 0·1, 0·01. The regression r^2 was 0·9269. After using a backward elimination procedure to select the most important variables, the model retained the charge, pK_a, and molecular weight, with F values of 32·62, 13·91, and 6·21, respectively. The F values were all statistically significant at the 0·05 level ($F(0·95, 1, 6) = 5·99$). The regression r^2 was then 0·8979. Most of the variances in the regression analysis can be explained by these three variables. We further reduced the independent variables to pK_a and charge, because the F value of the molecular weight was only marginally significant. The regression r^2 then became 0·7921, which was only marginally less than the commonly acceptable level of 0·8. The ionic charge appears to be the most significant variable in the determination of intestinal clearance of these compounds.

Discussion

Barbituric acid and salicylic acid are acidic compounds of small molecular weight that differ in serum protein binding and lipophilicity. Both compounds are negatively charged at physiological pH. The two compounds show low CL_i when they are compared with neutral compounds of similar molecular weight. Frusemide, another acidic compound, also shows a low intestinal clearance in-vivo (Valentine et al 1986). On the other hand, *S*-disopyramide and quinidine, both positively charged in physiological fluids, showed a

high CL_i in this study compared with other compounds. Ionic charge at physiological pH appears to be a major factor determining the intestinal clearance of drugs. The ratio, in-vitro, of transepithelial flux from the mucosal to the serosal side against the flux from the serosal to the mucosal side, deviates from unity for a number of organic acids and bases (Jackson & Morgan 1975). Our results are consistent with these earlier findings.

Considering the luminal flow as the sum of laminar flows, the flow rate near the intestinal brush border is slow and the fluid layer at the membrane boundary is essentially unstirred (Thomson & Dietschy 1984). This layer can often be the major resistance to the intestinal absorption of a solute (Westergaard & Dietschy 1974). An acidic microclimate-pH has been measured within the unstirred water layer (Lucas et al 1975) and the existence of low pH in the layer may, in part, explain the high clearance of basic compounds and low clearance of acidic compounds in terms of ion-trapping.

The microclimate-pH estimates of Lucas & Blair (1978) of the unstirred water layer were 5·7 in duodenum with higher values in the jejunum and the ileum. The unbound, unionized drug concentration in the unstirred water layer is assumed to be the same as that in serum at steady state. If the average microclimate-pH is one unit lower than the serum pH, the ionized concentration of the basic compounds such as *S*-disopyramide and quinidine will be ten-fold higher in the unstirred water layer. If the rate of drug exsorption is limited by the diffusion of the compound (ionized plus unionized form) through the unstirred water layer, a clearance estimate based on the serum concentration will be high. The intestinal clearance of acidic compounds such as barbituric acid or salicylic acid calculated on the serum drug concentration will be correspondingly small.

The theory of ion trapping in the unstirred water layer may explain the discrepancies in CL_i between these acidic and basic compounds, but it does not explain why theophylline also showed a high intestinal clearance. In addition, the intestinal clearance of quinidine seems too high to be explained by ion-trapping alone. Secretion of organic bases and quaternary ammonium compounds has been reported (Holland & Quay 1976; Turnheim & Lauterbach 1980) and there may be some non-diffusional pathways in exsorption of theophylline and the basic compounds to account for these high clearances.

The intestinal clearance of theophylline was estimated to be $83{\cdot}5 \pm 18{\cdot}1$ mL h^{-1} kg^{-1} in this study. This is close to the value reported by Arimori & Nakano (1988), although a much smaller rate of perfusion was used in our experiment. Theophylline is not ionized at the normal physiological pH. No ion-trapping can be expected. Although Arimori & Nakano (1988) reported no dose dependence for theophylline intestinal clearance, the possibility of active transport cannot be excluded and needs verification.

Acknowledgments

This study was supported by grants NSC77-0414-B006-02Z and DOH77-30 from the National Sciences Council and Department of Health, Republic of China.

References

Adler, L. J., Waters, D. H., Gwilt, P. R. (1986) The effect of activated charcoal on mouse sleep times induced by intravenously administered hypnotics. Biopharm. Drug Dispos. 7: 421–429

Allen, E. M., Buss, D. C., Williams, J., Routledge, P. A. (1987) The effect of charcoal on mefenamic acid elimination. Br. J. Clin. Pharmacol. 24: 830–832

Arimori, K., Nakano, M. (1985) Transport of theophylline from blood to the intestinal lumen following i.v. administration to rats. J. Pharmacobio-Dyn. 8: 324–327

Arimori, K. Nakano, M. (1986) Accelerated clearance of intravenously administered theophylline and phenobarbital by oral doses of activated charcoal in rats. A possibility of the intestinal dialysis. Ibid. 9: 437–441

Arimori, K., Nakano, M. (1988) Dose-dependency in the exsorption of theophylline and the intestinal dialysis of theophylline by oral activated charcoal. J. Pharm. Pharmacol. 40: 101–105

Berg, M. J., Berlinger, W. G., Goldberg, M. J., Spector, R., Johnson, G. F. (1982) Acceleration of the body clearance of phenobarbital by oral activated charcoal. N. Engl. J. Med. 307: 642–644

Burke, T. R. Jr., Nelson, W. L., Mangion, M., Hite, G. J., Mokler, C. M., Ruenitz, P. C. (1980) Resolution, absolute configuration, and antiarrhythmic properties of the enantiomers of disopyramide, 4-(diisopropylamino)-2-(2-pyridyl)-2-phenylbutyramide. J. Med. Chem. 23: 1044–1048

Dayton, P. G., Israili, Z. H., Henderson, J. D. (1983) Elimination of drugs by passive diffusion from blood to intestinal lumen: factors influencing nonbiliary excretion by the intestinal tract. Drug Metab. Rev. 14: 1193–1206

Holland, D. R., Quay, J. F. (1976) Intestinal secretion of erythromycin base. J. Pharm. Sci. 65: 417–419

Huang, J. D. (1983) Errors in estimating the unbound fraction of drugs due to the volume shift in equilibrium dialysis. Ibid. 72: 1368–1369

Huang, J. D. (1987) Kinetics of theophylline clearance in gastrointestinal dialysis. Ibid. 76: 525–527

Huang, J. D. (1988) Stereoselective gastrointestinal clearance of disopyramide in rabbits treated with activated charcoal. Ibid. 77: 959–962

Huang, J. D., Øie, S. (1983) Comparison of electrocardiographic response and disposition of *R*- and *S*-disopyramide in the rabbit. Res. Commu. Pathol. Pharmacol. 41: 227–241

Israili, Z. H., Dayton, P. G. (1984) Enhancement of xenobiotic elimination: role of intestinal excretion. Drug Metab. Rev. 15: 1123–1159

Jackson, M. J., Morgan, B. N. (1975) Relations of weak-electrolyte transport and acid-base metabolism in rat small intestine in vitro. Am. J. Physiol. 228: 482–487

Levy, G. (1982) Gastrointestinal clearance of drugs with activated charcoal. N. Engl. J. Med. 307: 676–678

Lucas, M. L., Blair, J. A. (1978) The magnitude and distribution of the acid microclimate in proximal jejunum and its relation to luminal acidification. Proc. R. Soc. Lond. 200: 27–41

Lucas, M. L., Schneider, W., Haberrich, F. J., Blair, J. A. (1975) Direct measurement by pH-microelectrode of the pH-microclimate in rat proximal jejunum. Ibid. 192: 39–48

Neuvonen, P. J., Elonen, E. (1980) Effect of activated charcoal on absorption and elimination of phenobarbitone, carbamazepine and phenylbutazone in man. Eur. J. Clin. Pharmacol. 17: 51–57

Rozman, K. (1986) Fecal excretion of toxic substances. In: Rozman, K., Hänninen, O. (eds) Gastrointestinal Toxicology. Elsevier, New York, pp 119–145

Thomson, A. B. R., Dietschy, J. M. (1984) The role of the unstirred water layer in intestinal permeation. In: Csáky, T. Z. (ed) Pharmacology of Intestinal Permeation II. Springer-Verlag, New York, pp 165–269

Turnheim, K., Lauterbach, F. (1980) Interaction between intestinal absorption and secretion of monoquaternary ammonium compounds in guinea pigs- a concept for the absorption kinetics of organic cations. J. Pharmacol. Exp. Ther. 212: 418–424

Valentine, J. F., Brater, D. C., Krejs, G. J. (1986) Clearance of furosemide by the gastrointestinal tract. Ibid. 236: 177–180

Westergaard, H., Dietschy, J. M. (1974) Delineation of the dimensions and permeability characteristics of the two major diffusion barriers to passive mucosal uptake in the rabbit intestine. J. Clin. Invest. 54: 718–732