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Gastrointestinal and hepatic first-pass metabolism of aspirin in rats

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The first-pass effect of aspirin was measured in male Wistar rats by comparing the plasma concentration after intravenous, oral or intraportal administration (10 mg kg^{-1}) of the drug. Approximately 88 and 86% of the dose was excreted mostly as salicylic acid and its conjugated forms, glucuronide and sulphate, in urine within 48 h of i.v. or oral administration, respectively. This suggests that the gastrointestinal absorption of aspirin was essentially complete in rats. On the average, the area under the plasma concentration-time curve for unchanged aspirin following oral dosing (AUC_o) was 0·35 of that obtained following i.v. administration ($AUC_{i.v.}$) and 0·53 of that following intraportal administration (AUC_p). Therefore, orally administered aspirin is subject to first-pass metabolism both in the gut and in the liver of rats. The gastrointestinal first-pass effect is estimated to be relatively more important than the hepatic effect.

Reduced systemic availability of drugs following oral administration, even in the presence of complete absorption, has been termed the 'first-pass effect'. The two processes responsible for this effect are gastrointestinal and hepatic extraction and/or metabolism. To differentiate these in animal studies a drug has either been directly administered into the hepato-portal vein whereby the extraction and/or metabolism component in the gut is bypassed (Harris & Riegelman 1969; Iwamoto & Klaassen 1977a,b; Back et al 1978) or a portacaval shunt that bypasses the liver is created (Gugler et al 1975). Recently, a pharmacokinetic model to differentiate presystemic (preabsorptive, gut epithelial and hepatic) first-pass metabolism has been developed (Colburn & Gibaldi 1978; Colburn 1979) and has been tested using literature data for phenacetin given to rats (Welch et al 1976). The relative contribution of the intestinal mucosa, liver and lung in the in vivo disposition of phenol in rats has also been evaluated by Cassidy & Houston (1980).

Aspirin is the first drug that has been pharmacokinetically demonstrated to have the first-pass effect and is also a drug with its response dependent on route of the administration. Rowland et al (1967) and Harris & Riegelman (1979) have shown that both the gut and the liver produce the first-pass effect on aspirin disposition in man and in dogs. We have evaluated the effect of route of administration upon the pharmacokinetics of aspirin in rats, to estimate the relative importance of the gut and the liver in producing the first-pass effect and to compare this effect in rats with that reported in dogs (Harris & Riegelman 1969).

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MATERIALS AND METHODS

Materials

Aspirin, ASA(JSP IX) was purchased from Maruishi Seiyaku Co. Ltd, Nagoya, Japan. Its chemical purity was more than 99·5%. The drug solution was prepared by wetting ASA with a small amount of ethanol and then dissolving it in ice-chilled 0·9% NaCl containing ethanol 1·5%. [carboxyl-^{14}C]-Aspirin was purchased from New England Nuclear, Boston, Mass., U.S.A. (spec. act. 33·35 mCi $mmol^{-1}$ radiochemical purity >99·0%). Trypsin (from bovine pancreas, Wako Pure Chemical Ind. Ltd, Nagoya, Japan) was used for acid hydrolysis of glycine conjugate. β-Glucuronidase/arylsulfatase (from *Helix pomatia*, Boehringers Mannheim, GmbH, West Germany) was used for enzymatic hydrolysis of the glucuronized and sulphate metabolites. All other chemicals used were of analytical grade.

Animal experiments

Male Wistar rats, 206–256 g, were chronically cannulated into the right jugular vein with silicone polymer tubing (i.d. 1·0 mm; o.d. 1·5 mm, Dow Corning, Tokyo, Japan) by a modified method of Upton (1975). These rats were used for bolus i.v. or oral administration. Animal care was as reported by Iwamoto & Klaassen (1977a,b) except that the fasting interval was about 15 h. Experiments with

the bolus dose were made 2 days after cannulation. Groups of four rats, unanaesthetized, were either given 10 mg kg^{-1} of ASA via the cannula in 10 s or orally by gastric intubation. The rats were then housed individually in metabolic cages to collect the urine and faeces separately. Urine was collected at 4, 8, 24 and 48 h, faeces were sampled daily; cage washings were added to the urine samples. Samples were kept frozen until urine samples were diluted with distilled water and faecal samples dried and ground for analysis.

Other groups (n = 6) chronically cannulated and unanaesthetized rats were given the same dose as described above. Sequential blood samples (about 0·25 ml) were withdrawn from the jugular vein cannula over 60 min. These were immediately added to chilled small tubes containing 1 unit of heparin and 125 μg of potassium fluoride on ice to prevent the in vitro hydrolysis of ASA in the whole blood (Rowland & Riegelman 1967). Hydrolytic degradation of ASA to salicylic acid(SA) was <1% in the whole blood so treated. The mixture was centrifuged at 3000 rev min^{-1} for 15 min and the plasma immediately extracted for analysis of unchanged ASA.

For systemic or intraportal administration by a constant rate infusion, other groups of rats (242–256 g) were cannulated into the femoral artery and vein with PE-50 tubing (i.d. 0·58 mm; o.d. 0·965 mm, Intramedic, Clay Adams, Parsippany, N.J., U.S.A.), and into the ileocolic vein and bile duct with PE-10 tubing (i.d. 0·28 mm; o.d. 0·61 mm) under light anaesthesia with ether. Drug solution was infused at 2 mg kg^{-1} min^{-1} for 5 min into the femoral (systemic) vein or ileocolic (portal) vein of the rats aroused from ether anaesthesia.

To reevaluate an extremely low rate of biliary excretion of total salicylates (ASA + SA + conjugates), 10 μCi kg^{-1} of [^{14}C]ASA (10 mg kg^{-1} ASA) was infused for 5 min as above. Sequential blood samples were collected over 60 min from the femoral artery while the cumulative bile was collected over 120 min. Blood samples were treated in the same way as described above.

Analytical procedures

ASA and its metabolites in various biological specimens were determined spectrofluorometrically as SA except the radioactive samples.

ASA in plasma and urine. The procedure for ASA in plasma was a slight modification of the method of Cotty & Ederma (1966). An aliquot (100 μl) of the plasma was immediately mixed with 100 μl of 5% $KHSO_4$ solution on ice and then extracted with 6 ml of ether. The ethereal extract was shaken with 1 ml of 4% ceric ammonium nitrate solution for 1 h. The separated ethereal layer (5 ml) was extracted with 1 ml of 1% $NaHCO_3$ solution and 500 μl of the $NaHCO_3$ layer was incubated with 200 μl of 1 M NaOH solution at 37 °C for 1 h. The resultant mixture was analysed spectrofluorometrically at excitation wavelength 294 nm and emission wavelength 403 nm. The reference plasma obtained before dosing was similarly treated.

Appropriately diluted urine (100 μl) was similarly treated except that heparin was not added to the tube.

ASA and SA in urine. To diluted urine (100 μl) was added 200 μl of 2·5% $KHSO_4$ solution and the mixture extracted with 5 ml of ether. The ethereal layer (4 ml) was then extracted with 2 ml of 1% $NaHCO_3$ solution and the separated alkaline layer (1 ml) was incubated with 1 M NaOH solution (500 μl). The sum of ASA and SA was determined spectrofluorometrically.

ASA, SA and their conjugates in urine, faeces and bile. Preliminary experiments with trypsin hydrolysis of the urine or bile samples obtained from the rats which were given ASA, (10 mg kg^{-1}) intravenously or orally, demonstrated that the glycine conjugate of SA was negligible both in urine (<1·2% of the dose by 48 h) and bile (<0·025% of the dose by 120 min). Therefore, diluted urine (100 μl) or an aliquot (50 μl) of bile was incubated with 200 μl of 20-fold diluted β-glucuronidase/arylsulfatase solution prepared in 0·05 M acetate buffer (pH 5·0) at 37 °C for 24 h. After 400 μl of 5% $KHSO_4$ was added to the incubated solution, the mixture was extracted with 5 ml of ether and the ethereal layer (4 ml) treated with 1% $NaHCO_3$ solution as above. Ground faecal samples (50 mg) were suspended in 1 ml of 30-fold diluted enzyme solution, incubated at 37 °C for 24 h, centrifuged at 3000 rev min^{-1} for 10 min and the supernatant (200 μl) added to 800 μl of 5% $KHSO_4$ solution. The mixture was extracted with ether and then reextracted with alkaline solution as above.

Total radioactivity in bile. An aliquot (50 μl) of bile was directly determined with 10 ml of toluene-Triton X-100 liquid scintillator (PPO 5 g, POPOP 300 mg, toluene 700 ml, Triton X-100 300 ml) (Mark II liquid scintillation spectrometer, Nuclear-Chicago Corporation, Des Plaines, Ill., U.S.A.). The counting efficiencies were automatically determined by a ^{133}Ba external standardization method and counts min^{-1} were converted to d min^{-1}.

Calculation of pharmacokinetic parameters. Plasma elimination curves obtained after bolus i.v. and oral

administration and constant rate infusion into the peripheral or portal vein were analysed by least-squares regression analysis. The area under the plasma concentration-time curve when plotted arithmetically ($AUC_{0-\infty}$) was calculated by the trapezoidal rule for the observed values ($t = 0 - t$) and then extrapolated to infinity ($t = t - \infty$). All data were tested for statistically significant differences using Student's *t*-test.

RESULTS AND DISCUSSION

Unchanged ASA was not detected in all the cumulative urine samples collected. Table 1 summarizes cumulative percentage of SA and conjugates (glucuronide plus sulphate) excreted with time and dose into the urine or via faeces. There were no significant differences between cumulative fractions of the dose excreted as SA or its conjugates following i.v. and oral administration ($P > 0.1$). About 88 and 86% of the dose were recovered as SA and its conjugates (glucuronide and sulphate) in the urine over 48 h after i.v. and oral adminstration, respectively. Therefore, the gastrointestinal absorption of ASA was considered to be almost complete in rats. Total faecal excretion over 48 h following i.v. and oral administration was less than 3 and 2% of the dose, respectively. Cumulative biliary excretion of total salicylates (ASA + SA + conjugates) was less than 0·25 and 0·22% of the dose at 2 h following systemic and intraportal administration, respectively. Biliary excretion of salicylates has also been reported to be low in dogs (i.e. 1 to 2% of the i.v. dose of 100 mg kg^{-1}, Rutishauser & Stone 1975). Since our main aim was to evaluate the participation of the gut and liver in producing the first-pass effect of ASA given in relatively low dose (10 mg kg^{-1}) to rats, it seemed unsuitable to use such high doses as 100 (Rutishauser & Stone 1975) or 400 (Bullock et al 1970) mg kg^{-1}, as these would cause a saturation in the first-pass metabolism of the drug. At 120 min after both infusion methods, the extent of biliary excretion of total salicylates determined by total radioactivity was still less than 0·3% of the dose and was not significantly different from the values mentioned above ($P > 0.1$). Thus, it was suggested that biliary excretion and enterohepatic circulation of the salicylates was almost negligible.

Computer-fitted plasma elimination curves of unchanged ASA after bolus i.v. and oral administration, or constant rate infusion into the systemic or portal vein are shown in Figs 1 and 2. The calculated

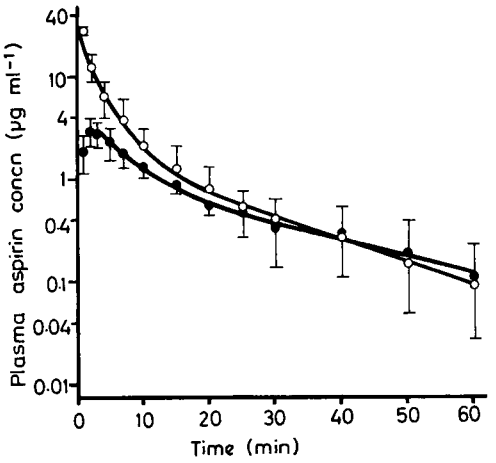

FIG. 1. Plasma concentration-time curves for unchanged aspirin in rats receiving aspirin (10 mg kg^{-1}) by bolus i.v. (○, n = 6) and oral (●, n = 6) administration, each point with vertical bar representing mean data point with standard deviation.

Table 1. Cumulative urinary and faecal excretion of salicylic acid(SA) and its glucuronide(G) and sulphate(S) following intravenous or oral administration (10 mg kg^{-1}) of aspirin to rats.

	Cumulative % of dose following							
	i.v. administration (n = 4)				oral administration (n = 4)			
Time after admin (h)	Urinary			Faecal	Urinary			Faecal
	SA	G + S	Total (SA + G + S)	SA + G + S	SA	G + S	Total (SA + G + S)	SA + G + S
4	12·2 (5·33)[b]	23·6 (6·92)	35·8 (8·52)	n.d.[a]	11·7 (10·4)	7·50 (9·11)	19·2 (7·82)	n.d.
8	16·4 (4·02)	37·3 (4·26)	53·7 (4·65)	n.d.	17·5 (11·3)	21·1 (10·6)	38·5 (11·3)	n.d.
24	37·4 (4·93)	46·6 (4·38)	84·0 (3·94)	1·76 (0·841)	24·7 (16·1)	44·7 (14·8)	69·4 (13·5)	1·48 (1·46)
48	40·1 (5·32)	47·4 (3·99)	87·5 (2·05)	2·73 (1·29)	26·2 (15·7)	59·3 (10·9)	85·5 (6·74)	1·96 (1·80)

[a] Not determined.
[b] Standard deviation.

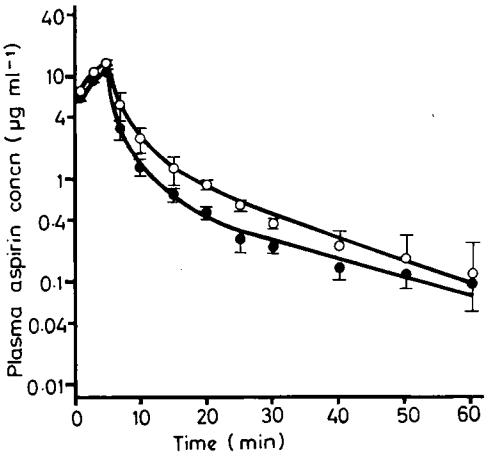

FIG. 2. Plasma-concentration-time curves for unchanged aspirin in rats receiving aspirin by constant rate infusion (2 mg kg^{-1} min^{-1}) for 5 min into femoral (systemic) vein (○, n = 4) and ileocolic (portal) vein (●, n = 4), each point with vertical bar representing mean data point with standard deviation.

pharmacokinetic parameters for the unchanged ASA are summarized in Table 2. The values of α and β for oral administration were significantly smaller than those for i.v. dosing ($P < 0{\cdot}001$ and $P < 0{\cdot}01$, respectively). There was no significant difference between AUC_{TR} (trapezoidal AUC) for the i.v. administrations with bolus and with constant rate infusion ($P > 0{\cdot}1$), whereas oral ($P < 0{\cdot}001$) and intraportal ($P < 0{\cdot}01$) administration of ASA gave much smaller AUC_{TR} than that for i.v. dosing. Thus, by comparing AUC_{TR} with that following i.v. administration, systemic availability after oral and intraportal administration was calculated to be about 35 and 67% of the dose, respectively. The assumption that the gastrointestinal absorption of ASA is complete was verified by the cumulative urinary excretion data. The peak of the plasma ASA levels which appeared within the first 5 min following oral administration (Fig.1) indicated that absorption was predominantly from the stomach. However, a significantly smaller value of β for oral than i.v. administration (Table 2) would suggest that absorption from duodenum or upper jejunum as well as from stomach still occurs during the decline of the plasma ASA levels.

Reduced systemic availability observed following both oral and intraportal administration indicates that aspirin is subject to the first-pass metabolism both in the gut and in the liver of rats. The fraction of gastrointestinal (f_g) or hepatic (f_h) extraction and/or metabolism to the given dose could be calculated according to the equations in Table 3 (Harris & Riegelman 1969) which summarizes the estimated values for f_g and f_h along with those previously obtained with dogs (Harris & Riegelman 1966). The value of f_h obtained in rats was similar to that estimated in dogs. In contrast with the results in dogs, in rats f_g was larger than f_h suggesting that the gastrointestinal tract plays a greater role in producing the first-pass effect of orally administered ASA than the liver. Furthermore, the overall first-pass effect as indicated in the value of $\{1 - (1 - f_g)(1 - f_h)\}$ as well as the gastrointestinal effect (f_g) seemed to have a species difference between rats and dogs.

Table 2. Pharmacokinetic parameters for aspirin following intravenous, oral or intraportal administration (10 mg kg^{-1}) of aspirin to rats.

Parameters	i.v. Bolus (n = 12)[a]	Constant rate infusion (n = 9)	Oral (n = 10)	Intraportal (n = 9)
A (μg ml^{-1})	23·1(9·37)[b]	n.e.[c]	n.e.	n.e.
B μg ml^{-1})	1·97(0·988)	n.e.	n.e.	n.e.
α (min^{-1})	0·343 (0·113)	0·373 (0·0571)	0·177 (0·0272)	0·353 (0·0671)
β (min^{-1})	0·0513 (0·0143)	0·0571 (0·00662)	0·0345 (0·00797)	0·0449 (0·00970)
V' d extrap[d] (1 kg^{-1})	5·08	n.e.	n.e.	n.e.
t β (min)	13·5	12·1	20·1	15·3
AUC[e] (μg min ml^{-1})	116	n.e.	n.e.	n.e.
AUC_{TR}[f] (μg min ml^{-1})	120 (18·3)	112 (10·6)	42·3 (10·1)	80·1 (7·69)

[a] Number of mean data points used for the least-squares regression analysis.
[b] Standard deviation.
[c] Not estimated.
[d] Apparent volume of distribution estimated using extrapolated B value.
[e] AUC value estimated by the equation, $A/\alpha + B/\beta$.
[f] AUC value estimated by the trapezoidal rule.

Table 3. Fractions of dose being subject to gastrointestinal (f_g) and hepatic (f_h) extraction and/or metabolism of aspirin in rats.

Fraction	In rats	(In dogs)[a]
f_g[b]	0·472	(0·280)
f_h[c]	0·331	(0·363)
$1 - (1-f_g)(1 - f_h)$[d]	0·647	(0·541)

[a] Calculated from the data of Harris & Riegelman (1969).

[b] Estimated by the equation, $f_g = 1 - \frac{AUC_p}{AUC_p}$.

[c] Estimated by the equation, $f_h = 1 - \frac{AUC_p}{AUC_{i.v.}}$.

[d] Overall fraction of dose which is subject to gastrointestinal and hepatic extraction and/or metabolism and is estimated by the equation,

$$1 - (1 - f_g)(1 - f_h) = 1 - \frac{AUC_o}{AUC_{i.v.}}$$

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