J. Pharm. Pharmacol. 1983, 35: 821–822
Communicated June 8, 1983

Limited capacity of jejunal segments to effect glucuronidation and sulphation of aspirin and salicylamide in rats

KIKUO IWAMOTO*, JUN WATANABE, *Department of Biopharmaceutics, Faculty of Pharmaceutical Sciences, Nagoya City University, Mizuho-ku, Nagoya 467, Japan*

We have previously demonstrated that both aspirin and salicylamide, after oral administration to rats, are subject to the first-pass effect in the gastrointestinal tract as well as in the liver and that gastrointestinal first-pass effect of aspirin is relatively large compared with the hepatic effect whilst with salicylamide this was reversed (Iwamoto et al 1982, 1983). It has been implied that in man (Rowland et al 1967) and dogs (Harris & Riegelman 1969), most of the hydrolysis of aspirin occurs in the liver the remainder taking place across the intestinal mucosa. The site at which salicylamide is conjugated with ethereal sulphate has not been clarified in man (Levy & Matsuzawa 1967), but it has been shown that rabbit ileum is able to effect the glucuronidation of salicylamide (Barr & Riegelman 1970). This approach to the determination of conjugated metabolites immediately after their formation by continuous collection of mesenteric venous blood samples from rabbits (Barr & Riegelman 1970) has been recently applied to rats, indicating that both morphine and nalorphine are subject to capacity-limited jejunal glucuronidation in-vivo (Iwamoto & Klaassen 1977) and that salicylamide is exclusively glucuronated in the in-situ ileal portion (Shibazaki et al 1981). The effect of dose on in-vivo intestinal conjugation of aspirin and salicylamide is now reported in this communication which describes and compares for the first time the capacity-limited conjugation (glucuronidation and sulphation) of aspirin and salicylamide in rats by direct mesurement of the amounts of both unchanged and conjugated drugs in the mesenteric venous blood immediately after absorption.

Methods

The second or third jejunal segment of male Wistar rats, 315–330 g (anaesthetized with 800 mg kg^{-1} urethane, i.p.), with a corresponding mesenteric vein, was prepared by a modification of the method for rabbits described by Barr & Riegelman (1970). The vein was cannulated with PE-50 tubing while the arterial blood supply was kept intact. The blood lost from the vein was continuously replaced by constant infusion via the femoral vein with an approximately equal volume of heparinized whole blood previously collected from donor animals. The segmental lumen was rinsed gently with 10 ml of warm 0·9% NaCl (saline). The drug solution (1 ml in saline) containing aspirin (Maruishi Seiyaku Co., Nagoya), salicylamide, (Tokyo Kasei Chemicals Co., Tokyo) or [carboxyl-^{14}C]asprin (spec. act. of 33·4 mCi $mmol^{-1}$ and radiochem. purity of more than 99·0%, New England Nuclear, Boston) was then placed in the closed loop which was tied at both ends. The mesenteric blood samples were collected continuously over 30 min after the drug administration. In preliminary experiments blood samples from the femoral artery were also collected periodically to examine whether the radioactivity had entered the systemic circulation. The cannulated rats were maintained in a manner similar to that used by Barr & Reigelman (1970) for rabbits.

Spectrofluorometric assay of aspirin and its metabolites (salicylic acid and conjugates) or salicylamide and its metabolites (conjugates) in the mesenteric venous plasma and in the remaining luminal drug solution was as described by Iwamoto et al (1982, 1983). The plasma concentration was then converted to the amount of each component by multiplication by the plasma volume estimated from the haematocrit value. Total radioactivity in the arterial blood was measured by applying 1 ml of Soluene-350 (Packard Instruments, Downers Grove, Ill., U.S.A.) to the sample (50 or 100 µl) before mixing with 10 ml of scintillator (Toleuene-Triton X-100).

Results and discussion

The mesenteric venous blood flow ranged from 0·45 to 0·62 ml min^{-1} for the rats used. Since arteriovenous

Table 1. Fraction of the jejunal metabolism to the total amount absorbed at various intraluminal drug concentrations.

Initial intraluminal concn mM	Aspirin (n = 3)	Salicylamide (n = 3)	Significance level
0·5	0·788 ± 0·038[a]	0·647 ± 0·035	$P<0·01$
1·0	0·792 ± 0·063	0·546 ± 0·058	$P<0·01$
5·0	0·768 ± 0·044	0·229 ± 0·029	$P<0·001$
10·0	0·603 ± 0·097	0·126 ± 0·043	$P<0·01$

[a] These values (mean ± s.d.) were calculated by applying the following equation to the cumulative amounts by 30 min after intrajejunal administration, 1−(amount of unchanged drug) (amount of total drug)$^{-1}$.

* Correspondence.

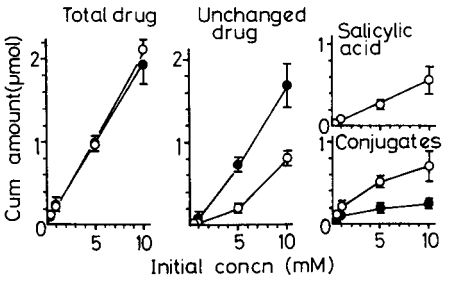

FIG. 1. Dose-dependence in jejunal transport and metabolism of aspirin and salicylamide. Cumulative amount of each component (total, unchanged drug or metabolites) in the mesenteric venous plasma by 30 min after dosing with aspirin (○) and salicylamide (●) was plotted against the initial luminal concentration. Vertical bars represent s.d. of the mean data points from three rats.

anastomoses are known to be present in the gastrointestinal circulation (Chambers & Zweifach 1944; Zweifach 1961), the preliminary study using labelled aspirin was made to establish that the amount of drug reaching the systemic circulation was negligible. The radioactivities recovered in arterial blood samples within 30 min were insiginificant (less than 0·01% of that in the venous effluent). For both drugs, the amount of any metabolite in the luminal solution at 30 min was almost negligible compared with that in the mesenteric venous plasma.

At all doses tested, the time course (0–30 min) of accumulation of each component in the mesenteric venous plasma indicated that the maximum in the rate of appearance of the total drug or each component in the mesenteric circulation occurred during the first 6 to 12 min. This suggested a rapid absorption followed by immediate metabolism during passage across the jejunal wall. The cumulative amounts of each component in the mesenteric venous plasma over 30 min are compared for the two drugs in Fig. 1. There was significant linearity of the cumulative total amounts with dose for both salicylamide ($P < 0·001$) and aspirin ($P < 0·01$). However, the extent of absorption over 30 min from the closed jejunal loop was only about 30% of the dose of either drug. Thus, the in-situ preparation appeared to be limited in its ability to absorb the drugs even though complete absorption in-vivo of aspirin and salicylamide from the gut of rats has been reported (Iwamoto et al 1982, 1983). Although a fair linearity ($P < 0·05$) was obtained with salicylic acid, a clear non-linearity was observed with both unchanged and conjugated components of both drugs. These findings, especially the typical concave curves with the conjugates, suggest the existence of a saturation mechanism for the glucuronidation and sulphation of aspirin and salicylamide. A limited capacity for conjugation of salicylamide with glucuronic acid has been reported in rabbit ileum (Barr & Riegelman 1969), and for morphine and nalorphine with glucuronic acid in rat jejunum (Iwamoto & Klaassen 1977). The cumulative amount of aspirin-conjugates at 30 min was significantly larger at all doses than the amount of salicylamide-conjugates. The proportions of intestinal extraction or metabolism, or both, of the dose given, calculated according to the equation of Harris & Riegelman (1969), were 0·472 and 0·354 for aspirin (given at 10 mg kg^{-1}) and salicylamide (given at 30 mg kg^{-1}) respectively (Iwamoto et al 1982, 1983), which is fairly consistent with the significant differences in the proportions of intestinal metabolism to the total absorbed amount of the drugs determined in the mesenteric venous plasma following their intrajejunal administration (Table 1).

The data thus show that using the isolated in-situ intestinal loop with complete venous collection it is possible to establish the extent of the intestinal participation in the first-pass effect of drugs.

This study was supported by funds from Ministry of Education, Sciences and Culture of Japan Grant D 56379.

REFERENCES

Barr, W. H., Riegelman, S. (1970) J. Pharm. Sci 59: 154–163

Chambers, R., Zweifach, B. W. (1944) Am. J. Anat. 75: 173–193

Harris, P. A., Riegelman, S. (1969) J. Pharm. Sci. 58: 71–75

Iwamoto, K., Klaassen, C. D. (1977) J. Pharm. Exp. Ther. 203: 365–376

Iwamoto, K., Takei, M., Watanabe, J. (1982) J. Pharm. Pharmacol. 34: 176–180

Iwamoto, K., Arakawa, Y., Watanabe, J. (1983) Ibid. 35: in the press

Levy, G., Matsuzawa, T. (1967) J. Pharmacol. Exp. Ther. 156: 285–293

Rowland, M., Riegelman, S., Harris, P. A., Sholkoff, S. D., Eyring, E. J. (1967) Nature (London) 215: 413–414

Shibazaki, J., Konishi, R., Koike, M., Imamura, A., Sueyasu, M. (1981) J. Pharm. Dyn. 4: 91–100

Zweifach, B. W. (1961) in: Thomas, C. C. (ed.) Functional Behavior of the Microcirculation. Springfield, Ill., U.S.A. pp 173–183