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Renal Handling of Iodobenzoates in Rats

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Abstract

Renal elimination pathways of three positional isomers of iodobenzoic acid (2-iodobenzoate, 3-iodobenzoate and 4-iodobenzoate radiolabelled with ^{125}I) were compared using the perfused rat kidney in-situ.

All agents were eliminated both in a parent form (involving all renal elimination mechanisms i.e. glomerular filtration, tubular secretion, and tubular reabsorption) and also metabolized to a large extent in the kidney. After 3-iodobenzoate and 4-iodobenzoate administration, the major fractions of radioactivity found in urine were in the form of their metabolites, whereas 2-iodobenzoate was eliminated into urine mostly as the parent compound. Proportions of the individual metabolites in the urine of the perfused rat kidney were similar to those in intact rats for all agents.

The results suggest that the kidney is the major organ for both the excretion and metabolism of iodobenzoates in rats. The principal renal metabolic reaction for all compounds under study was conjugation with glycine to produce the corresponding hippuric acid derivatives.

Studies of structure–pharmacokinetic relationships can be of considerable importance in new drug development because it may simplify and accelerate drug selection and testing processes (Hathway 1982; D'Souza & Boxenbaum 1988). In interdrug comparisons, the elimination parameters, clearance and elimination half-life of structurally similar drugs can be reasonably well predicted from their physicochemical characteristics (Seydel & Schaper 1982; Herman & Veng-Pedersen 1994), but such relationships are mostly successful for only those drugs whose elimination pathways are similar.

A more precise approach might be based on an analysis of contributions of different excretion and metabolic processes to the overall elimination of each drug, with consequent correlations of these individual mechanisms within the drug group. This approach is especially important in quantitative structure–pharmacokinetic studies, when the total rate of elimination is related to physicochemical and structural characteristics (van Rossum et al 1989; Boxenbaum 1992).

It has been shown in previous studies that some acidic drugs, for example salicylic acid, are metabolized primarily in the kidney in rats (Bekersky et al 1980; Laznicek & Laznickova 1994) and rabbits (Fukasawa et al 1982). In this study, the excretion and biotransformation mechanisms of position isomers of iodobenzoic acid (radiolabelled with ^{125}I) were investigated in the perfused kidney of rats to determine the effect of the structure on individual elimination pathways. Radiolabelling makes a simple detection of both the parent compounds and all subsequent metabolites in biological fluids possible.

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Materials and Methods

Chemicals

2-Iodobenzoate, 3-iodobenzoate, 4-iodobenzoate, and 2-iodohippurate (a marker of the effective renal plasma flow measurement) radiolabelled with ^{125}I were obtained from the Nuclear Reserch Centre (Rez, Czech Republic). The specific activity was $4-12\,GBq\,g^{-1}$ and radiochemical purity was over 98%. ^{99m}Tc-Diethylenetriaminepentaacetic acid (DTPA; prepared from a kit from the Nuclear Research Centre, Rez) was used as a marker of the glomerular filtration rate measurement. All other non-labelled chemicals used were of the finest grade available.

Animals

Male Wistar rats, 220–280 g, were used for the experiments. The animals were fasted overnight before the experiment but had free access to water.

All animal experiments were approved by the Faculty of Pharmacy Ethics Committee.

Rat kidney perfusion experiments

Recirculating rat kidney in-situ perfusions were carried out as outlined in a previous study (Laznicek & Laznickova 1994). Animals were anaesthetized with pentobarbital (27 mg kg^{-1}, i.p.), and the renal artery and the ureter were cannulated with polyethylene tubing. The kidney perfusate consisted of 5% (w/v) bovine serum albumin in a Krebs–Henseleit buffer solution (pH 7·4) equilibrated with 95% O_2 and 5% CO_2 at 37°C. The additional substrates used were glucose (1 g L^{-1}) and the L-amino acids: methionine (0·5 mM), alanine (2 mM), serine (2 mM), arginine (1 mM), proline (2 mM), isoleucine (1 mM), aspartic acid (3 mM), and glycine (2 mM). After a 20-min equilibration period, the perfusion pressure was maintained at 90 ± 10 mmHg and radiolabelled iodobenzoate (0·1 mg) was introduced to a 50-mL reservoir solution. Urine was collected continually during four 20-min periods. Input perfusate was sampled at the midpoint of each urine collection. In separate experiments, the glomerular filtration rate and the effective renal plasma flow values were determined in the same manner after administration of ^{99m}Tc-DTPA and 2-iodohippurate as the references.

Elimination studies

The agents under study were administered to rats intravenously via the tail vein at a dose of 0·1 mg (0·2 mL) and the animals were placed singly in glass metabolic cages. Two hours post-injection, the rats were forced to empty their bladders by handling (immobilization by holding the animal's shoulders) and the urine was collected.

Determination of metabolites in urine

Thin-layer chromatography on silica-gel was used for the separation of the metabolites of the compounds (glycine and glucuronic acid conjugates). The mobile phase was benzene : acetic acid : water at various ratios (from 65 : 25 : 1 to 80 : 25 : 1) depending on the lipophilicity of the compound (Laznicek & Kvetina 1988).

Protein binding determination

Binding of model drugs to proteins in the perfusate was determined by equilibrium dialysis at 37°C (Laznicek & Senius 1986). The initial concentration of all agents was 1 mg L^{-1}.

Distribution coefficient between octanol and aqueous phase

Lipophilicity of the compounds under study was characterized by the distribution ratio between octanol and water, determined by direct measurement of the distribution of the compounds in the mixture of 1-octanol and phosphate buffer, pH 7·40.

Calculation of results

The renal clearance value of 2-iodohippurate was used as the measure of the rate of the effective renal plasma flow (ERPF) and the renal clearance value of ^{99m}Tc-DTPA was taken to represent glomerular filtration rate (GFR). The elimination rate of a drug by glomerular filtration, CL_{GF}, was calculated according to the following equation:

$$CL_{GF} = GFR \cdot f_u \quad (1)$$

where f_u represents the free drug fraction in perfusate.

Net secretion clearance, CL_{NS} (the difference between renal tubular secretion and renal tubular reabsorption) was calculated as:

$$CL_{NS} = CL_T - CL_{GF} \quad (2)$$

where CL_T represents total renal clearance of the drug.

Results

Values of the functional characteristics of the perfused rat kidney are presented in Table 1. The relative lipophilicity of the compounds under study, characterized by the distribution coefficient between octanol and aqueous phase at pH 7·40, was significantly lower (by a factor of ten) for 2-iodobenzoate compared with the other compounds. The difference between the lipophilicities of 3-iodobenzoate and 4-iodobenzoate was not statistically significant (Table 2).

Table 1. Functional characteristics of the perfused rat kidney.

Glomerular filtration rate	$0·045 \pm 0·017$
Effective renal plasma flow	$0·209 \pm 0·053$
Urine flow	$0·072 \pm 0·028$

Each value (mL min^{-1}) represents the mean ± s.d. of four independent experiments.

Table 2. The distribution between octanol and aqueous phase at pH 7·40.

Compound	Distribution coefficient
2-Iodobenzoate	0·054 ± 0·006
3-Iodobenzoate	0·563 ± 0·112
4-Iodobenzoate	0·583 ± 0·162

Values are means ± s.d. of four experiments.

The elimination parameters of iodobenzoates in the perfused rat kidney are shown in Table 3. The values of renal clearance of all the agents were lower than the glomerular filtration rate. Due to their high protein binding in the perfusate (Table 3) only a fraction of these compounds was eliminated by glomerular filtration and the remainder was secreted by the tubules. The values of net secretion, however, represent the difference between tubular secretion and tubular reabsorption. The free (non-protein bound) fraction in the perfusate was significantly higher for 2-iodobenzoate when compared with the other compounds, which is in agreement with the differences in their lipophilicity.

Relative proportions of parent compounds and their metabolites (conjugates with glycine and glucuronic acid) in urine are presented in Table 4. In in-vivo experiments, more then 85% of administered activity was eliminated into urine during the first 2 h after dosing. 2-Iodobenzoate was eliminated into urine mostly as the parent drug, the other isomers were eliminated mostly in the form of conjugates with glycine. The overall renal disposition of the agents is summarized in Figure 1.

Discussion

A complexity of factors determines the disposition of a drug. The blood clearance of a drug is influenced by the volume of distribution, degree of

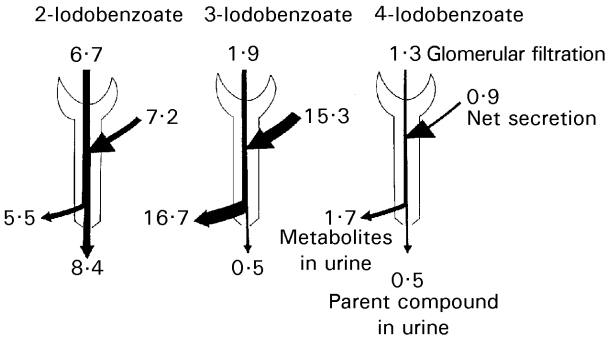

Figure 1. Schematic presentation of the renal handling of iodobenzoates in rats. The numbers represent the contribution of each elimination mechanism to the compound disposition in the kidney. The value of effective renal plasma flow equals 100.

Table 3. Elimination parameters of [^{125}I]iodobenzoates in the perfused rat kidney (total eliminated activity of ^{125}I calculated with respect to the parent compound).

Compound	CL_T (mL min^{-1})	CL_{GF} (mL min^{-1})	CL_{NS} (mL min^{-1})	Free fraction in perfusate
2-Iodobenzoate	0·029 ± 0·009	0·014 ± 0·007	0·015 ± 0·003	0·32 ± 0·07
3-Iodobenzoate	0·036 ± 0·011	0·004 ± 0·002	0·032 ± 0·009	0·09 ± 0·02
4-Iodobenzoate	0·0045 ± 0·0020	0·0027 ± 0·0011	0·0018 ± 0·0008	0·06 ± 0·02

CL_T represents the total renal clearance value, CL_{GF} the value of clearance by glomerular filtration and CL_{NS} the net secretion clearance value (the difference between tubular secretion and tubular reabsorption). Each value represents the mean ± s.d. of four experiments.

Table 4. Relative proportion (%) of the parent drug and individual metabolites in the urine of intact rats and in the urine of the perfused rat kidney.

Compound	Urine of intact rats			Urine of perfused rat kidney		
	Parent compound	Glycine conjugate	Glucuronide conjugate	Parent compound	Glycine conjugate	Glucuronide conjugate
2-Iodobenzoate	48·8 ± 11·2	27·9 ± 6·3	23·3 ± 7·7	60·2 ± 9·9	26·1 ± 8·3	13·7 ± 5·2
3-Iodobenzoate	1·5 ± 1·1	90·6 ± 6·8	7·9 ± 6·7	2·8 ± 1·1	91·1 ± 4·0	6·1 ± 3·6
4-Iodobenzoate	3·9 ± 1·8	73·2 ± 8·2	22·9 ± 9·2	23·9 ± 10·4	63·4 ± 10·1	12·7 ± 6·3

Each value represents the mean ± s.d. of four experiments.

binding to plasma proteins or tissues, the extent of biotransformation, and the effectiveness of both renal and hepatobiliary excretion. In previous reports, the effect of the structure of a group of benzoic acid derivatives on pharmacokinetic parameters in different species was tested, and the principal role of the kidney in the elimination of the compounds was determined (Laznicek & Kvetina 1988; Laznicek 1992). The results presented in Table 3 indicated that all three iodobenzoic acid derivatives were secreted by renal tubular systems (as their total renal clearance markedly exceeded the glomerular filtration clearance) and consequently partly reabsorbed in the kidney as their renal clearance was significantly reduced by probenecid (unpublished results). It has been suggested that substituted benzoates exhibit affinity to the *p*-aminohippurate transport system, and hydrophobic and electronic parameters are the main determinants of affinity of these compounds for active secretion by the mammalian kidney (Russel et al 1991). Also, salicylate (*o*-hydroxybenzoate) is actively secreted and subsequently reabsorbed by carrier-mediated mechanisms in renal tubules (Ferrier et al 1983).

In Table 4, relative proportions of the parent drug and individual metabolites found in the urine of intact rats and that of the perfused rat kidney are compared. It is clear that in the kidney significant biotransformations of iodobenzoates took place. When the ratios of iodohippurate-to-parent drug in the urine of intact rats and the perfused kidney were compared, similar values were obtained for all three iodobenzoates. The ratios of iodobenzoylglucuronide-to-benzoate were substantially lower in the urine of the perfused kidney than that of the intact rats. That means that conjugates with glycine (iodohippurates) were formed mainly in the kidney. In glucuronide formation, both the liver and kidney were involved. It is well known that the liver has high blood perfusion rates and it is a particularly rich site for metabolizing enzymes; thus it is generally believed that drug metabolism is primarily a liver function and the contribution of other extrahepatic systems (kidney, lung, gastrointestinal tissues and others) to total drug metabolism is usually considered to be minor. At variance with this general principle, the kidney has been previously indicated as a major site of salicylate metabolism, namely for conjugation with glycine (Wan & Riegelman 1972; Bekersky et al 1980; Laznicek & Laznickova 1994). Results obtained in this report for iodobenzoic acid isomers are in agreement with those findings. Even if the liver is beyond doubt the most important drug-metabolizing organ, the substrate availability is also a determinant of the rate of biotransformation reactions. It has been found that the concentration of iodobenzoates in the kidney is tenfold that in the liver (Laznicek & Kvetina 1988); this is probably the reason for the dominant role of the kidney in the metabolism of iodobenzoates.

It has been shown that renal clearance of the parent drug in a group of substituted benzoates is related to its lipophilicity (Laznicek 1992). However, no significant relationships between total plasma clearance and lipophilicity for this group of agents were found (Laznicek & Kvetina 1988). Results obtained in this study could assist in the explanation of these findings. The parent drug which is primarily eliminated in the kidney (both by glomerular filtration and tubular secretion) is partly metabolized and partly reabsorbed in the renal tubules. The rate of glomerular filtration of a drug is protein-binding dependent and also lipophilicity-dependent. Its elimination by tubular secretion as well as its rate of biotransformation, however, are structure-dependent. The tubular reabsorption of the parent drug may also be lipophilicity-dependent (very hydrophilic conjugates with glycine and glucuronic acid may not be reabsorbed at all). As all these processes are generally involved in the total renal elimination rate of drugs and participate in the elimination of the agents under study via kidney, no simple relationship between elimination characteristics and physicochemical parameters may be achieved.

In conclusion, all agents were excreted (involving glomerular filtration, tubular secretion and reabsorption) and metabolized to a large extent in the kidney. Whereas the conjugation with glycine takes place primarily in the kidney, the kidney is involved only in part in the overall conjugation with glucuronic acid. Even if plasma protein binding and renal excretion of the parent drug are lipophilicity-dependent, no relationship between the total rate of elimination and physicochemical characteristics can be expected since different pathways participate to different degrees in the disposition of the compounds studied.

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References

Bekersky, I., Fishman, L., Kaplan, S. A., Colburn, W. A. (1980) Renal clearance of salicylic acid and salicyluric acid in the rat and the isolated perfused rat kidney. J. Pharmacol. Exp. Ther. 212: 309–314

Boxenbaum, H. (1992) Pharmacokinetics: philosophy of modeling. Drug Metab. Rev. 24: 89–120

D'Souza, R. W., Boxenbaum, H. (1988) Physiological pharmacokinetic models: some aspects of theory, practice and potential. Toxicol. Industrial Health 4: 151–171

Ferrier, B., Martin, M., Roch-Ramel, F. (1983) Effects of p-aminohippurate and pyrazinoate on the renal excretion of salicylate in the rat: a micropuncture study. J. Pharmacol. Exp. Ther. 224: 451–458

Fukasawa, K., Nishida, M., Ichida, R., Kato, Y. (1982) Effects of phenylbutazone analogues on the plasma concentration and renal excretion of salicylate and its metabolites in rabbits. Chem. Pharm. Bull. 30: 249–256

Hathway, D. E. (1982) Structure–activity considerations: a synthesis of ideas. Chem.–Biol. Interactions 42: 1–26

Herman, R. A., Veng-Pedersen, P. (1994) Quantitative structure–pharmacokinetic relationships for systemic drug distribution kinetics not confined to a congeneric series. J. Pharm. Sci. 83: 423–428

Laznicek, M. (1992) Renal clearance–lipophilicity relationships of some organic acids in rabbits, rats and mice. J. Pharm. Pharmacol. 44: 255–257

Laznicek, M., Kvetina, J. (1988) The effect of molecular structure on the distribution and elimination of some organic acids in rats. Quant. Struct. Act. Relat. 7: 234–239

Laznicek, M., Laznickova, A. (1994) Kidney and liver contributions to salicylate metabolism in rats. Eur. J. Drug Metab. Pharmacokinet. 19: 21–26

Laznicek, M., Senius, K. E. O. (1986) Protein binding of tolfenamic acid in the plasma from patients with renal and hepatic disease. Eur. J. Clin. Pharmacol. 30: 591–596

Russel, F. G. M., Heijn, M., de Laet, R. C., an Ginneken, C. A. M. (1991) Effect of substituted benzoates on p-aminohippurate transport in dog membrane vesicles. Naunyn Schmiedebergs Arch. Pharmacol. 343: 102–107

Seydel, J. K., Schaper, K.-J. (1982) Quantitative structure–pharmacokinetic relationships and drug design. Pharmacol. Ther. 15: 131–182

van Rossum, J. .M., de Bie, J. E. G. M., van Lingen, G., Teeuwen, H. W. A. (1989) Perspectives in pharmacokinetics: pharmacokinetics from a dynamical point of view. J. Pharmacokin. Biopharm. 17: 365–391

Wan, S. H., Riegelman, S. (1972) Renal contribution to overall metabolism of drugs I: conversion of benzoic acid to hippuric acid. J. Pharm. Sci. 61: 1278–1292