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BIOPHARMACEUTICS OF RECTAL ADMINISTRATION OF DRUGS IN MAN
II. EFFECT OF PARTICLE SIZE ON ABSORPTION RATE AND BIOAVAILABILITY

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SUMMARY

Particle size of readily water-soluble drugs has a significant influence on the release rate from a fatty suppository base in vitro. In a rectal investigation this phenomenon was studied in ten healthy volunteers, by using sodium benzoate as a test drug. The absorption process of benzoate was followed by determination of urine levels of hippurate. After insertion of suppositories containing a coarse powder (sieve fraction 125–250 μm), the absorption rate was significantly faster during 60 min as compared with a micronized powder (<20 μm); the transportation through the base was rate limiting. Absorption rate was maximal after administration of a rectal solution.

The influence of particle size of slightly soluble drugs (in this study benzoic acid) on the absorption rate proved to be much less than in the case of readily water-soluble drugs.

In the case of a micronized fraction the spreadability of the suppository mass can favour the absorption process.

As compared with the sodium salt, benzoic acid was absorbed more rapidly and with less inter-individual variation from a fatty base; this finding is in agreement with the pH-partition hypothesis.

In spite of all differences in absorption rate, the relative bioavailability of all rectal dosage forms in this study was the same (95% confidence).

Release from a fatty suppository base involves a two step mechanism. Firstly, the drug has to be transported to the lipid–water interface before entering the water phase. Most of the time drug particles are suspended in a suppository base and are readily soluble in water; consequently the drug is almost insoluble in lipid: this means that the transportation through the base is not (or to a very limited extent) taking place by diffusion, but by some mechanical process such as sedimentation of the suspended particles. In earlier studies (Schoonen et al., 1976), it was shown that for sodium salts of phenobarbital and salicylic acid, which are both very soluble in water, the transport through the base was the rate limiting step in vitro. Due to sedimentation, a coarse powder (sieve fraction

125–250 μm) was released considerably faster than a fine powder (particle size <20 μm).

The acids themselves are moderately soluble in lipid and therefore both sedimentation and diffusion mechanisms may operate. Also, since the acids are only moderately soluble in water, then in this case their dissolution in water must be the rate limiting step; in fact there was not much difference in release of both particle sizes from the suppository base.

The influence of particle size on the rectal absorption in man was investigated for pentobarbital sodium (Rutten-Kingma, 1977), aspirin (Parrot, 1975) and for thiazinamium methylsulphate (Kerckhoffs and Huizinga, 1976). Since the aqueous solubility of the particular drugs is rather different, and since each time another particle size was studied, it is difficult to generalize the results of these reports. Besides, the results were not always evaluated statistically, the number of volunteers was rather small and it was uncertain whether one of the two processes, absorption or release, was rate determining.

The purpose of this present study was to determine the influence of lipid solubility and particle size of drugs on absorption rate and bioavailability from a fatty suppository base in man. Benzoic acid was used as a test drug to study some parameters regulating release and absorption processes from rectal dosage forms; benzoic acid is lipid-soluble and moderately water-soluble, while sodium benzoate is an example of a drug that is insoluble in water. For both drugs the release rate of a fine powder (particle size <20 μm) was compared with a coarse powder (sieve fraction 125–250 μm).

Since a good correlation was established between plasma and urine levels of hippurate formed by an extremely rapid conjugation of benzoate in the liver, urine excretion data were used to measure the absorption rate and the bioavailability. A cross-over investigation was conducted in which absorption rate and bioavailability of the two test drugs for the above-mentioned particle sizes were studied in healthy volunteers. To establish the maximal pharmaceutical availability a solution of sodium benzoate in water (micro-enema) was administered rectally.

MATERIALS AND METHODS

Dosage forms

The drugs used in this study were benzoic acid (USP XVIII)[1] and sodium benzoate (Ned. Farm. ed. VII)[1].

A coarse powder (125–250 μm) was separated by sieving and a fine powder (particle size <20 μm) was prepared by grinding the drugs in a jet mill. The powders were mixed with the molten base Witepsol H 15[1], poured into brass moulds (4 ml) and stored in the refrigerator for at least one night before use. The suppositories contained 10% of benzoic acid or the equivalent amount of sodium benzoate (11.8%) and weighed 4.0 g.

The micro-enema was a solution of 472 mg of sodium benzoate in 10 ml of medium (0.5% methylcellulose 400 cP (Ned. Farm. ed. VI)[1] in distilled water). The pH was 7.7. This amount was administered rectally using a 10 ml plastic disposable syringe to which a plastic application tube was connected.

[1] Obtained from Interpharm ('s Hertogenbosch)

In vitro determinations

The release of the substances under study from the various suppositories at 37 ± 0.5°C was determined using the apparatus described by Schoonen et al. (1976). The absorbance of the samples was measured at 224 nm on a UV-VIS spectrophotometer (Beckmann 25). The results are mean values of four runs.

The viscosity of the base Witepsol H 15 and of the drug suspensions was measured with a Haake rotovisco 3 at a temperature of 37 ± 0.1°C. The viscosities of the masses showed a Newtonian character.

The solubility of benzoic acid and sodium benzoate in the base was determined by shaking (200 rpm) a saturated solution of both drugs in Witepsol H 15 during two days in a constant temperature air bath at 37°C. The mixture was centrifuged and about 5 g of the clear solution was weighed accurately and dissolved in 25 ml petroleum ether (40–60 ratio)[2]. After shaking with a 0.1 N solution of sodium hydroxide the absorbance was measured at 224 nm.

The melting points and the melting times at 37 ± 0.1°C of the base and the various drug suspensions were measured with the aid of the apparatus of Setniker and Fantelli (1962). To obtain some information about the spreading behaviour of the various suppositories, they were allowed to melt in an oven at 37°C on glass plates.

Human experiments

Protocol

Ten healthy human subjects, female and male, ranging in age from 22 tc 30 years and of body-weight 50–83 kg, participated in this study.

No drugs were taken for two weeks prior to or during the study. The investigation was performed as a cross-over study with intervals of at least two days. The experiments were started in the morning after an overnight fast and food was withheld during the time of the experiment. At least one hour before administration of the dosage forms the volunteers started drinking 200 ml of water every 15 min to enable collection of urine, also at 15 min intervals. At time zero a blank urine sample was collected and the dosage form was administered; urine levels were followed during a period of 4 h. Also in order to obtain some information about the intra-subject variation, the dosage forms were administered twice to the same volunteer.

Analytical determination

The analysis of hippurate in urine samples was modified from the method described by Amsel and Levy (1969). The amount excreted in 15 min was expressed in mg of benzoate.

Statistical treatment of the results

Differences in urine hippurate levels after administration of the various dosage forms were tested for statistical significance by Student's '*t*' test ($P = 0.05$ two sided). In pairs,

[2] Obtained from Baker Chemical N.V. (Deventer)

the excreted amounts of hippurate, expressed as benzoate, were compared after it had been established that these amounts were normally distributed applying Shapiro–Wilk's test ($P = 0.05$). These data afforded the relative absorption rate after administration of different dosage forms.

To investigate whether these differences in urine hippurate levels were due to administration of different dosage forms, an analysis of variance was carried out on the results. This operation made it possible to separate variances due to different physiological behaviour of the volunteers, of the dosage forms and of a possible interaction between the physiological behaviour and the dosage forms. The analysis of variance ($P = 0.05$) was applied to the cumulative amounts excreted in the first hour, since differences between absorption rates especially occurred during this period.

The bioavailability of sodium benzoate and benzoic acid from different dosage forms was calculated from the cumulative amount excreted in 4 h and tested in pairs for statistically significant differences by the Student's '*t*' test ($P = 0.05$).

To establish differences in the inter-subject variation with respect to benzoate absorption from the various dosage forms, the variance, for example, of the maximum urine level (C_{max}) after administration of a dosage form was compared to the variance of C_{max} after administration of a micro-enema. To prove a significant difference in variance, the test statistic

$$\frac{(n-1) \times s^2_{\text{dosage form}}}{s^2_{\text{micro-enema}}}$$

in which n is the number of experiments, has to be greater than the critical value $\chi^2_{\nu;\alpha}$ ($P = 0.05$ single-sided). This treatment is possible only if the urine data have been normally distributed; in the present study this was checked by the Shapiro–Wilk test ($P = 0.05$).

RESULTS AND DISCUSSION

In vitro experiments

The release of sodium benzoate and benzoic acid from suppositories was in agreement with the results found for sodium salicylate and salicylic acid. Reduction in particle size decreased the release rate of sodium benzoate, but gave only a small difference in the release rate of benzoic acid (Fig. 1). Since, with respect to the dosage forms, the differences in release rate could not be explained by the physical parameters in Table 1, it was concluded that they were caused by the differences in particle size and water and lipid solubilities.

For sodium benzoate, which is insoluble in Witepsol H 15 and is very soluble in water (1 g in 1.8 g), sedimentation in the base was the rate limiting step for release in vitro.

Sedimentation rate is proportional to the square of particle size (Stokes). This implies that a coarse powder is released faster than a fine one. Particle size reduction of benzoic acid, which is soluble in Witepsol H 15 (1 g in 16 g), gave only small differences; the dissolution rate of benzoic acid (1 g in 400 g) in water is probably rate limiting. Particle size also affected the spreading behaviour: a suppository containing the fine powde

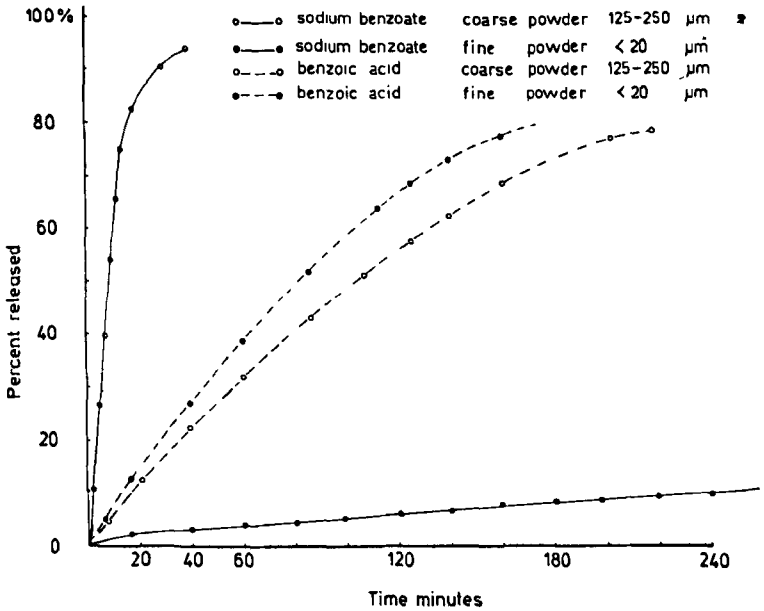

Fig. 1. Effect of particle size on release of sodium benzoate and benzoic acid from Witepsol H 15.

melted as a homogeneous mass, whereas in the molten mass containing the coarse fraction, sedimentated particles could be seen, which were not carried along by the base.

In vivo experiments

Comparison of a micro-enema and suppositories containing sodium benzoate

To determine the meaning of these findings with respect to absorption rate and bio-

TABLE 1

PHYSICAL PARAMETERS AND PERCENTAGES OF DRUG OF THE DIFFERENT SUPPOSITORIES

	Viscosity (37°C) (cP)	Melting time ±S.D. (37.5 ± 0.1°C) (min)	Melting point ±S.D. (°C)	Percentage of drug ± S.D. (%)
Witepsol H 15	37.2	9.59 ± 0.05	34.5 ± 0.1	
Witepsol H 15 + 11.8% sodium benzoate (<20 µm)	56.0	11.22 ± 0.12	34.5 ± 0.1	10.06 ± 0.23
Witepsol H 15 + 11.8% sodium benzoate (125–250 µm)	42.3	13.25 ± 0.04	34.9 ± 0.1	9.76 ± 0.26
Witepsol H 15 + 10% benzoic acid (<20 µm)	40.6	8.09 ± 0.07	33.2 ± 0.1	10.02 ± 0.40
Witepsol H 15 + 10% benzoic acid (125–250 µm)	38.4	9.32 ± 0.10	33.6 ± 0.1	10.01 ± 0.33

availability, sodium benzoate suppositories with the two particles sizes were administered to ten volunteers. Since it has been established that rectal absorption of this amount of benzoate was not the rate-limiting step, and since the dosage forms were given under identical circumstances, differences in excretion of hippurate in urine must occur mainly as a result of differences in pharmaceutical availability of benzoate. For a valid comparison all volunteers were given a rectal solution with the same amount of sodium benzoate. In this way maximal pharmaceutical availability was obtained.

Mean urine excretion data of hippurate (expressed as benzoate) are given in Table 2 and in Fig. 2. The following conclusions may be drawn from statistical evaluation of these experimental data:

(i) after administration of the coarse sodium benzoate suppository, urine levels are significantly higher during 60 min (four periods) than after administration of the fine powder suppository;

(ii) administration of a rectal solution of sodium benzoate resulted in significantly higher urine levels than after administration of the coarse sodium benzoate during 30 min (two periods); and

(iii) the cumulative amounts excreted in the urine during 4 h after administration of the three dosage forms did not differ significantly.

It is obvious that the rate differences observed during the first two periods between the micro-clysma and the coarse sodium benzoate suppositories were due to the melting

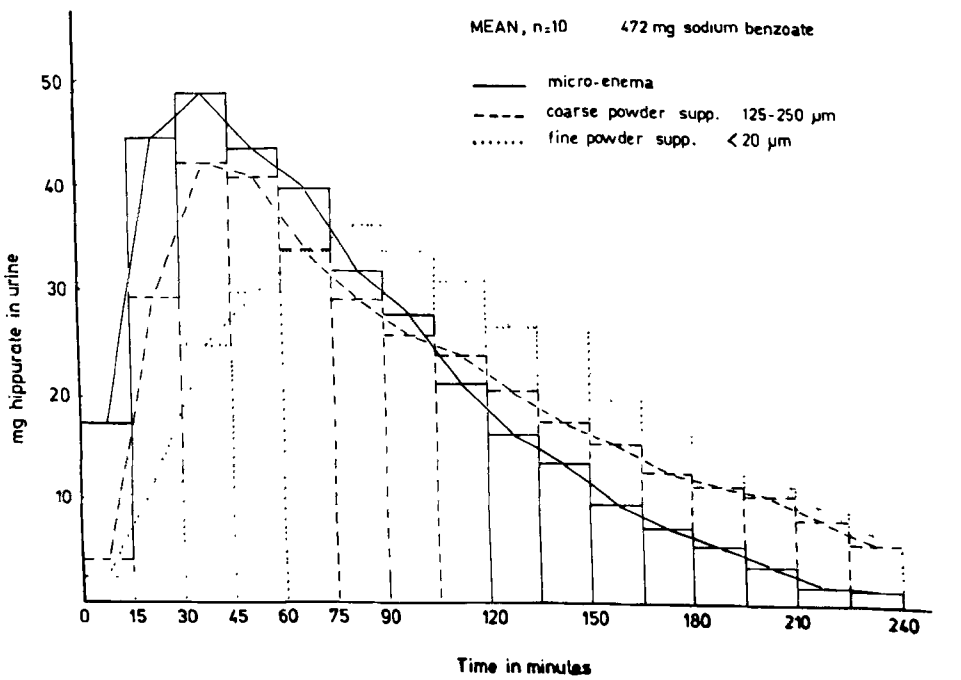

Fig. 2. Mean hippurate urine concentration curve (expressed as benzoate) after rectal administration of sodium benzoate in different dosage forms. For convenience in presentation a line has been drawn through the mid-points of the urine-block diagrams.

TABLE 2

MEAN URINE EXCRETION DATA OF HIPPURATE EXPRESSED AS BENZOATE AFTER RECTAL ADMINISTRATION OF SODIUM BENZOATE AND BENZOIC ACID

Period (min)	472 mg sodium benzoate						400 mg benzoic acid	
	Fine powder (<20 μm)		Coarse powder (125–250 μm)		Micro-enema		Fine powder (<20 μm)	
	Mean ± S.D. (mg)	(%) [a]	Mean ± S.D. (mg)	(%) [a]	Mean ± S.D. (mg)	(%) [a]	Mean ± S.D. (mg)	(%) [a]
P_1 (0–15)	2.3 ± 2.6	(114.4)	4.0 ± 3.2	(81.1)	17.2 ± 6.7	(39.1)	10.9 ± 4.5	(41.9)
P_2 (15–30)	11.7 ± 7.1	(61.0)	29.1 ± 14.5	(49.8)	44.5 ± 9.7	(21.9)	43.1 ± 10.7	(24.9)
P_3 (30–45)	24.6 ± 11.4	(46.4)	42.1 ± 14.0	(33.3)	48.9 ± 8.3	(16.9)	46.6 ± 9.1	(19.5)
P_4 (45–60)	29.8 ± 11.6	(39.0)	40.7 ± 13.6	(33.5)	43.4 ± 7.2	(16.5)	44.4 ± 8.6	(19.4)
P_5 (60–75)	33.5 ± 10.7	(32.0)	33.5 ± 10.0	(29.8)	39.5 ± 8.8	(22.4)	37.0 ± 8.3	(22.3)
P_6 (75–90)	36.0 ± 10.3	(28.6)	28.9 ± 10.4	(36.3)	31.6 ± 6.0	(19.1)	32.7 ± 8.2	(25.0)
P_7 (90–105)	33.2 ± 10.2	(30.7)	25.5 ± 6.8	(26.7)	27.5 ± 5.2	(18.9)	24.6 ± 6.4	(26.1)
P_8 (105–120)	30.6 ± 11.3	(36.8)	23.7 ± 8.4	(35.5)	21.1 ± 6.6	(31.4)	20.6 ± 5.4	(26.1)
P_9 (120–135)	26.3 ± 9.8	(37.4)	20.3 ± 6.0	(29.7)	16.2 ± 6.3	(38.6)	17.3 ± 5.7	(33.1)
P_{10} (135–150)	25.9 ± 9.3	(36.1)	17.3 ± 4.6	(26.8)	13.3 ± 5.9	(44.6)	15.8 ± 5.3	(33.2)
P_{11} (150–165)	19.3 ± 8.2	(42.5)	15.1 ± 6.1	(40.5)	9.5 ± 5.0	(52.5)	11.3 ± 4.9	(43.4)
P_{12} (165–180)	16.8 ± 7.4	(44.3)	12.5 ± 6.1	(48.7)	7.2 ± 4.4	(61.2)	10.0 ± 4.5	(44.7)
Cumulative amount in 4 h	320.5 ± 70.5	(22.0)	330.0 ± 60.4	(18.3)	330.8 ± 33.8	(10.2)	341.4 ± 40.1	(11.7)

[a] Variation coefficient.

process of the fatty suppositories. After this lag time the urine data showed similar profiles.

It is evident that the influence of particle size reduction on the release of suppositories is of importance with regard to rectal administration of sodium benzoate. It is remarkable that bioavailability in 4 h was the same after administration of both solid dosage forms (95% confidence), although in vitro only 15% of the fine powder was released in 6 h. Obviously there are factors other than sedimentation in the physiological milieu, which determine transportation of lipid-insoluble drugs into the rectal fluid.

The spreading behaviour of the suppository, in which particles have been suspended, may be important in this respect, especially with regard to the micronized fraction of lipid-insoluble drugs. This increase in area together with an intra-abdominal pressure of the rectum wall on the suppository mass, which facilitates the transportation of the drug into the rectal fluid, might explain the bioequivalence.

Comparison of suppositories containing benzoic acid

In a preliminary investigation in which eight volunteers participated, suppositories containing benzoic acid with both particle sizes were compared. Mean renal excretion profiles are given in Fig. 3. There appeared to be a difference in rate during the first 30 min, to the advantage of the fine fraction of benzoic acid. However, this difference was not significant. Differences in transportation of the drug through the base and in spreading behaviour are both covered by the slow dissolution rate of benzoic acid in water. No differences were found in the cumulative amount excreted after 4 h.

Comparison of suppositories containing sodium benzoate and benzoic acid

There was a substantial difference in release rate in vitro between benzoic acid and its sodium salt, the latter releasing at least twice as fast. To show possible differences in

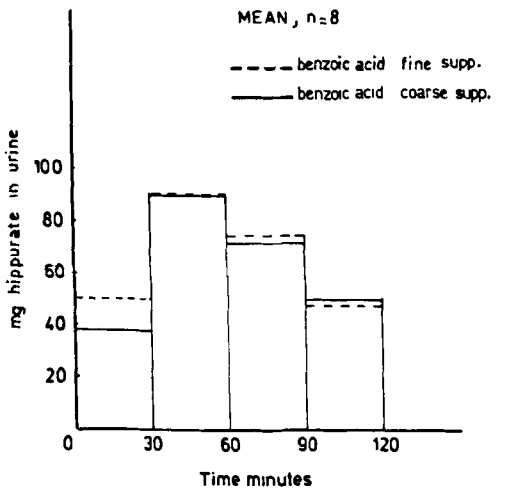

Fig. 3. Mean hippurate urine concentration curve (expressed as benzoate) after rectal administration of suppositories with benzoic acid of different particle size.

absorption rate between suppositories containing sodium benzoate or the free benzoic acid, a cross-over study was performed with that particle size of the drugs which previously resulted in the higher urine levels. For this reason, suppositories containing the fine fraction of benzoic acid were administered to seven volunteers of the sodium benzoate study. Mean excretion data are given in Table 2 and in Fig. 4. During 30 min (two periods) urine levels were significantly higher after administration of the fine benzoic acid suppository. However, there were no significant differences in the cumulative amount in 4 h.

When the free acid enters the rectal fluid it will cause a drop in the pH; for this reason there will be a high concentration of the non-ionized moiety of benzoic acid in the rectal fluid ($pK_a = 4.2$). If it is assumed that this concentration is the driving force in absorption it can be rationalized that a higher concentration of the sodium salt in the rectal fluid compared to the acid will not be measured in vivo. A limiting step is the small amount of rectal fluid; administration of a micro-enema in which benzoic acid is suspended might result in even higher levels, but a strong pH reduction (to about pH 3.0) is somewhat uncomfortable for volunteers.

Analysis of variance

In all comparisons between the various rectal dosage forms the influence of the dosage form on the excretion was significant.

Inter-subject and intra-subject variation

Since there was a good correlation between hippurate levels in plasma and urine, values of P_{max} (period in which the highest amount of hippurate is excreted in urine) and C_{max}

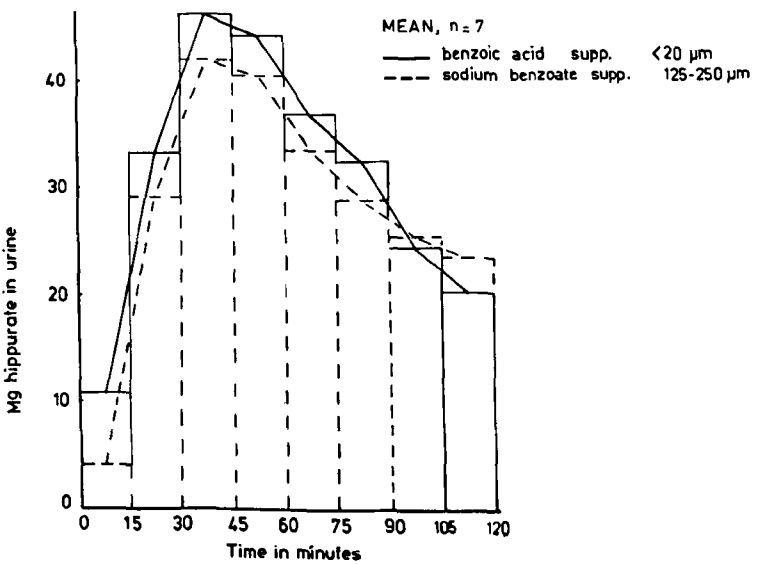

Fig. 4. Mean hippurate urine concentration curve expressed as benzoate after rectal administration of suppositories with benzoic acid and sodium benzoate. For convenience in presentation a line has been drawn through the mid-points of the urine-block diagrams.

TABLE 3

INDIVIDUAL ABSORPTION CHARACTERISTICS OF SODIUM BENZOATE AND BENZOIC ACID AFTER RECTAL ADMINISTRATION OF DIFFERENT DOSAGE FORMS

Subject	472 mg sodium benzoate												400 mg benzoic acid			
	Fine powder (<20 μm)				Coarse powder (125–250 μm)				Micro-enema				Fine powder (<20 μm)			
	P_{max} [a]		C_{max} (mg)		P_{max} [a]		C_{max} (mg)		P_{max} [a]		C_{max} (mg)		P_{max} [a]		C_{max} (mg)	
	I	II [b]	I	II [b]	I	II [b]	I	II [b]	I	II [b]	I	II [b]	I	II [b]	I	II [b]
1	P_4	P_6	53.5	53.3	P_3	P_4	53.1	31.3	P_2	P_4	59.9	46.6	P_5	P_4	47.3	58.5
2	P_5	P_8	35.9	43.9	P_4		43.6		P_3	P_3	64.1	57.1	P_2	P_3	44.5	54.8
3	P_6		25.7		P_4	P_3	34.5	32.1	P_5	P_4	47.5	46.6	P_2	P_3	39.9	56.0
4	P_6		36.5		P_2	P_3	49.7	34.5	P_2	P_2	47.5	42.2	P_2	P_2	51.0	60.0
5	P_{12}	P_7	24.4	30.8	P_3		44.5		P_4	P_3	49.9	37.2	P_2	P_3	40.3	45.1
6	P_8		52.0		P_3	P_4	49.4	75.1	P_3	P_3	54.7	59.3	P_5	P_4	50.4	56.1
7	P_4	P_5	48.3	42.2	P_3	P_3	78.4	57.1	P_3	P_3	56.5	60.9	P_2	P_3	61.4	46.1
8	P_7		45.9		P_4		51.2		P_3	P_2	44.3	45.7				
9	P_7		42.5		P_4	P_6	65.8	35.3	P_3		59.1					
10	P_3		25.7		P_4		37.1		P_4	P_3	50.3	42.7				
Mean ±S.D.	P_7		40.0 ± 10.4		P_4		48.3 ± 14.8		P_4		51.1 ± 7.6		P_3		50.8 ± 7.1	
Variance			107.6				220.4				57.8				51.0	

[a] P_{max} refers to the period in which the maximum urine concentration (C_{max}) is reached.
[b] Repeated administration to determined the intra-subject variation.

(the highest amount of hippurate expressed as benzoate, in period P_{max}) are a measure for the absorption process (Table 3). It was observed from individual excretion characteristics of the different dosage forms that there was a substantial inter-subject variation with respect to sodium benzoate and benzoic acid absorption from suppositories.

After administration of the coarse sodium benzoate suppository or the rectal solution form there were only small differences between the values of the mean C_{max} and P_{max} (respectively 48.3 mg and 51.1 mg both in period 4). Statistical analysis, however, showed a significantly smaller inter-subject variation after administration of the rectal solution form.

The variation in P_{max} was very substantial for suppositories with the fine sodium benzoate fraction; this is probably due to the transportation through the base which is the rate limiting step. This process is strongly influenced by the spreadability of the suppository mass.

After repeated administration of the same dosages forms to the same volunteers under identical conditions, it is concluded that a considerable intra-subject variation exists; a smaller intra-subject variation for the rectal solution form was also observed (Table 3).

CONCLUSION

Further studies with other drugs for which a sufficiently fast rectal absorption has been established, should determine whether the results from this present study are applicable in a more general context, in particular to determine whether these results can be generalized to the following conclusions: whether an aqueous solution or suspension of a drug is required if speed of action and exact dosage are desired. In the case of suppositories, the particle size of the suspended drug in a suppository base affects the absorption rate in man; thus a readily water-soluble drug should be used in a coarse powder if a fast effect is required; while for a sustained release effect such drugs should be used in a micronized powder form. Furthermore, if dissolution of the drug in water is slow, then particle size reduction will reduce the absorption rate. A further point of importance is that when the drug is micronized, the absorption rate is favoured by the ability of the suppository mass to spread in the rectum.

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