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COMMUNICATIONS

Renal clearance–lipophilicity relationships of some organic acids in rabbits, rats and mice

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Abstract—The effect of lipophilicity on the renal clearance for a group of weak organic acids (benzoic, phenylacetic and hippuric acid derivatives) was studied in rabbits, rats and mice. These compounds are eliminated in the kidney by glomerular filtration and undergo both tubular secretion and tubular reabsorption. For quantification of the effect of lipophilicity, an equation

$$\log\left(\frac{ERPF}{CL_R}-1\right)=a+b\log D$$

was employed, where CL_R represents renal clearance of the parent drug, ERPF is effective renal plasma flow, D is the partition coefficient of the acids between octanol and water, and a and b are constants. In interspecies comparison, the values of parameters a and b are similar indicating no significant interspecies differences in this route of elimination.

An attractive approach to the correlation of drug pharmacokinetics to physicochemical or structural characteristics is the development of quantitative relationships which allow a prediction of pharmacokinetic properties before synthesis (Seydel 1984; Mayer & Van de Waterbeemd 1985). We have shown previously that total plasma clearance of some acidic compounds (iododerivatives of benzoic, phenylacetic and hippuric acids) is predominantly dependent on their molecular structure in three animal species (Lázníček et al 1990). Because the value of total plasma clearance is the sum of the clearances that occur by different routes, in the present paper we have focused on the relationships between renal clearance of unchanged compound and the characteristics of a group of organic acids in rabbits, rats and mice.

Materials and methods

Compounds. All iodinated compounds labelled with ^{125}I were obtained from the Nuclear Research Centre (Řež, Czechoslovakia). The radiochemical purity was over 97%. [Carboxy-^{14}C]salicylic acid (2-hydroxybenzoic acid) was obtained from V/O Izotop (Moscow, USSR). [^{3}H]Amino hippuric acid was obtained from Amersham International, UK.

Animals. Male grey Chinchilla rabbits, 3·0–3·5 kg, male Wistar rats, 170–220 g, and male mice, strain H, Konárovice, 18–25 g were fasted 18–24 h before the experiment, with free access to water.

Pharmacokinetic studies. The determination of total plasma clearance has been described previously (Lázníček et al 1990). The pharmacokinetics were examined after intravenous administration of compounds under study at 0·1 mg kg^{-1} to rabbits, 0·5 mg kg^{-1} to rats, and 1 mg kg^{-1} to mice. The plasma concentration-time data were analysed by means of the MULTI program (Yamaoka et al 1981) with weighting of data according to 1/concentration, using a two-compartment open pharmacokinetic model for the description. The renal clearance of parent compound (CL_R) was calculated according to the equation:

$$CL_R=\frac{Ae_{(0-t)}}{AUC_{(0-t)}} \quad (1)$$

where $Ae_{(0-t)}$ is the amount of unchanged compound excreted into urine from zero to time t, and $AUC_{(0-t)}$ is the area under the concentration-time curve from zero to time t. For the determination of urine elimination, the rats and mice were placed immediately after administration of the compounds into metabolic cages, the construction of which allowed reliable separation of urine from solid excreta. The collection period of the urine was at least 24 h and animals had free access to standard diet and water. For rabbits, the urine was withdrawn from the urinary bladder at the end of the pharmacokinetic study after killing the animal.

Separation of metabolites. To separate metabolites of the compounds from biological fluids, thin-layer chromatography in benzene:acetic acid:water (from 65:25:1 to 90:25:1 depending on compound lipophilicity) was used (Lázníček & Květina 1988).

Determination of lipophilicity. Lipophilicity was characterized by the logarithm of the partition coefficient between octanol and water (log D) determined by direct measurement of the distribution of the purified compounds in a mixture of 1-octanol and 0·5 M HCl as described previously (Lázníček & Květina 1988).

Plasma protein binding. Binding of the compounds to plasma proteins was determined by equilibrium dialysis at 37°C by the method described previously (Lázníček & Senius 1986).

Results

The values of renal clearances of compounds under study, their plasma protein binding, and lipophilicity are shown in Table 1. The renal clearance of parent compounds decreases with increasing lipophilicity; for quantitative evaluation of relationships between renal clearance and lipophilicity, equation 2 was employed:

$$\log\left(\frac{ERPF}{CL_R}-1\right)=a+b\log D \quad (2)$$

where ERPF represents effective renal plasma flow (determined as clearance of *p*-aminohippurate), and a and b are constants.

Table 1. Renal clearance of parent compounds (CL_R, mL h^{-1} kg^{-1}), fraction unbound in plasma (f_u), and lipophilicity (log D) of weak organic acids (mean of at least four values).

	Rabbits		Rats		Mice		
Compound	CL_R	f_u	CL_R	f_u	CL_R	f_u	log D
o-Iodohippurate	908	0·47	702	0·51	2484	0·80	0·973
m-Iodiohippurate	887	0·06	630	0·12	1962	0·25	1·883
p-Iodohippurate	529	0·05	485	0·10	2250	0·31	1·940
o-Iodobenzoate	194	0·15	89·4	0·42	105	0·65	2·621
m-Iodobenzoate	2·9	0·06	3·4	0·07	48·0	0·10	3·171
p-Iodobenzoate	2·3	0·04	1·7	0·06	18·4	0·10	3·164
Salicylate	63·7	0·08	52·0	0·22	143	0·42	2·240
o-Iodophenylacetate	14·7	0·06	31·8	0·28	50·2	0·66	2·327
m-Iodophenylacetate	4·9	0·03	3·7	0·07	20·5	0·30	2·694
p-Iodophenylacetate	1·5	0·03	1·6	0·12	1·6	0·15	2·882
Effective renal plasma flow	920		772		2652		

This equation adequately describes the renal clearance-lipophilicity relationship over the whole range of lipophilicity, i.e. it predicts the minimal and maximal value the renal clearance can achieve (from zero to effective renal plasma flow). Relationships for individual species are given by equations 3 to 5:

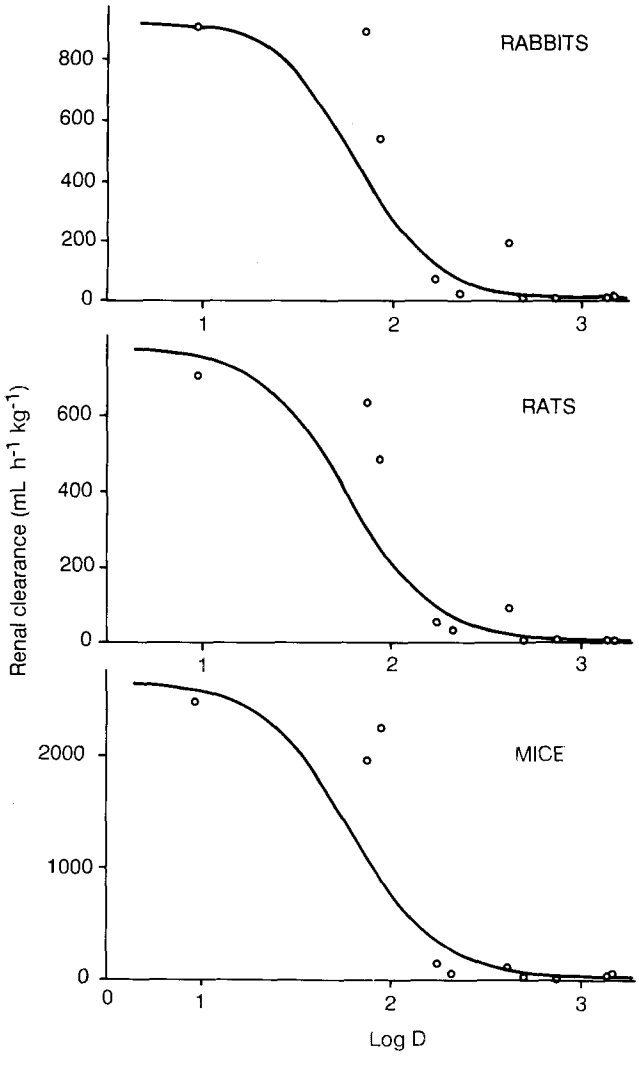

FIG. 1. Relationships between renal clearance of parent compound and lipophilicity of weak organic acids in rabbits, rats and mice.

$$\text{rabbit:}\quad \log\left(\frac{ERPF}{CL_R}-1\right)=-3{\cdot}641+2{\cdot}004\log D \qquad (3)$$
$$n=10 \quad r=0{\cdot}891 \quad s=0{\cdot}731 \quad F=34{\cdot}52$$

$$\text{rat:}\quad \log\left(\frac{ERPF}{CL_R}-1\right)=-3{\cdot}357+1{\cdot}887\log D \qquad (4)$$
$$n=10 \quad r=0{\cdot}918 \quad s=0{\cdot}585 \quad F=46{\cdot}47$$

$$\text{mouse:}\quad \log\left(\frac{ERPF}{CL_R}-1\right)=-3{\cdot}264+1{\cdot}834\log D \qquad (5)$$
$$n=10 \quad r=0{\cdot}863 \quad s=0{\cdot}768 \quad F=27{\cdot}02$$

For a graphical illustration of these relationships, more suitable plots of renal clearance vs lipophilicity are shown in Fig. 1.

Renal clearance values of most substances studied, clearly exceed their glomerular filtration (counted as glomerular filtration rate times free fraction in plasma), indicating additional tubular excretion of these compounds.

Discussion

The effect of lipophilicity often significantly contributes to structure-activity and structure-pharmacokinetic relationships of drugs. For elimination parameters, a positive dependency of clearance on lipophilicity has been observed for penicillins (Seydel 1984). In contrast to this result, the clearance of some sulphonamides is negatively dependent on lipophilicity (Seydel 1984). As sulphonamides are known to be predominantly excreted by glomerular filtration, this negative sign is attributed to a plasma protein binding-lipophilicity relationship, as only free drugs in plasma are glomerularly filtered. Renal clearance generally involves, however, three processes—glomerular filtration, tubular secretion and tubular reabsorption—and probably all three processes take part in the elimination of these weak acids. With the exception of glomerular filtration, these compounds are also eliminated by carrier-mediated transport probably using the *p*-aminohippurate carrier. In addition, there may be a certain degree of tubular reabsorption by passive diffusion, which is counteracted by decreasing lipophilicity of the drug molecule. The rate-limiting step for this process is compound transfer across the cell membrane; according to general theory the rate of this movement decreases with decreasing lipophilicity of the molecule. As there is competition between reabsorption and elimination into urine in the renal tubule, a decreased rate of transfer of the drug across the tubule membrane will result in an increase in the amount of the drug ultimately eliminated into the

urine. For this reason, the lipophilicity is the primary criterion affecting the renal pathway of structurally similar compounds.

For the three species studied, the values of parameters a and b in the renal clearance-lipophilicity equations are surprisingly similar, indicating no significant interspecies differences in dependency of renal clearance on lipophilicity. Even if correlation coefficients are sufficient for biological experiments, their values are not too high indicating that not only lipophilicity, but also other factors (molecular structure, pK_a, biotransformation, etc.) can affect to a certain degree the renal elimination of these compounds. Nevertheless, the approach to lipophilicity-renal clearance profiles from three different species into a unique species-independent profile by using equation 2 demonstrates the similarity of renal elimination of these organic acids among rabbits, rats, and mice, and perhaps it could provide the foundation for interspecies scaling of this route of elimination in mammals.

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Evidence against Substance P as a neurotransmitter at the neuroepithelial junction in rat colonic mucosa

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Abstract—Substance P (SP) caused a concentration-dependent increase in short-circuit current of rat isolated colonic mucosal preparations (ED50 10 nM). The SP antagonist [D-Arg^1, D-Pro^2, D-Trp^{7,9}, Leu^{11}]SP (50 μM) did not increase short-circuit current. Tetrodotoxin (3·1 μM) reduced the effect of a maximum concentration of SP (300 nM). This reduction was increased when tetrodotoxin was given with [D-Arg^1, D-Pro^2, D-Trp^{7,9}, Leu^{11}]SP. Increases in short-circuit current produced by electrical field stimulation were not reduced by [D-Arg^1, D-Pro^2, D-Trp^{7,9}, Leu^{11}]SP. It is concluded that SP is not a transmitter at the neuroepithelial junction in rat colonic mucosa.

Substance P (SP)-like material has been detected by radioimmunoassay in the intestinal mucosa of a number of species (Keast et al 1985a). Such fibres may innervate enterocytes, blood vessels or other nerves. In-vitro studies, using intact sheets of ileal mucosa, have shown SP to produce an increase in short-circuit current (s.c.c.). This effect was mediated by a combination of actions on secretomotor neurones and enterocytes (Keast et al 1985b) or histamine-releasing cells (Kuwahara & Cooke 1990).

There is conflicting evidence as to whether SP is a secretomotor neurotransmitter in the intestine. In rabbit ileal mucosa the s.c.c. response to nerve stimulation was unaltered by desensitization to SP (Hubel 1984), whereas in guinea-pig ileal mucosa, SP desensitization, SP antibodies (Perdue et al 1987) and SP antagonists (Keast et al 1985c; Kuwahara & Cooke 1990) reduced electrically-evoked increases in s.c.c.

The purpose of the present investigation was to determine whether SP might function as a secretory neurotransmitter at the neuroepithelial junction in rat colonic mucosa.

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Methods

Segments of large bowel were obtained from male Wistar rats, 180–250 g. The segment consisted of an approximately 10 cm length extending from proximal colon to distal rectum. The segments were opened longitudinally along the anti-mesenteric border and pinned mucosa downwards in Krebs-Henseleit solution. Muscle-stripped preparations consisting of mucosa, muscularis mucosa and submucosa were prepared by dissection under a binocular microscope. The striated part of the proximal colon was not used. Mucosal sheets were mounted in Ussing chambers (0·64 cm^2 window area). Five mL of Krebs solution bathed each side of the preparation, this was reduced to 3 mL each side when using the SP antagonist.

Electrical field stimulation (1 ms, 150 pulses at 1 or 10Hz) of mucosal nerves was achieved by passing monophasic pulses across the tissue using a Grass S88 stimulator connected to two aluminium foil electrodes. One foil was placed either side of the preparation to reduce short-circuiting through the bathing fluid (Hubel, personal communication). Some tissue was fixed in 10% phosphate buffered formalin after which haematoxylin- and eosin-stained sections were prepared for histological examination. Composition of the Krebs fluid was (mM): NaCl 118, glucose 11·5, $NaHCO_3$ 25, KCl 4·7, $MgSO_4$ 1·2, KH_2PO_4 1·2, $CaCl_2$ 2·5.

Transmucosal short circuit current was measured with a high impedance voltage clamp (DCV-1000, World Precision Instruments). Compensation was made for fluid resistance between the tips of the voltage electrodes. Both current-passing and voltage-detecting electrodes utilized a system of Ag/AgCl half cells which screw into short, large-diameter tubes filled with 4% agar in 3 M KCl. Short circuit current was continuously displayed on pen recorders. All electrical values quoted are calculated for an