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Influence of physicochemical interactions on the properties of suppositories IV. Factors influencing the in vivo release of ketoprofen and metronidazole from fatty suppository bases

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Summary

The advantages and disadvantages of animal models employed for the testing of suppositories in vivo are discussed and the rat has been selected for the present work. Some of the important variables involved in these tests have been investigated using fatty suppositories consisting of binary mixtures of pure mono-acid triglycerides. The drugs employed, ketoprofen and metronidazole, give solution and suspension suppositories, respectively. Using Kendall's method of rank correlation, the bioavailability of ketoprofen or metronidazole has been found to correlate significantly with the rate of spreading of fatty suppositories but not with the plastic viscosity of the base, determined in a rotational viscometer. Accurate prediction of the plots of drug plasma levels vs time from individual mean data points from a number of rats is found to be difficult as reflected by the wide 95% confidence limits. The main use of such plots is to indicate trends. The highest blood level profiles for both the solution and suspension suppositories are found to be given by formulations containing the binary triglyceride mixture consisting of 60% w/w tricaprin and 40% w/w trilaurin as constituents of the base.

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Introduction

There appears at present to be no generally accepted method for determining the in vitro release of drugs from suppositories. Although a number of attempts have been made to develop such methods, each is fraught with problems or drawbacks. The technique used must be tailored to each individual drug and excipient. Before designing an in vitro method, in vivo experiments must be performed to define the parameters and constraints that are likely to be involved.

The most important variable in vivo is the nature of the experimental animal. Ideally, the human volunteer is the best choice but is clearly out of the question in the early stage of research, although some workers have used humans in tests of the bioavailability of certain drugs from suppositories (Möes and Jaminet, 1976; Caillé et al., 1980; Bergen and Arnold, 1980). Human studies were impractical in the present work, because of legal, ethical and economic considerations.

The results obtained in animal studies may not be applicable to humans on account of differences in: (a) the relative concentrations of the drug in the various tissues; and (b) the physiology of the rectum. The differences in rectal physiology will generally have a pronounced effect on the bioavailability of a drug for the following reasons. (i) The amount of liquid present in the rectum will vary from species to species and hence the amount of drug in solution and available for absorption will also vary. (ii) The pressure and peristaltic movement in the rectum, which together control spreading, will vary from species to species. (iii) The anatomy of the rectum will vary, so that absorption through the membrane and transport away from the membrane will vary and may therefore affect the first-pass mechanism which is frequently encountered in the administration of suppositories (Jonkermann et al., 1979). (iv) The liquefaction time of the suppository will differ on account of differences in suppository size required for different species. For example, a suppository suitable for a dog would have to be made smaller to be accommodated by a rat and would then melt more quickly, corresponding to a shorter liquefaction time and a more rapid onset of drug absorption. (v) The temperature and temperature gradient of the rectum may vary between different animal species and man. (vi) Most animals are anaesthetized prior to insertion of a suppository which is then sealed within the rectum by means of anal clipping or anal adhesion. The administered anaesthetic then reduces body temperature, blood flow to the rectum and peristaltic movement, effects which may combine to give a reduced absorption. However, even with these constraints, in vivo data obtained using animals can be valuable because it can lead to more refined human studies.

A survey of the animals used in rectal work highlights 5 species, namely, rabbits, rats, dogs, guinea pigs and mice. The dog is the preferred animal model because its rectum is similar to that of man (Lowenthal et al., 1970; Borzelleca and Lowenthal, 1966; Miller et al., 1964). Suppositories of normal commercial size can be applied to dogs, so that spreading and liquefaction time should be similar to those in humans facilitating correlation of canine results with human data. The disadvantages of the dog are high maintenance expenses and certain legal restrictions. Mice are very inexpensive, but their small size gives rise to the following disadvantages: (a) the

small suppository used in mice has a liquefaction time quite different from that used for humans; (b) the amount of blood available for assay (up to 1 ml) is very much smaller than from man. The guinea pig is relatively inexpensive and is large enough to enable suitable quantities of blood to be taken for assay, but has the disadvantage (a) above. Furthermore, the guinea pig has no tail and a very loose coat, making handling of the animal and insertion of the suppository very difficult. The rabbit has the advantage of requiring a suppository of a more representative size and the capacity for providing plenty of blood for assay. The disadvantages of rabbits are that they are relatively expensive and difficult to handle. The rat has the advantage of cheapness, of being large enough to provide sufficient blood for assay and of ease of handling. The disadvantage of the rat is the unrepresentative size of the suppository used. Although the rabbit might normally offer the best compromise, the rat was selected for the present in vivo bioavailability work, because funding for the research was limited.

Previous papers in this series (Liversidge et al., 1981; Liversidge and Grant, 1982; Grant and Liversidge, 1982) have described thermal analytical, solubility and rheological investigations of the physicochemical interactions in a series of proposed new suppository formulations based on binary mixtures of triglycerides. The drugs chosen were the relatively hydrophilic drug, metronidazole, which forms suspension suppositories, and the relatively hydrophobic drug, ketoprofen, which forms solution suppositories in triglyceride bases. The present paper reports the in vivo plasma levels of these drugs from the fatty suppository formulations and relates them to the physicochemical and rheological parameters previously investigated.

Materials and methods

The materials used and the preparation and constituents of the suppository formulations, which are summarized in Table 1, have been described by Liversidge et al. (1981) and by Liversidge and Grant (1982). For each formulation the subscript number indicates the composition of the triglyceride base in the formulation, whereas the subscript letter indicates the minor component in the formulation, if any, i.e., D = dye, m = metronidazole, k = ketoprofen. The preferred in vivo purging method used in all the in vivo work has been described by Grant and Liversidge (1982). This method exploits the fact that rats defecate when frightened. Handling-induced defecation was repeated after 120 min to ensure that all fecal pellets had been expelled.

Suppository ingression, i.e. spreading, in the rat rectum was studied by the method of Grant and Liversidge (1982). A suppository containing Oil Blue dye was inserted into the rectum which was then sealed with cyanoacrylate adhesive. After the allotted time, the animal was sacrificed and dissected. The distance of ingression of the coloured suppository mass was measured along the gastrointestinal tract from the external anal sphincter and was of the order 10 cm over a period of 10 min.

The absorption of ketoprofen or metronidazole was studied following insertion of each drug-containing suppository formulation into 17 rats. Five rats were sacrificed

TABLE 1

COMPOSITION OF THE SUPPOSITORIES USED IN THE IN VIVO EXPERIMENTS

Formulation code [a]	Composition of a 200 mg suppository		
	Drug or dye (mg)	Excipients expressed as % w/w ratio (and in mg)	
		Tricaprin	Other triglyceride
tc_1	none	60 (120.0)	trilaurin 40 (80.0)
tc_2	none	40 (80.0)	trilaurin 60 (120.0)
tc_3	none	75 (150.0)	trimyristin 25 (50.0)
tc_4	none	90 (180.0)	tripalmitin 10 (20.0)
tc_5	none	92 (184.0)	tristearin 8 (16.0)
tc_{1D}	Oil Blue dye (20.0)	60 (108.0)	trilaurin 40 (72.0)
tc_{2D}	Oil Blue dye (20.0)	40 (72.0)	trilaurin 60 (108.0)
tc_{3D}	Oil Blue dye (20.0)	75 (135.0)	trimyristin 25 (45.0)
tc_{4D}	Oil Blue dye (20.0)	90 (162.0)	tripalmitin 10 (18.0)
tc_{5D}	Oil Blue dye (20.0)	92 (165.0)	tristearin 8 (14.4)
tc_{1m}	metronidazole (46.0)	60 (92.4)	trilaurin 40 (61.6)
tc_{2m}	metronidazole (46.0)	40 (61.6)	trilaurin 60 (92.4)
tc_{3m}	metronidazole (46.0)	75 (127.8)	trimyristin 25 (26.2)
tc_{4m}	metronidazole (46.0)	90 (144.8)	tripalmitin 10 (9.2)
tc_{5m}	metronidazole (46.0)	92 (146.3)	tristearin 8 (7.7)
tc_{1k}	ketoprofen (7.4)	60 (115.6)	trilaurin 40 (77.0)
tc_{2k}	ketoprofen (7.4)	40 (77.0)	trilaurin 60 (115.6)
tc_{3k}	ketoprofen (7.4)	75 (159.9)	trimyristin 25 (32.7)
tc_{4k}	ketoprofen (7.4)	90 (181.1)	tripalmitin 10 (11.6)
tc_{5k}	ketoprofen (7.4)	92 (183.0)	tristearin 8 (9.6)

[a] These codes are referred to in the text and in Tables 2, 3 and 4.

by humane asphyxiation each at 10, 30 and 60 min, one rat was sacrificed each at 90 and 120 min and blood samples were taken immediately by cardiac puncture. Ketoprofen levels in plasma samples were determined by the method of Kaye et al. (1981) using 2-(5-keto-10,11-dihydro-5H-dibenzo[a,d]-2-cycloheptenyl)propanoic acid as the internal standard. Plasma samples were analyzed for metronidazole by the method of Kaye et al. (1980). The assay of each drug is highly specific for that compound and is not influenced significantly by the presence of normal quantities of any metabolite.

Results and discussion

The drug plasma levels at various times after administration are summarized in Tables 2 and 3 and the area under the plasma–time curve from time zero to 60 min ($AUC_{0-60min}$) are stated in Table 4. The data were analyzed statistically using the

TABLE 2

RAT PLASMA LEVELS OF METRONIDAZOLE FROM 200 mg SUPPOSITORY FORMULATIONS CONTAINING 46 mg OF METRONIDAZOLE

Time (min)	Formulation [a] tc_{1m}			Formulation [a] tc_{2m}			Formulation [a] tc_{3m}			Formulation [a] tc_{4m}			Formulation [a] tc_{5m}		
	mean (μg/ml)	S_x [b] (μg/ml)	C.L. [c] (μg/ml)	mean (μg/ml)	S_x [b] (μg/ml)	C.L. [c] (μg/ml)	mean (μg/ml)	S_x [b] (μg/ml)	C.L. [c] (μg/ml)	mean (μg/ml)	S_x [b] (μg/ml)	C.L. [c] (μg/ml)	mean (μg/ml)	S_x [b] (μg/ml)	C.L. [c] (μg/ml)
10	33.9	11.1	13.8	7.5	2.7	3.4	21.2	3.1	3.9	25.8	4.9	6.1	36.6	5.8	7.2
30	47.0	11.3	14.0	11.6	4.3	5.3	33.7	5.6	7.0	49.0	11.6	14.5	38.4	6.1	7.6
60	61.8	18.6	23.1	33.9	13.1	16.3	30.2	2.9	3.7	67.0	10.9	13.5	55.7	7.9	9.8
90	43.0	–	–	22.1	–	–	31.0	–	–	17.6	–	–	50.3	–	–
120	44.5	–	–	26.2	–	–	25.0	–	–	28.6	–	–	40.1	–	–

[a] For Formulation Code, see Table 1.

[b] S_x = standard deviation.

[c] C.L. = 95% confidence limits = $\pm S_x \times t_{0.05}/\sqrt{n}$.

TABLE 3

RAT PLASMA LEVELS OF KETOPROFEN FROM 200 mg SUPPOSITORY FORMULATIONS CONTAINING 7.4 mg OF KETOPROFEN

Time (min)	Formulation [a] tc_{1k}			Formulation [a] tc_{2k}			Formulation [a] tc_{3k}			Formulation [a] tc_{4k}			Formulation [a] tc_{5k}		
	mean (μg/ml)	S_x [b] (μg/ml)	C.L. [c] (μg/ml)	mean (μg/ml)	S_x [b] (μg/ml)	C.L. [c] (μg/ml)	mean (μg/ml)	S_x [b] (μg/ml)	C.L. [c] (μg/ml)	mean (μg/ml)	S_x [b] (μg/ml)	C.L. [c] (μg/ml)	mean (μg/ml)	S_x [b] (μg/ml)	C.L. [c] (μg/ml)
10	61.3	12.2	15.1	21.6	8.7	10.8	41.9	10.3	12.8	59.3	13.0	16.1	55.4	1.7	2.1
30	60.8	13.2	16.4	38.5	16.8	20.9	58.2	9.8	12.2	63.7	5.6	6.9	66.4	9.4	11.7
60	67.0	10.6	13.2	41.9	4.5	5.6	50.7	2.9	3.6	61.0	10.9	13.5	59.7	7.7	9.6
90	73.0	-	-	68.8	-	-	60.5	-	-	44.5	-	-	76.1	-	-
120	41.6	-	-	54.0	-	-	50.7	-	-	44.7	-	-	36.2	-	-

[a] For Formulation Code, see Table 1.

[b] S_x = standard deviation.

[c] C.L. = 95% confidence limits = $\pm S_x \times t_{0.05}/\sqrt{n}$.

Student's (1908) *t*-test, where S_x is the standard deviation, and both the upper and lower levels of certainty at 95% confidence limits of the mean are expressed as ± standard error of the mean (S.E.M.) × $t_{0.05}$ for 4 degrees of freedom.

For metronidazole suppositories the following conclusions can be drawn from Tables 2 and 4: (a) if rapid absorption of metronidazole is required formulation tc_{1m} and tc_{5m} are preferred; (b) the highest plasma levels can be achieved by formulations tc_{1m} and tc_{4m}; (c) high $AUC_{0-60min}$ is achieved by formulations tc_{1m}, tc_{4m} and tc_{5m}. Formulation tc_{1m} gives the greatest bioavailability according to all these criteria.

As discussed previously (Liversidge et al., 1981), the initial absorption is usually highly dependent on the liquefaction time of the suppository. Accordingly, the suppositories with the lowest liquefaction time will give the highest initial rates of absorption, as is the case of formulations tc_{1m} and tc_{5m}.

The area under the plasma–time curve is a measure of the actual amount of metronidazole absorbed and may be related to the viscosity and spreading in the rectum, which have been measured and discussed by Grant and Liversidge (1982). Comparison of the rate of ingression up the rectum (which is in the rank order, formulation $tc_{2D} < tc_{3D} < tc_{4D} < tc_{5D} < tc_{1D}$) with $AUC_{0-60\,min}$ (which is in the rank order $tc_{2m} < tc_{3m} < tc_{5m} < tc_{1m} < tc_{4m}$), using the Kendall (1970) method of rank correlation, indicates that the correlation coefficient is 0.6 with a probability better than 0.001. This indicates a significant correlation between spreading and absorption.

A rank order correlation between the plastic viscosity, η_{pl}, of metronidazole suppositories determined in a rotational viscometer (Grant and Liversidge, 1982) and $AUC_{0-60min}$ (Table 4) can be examined, if the following assumptions are made: (i) the rectal pressure exceeds 67 $N \cdot m^{-2}$ (Grant and Liversidge, 1982); (ii) the incorporation of the drug affects the viscosity of each base in the same proportion (Rutten-Kingma, 1973; Rutten-Kingma et al., 1979); (iii) the plastic viscosities in the rectum are the same as η_{pl}. The rank order of η_{pl} is formulation $tc_5 < tc_3 < tc_4 < tc_2$

TABLE 4

AREA UNDER THE CURVE FOR PLOTS OF DRUG PLASMA LEVEL AGAINST TIME, OVER A PERIOD OF 60 min AFTER ADMINISTRATION ($AUC_{0-60min}$) OF METRONIDAZOLE OR KETOPROFEN SUPPOSITORIES TO RATS

Metronidazole suppository formulation [a]	$AUC_{0-60min}$ ($\mu g \cdot ml^{-1} \cdot h$)	Ketoprofen suppository formulation [a]	$AUC_{0-60min}$ ($\mu g \cdot ml^{-1} \cdot h$)
tc_{1m}	2613	tc_{1k}	3445
tc_{2m}	91	tc_{2k}	1915
tc_{3m}	161	tc_{3k}	2851
tc_{4m}	2616	tc_{4k}	3396
tc_{5m}	2344	tc_{5k}	3386

[a] For Formulation Code, see Table 1.

$< tc_1$. When Kendall's (1970) method of rank correlation is applied to compare η_{pl} with $AUC_{0-60\ min}$, zero correlation is found. Thus, the plastic viscosity cannot be correlated with the absorption of metronidazole. This lack of relationship may arise from errors in the assumptions (i)–(iii) stated above, or may result from differences in drug transport, rectal temperature or body movement from one individual animal to another. In view of the satisfactory correlations between suppository spreading and drug absorption, the authors suggest that a certain variability in body movement or peristalsis may account for a lack of correlation with plastic viscosity.

For ketoprofen suppositories the following conclusions can be drawn from Tables 3 and 4: (a) if rapid absorption of ketoprofen is required formulations tc_{1k}, tc_{4k} and tc_{5k} are preferred; (b) high plasma levels can be achieved by formulations tc_{1k}, tc_{4k} and tc_{5k}; (c) high $AUC_{0-60min}$ is achieved by formulations tc_{1k}, tc_{4k} and tc_{5k}. Formulations tc_{1k}, tc_{4k} and tc_{5k} give the greatest bioavailability according to all these criteria.

The amount of ketoprofen absorbed from fatty suppositories (Table 4) is found to bear relationships to the viscosity and spreading in the rectum similar to those for metronidazole. Analysis of the rank order of the spreading rate (formulation $tc_{2D} < tc_{3D} < tc_{4D} < tc_{5D} < tc_{1D}$; Grant and Liversidge, 1982) with the rank order for $AUC_{0-60min}$ (formulation $tc_{2k} < tc_{3k} < tc_{5k} < tc_{4k} < tc_{1k}$, Table 4) using Kendall's (1970) method of rank correlation shows a correlation coefficient of 0.8 with a probability better than 0.001. This indicates a highly significant correlation between spreading and absorption.

A rank order correlation between the plastic viscosities, η_{pl}, of ketoprofen suppositories (Grant and Liversidge, 1982) and the $AUC_{0-60min}$ (Table 4) can be attempted if the above assumptions (i)–(iii) for metronidazole suppositories are also applied to ketoprofen suppositories. Using Kendall's (1970) method of rank correlation the correlation coefficient is -0.1 with a probability worse than 0.5. Thus, there is no significant correlation between plastic viscosity and the amount of ketoprofen absorbed, for which we offer the same explanation as in the case of metronidazole considered above.

In conclusion, the area under the curve of concentration of ketoprofen or metronidazole in plasma plotted against time correlates significantly with the rate of ingression of the suppository mass up to gastrointestinal tract but not with the plastic viscosity of the triglyceride base. The base tc_1, consisting of 60% w/w tricaprin and 40% w/w trilaurin, gives the greatest area under the curve for absorption of either drug.

The next paper in this series will compare various methods of determining drug release in vitro with and without membranes and will assess the extent to which the results obtained correlate with the in vivo data presented here.

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References

Bergen, T., and Arnold, E., Pharmacokinetics of metronidazole in healthy adult volunteers after tablets and suppositories. Chemotherapy, 26 (1980) 231–241.

Borzelleca, J.P. and Lowenthal, W., Drug absorption from the rectum. II. Rates of absorption and elimination from the blood for various suppository bases. J. Pharm. Sci., 55 (1966) 151–154.

Caillé, G., Besner, J.G., Lacasse, Y. and Vezina, M., Pharmacokinetic characteristics of ketoprofen suppositories. Biopharm. Drug Disp., 1 (1980) 195–201.

Grant, D.J.W. and Liversidge, G.G., Influence of physicochemical interactions on the properties of suppositories. III. Rheological behaviour of fatty suppository bases and its effect on spreading in rats. Drug Dev. Ind. Pharm., (1982) in the press.

Jonkermann, J.H.G., Van Book, L.E., Wysbeek, J., Bothuis-De Vries, A.S., De Zeeuw, R.A., Orie, N.G.M. and Cox, H.L.M., First-pass effect after rectal administration of thiazinium methylsulfate. J. Pharm. Sci., 68 (1979) 69–72.

Kaye, C.M., Sankey, M.G. and Thomas, L.A., A rapid and specific semimicro method involving high pressure liquid chromatography for the assay of metronidazole in plasma, saliva, serum, urine and whole blood. Br. J. Clin. Pharmacol., 9 (1980) 528–529.

Kaye, C.M., Sankey, M.G. and Holt, J.E., A high pressure liquid chromatographic method for the assay of ketoprofen in plasma and urine, and its application to determine the urinary excretion of free and conjugated ketoprofen following oral administration of Oridis® to man. Br. J. Clin. Pharmacol., 11 (1981) 395–398.

Kendall, M.G., Rank Correlation Methods, 4th Edn., Griffin, London, 1970.

Liversidge, G.G. and Grant, D.J.W., Influence of physicochemical interactions on the properties of suppositories. II. Interactions between the constituents of fatty suppository bases and ketoprofen or metronidazole. Drug Dev. Ind. Pharm., (1982) in the press.

Liversidge, G.G., Grant, D.J.W. and Padfield, J.M., Influence of physicochemical interactions on the properties of suppositories. I. Interactions between the constituents of fatty suppository bases. Int. J. Pharm., 7 (1981) 211–223.

Lowenthal, W., Borzelleca, J.F. and Corder, C.D., Drug absorption from the rectum. III. Aspirin and some aspirin derivatives. J. Pharm. Sci., 59 (1970) 1353–1355.

Miller, M.E., Christensen, G.C. and Evans, H.H., Anatomy of the Dog, W.B. Saunders, Philadelphia, PA, 1964 p. 689.

Moes, A. and Jaminet, F., Influence of ageing of suppositories on rectal absorption of paracetamol. Pharm. Acta Helv., 51 (1976) 119–125.

Rutten-Kingma, J.J., Biofarmaceutisch onderzoek van suspensizetpillen. Ph.D. Thesis, Leiden, The Netherlands, 1973.

Rutten-Kingma, J.J., Polderman, J. and DeBlaey, C.J., Biopharmaceutical studies of fatty suspension suppositories. I. Spreading in situ. Int. J. Pharm., 3 (1979) 39–59.

Student, The probable error of a mean. Biometrika, 6 (1908) 1.