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BIOPHARMACEUTICS OF RECTAL ADMINISTRATION OF DRUGS IN MAN
I. INTRODUCTION OF BENZOIC ACID AS A TEST DRUG

F. MOOLENAAR, A.G.G. STUURMAN-BIEZE, J. VISSER and T. HUIZINGA

Laboratorium voor Farmacotherapie en Recepteur, Ant. Deusinglaan 2, Groningen (The Netherlands)

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SUMMARY

In this report it will be shown that benzoic acid is a suitable test drug to study the biopharmaceutics of rectally administered dosage forms in man. After intravenous administration of sodium benzoate it can be seen from pharmacokinetic parameters that biotransformation to hippurate is extremely rapid. The clearance of hippurate occurs exclusively by renal excretion. At relatively low concentrations, as used in our rectal experiments, elimination of hippurate is not capacity limited and therefore linear pharmacokinetics is assumed, according to a multi-compartment model.

After rectal administration the absorption of sodium benzoate is fast and complete. Bioavailability after 4 h has been found in the same range for both oral and rectal routes. There was an excellent correlation between plasma and urine levels of hippurate.

It is concluded that, by measuring hippurate levels in plasma or urine, the influence on absorption rate and bioavailability of formulation factors using rectal dosage forms with sodium benzoate and benzoic acid can be determined.

INTRODUCTION

In order to study the biopharmaceutical aspects of rectal drug administration, it is a prerequisite that absorption should be fast compared to the release process. Absorption from the rectum is more rapid and more complete when drugs are administered rectally in the form of a suspension or a solution (micro-enema) than in the form of a suppository (Wagner, 1971). For this reason it is remarkable that only a few authors have paid attention to this first dosage form (Yunginer et al., 1966; Lilehei, 1968; Wagner, 1971). However, with respect to human studies, Wagner (1971) concluded that the results obtained by rectal administration of some antibiotics in solution form were quite poor compared with the results achieved by the oral route. Nevertheless, from a biopharmaceutical point

of view, formulation factors which are connected with the rectal absorption from a micro-enema, such as the volume, the concentration and the pH of the aqueous suspension or solution, as well as the nature of the drug, such as the pK_a value and the partition coefficient, are often not considered.

Considerable attention has been given to those physicochemical parameters which are associated with the disintegration of suppositories and the dissolution of the drug in the rectal fluid (Bevernage and Polderman, 1973; Rutten-Kingma, 1977). However, in man there is little agreement about the influence of some of these factors, such as particle size of the drug and the spreadability of a suppository, on the absorption rate and bioavailability. Human studies were therefore planned to determine the nature of the absorption process and some formulation factors which might be of importance to the absorption process. For this purpose it was necessary to introduce a test drug with relatively non-toxic properties and a fast absorption profile in proportion to the elimination. In addition, from a biopharmaceutical point of view, it was desirable to consider slightly soluble and reasonably soluble drugs. There also had to be an adequate method for bioanalysis. In this context benzoic acid and the corresponding salt have been discussed, to evaluate the use of these substances in biopharmaceutics in man.

Benzoic acid is added as a preservative to many foodstuffs. After giving sodium benzoate by mouth, devised by Quick (1933) as a test of liver function, absorption is rapid. The major route of biotransformation of benzoate in man is conjugation with glycine, resulting in the formation of hippurate (Amsel and Levy, 1969). As the formation of hippurate is extremely fast (Wu and Elliot, 1961) and hippurate is almost quantitatively recovered from urine (except for a minor fraction, less than 1%, which is excreted via the urine as benzoyl glucuronide) it was reasoned that the absorption rate of benzoate would be reflected in hippurate plasma levels. Only traces of free benzoic acid are detected in urine.

In this study the pharmacokinetics of benzoate and hippurate after intravenous administration in man are reported. Absorption rate and bioavailability after rectal administration of a solution form with different concentrations of sodium benzoate are discussed and compared to those after oral administration.

MATERIALS AND METHODS

Determination of benzoate in plasma

Benzoic acid was quantitatively converted to the more volatile trimethylsilyl derivative and assayed by the GLC-method of Wan and Riegelman (1972).

Procedure. Plasma was acidified to pH 2.8 ± 0.2 by adding a 50 µl saturated solution of potassium bisulfate [1] to 1.0 ml of plasma and extracted with 10.0 ml freshly distilled diethyl ether [2]. An 8 ml aliquot of the ether fraction was pipetted off and evaporated to nearly dryness at about 50°C. To the residue 1.0 ml of a carbon disulfide [3] solution was

[1] Merck (G.R.)
[2] Merck (UVASOL)
[3] Merck (G.R.)

added, containing 5 μg/ml diethyldiethylmalonate [4] as external standard and 5 μl bis-(trimethylsilyl)trifluoroacetamide [5].

The test tube was thoroughly rinsed with the carbon disulfide mixture. The solution was evaporated again at about 50°C to a residual volume of 50 μl. Within 2 h 1.0 μl was injected into a gas chromatograph (Perkin Elmer F 17) fitted with a 1.8 m X 0.3 cm glass column, and a flame-ionization detector (FID). The packing materials consisted of 3% OV-1 coated on Chromosorb W AW-HMDS, 80–100 mesh. The operation conditions were: injection/detection temperature, 175°C; oven temperature, 100°C; nitrogen, hydrogen and air flows were 30, 30 and 350 ml/min respectively.

The retention times of benzoic acid and diethyldiethylmalonate were 3 and 4 min, respectively. The amount of benzoic acid was measured by comparing the peak height ratios of benzoic acid and the external standard. This GLC method is accurate to a concentration of 0.3 μg/ml plasma, with a mean coefficient of variation of 5% measured on 7 runs. Diethyl ether must be distilled to remove the anti-oxidants which disturb the analysis. It was found necessary to saturate the GLC column with trimethylsilyl benzoate every day before drug estimation.

Determination of hippurate in plasma

Hippurate was first hydrolyzed to benzoic acid, extracted and measured by the GLC method above.

Procedure. One ml of plasma and 1.0 ml of 6 N hydrochloric acid [6] were pipetted into a test tube which was securely capped and heated at 100°C in an oven for 16 h. After cooling, 2.0 ml of 4 N sodium hydroxide was added and the mixture was then extracted with 10.0 ml carbon tetrachloride [7]. Three ml of the water layer was pipetted off into another test tube; 2.0 ml of 1 N hydrochloric acid was added and extracted with 10.0 ml distilled diethyl ether using the same procedure as described for benzoate in plasma. This procedure is accurate to concentrations of 1.0 μg/ml, with a mean coefficient of variation of 6% measured on 7 runs.

By subtracting the concentration of unmetabolized benzoate (expressed in μg/ml hippurate) from the total concentration of hippurate in plasma, it was possible to find the total metabolized hippurate per ml plasma.

Determination of benzoate in urine

The analysis of benzoate in urine was assayed by the spectrophotometric method of Amsel and Levy (1969).

Procedure. One ml of urine was acidified by 1.0 ml of 6 N hydrochloric acid and extracted with 15.0 ml of carbon tetrachloride. After removing the upper aqueous layer, 5.0 ml of the organic phase was pipetted off and extracted with 5.0 ml of a 5% solution of sodium bicarbonate [8]. To 3 ml of the latter solution 1.0 ml of concentrated hydro-

[4] Merck (for synthesis)
[5] Pierce Chemicals (BSTFA)
[6] Merck (G.R. 37%)
[7] Merck (G.R.)
[8] Merck (G.R.)

chloric acid was added. The solution was shaken in a test tube until the carbon dioxide had disappeared. The absorbance of the solution was measured at 231 nm on a UV-VIS spectrophotometer (Beckman 25) against a blank of 3.0 ml of the sodium bicarbonate solution with 1.0 ml concentrated hydrochloric acid. The amounts of excreted benzoate were calculated from a calibration curve which was made by adding known amounts of benzoate to water, and by multiplying with the volume of urine. This procedure was accurate to a minimum concentration of 25 μg/ml with a mean coefficient of variation of 5%.

Determination of hippurate in urine

Hippurate was first hydrolyzed to benzoic acid which was extracted and quantitated by the spectrophotometric method mentioned above.

Procedure. Five ml of urine and 5.0 ml of concentrated hydrochloric acid were pipetted into a test tube which was securely capped and heated in an oven at 100°C for 16 h. After cooling 1.0 ml of the mixture was extracted with 15.0 ml of carbon tetrachloride, using the same procedure as described for benzoate in urine. This procedure is accurate to a minimum concentration of 25 μg/ml with a mean coefficient of variation of 5%. The recoveries of benzoic acid and hippuric acid at various concentrations were 98–100%.

By subtracting the endogenous amount of hippurate at $t = 0$ from the amount excreted in urine every 15 min, the quantity of absorbed benzoate (as hippurate) was obtained.

Intravenous administration

Here 660 mg sodium hippurate [9], equivalent to 400 mg benzoic acid, was dissolved in 10 ml distilled water, and 472 mg sodium benzoate [10], equivalent to 400 mg benzoic acid was dissolved in 10 ml distilled water. The solutions were filtered and heated at 100°C during 30 min. Three healthy volunteers, ranging in age from 25 to 32 years and in body weight from 57 to 95 kg, participated in the study.

No drugs were taken for 2 weeks prior to or during the study. The experiments were initiated in the course of the morning (at 11.00 h) after an overnight fast. By that time endogenous hippurate levels in plasma and urine were negligible. A 5 ml sample of blood was taken from a forearm vein by means of an intermittent infusion set with reseal injection site [11] and a three-way stopcock [12]; 10 ml of the sodium hippurate solution was injected intravenously in the other arm during 1 min. During 4 h about 20 blood samples were taken. They were heparinized and centrifuged, after which plasma was separated and frozen. The three subjects followed the same protocol after a week for the intravenous injection of sodium benzoate.

[9] Merck (for synthesis)
[10] Lamers and Indemans (for synthesis)
[11] Abbott Butterfly-19, int.
[12] Pharmaseal K-75a Luer

Rectal administration

An amount of 472 mg sodium benzoate was dissolved in 10 ml enema-medium (0.5% methylcellulose 400 cps in distilled water); this amount was rectally administered to 7 subjects, using a 10 ml plastic disposable syringe [13] with a plastic application tube. The age of the subjects varied between 24 and 34 years, and their weight between 57 and 95 kg. They used no other drugs.

Before administration of the micro-enema, subjects started drinking 200 ml of water every 15 min. At time zero a blank urine sample was collected, a blood-sample was taking using Venoject tubes [14] with 1.0 ml 3.8% sodium citrate and the micro-enema was administered. Every 15 min a blood sample was taken, urine was collected and 200 ml water were ingested. During 4 h the plasma and urine levels were followed. To obtain an estimate of the absorption capacity, micro-enemas with different doses of sodium benzoate (equivalent to 200, 400, 800 and 2000 mg benzoic acid) were administered to one subject; these amounts were dissolved in 10 ml enema-medium; the pH of the solutions ranged from 7.15 (400 mg) to 7.70 (2000 mg).

Oral administration

The 7 subjects were given 472 mg sodium benzoate dissolved in 50 ml of water. The plasma levels of benzoate and hippurate were followed during 4 h. The procedure of blood sampling was similar to that described for the intravenous administration.

RESULTS AND DISCUSSION

The plasma curve after an intravenous bolus injection of *sodium hippurate* is shown in Fig. 1. The semilogarithmic plot of the plasma concentration time curve exhibits 3 distinct phases: a phase consisting of an early drop with a steep slope, an intermediate phase with a relatively more gradual slope, and an elimination phase having a half-life of at least 2 h. Elimination from a 3-compartment system was assumed to explain this pharmacokinetic pattern. The first compartment may be partly explained as a dilution phase, the second as a distribution phase, and finally there is an elimination phase in which the renal clearance of hippurate appears as a first-order process. The pharmacokinetic parameters of hippurate in 3 volunteers are given in Table 1. It can be concluded that hippurate has a volume of distribution which is approximately equal to the volume of total body-water (30-40 litres). The clearance of hippurate occurs exclusively by renal excretion. Since, apart from the renal excretion, no further biotransformation occurs, the total-body clearance constant (about 300 ml/min) is high in proportion to the glomerular filtration rate (125 ml/min) which is probably caused by tubular secretion. Such secretion processes are limited by a certain fixed maximal transport value and are principally non-linear in nature. At relatively low concentrations, as used in our rectal experiments, elimination of hippurate is not capacity limited and therefore linear pharmacokinetics has been assumed. This will be proved in Fig. 1.

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[13] B-D (Luer-Lok Tip)
[14] Terumo Corporation

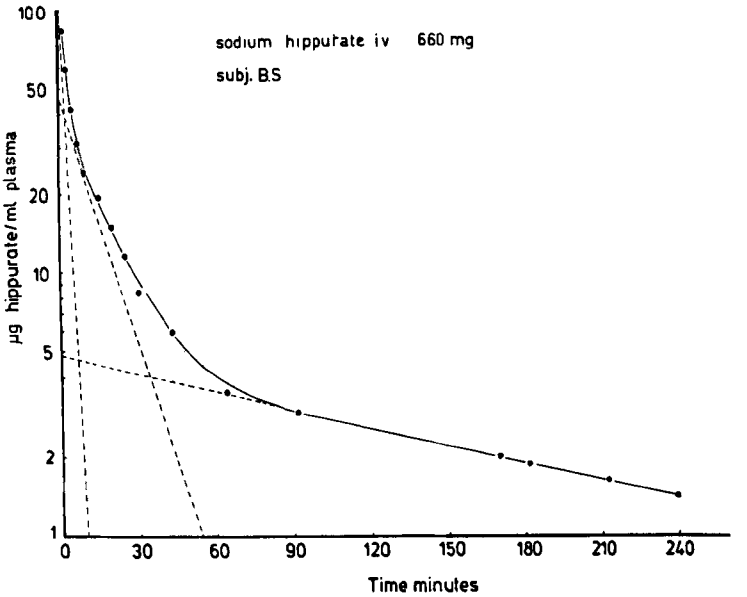

Fig. 1. Hippurate plasma concentrations (µg/ml) on semi-logarithmic scale after intravenous injection of a dose of 660 mg sodium hippurate to subject B.S. The curve was calculated from the experimental data (see Table 1).

TABLE 1

PHARMACOKINETIC PARAMETERS OF HIPPURATE AFTER INTRAVENOUS ADMINISTRATION OF SODIUM HIPPURATE TO 3 HEALTHY VOLUNTEERS, ACCORDING TO A 3-COMPARTMENT OPEN MODEL

A_1 = intercept of the calculated declining line of the first phase; α_1 = distribution rate constant; A_2 = intercept of the calculated declining line of the second phase; α_2 = distribution rate constant; A_3 = intercept of the calculated declining line of the third phase; α_3 = distribution rate constant; B = intercept of the calculated declining line of the elimination phase; β = elimination rate constant; $(t_{1/2})_\beta$ = biological half-life; V_c = volume of distribution of the central compartment; V_f = total volume of distribution; Cl_{tot} = total clearance.

	Name		
	B.S.	L.U.	S.B.
Dose (free acid; mg)	588	588	588
Dose/kg (mg)	6.2	8.2	10.3
A_1 (µg/ml)	100	30	18
α_1 (min^{-1})	0.45	0.35	0.09
A_2 (µg/ml)	45	50	56
α_2 (min^{-1})	0.07	0.06	0.07
B (µg/ml)	5	5	9
β (min^{-1})	0.005	0.004	0.005
$(t_{1/2})_\beta$ (min)	130	175	135
V_c (litres)	3.9	6.7	7.2
V_f (litres)	33.3	39	26.7
V_f/kg (l/kg)	0.35	0.54	0.46
Cl_{tot} (ml/min)	350	268	212

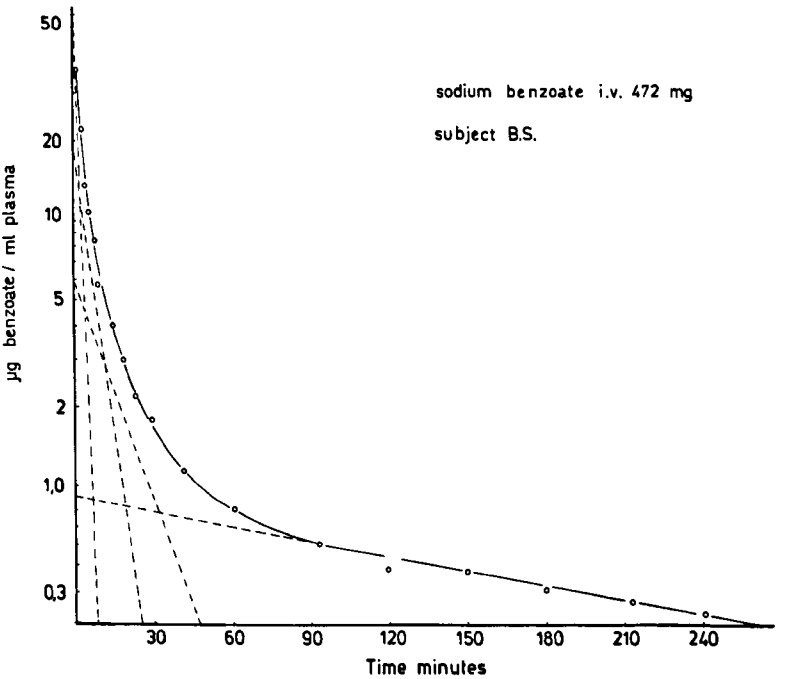

Fig. 2. Benzoate plasma concentrations (µg/ml) on semi-logarithmic scale after intravenous injection of a dose of 472 mg sodium benzoate to subject B.S. The curve was calculated from the experimental data (see Table 2).

TABLE 2

PHARMACOKINETIC PARAMETERS OF BENZOATE AFTER INTRAVENOUS ADMINISTRATION OF SODIUM BENZOATE TO 3 HEALTHY VOLUNTEERS, ACCORDING TO A 4-COMPARTMENT OPEN MODEL

	Name		
	B.S.	L.U.	S.B.
Dose (free acid; mg)	400	400	400
Dose/kg (mg)	4.2	5.5	7.0
A_1 (µg/ml)	56	30	45
α_1 (min^{-1})	0.67	0.12	0.71
A_2 (µg/ml)	18	7.5	35
α_2 (min^{-1})	0.15	0.05	0.12
A_3 (µg/ml)	5.8	3.1	3.6
α_3 (min^{-1})	0.06	0.01	0.02
B (µg/ml)	0.9	0.8	0.8
β (min^{-1})	0.004	0.005	0.004
$(t_{1/2})\beta$ (min)	160	150	160
V_c (litres)	5.0	9.6	4.7
V_fx (litres)	80.0	24	42
V_f/kg (l/kg)	0.84	0.33	0.74
Cl_{tot} (ml/min)	798	479	574

A representative example of the plasma level after an intravenous bolus injection of *sodium benzoate* is given in Fig. 2. The concentration curve consists of an extremely rapid drop with a steep slope and a latter phase with a more gradual slope. The profile of the rapid distribution phase of benzoate, calculated as a tri-exponential decay, is likely to be determined by the extremely rapid biotransformation to hippurate. The pharmacokinetic parameters of benzoate in the same 3 volunteers are given in Table 2, according to a multi-compartment model. Just as in the case of hippurate, it is difficult to unravel the different phases in terms of separate compartments. Frequent sampling of blood (10 samples in the first 25 min) made it possible to establish the distribution to a number of compartments which usually are considered to belong to the central compartment. It should be noted that in comparison with hippurate, larger estimates for the overall apparent volume of distribution of benzoate were found, according to the more lipophilic character of the drug. Since it was proved that the blood cells contained only negligible amounts of benzoate, it was possible to compare the clearance constant with the plasma flow through the liver, which was approximately 750 ml/min. Assuming that the metabolism predominantly takes place in the liver, a substantial first-pass effect after oral administration should be expected. In contrast, the contribution of renal excretion is negligible; no free benzoate could be detected in the urine.

A representative example of the relationship between the concentration pattern of the unmetabolized benzoate fraction (expressed in μg/ml) and the simultaneous development of metabolized benzoate to hippurate after an intravenous injection of sodium benzoate is given in Fig. 3; as a result of the extremely rapid biotransformation the maximal hippurate level in plasma was reached within 10 min.

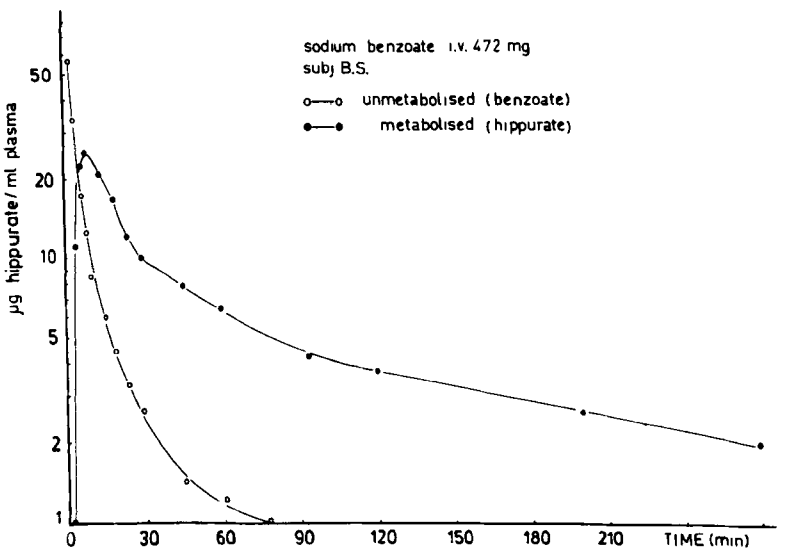

Fig. 3. Benzoate plasma concentrations (expressed as μg hippurate/ml plasma) and hippurate plasma concentrations (μg/ml plasma) on semi-logarithmic scale after intravenous injection of a dose of 472 mg sodium benzoate to subject B.S. The curve was calculated from the experimental data (see Table 2).

TABLE 3

HIPPURATE CONCENTRATIONS, CHARACTERIZING THE ABSORPTION PROCESS OF SODIUM BENZOATE AFTER RECTAL AND ORAL ADMINISTRATION OF A DOSE OF 472 mg (EQUIVALENT WITH 400 mg FREE ACID) TO 7 VOLUNTEERS

	Subjects	Mean t_{max} (min) in plasma	Mean C_{max} (μg/ml) in plasma	Mean AUC (mg · min/l)	F (%) [a]	% dose [b]
Rectal	7	42 ± 14	8.6 ± 1.3	882 ± 162	81	82
Oral	7	10 ± 3	31 ±5.2	1042 ± 175	92	91

[a] F (bioavailability) was obtained by: AUC dosage form/AUC intravenously · 100%.
[b] From the cumulative urine data after 4 h.

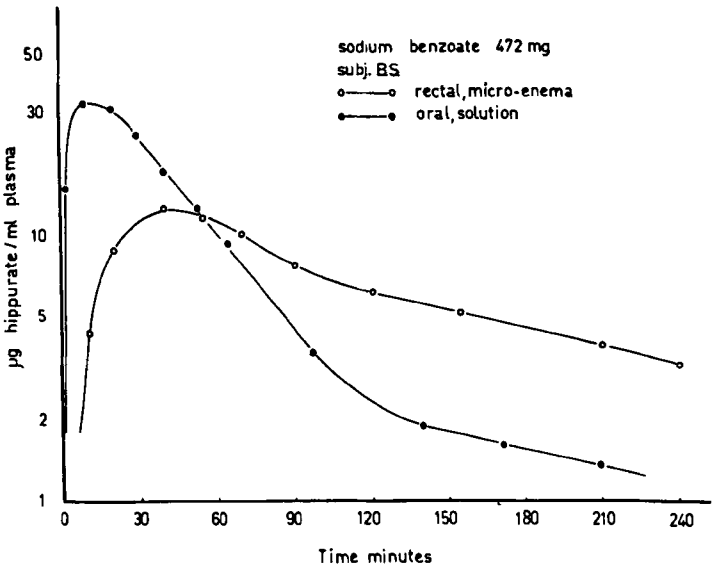

Fig. 4. Hippurate plasma concentrations (µg/ml) after oral and rectal administration of a dose of 472 mg sodium benzoate in solution form to subject B.S.

Sodium benzoate was given in the form of a solution (micro-enema) to 7 volunteers, who participated in a rectal study; no discomfort following application of this dosage form was reported. From Table 3 and Fig. 4 it can be seen that absorption was fast; the mean t_{max} in plasma being reached within 45 min. When compared with oral administration of an equal dose of benzoate it is clear that oral absorption proceeds more rapidly, in accordance with generally accepted theories concerning gastrointestinal absorption of organic acids by passive diffusion. After oral administration sodium benzoate is protonated and absorption of the unionized acid (pK = 4.2) occurs rapidly. The pH in the rectum is about 7.5, however, which is relatively unfavourable for absorption. In addition, there are other rate-determining parameters, such as the variation in the amount of faecal matter and the limited absorption surface (15–19 cm). Virtually no villi are present on the internal surface, there is no motility of the rectum and there is only little secretion of mucus.

Yet, with respect to the bioavailability of sodium benzoate, rectal absorption is almost complete. This can be concluded from the AUCs (Area Under the Cruve from t = 0 to t = 4) and the cumulative urine data. Bioavailability (F%) after 4 h was about 80% (after oral administration about 90%) and the same percentages were excreted via the urine. As a consequence, bioavailability has been found in the same range for the oral and rectal route.

After rectal administration of different concentrations of sodium benzoate (equivalent to 200, 400, 800 and 2000 mg benzoic acid per 10 ml micro-enema medium) it appears that there is an excellent correlation between plasma and urine levels of hippurate. This phenomenon is demonstrated in Fig. 5. The plot of the excretion rate of hippurate

TABLE 4

BENZOATE CONCENTRATIONS, CHARACTERIZING THE ABSORPTION PROCESS OF SODIUM BENZOATE AFTER RECTAL AND ORAL ADMINISTRATION OF A DOSE OF 472 mg (EQUIVALENT WITH 400 mg FREE ACID) TO 3 VOLUNTEERS

	Subjects	Mean t_{max} (min) in plasma	Mean C_{max} (μg/ml) in plasma	Mean AUC (mg · min/l)	F (%)	% dose [a]
Rectal	3	–	<1	35 ± 8.4	<10	–
Oral	3	9 ± 3	5.2 ± 1.4	80 ± 12.6	31	–

[a] No free benzoate could be detected in the urine.

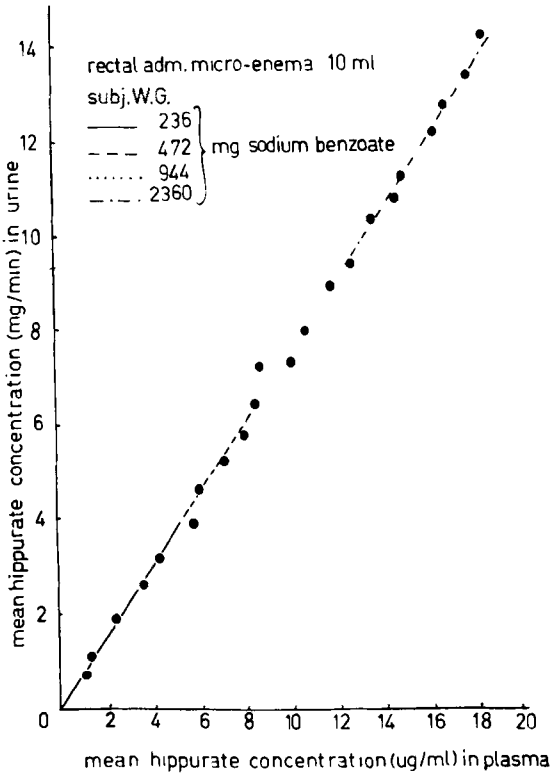

Fig. 5. Plot of excretion rate (mg hippurate/min) versus hippurate plasma concentrations (μg/ml) after rectal administration of different doses sodium benzoate in solution form to subject W.G.

against the plasma concentrations shows a linear correlation, even for the rectal solution of 2 g of benzoate. In this context it should be mentioned that after oral doses of more than 2 g of sodium benzoate the formation of hippurate is determined by the limited availability of glycine (Quick, 1933). In the present experiments linear pharmacokinetics can be assumed and urine excretion data can be used to determine absorption rate and bioavailability of dosage forms containing sodium benzoate. The concentration of unmetabolized benzoate in plasma after oral administration is small as a consequence of a substantial first-pass effect. After rectal administration only negligible concentrations of benzoate could be detected (Table 4). The argument of the supposed advantage of the rectal route to bypass the liver does not hold in the case that biotransformation is rapid relative to absorption. Only from hippurate levels it is relevant to follow the absorption process of benzoate.

Studies on the biopharmaceutics of rectal drug administration require rather large groups of volunteers who are required to provide frequent blood samples. This is often inconvenient to the volunteers, so it is important to consider other body-fluids. Especially in the case of benzoate, urine seems to be an excellent alternative. However, it is necessary to drink large quantities of water so that sufficient volumes of urine may be co

TABLE 5

SOME PHARMACOKINETIC PARAMETERS AFTER INTRAVENOUS ADMINISTRATION OF SODIUM BENZOATE TO SUBJECT B.S., WITHOUT DRINKING AND DRINKING ABOUT 8 LITERS OF WATER

Symbols are as defined in Table 1.

Name: B.S.	Without drinking	Drinking
Dose (free acid; mg)	400	400
V_d (litres)	80.0	72
Cl_{tot} (ml/min)	800	725
C_{max} hippurate (μg/ml)	26	23
t_{max} hippurate (min)	7	9
$t_{1/2}$ hippurate	150	155

lected every 15 min. It is possible that this hydration can interfere with some physiological parameters such as the blood flow through liver and kidney. However, from the data presented in Table 5 it may be concluded that drinking a large quantity of water (8 litres of water in 4 h) does not have a significant influence on the pharmacokinetic parameters of sodium benzoate.

CONCLUSION

From intravenous data is is concluded that the biotransformation of benzoate to hippurate is extremely rapid; at low drug concentrations pharmacokinetics appear to be linear. In addition, there is a linear correlation between plasma and urine levels.

After rectal administration of sodium benzoate in solution form, absorption is rather fast and complete; consequently hippurate plasma and urine levels give excellent information on the absorption process. Compared with oral data, pH environment and absorption surface in the rectum limit the absorption capacity. However, bioavailability is about the same.

In conclusion it can be said that sodium benzoate is a suitable drug to study the parameters which are regulating the release process from a dosage form in man by measuring hippurate levels in plasma or urine.

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