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BIOPHARMACEUTICAL STUDIES OF FATTY SUSPENSION SUPPOSITORIES III. INFLUENCE OF PARTICLE SIZE AND CONCENTRATION ON BIOAVAILABILITY OF LITHIUM SULPHATE IN RATS

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SUMMARY

The influence of particle size and concentration of lithium sulphate on the bioavailability in rats is reported. Particles <10 μm exhibit a retardation in absorption as compared with larger ones (45–55 μm, 90–125 μm and 500–630 μm). This is more evident in suppositories containing 20% of the compound than in suppositories containing 5 and 10%, respectively. The results fit a model in which the transport of suspended particles in the suppository vehicle towards the interface with the rectal fluid is rate-limiting. It is therefore suggested to set a lower limit for particles in suspension suppositories, especially for readily water-soluble, hydrophilic, drugs.

INTRODUCTION

In foregoing parts of this series the spreading of suspension suppositories was studied in the rat (Rutten-Kingma et al., 1979a) and subsequently the influence of particle size and concentration of the in vitro release of readily water-soluble compounds (Rutten-Kingma et al., 1979b). In the present paper the in vivo results studying the same particle parameters are presented.

Before testing the bioavailability of dosage forms in animals the choice of the species is a major consideration. Barr (1972) considered the small blood volume in small laboratory animals as an important disadvantage, especially for blood level measurements. By applying modern analytical techniques the sample volume may be reduced considerably, however. Furthermore, relatively large blood samples (up to 1 ml) may be withdrawn

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from rats if the blood is replaced by a physiological salt solution. In this way Agrelo and Miliozzi (1974) followed the blood level in rats during 4 h, taking 5 ml blood in 1 ml samples. In other studies several authors obtained valuable information on the bioavailability after oral administration using rats, thanks to the physiological and anatomical resemblances of the rat and human intestine (e.g. Riegelman and Crowell, 1958; Tardos et al., 1959; Brenner, 1968; Ayres et al., 1976). With respect to rectal administration, histologically as well as anatomically different situations exist in rats and in man, but this does not seem to be predominant over the apparent advantages. Large homogeneous populations of rats are available thus reducing the interindividual variation and hence the number of experiments required.

MATERIALS AND METHODS

Suppositories, weighing about 50 mg, were prepared as described in the previous papers (Rutten-Kingma et al., 1979a, b), using Witepsol H5. They measured 1.5 cm in length and approximately 2 mm in diameter. Lithium sulphate was classified with an air jet sieve (Alpine) in classes of <90 μm, 90–125 μm and 500–630 μm. The smallest class was further subdivided with a centrifugal classifier (Alpine), and the classes <10 μm and 45–55 μm were selected for further use. All particles in the class <10 μm obeyed the size limits, while in the other classes at least 85% did. The mean contents of the suppositories, and standard deviations, are given in Table 1.

The different series will be indicated as 5, 10 and 20% m/m respectively, although the real concentrations will be somewhat different (see Table 1).

During the experimentation special attention was paid to reduce the faecal output of the rats as much as possible by feeding a low residue diet (Vivonex) for at least two days. Lithium sulphate was dosed on a body weight basis by cutting an appropriate part from the rod-shaped suppositories. After administration at full consciousness the anus was

TABLE 1

MEAN CONTENT (mg AND m/m%), WITH STANDARD DEVIATION, OF LITHIUM SULPHATE SUPPOSITORIES (n = 10)

Class	Content (mg)	m/m (%)	S.D. (mg)	s_{rel} (%)
<10 μm	2.47	4.9	0.044	1.8
	5.18	10.3	0.177	3.4
	9.49	18.3	0.317	3.3
45–55 μm	5.67	9.9	0.046	0.8
	8.78	16.8	0.436	5.0
90–125 μm	2.43	4.8	0.093	3.8
	4.70	9.2	0.189	4.0
	9.60	18.8	0.311	3.2
500–630 μm	4.72	9.2	0.472	10.0
	8.50	17.0	0.614	7.8

closed by clips. Blood samples were taken from the venous plexus behind the eye under brief ether anaesthesia, and the blood concentration determined by flame ionization. After centrifugation at 3000 rpm, 0.1 ml of plasma was diluted to 1.0 ml with a solution of 3.00 g/litre cesium chloride in water containing 6% butanol. All data points were corrected for the blank level, equal to 0.2 mg/litre lithium. In all series data were collected on at least 10 rats who did not expel the suppository during the experiment. As a control to every series, 10 rats were given an intraperitoneal injection containing the equivalent amount of lithium. The relative bioavailability was calculated from the AUCs following administration of the i.p. injection and the suppositories, respectively, with correction for the undetermined area to infinity.

RESULTS AND DISCUSSION

The intraperitoneal injections were given for two reasons. Firstly, three doses, i.e. 2.5, 5 and 10 mg lithium sulphate per 200 g body weight, were administered to find out whether the amount absorbed would be linearly related with the dose. This proved to be so, thus permitting correction for differences in administered amount between the series. Secondly, we came to the conclusion that the intra-group variation was acceptable, but that different groups of rats used in the same experiment with an interval of a few months could give different blood levels, making a standard (as the i.p. injection) necessary for every population.

The plasma levels are presented as measured, i.e. not corrected for the dose. Thus for quantitative interpretation the curves as well as the tables have to be used.

In Fig. 1 the results of experiments comparing particles <10 μm and 90–125 μm are given for the 20% series. At 5, 10 and 20 min after administration the blood levels differ

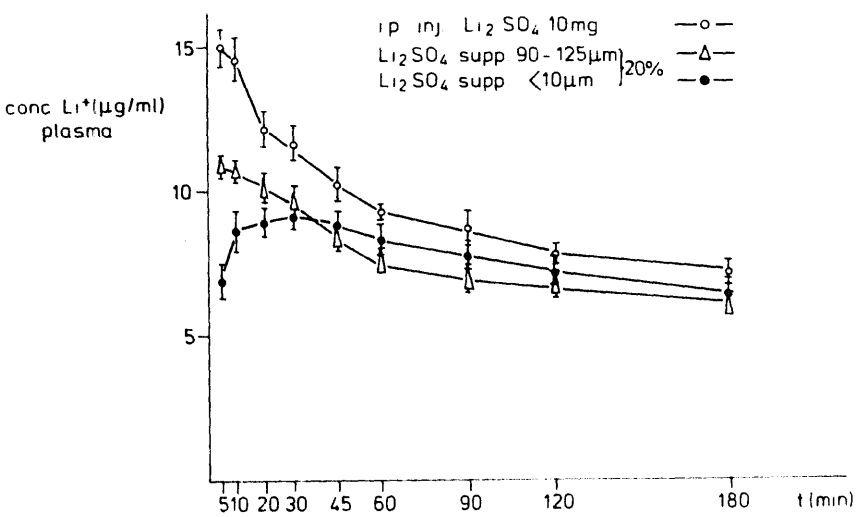

Fig. 1. Mean plasma levels, with standard deviation, obtained in 10 rats following an i.p. injection (o———o) and administration of suppositories containing 20% lithium sulphate of different particle sizes (Δ———Δ, 90–125 μm; •———•, <10 μm).

significantly ($P \leqslant 0.02$). The levels obtained with the 90–125 μm particles reached a maximum within 5 min, while the small particles (<10 μm) took as long as 30 min to do this. The relative amount absorbed for both particle sizes is equal, and somewhat lower than that after an i.p. injection. These data are summarized in Table 2 together with the data obtained in the other series.

If the data from Fig. 1 are plotted on a semi-logarithmic scale, the elimination phase proves to be linear, with a half-life of approximately 5 h. This value is in good agreement with Schou (1958) who reported a half-life of 6 h after i.v. injection. The route and particular form of administration did not alter the value for the half-life given here.

In Fig. 2 another series of experiments with the 20% series is shown, i.e. particle sizes of 45–55 μm and 500–630 μm compared with an i.p. injection. The 500–630 μm particles resulted in somewhat higher blood levels than the 45–55 μm particles (paired *t*-test, $P < 0.01$). It should be borne in mind, however, that the content uniformity for the largest particles is poor (Table 1), which might have distorted the results. No difference was observed in the rate of absorption as reflected by the t_{max}. The relative bioavailability for the 45–55 μm particles is not much different from that for <10 μm and 90–125 μm particles, whereas that for the largest particles is higher.

Thus for the 20% series the main conclusion has to be that the smallest particles (<10 μm) show a retarded absorption as compared with the other ones studied, and that the amount absorbed was not affected.

The results for the 10% series are given in Fig. 3 for all particle sizes and this time in one i.p. series only, since they were performed within a short interval, in which the i.p. series overlapped. The curve obtained with the smallest particles is lower than the others at all times. No differences exist between the times at which the maximal blood levels are observed, indicating that the particle size does not affect the absorption rate in contrast with the 20% series. The amount absorbed, however, shows more variation. Especially both the smaller fractions (<10 μm and 45–55 μm) give lower values here (Table 2, column F_{rel} corrected), which provides an indication that the absorption is retarded nevertheless. The larger particles do not show the behaviour as in the 20% series.

Since the differences observed in the 10% series were already less outstanding than in the 20% series, the 5% series was only studied with particles of <10 μm and 90–125 μm, which are two extremes likely to be encountered in suppository formulation. The results, shown in Fig. 4, show very little difference between the particles sizes. Strictly speaking, the larger particles exhibited a higher t_{max}, but the reliability of the assessment of t_{max} in such flat curves is questionable. If so, the effect would be in contradiction with the one observed in the 10% and 20% series. The relative bioavailability of the smallest particles is definitely lower (~70%) than for the larger ones (~95%), which would indicate a retarded absorption. This would not comply with the t_{max} values. It is apparent, however, that at all sampling times (except 20 min) the smaller particles exhibit the lower blood levels, which cannot be attributed for by the doses given. This supports a retardation in absorption.

The role of particle concentration can be read from Table 2. Little influence is observed, except for the smallest particles. The absorption is somewhat retarded in the 20% series compared with the 5% and 10% series. A generalization therefore does not seem justified as yet. The bioavailability, under influence of concentration, shows a higher

TABLE 2

MEAN KINETIC DATA IN 10 RATS ON LITHIUM SULPHATE SUPPOSITORIES, CONTAINING DIFFERENT PARTICLE SIZES AND DIFFERENT PARTICLE CONCENTRATIONS

Series	Particle size (μm)	Dose (mg)	C_{max} (μg/ml)	t_{max} (min.)	AUC 0–180 (μg · min/ml)	AUC 0–∞ (μν · min/ml)	F_{rel} 0–180 (%)	F_{rel} 0–∞ (%)	F_{rel} corr. [a] (%)
20%	i.p.	10	14.6	5	1550	3510	100	100	100
	<10	9.5	9.1	30	1380	3270	89	90	95
	45–55	8.8	10.7	10	1380	3180	89	90	101
	90–125	9.6	10.6	5	1380	3180	89	90	94
	500–630	8.5	12.0	10	1560	3630	100	103	120
10%	i.p.	5.0	6.9	5	705	1545	100	100	100
	<10	5.2	3.0	20	395	1025	57	66	64
	45–55	5.7	4.9	20	655	1425	93	94	80
	90–125	4.7	3.6	20	530	1310	76	87	90
	500–630	4.7	4.4	20	530	1340	76	89	93
5%	i.p.	2.5	3.6	5	375	855	100	100	100
	<10	2.5	1.9	20	245	625	63	72	72
	90–125	2.4	1.9	45	305	795	80	92	95

[a] Corrected for the dose.

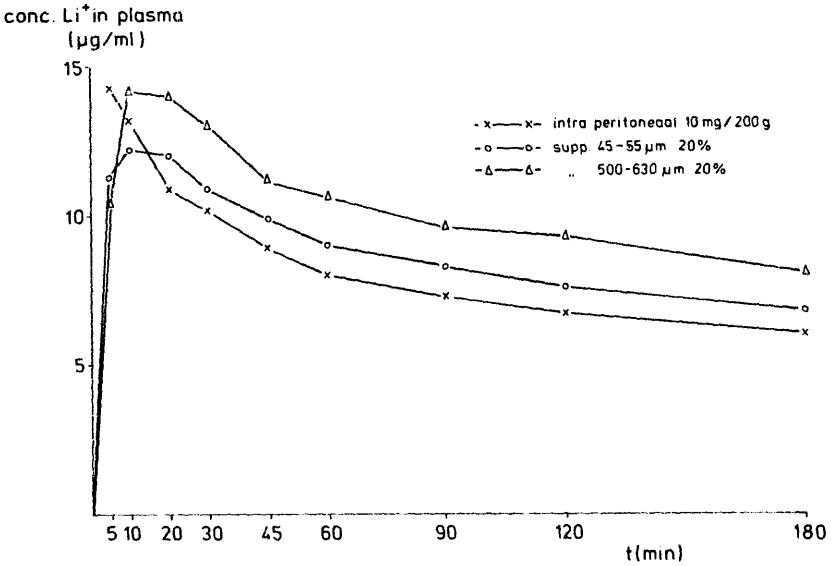

Fig. 2. Mean plasma levels obtained in 10 rats following an i.p. injection (x———x) and administration of suppositories containing 20% lithium sulphate of different particle sizes (o———o, 45–55 µm; Δ———Δ, 500–630 µm).

value in the 20% series for the <10 µm particles, the same effect being found for the 45–55 µm ones. No explanation can be offered for this phenomenon. In conclusion, the effects observed in the in vivo experiments presented here seem to be in agreement with the findings in vitro presented in our previous paper (Rutten-Kingma et al. 1979b), as far as the influence of particle size is concerned. The effect of particle concentration

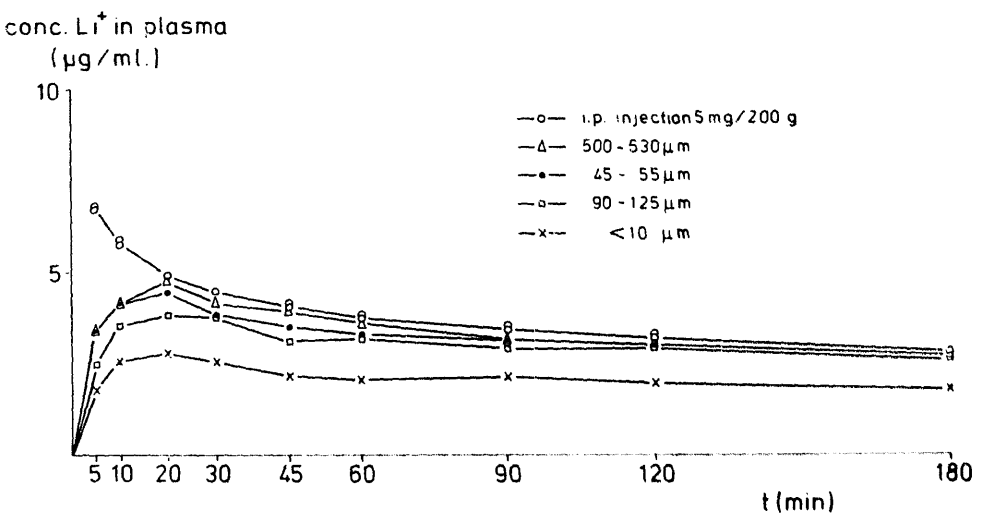

Fig. 3. Mean plasma levels obtained in 10 rats following an i.p. injection (o———o) and administration of suppositories containing 10% lithium sulphate of different particle sizes (Δ———Δ, 500–630 µm; □———□, 90–125 µm; •———•, 45–55 µm; x———x, <10 µm).

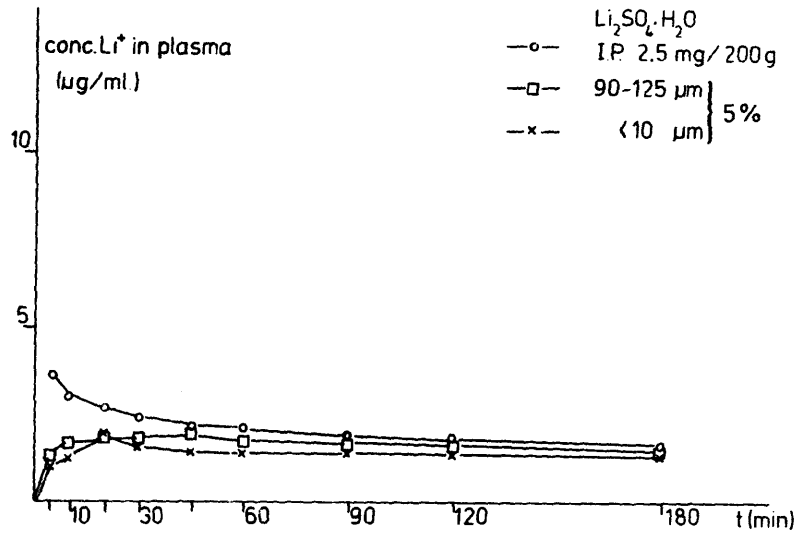

Fig. 4. Mean plasma levels obtained in 10 rats following an i.p. injection (o———o) and administration of suppositories containing 5% lithium sulphate of different particle sizes (□———□, 90–125 µm; x———x, <10 µm).

is more evident in vitro and less clear in vivo. There is a tendency to show more effect of particle size, in vitro as well as in vivo, as the particle concentration increases. This suggests a possible role of the apparent viscosity of the suppositories at 37°C.

The results seem to fit the release model proposed, in which transport of suspended particles towards the suppository/rectal fluid interface may act as a rate-limiting step in the absorption process. Since, for the readily water-soluble compound studied, dissolution will be rapid enough, and since also wetting by the aqueous rectal fluid (or dissolution medium) is not likely to be very important, this automatically leaves transport within the suppository as the most important step. This will become more so, as this step becomes slower in comparison with the subsequent wetting and dissolution, which will occur in the case of an increasing vehicle viscosity.

The influence of particle size on transport within the suppository may be approximated through a sedimentation process. Although Stokes' law cannot be more than a rather rough indication in these systems, it would explain why the influence of particle size in vitro (layer thickness ≈3 mm) is more evident than in vivo (layer thickness estimated close to 1 mm). Because of this it therefore seems not unreasonable to assume that particles >50 µm would exhibit sedimentation times of around 1 min only, whereas for <10 µm particles this would be more like 20 min. Thus only very small particles would be expected to show a retardation for this reason. Furthermore, it seems clear enough that the model proposed might be more fruitful in explaining the behaviour of drugs in suppositories than the model usually applied for ointments, which have too high a viscosity to exhibit sedimentation at all.

A factor of influence on the in vivo experiments may be the spreading of the suppositories. As shown in Rutten-Kingma et al. (1979a) this may happen quite irregularly,

which would certainly have a masking effect on the differences due to other factors.

In general it seems advisable to limit the particle size in suspension suppositories not only to, e.g., *below 150 μm* to avoid content uniformity problems, but also to, e.g., *above 50 μm* to avoid retardation in absorption. This latter point would be especially important for readily water-soluble, hydrophilic, drugs.

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