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Interspecies Pharmacokinetic Scaling of Some Iodinated Organic Acids

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Abstract—We have investigated the possibility of interspecies scaling of relationships between the structure and total plasma clearance in a group of nine organic acids (iododerivatives of benzoic, phenylacetic and hippuric acids) in rabbits, rats and mice. The intercompound comparison established the dependence of total plasma clearance predominantly on the molecular structure in all the animals under study, but the dependence on drug lipophilicity was also meaningful. For interspecies scaling of total plasma clearance, the use of a biological clock with an effective renal plasma flow as the unit seemed most suitable and is probably connected with the principal role of the kidney in the elimination of the compounds under study.

An understanding of the relationships between the pharmacokinetics and physicochemical characteristics of drugs makes preconditions for a rational design of new drugs and for pharmacokinetic predictions (Hathway 1982; Seydel & Schaper 1982; Seydel 1984; Boxenbaum 1984; Mayer & Van de Waterbeemd 1985). A study of interspecies differences in drug pharmacokinetics would help to clarify the problems inherent in the extrapolation of results from animal species to man (Mordenti 1985a, 1986; Boxenbaum 1986). Smaller mammals usually eliminate drugs faster than larger ones so considering that they have similar anatomy, physiology and biochemistry (Mordenti 1985b), it seems reasonable to surmise that interspecies pharmacokinetic scaling can be carried out.

Two approaches to pharmacokinetic scale-up have been used, an allometric scaling and a physiological one (Mordenti 1986). In the allometric scaling, the pharmacokinetic parameters are scaled by body weight and the allometric exponent. The physiological approach introduces a weight-dependent time scale. This new 'physiological' time is measured by biological clocks and uses internal, physiological parameters such as heart beats or blood circulation velocities as units of measurement.

In previous publications (Lázníček et al 1985; Lázníček & Květina 1988), relationships between pharmacokinetics and chemical structure of some model acidic drugs were studied in some laboratory animals. The present study was designed to investigate the possibility of interspecies scaling of some structure-pharmacokinetic relationships with a view to drug elimination in a group of organic acids, namely iododerivatives of benzoic, phenylacetic and hippuric acids. The criterion for model drug selection was simple detection and structural uniformity of the group tested.

Materials and Methods

Materials

All compounds (position isomers of iodobenzoate, iodophenylacetate, and iodohippurate) were labelled with ^{125}I and were obtained from the Nuclear Research Centre, Řež, Czechoslovakia. Radiochemical purity was over 98%.

^{99m}Tc-DTPA (diethylenetriamminopentaacetic acid) was prepared by mixing 0·1 mL 20 mmol L^{-1} $SnCl_2$ in 0·12 mol L^{-1} HCl with 1 mL 40 mmol L^{-1} DTPA in saline (pH adjusted to 7 by 1 mol L^{-1} NaOH) and addition of 2·8 mL pertechnetate eluate (activity 100–200 MBq mL^{-1}) from a ^{99}Mo–^{99m}Tc generator (Rotop, GDR). The radiochemical purity of the complex was over 99%.

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 used for pharmacokinetic experiments. The animals were fasted 18–24 h before the experiment, but had free access to water.

Pharmacokinetic studies

The performance of the biological experiments have been described by Lázníček et al (1985) and Lázníček & Květina (1988). Briefly, the compounds were dosed intravenously at 0·1 mg kg^{-1} into the marginal ear vein of rabbits, 0·5 mg kg^{-1} into the femoral vein of rats, and 1 mg kg^{-1} into the tail vein of mice. At selected times after dosing, blood samples were collected (in rabbits from the marginal vein of the opposite ear; the rats and mice were exsanguinated under ether anaesthesia). Heparinized blood samples were immediately centrifuged at 3000 rev min^{-1} and plasma was tested for the presence of metabolites by TLC after extraction of compounds in acidic form into diethylether (Lázníček & Květina 1988). The model drug concentrations were determined after measurement of ^{125}I activity with a beta-gamma spectrometer NE 8312 (Nuclear Enterprises Ltd, Edinburgh) and comparison with the activity of standard samples. The pharmacokinetics of ^{99m}Tc-DTPA (standard for glomerular filtration rate measurement) was examined similarly after intravenous administration of ^{99m}Tc-DTPA solution. The plasma concentration-time data were analysed using non-linear least-square regression analysis by means of the Gauss-Newton or Marquart method (Yamaoka et al 1981) weighting of data according to 1/y. Plasma concentration

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curves could be described adequately by a biexponential equation

$$C = C_1 \cdot \exp(-\lambda_1 t) + C_2 \cdot \exp(-\lambda_z t), \quad (1)$$

where C is the plasma concentration, t is the time after dosing, λ_1 and λ_z are the rate constants characterizing the distribution and elimination phases, respectively, and C_1 and C_2 are the hypothetical intercepts with ordinate. Plasma concentration was determined for the period until its value decreased below 1% of the initial concentration. The criterion for the model selection was the best agreement of the experimental values with the theoretical plasma concentration-time courses.

Only the principal elimination parameter (total plasma clearance) is presented comparatively. The comprehensive pharmacokinetic parameters were published previously (Lázníček et al 1985; Lázníček & Květina 1988).

The total plasma clearance values (CL) were calculated using the equation

$$CL = \frac{D}{AUC}, \quad (2)$$

where D is the dose and AUC is the area under the plasma concentration-time curve. In rabbits, the total plasma clearance value was calculated for each animal and the mean values and standard deviations from 5 to 6 rabbits are presented. In rats and mice, the mean plasma concentrations of model compounds (mean of 5–6 animals) were used for calculation of total plasma clearance values.

Protein binding

Plasma protein binding was determined by equilibrium dialysis at 37°C (Lázníček & Senius 1986). The initial concentrations of compounds under study were 1 mg L^{-1}.

Partition coefficient octanol: water

Data were taken from Lázníček & Květina (1988).

Structural-pharmacokinetic relationships

Relationships between total plasma clearance and lipophilicity were investigated by linear regression analysis. Free-Wilson analysis was performed using the program according to Purcell et al (1973).

Results

The principal elimination parameter that is not dependent on the drug distribution volume or the pharmacokinetic model

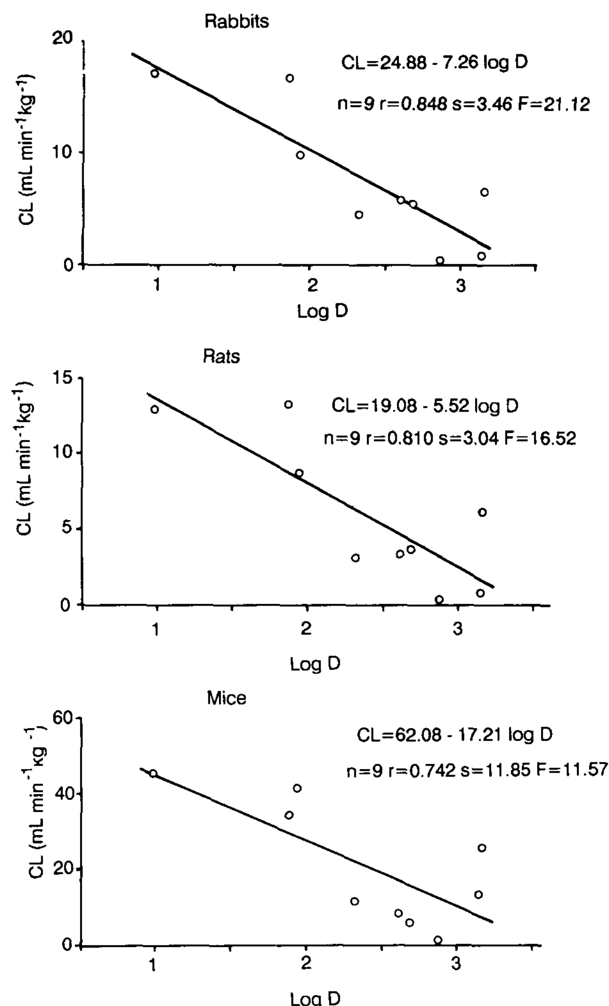

FIG. 1. Relationships between total plasma clearance (CL) and lipophilicity (log D) for the acidic compounds in rabbits, rats and mice.

Table 1. Total plasma clearance (CL), plasma protein binding (f_u = fraction unbound) and lipophilicity of compounds studied.

	Rabbits[a]		Rats[b]		Mice		
Compound	CL mL min^{-1} kg^{-1}	f_u	CL mL min^{-1} kg^{-1}	f_u	CL mL min^{-1} kg^{-1}	f_u	lipophilicity[b]
2-Iodobenzoate	5·82±2·31	0·145	3·36	0·421	7·94	0·654	2·621
3-Iodobenzoate	6·56±1·65	0·056	6·23	0·066	25·8	0·103	3·171
4-Iodobenzoate	0·76±0·22	0·037	0·751	0·061	13·3	0·097	3·164
2-Iodophenylacetate	4·45±1·26	0·060	3·07	0·275	11·5	0·660	2·327
3-Iodophenylacetate	5·44±0·96	0·027	3·72	0·074	5·89	0·304	2·694
4-Iodophenylacetate	0·13±0·02	0·027	0·241	0·123	0·319	0·151	2·882
2-Iodohippurate	17·0±4·1	0·467	12·9	0·512	45·6	0·804	0·973
3-Iodohippurate	16·8±4·4	0·061	13·3	0·124	34·3	0·254	1·883
4-Iodohippurate	9·65±2·98	0·055	8·58	0·098	41·2	0·309	1·940
Glomerular filtration rate (CL of ^{99m}TC-DTPA)	4·22±0·70		4·09 ±1·03		16·2 ±1·6		

[a] Partly cited from Lázníček et al (1985).
[b] Cited from Lázníček & Květina (1988).

selection is total plasma clearance. Its values were therefore employed for interdrug and interspecies scaling. Total plasma clearance values, free fractions of model drugs in plasma, and their lipophilicity together with glomerular filtration rate (total plasma clearance of ^{99m}Tc-DTPA) are summarized in Table 1.

All the compounds were eliminated either as parent compounds or as metabolites mainly (more than 90%) in urine. A comparison of their total plasma clearance values, corrected for plasma protein binding, to glomerular filtration rate suggests that their excretion was by active renal transport. For most of them, the plasma protein binding was not the elimination rate limiting step.

Quantitative evaluation of the total plasma clearance-lipophilicity relationships for the compounds are shown in Fig. 1. Allowing that the correlation coefficients of these relationships are relatively satisfactory for biological experiments and are statistically sound, significant deviations of derivatives substituted with iodine in the para position were found. This led to an attempt to analyse the values of total plasma clearance by Free-Wilson analysis seeking to answer the question of whether total plasma clearance value can be separated into a sum of contributions of the effects of the individual molecular fragments; the first characterizing the respective acid and the second characterizing the position of substitution of the benzene ring with iodine (Fig. 2). The results of this analysis are shown in Table 2. For these compounds, the value of total plasma clearance can be considered additive in the three species; the absolute values of contributions of molecular fragments, however, are different in individual species, even when corrected for body weight.

As to the molecular fragments characterizing the individual acids, the total plasma clearance value was decreased in the three species by the presence of molecular fragments characterizing benzoic and phenylacetic acids, but it was significantly increased by the presence of the molecular fragment characterizing hippuric acid. This is in agreement with the substantially lower lipophilicity of hippuric acid derivatives compared with those of benzoic and phenylacetic acids.

For those molecular fragments characterizing the substitution of the aromatic ring with iodine, a significant decrease in the total plasma clearance value was shown by substitution in the para position in the three species. The decrease cannot be explained by the difference in the lipophilicity of the individual position isomers.

For interspecies scaling, the suitability of different approaches was tested to find whether the calculation of total plasma clearance to the body surface, or application of an allometric equation, or an employment of biological clocks with different time units (heart beats, blood circulation velocities, glomerular filtration rate, effective renal plasma flow etc.) gave the best results. These were obtained for the biological clock with an effective renal plasma flow as the unit parameter (Table 3). The effective renal plasma flow was expressed in terms of total plasma clearance of 2-iodohippurate.

The values of parameters of relationships between total plasma clearance and lipophilicity recalculated as above are summarized in Table 4.

Table 2. Results of Free-Wilson analysis of total plasma clearance (calculated for body weight, mL min^{-1} kg^{-1}).

Parameter	Rabbits	Rats	Mice
Mean value	7·40	5·57	20·66
Contributions of molecular fragments:			
—COO^-	−3·02	−2·13	−4·96
—CH_2COO^-	−4·06	−3·23	−14·75
—$CONHCH_2COO^-$	7·08	5·36	19·71
2-I-	1·69	0·88	1·03
3-I-	2·20	1·51	1·35
4-I-	−3·89	−2·39	−2·37
Coefficient of multiple correlation	0·996	0·984	0·936
Amount of explained variance, %	99·2	96·8	87·7

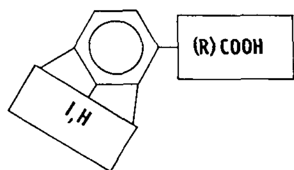

FIG. 2. Division of model drug structure into molecular fragments.

Table 3. Free-Wilson analysis of total plasma clearance calculated to effective renal plasma flow.

Parameter	Rabbits	Rats	Mice
Mean value	0·44	0·43	0·45
Contributions of molecular fragments:			
—COO^-	−0·18	−0·17	−0·11
—CH_2COO^-	−0·24	−0·25	−0·32
—$CONHCH_2COO^-$	0·42	0·42	0·43
2-I-	0·10	0·07	0·02
3-I-	0·13	0·12	0·03
4-I-	−0·23	−0·19	−0·05

Table 4. Parameters of relationships between total plasma clearance calculated to effective renal plasma flow (CL/ERPF) and lipophilicity (log D).

	CL/ERPF = a + b log D	
Species	a	b
Rabbits	1·463	−0·427
Rats	1·479	−0·428
Mice	1·361	−0·378

Discussion

The value of the total plasma clearance of the compounds is dependent not only on lipophilicity (Fig. 1) but also on the molecular structure (Table 2). Total plasma clearance is the sum of the clearances by each eliminating organ, i.e. its value is related partly to renal excretion (CL_R) and partly to the elimination by other organs (CL_{NR}). Most of the activity of the compounds studied was excreted via the urine; the kidney is therefore the most important organ for their elimination. Even if they were eliminated partly as conjugates, the metabolism of structurally similar drugs (benzoic acid

derivatives) was also mainly in the kidneys (Wan & Riegelman 1972a, b; Bekersky et al 1980).

Renal clearance is generally determined by the value of effective renal plasma flow (ERPF) and by the probability of the elimination of the drug during the passage through the kidneys, P:

$$CL_R = ERPF \cdot P \quad (3)$$

The excretion can take place by different mechanisms and the total probability of elimination P involves the combination of the probabilities of the individual processes:

$$P = [p_1 + (1 - p_1)p_2] \cdot [1 - p_3 (1 - p_4)] \quad (4)$$

where p_1 is the probability of elimination by glomerular filtration (plasma protein binding dependent), p_2 is the probability of elimination by tubular secretion (structure dependent), p_3 is the probability of tubular reabsorption (lipophilicity and/or structure dependent), p_4 is the probability of biotransformation in the kidney (structure dependent).

Extrarenal clearance includes, above all, biotransformation and elimination in the liver and also in other organs, and these processes are dependent primarily on the structure and lipophilicity of drugs.

The individual processes of elimination affect the rate of elimination for each compound to a different degree. For this reason, interdrug comparison of total plasma clearance establishes the dependence of its value mainly on the molecular structure, even though the dependence on lipophilicity is also meaningful. The unexplained deviation of the 4-iododerivatives is probably a position-dependent steric effect.

Qualitatively similar results obtained for the intercompound comparison in the three species suggests a similar manner of elimination of the compounds in those species.

For interspecies scaling, the principal role of the kidney in the elimination of the compounds suggested that renal functions could serve as a suitable unit for comparison. In agreement with equation 3, the biological clock with an effective renal plasma flow as the unit would seem to be the most suitable way of interspecies scaling of the model acidic compounds studied (Tables 3, 4). This unit is not universal. Different types of drugs eliminated by another route could be evaluated by applying such units as heart beat, breath cycles, or hepatic function. For the compounds studied, the use of renal function as the unit for comparison also results from the perfusion rate-limited model (D'Souza & Boxenbaum 1988) in which the kidney is assumed to be the elimination rate-limited compartment.

These results indicate the possibility of both interdrug and interspecies scaling of total plasma clearance in a group of organic acids. For interspecies scaling, a biological clock with an effective renal plasma flow as the unit follows from their way of elimination. An exact explanation of intercompound results would require a quantitative analysis of the relative proportion of the individual processes involved in the elimination of the compounds.

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