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Gastrointestinal and hepatic first-pass effects of salicylamide in rats

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The orally administered drug that exhibits reduced systemic availability even after being completely absorbed from the gastrointestinal tract is subject to first-pass metabolism. To identify where this occurs a drug is either directly administered into the hepatoportal vein, whereby the extraction and/or metabolism component in the gut is bypassed (Harris & Riegelman 1969; Iwamoto & Klaassen 1977a, b; Back et al 1978; Iwamoto et al 1982), or a portal systemic shunt that bypasses the liver is created (Gugler et al 1975).

Salicylamide has been recently excluded from several National Pharmacopoeias because of its lower efficacy by mouth compared with aspirin. Salicylamide was reported to have the first-pass effect during intestinal absorption in rabbits (Barr & Riegelman 1970a, b) and to have the effect both in the gut and in the liver of dogs (Gugler et al 1975). Shibazaki et al (1981) have also reported the relative importance of the intestine and the liver in the first-pass metabolism of salicylamide in rabbits and rats the intestinal segments of which were perfused in-situ. However, neither the intestinal or hepatic effects have been evaluated either completely or directly in intact animals.

We have examined the effects of routes of administration upon the pharmacokinetics of salicylamide in rats, to estimate the relative importance of the gut and the liver in producing the first-pass effect, to compare this effect in rats with those reported in dogs (Gugler et al 1975) and in rabbits (Shibazaki et al 1981) and to compare the first-pass metabolism of salicylamide with that of aspirin in rats (Iwamoto et al 1982).

Methods

Two groups of unanaesthetized male Wistar rats (204–240 g, n = 6) which were chronically cannulated (for 1 to 2 days) in the right external jugular vein in the same manner as described previously (Iwamoto et al 1982) were given 30 mg kg^{-1} of salicylamide (Tokyo Kasei Chemicals Co., Tokyo) via the cannula in 10 s or orally by gastric intubation after an overnight fast for 15 h. Both urine and faeces were collected at intervals. Each sample was treated as described by Iwamoto et al (1982).

Two other groups of chronically cannulated and unanaesthetized rats (226–248 g, n = 6) were given 30 mg kg^{-1} intravenously or orally as described above. Sequential blood samples (about 0·25 ml) were withdrawn from the jugular vein. The blood was heparinized and then centrifuged for plasma.

Two groups of the cannulated rats (232–252 g, n = 6) were prepared in the same way as reported by Iwamoto et al (1982). The drug solution was infused into the femoral (systemic) vein or ileocolic (portal) vein at 3 mg kg^{-1} min^{-1} for 10 min. Both blood and bile samples were collected at intervals from the femoral artery and from the bile duct, respectively. Blood samples were treated as described above.

The analytical procedure for salicylamide in plasma was a slightly modified method of Barr & Riegelman (1970a). An aliquot (0·1 ml) of the plasma was immediately mixed with 2 ml of McIlvain buffer solution (pH 4·3) and then extracted with 5 ml mixture of dichloroethane and cyclohexane (65:35, v/v). The organic layer (3 or 4 ml) was then shaken with 4 ml of 0·2 M NaOH solution for 20 min. The separated alkaline layer (3 ml) was analysed spectrofluorometrically at excitation wavelength 322 nm and emission wavelength 418 nm. Appropriately diluted urine (0·1 ml) was similarly treated. Total salicylamide (salicylamide and its conjugates) in urine or in bile was analysed as above after the hydrolysis of the sample with β-glucuronidase/arylsulfatase (Boehringers Mannheim, GmbH, West Germany) at 37 °C for 24 h. Ground faecal samples were similarly treated with the enzyme solution.

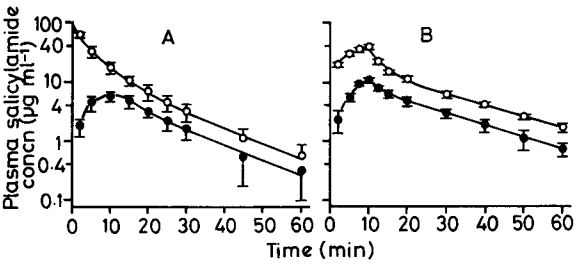

FIG. 1. Plasma concentration-time curves for unchanged salicylamide in rats (A) receiving salicylamide (30 mg kg^{-1}) by bolus i.v. (○, n = 6) and oral (●, n = 6) administration, (B) receiving salicylamide by constant rate infusion (3 mg kg^{-1} min^{-1}) for 10 min into femoral (systemic) vein (○, n = 6) and ileocolic (portal) vein (●, n = 6). Each point with vertical bar represents the mean data point with standard deviation.

* Correspondence.

Table 1. Pharmacokinetic parameters for salicylamide after intravenous, intraportal or oral administration (30 mg kg^{-1}) to rats.

Parameter	i.v. Bolus (n = 9)[a]	Constant rate infusion Systemic (n = 7)	Intraportal (n = 7)	Oral (n = 6)
A (μg ml^{-1})	61·0 (8·36)[c]	n.e.[b]	n.e.	n.e.
B (μg ml^{-1})	17·4 (3.94)	n.e.	n.e.	n.e.
α(min^{-1})	0·203 (0·036)	0·307 (0·130)	0·430 (0·277)	0·111 (0·038)
β (min^{-1})	0·0575 (0·0048)	0·0440 (0·0029)	0·0472 (0·0013)	0·0539 (0·0065)
$V'_{dextrap}$[d] ($l\ kg^{-1}$)	1·72 (0·454)	n.e.	n.e.	n.e.
$t_{1/2\,\beta}$ (min)	12·1 (2·55)	15·8 (1·17)	14·7 (0·98)	12·9 (3·06)
AUC[e] (μg min ml^{-1})	604 (88·1)	n.e.	n.e.	n.e.
AUC_{TR}[f] (μg min ml^{-1})	606 (51·1)	633 (36·8)	212 (22·3)	137 (40·5)

[a] Number of mean data points used for the least-squares regression analysis (all data points except those during the constant rate infusion and those to the peak after the oral administration).
[b] Not estimated.
[c] Standard deviation.
[d] Apparent volume of distribution estimated using B value.
[e] AUC value estimated by the equation, $A/\alpha + B/\beta$.
[f] AUC value calculated by the trapezoidal rule.

Results and discussion

Unchanged salicylamide excreted cumulatively in the urine by 48 h after i.v. (n = 6) and oral (n = 6) administration was only 1·8 and 1·5% of the dose, respectively. In contrast, approximately 95% of the dose was recovered as the conjugates in the urine over 48 h after i.v. and oral administration. Total faecal excretion for 48 h after i.v. and oral dosing was only about 3·1 and 4·6% of the dose, which was almost coincident with the cumulative biliary excretion 60 min after systemic (3·8%) and intraportal (4·7%) infusion, respectively. Since there was no significant difference between cumulative fractions of the dose excreted as salicylamide and the conjugates in the urine over 48 h after i.v. (97·1 ± 5·1%) and oral (95·5 ± 5·7%) administration ($P > 0·1$), the gastrointestinal absorption of the drug was considered to be essentially complete in rats.

The plasma concentration of the drug was much lower for the first 20 min ($P < 0·001$ to 0·01) and tended to be lower thereafter in rats that had received the drug orally rather than intravenously (Fig. 1A). The peak of the drug in plasma appeared 10 min after oral dosing. When the drug was given by a constant rate infusion to rats, the plasma concentration was much lower ($P < 0·001$) over 60 min after intraportal than systemic administration (Fig. 1B). The calculated pharmacokinetic parameters for the unchanged drug are summarized in Table 1. There was no significant difference between plasma elimination half-life ($t_{1/2\beta}$) values after any paired routes of administration ($P > 0·1$), ranging from 12·1 min after a bolus i.v. administration to 15·8 min after a constant rate infusion into the systemic vein. Furthermore, there was no significant difference between the AUC_{TR} (trapezoidal AUC) values for i.v. administration with bolus ($AUC_{i.v.}$) and with constant rate infusion ($P > 0·1$), whereas oral (AUC_o, $P < 0·001$) and intraportal (AUC_p, $P < 0·001$) administration of salicylamide produced much smaller AUC values than that obtained after i.v. administration.

Table 2. Fractions of dose being subject to gastrointestinal (f_g) and hepatic (f_h) extraction and/or metabolism of salicylamide in rats.

Fraction	In rats	(In dogs)[a]
f_g[b]	0·354	(0·562)
f_h[b]	0·650	(0·575)
$1-(1-f_g)(1-f_h)$	0·774	(0·782)

[a] Calculated from the data of Gugler et al (1975).
[b] Estimated by the equations, $f_g = 1 - \frac{AUC_o}{AUC_p}$ and $f_h = 1 - \frac{AUC_p}{AUC_{i.v.}}$
[c] Estimated by the equation, $1-(1-f_g)(1-f_h) = 1 - \frac{AUC_o}{AUC_{i.v.}}$.

Drugs that are subject to first-pass effect are characterized by extensive extraction and/or metabolism by the gastrointestinal wall or the liver during their first-passage through these organs. Table 2 summarizes the estimated values for the fraction of gastrointestinal (f_g) and hepatic (f_h) extraction and/or metabolism to the dose of salicylamide given to rats along with those in dogs which we calculated for the reported data (Gugler et al 1975) according to the equations in Table 2 (Harris & Riegelman 1969). Though f_g was almost equal to f_h in dogs, in rats f_h was much larger than f_g, suggesting that the liver plays a greater role in producing the first-pass effect of orally administered salicylamide than the gut. The extent of the overall first-pass effect as indicated by the value of $1-(1-f_g)(1-f_h)$ was almost the same in both species. Shibazaki et al (1981) have recently measured in-situ the overall first-pass effect of salicylamide as a fraction of the dose in rabbits and rats. They obtained an almost constant value close to 0·95 as the overall first-pass effect with both doses at 100 and 300 mg per rabbit. In contrast, approximate values of 0·69 and 0·56 were obtained when 10 and 30 mg salicylamide were given per rat, respectively. Interestingly, the dose 30 mg kg^{-1} in the present study, which is approximately 10 to 30% lower than the low dose (10 mg per head) of Shibazaki et al (1981), produced a larger value (0·774) as a fraction of overall first-pass effect than the values obtained by those authors. These results could be explained by the dose dependency of salicylamide metabolism (conjugation) that has been demonstrated in man (Levy & Matsuzawa 1967),

rabbits and rats (Shibazaki et al 1981).

Orally administered aspirin has been reported to be subject to first-pass metabolism both in the gut and liver of rats (Iwamoto et al 1982). In contrast to the results with aspirin, the hepatic effect was relatively greater than the gastrointestinal one in producing the first-pass effect of salicylamide in rats. Furthermore, it was demonstrated that the overall first-pass effect of salicylamide was more extensive than that of aspirin in both species, rats and dogs, even when considered on the same dose level basis.

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Influence of sodium ion-pair formation on transport kinetics of warfarin through octanol-impregnated membranes

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It is generally accepted that drugs in their ionized form cannot readily permeate through biological membranes, at least by means of a passive transport mechanism. This may be attributed to the fact that first, the solubility of ions in hydrophobic medium is much lower than the solubility of neutral uncharged analogues and second, an electromotive gradient opposing the diffusion gradient will be generated by the net transport of charged particles (at least in a closed system and in the absence of compensating mechanisms).

From recent research in our laboratory (Van Der Giesen & Janssen 1982) it appeared that the apparent partition coefficient of the anticoagulant warfarin at pH10·0 (warfarin having a pK of 5·0 being completely dissociated under these conditions), was increased by the presence of Na^+. A linear relationship was found between the logarithm of the apparent partition coefficient and the logarithm of the Na^+ concentration. This observation could be explained by assuming that ion pairs are formed between the anion of warfarin and Na^+. It also appeared that the lipophilicity of such an ion pair is comparable with the lipophilicity of the undissociated acid. In the present investigation we show that the presence of Na^+ stimulated the diffusion of warfarin at pH 11 through octanol-impregnated membranes.

* Correspondence.

Materials and methods

Sodium warfarin was purchased from ACF Chemiefarma (ACF, Maarssen, The Netherlands) and used as supplied. Octan-1-ol was purchased from Merck (Merck, Darmstadt, GFR) and purified by subsequent washings with an equal volume of 4 M NaOH, 2 M H_2SO_4 and 1 M Na_2CO_3. Finally the octanol was washed repeatedly with water until the water phase reacted neutral.

Membranes used in the transport studies were prepared by soaking Millipore ultrafiltration filters, type VSWP, pore size 0·025 μm, in water saturated octanol. Shortly before use the excess octanol was removed from the membranes by drying between filter paper.

To measure warfarin transport through these octanol-impregnated filters a two compartment cylinder assembly according to Kroon & Janssen (1982) was used. One compartment (A) was filled with 15 ml solution containing warfarin, NaOH and NaCl. The starting concentration warfarin was 1 mM and the pH was 11·0. The NaCl concentration was varied such that the total Na^+ concentration was between 0·01 and 5 M. The second compartment (B) was filled with 15 ml of the same solution as in A but without warfarin. The solutions in both compartments were presaturated with octanol. A spectrophotometric cell was placed on top of compartment B. Stirring was achieved by putting the whole on a roller-mixer such that the cylinder assembly