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THE EFFECTS OF VARIOUS SUSPENDING AGENTS ON THE BIOAVAILABILITIES OF ASPIRIN AND SALICYLIC ACID IN THE RABBIT

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SUMMARY

Bioavailability studies on aspirin suspensions in rabbits using an 8 × 8 latin square design of experiment have shown that significant differences in the amount of drug absorbed, but not in its rate of absorption, occur when different macromolecular suspending agents are used. Linear relationships between the logarithm of the apparent viscosity of the suspension medium at a shear rate of 100 s^{-1}, pH 1.2 and 37°C and the amount of drug absorbed in 9 h and its peak concentration in the blood have been established. It is suggested that the effect of viscosity on the gastric emptying rate is the reason for the variation in the bioavailability of aspirin from the suspensions.

Results obtained using suspensions of salicyclic acid in 1% w/v dispersions of sodium carboxymethylcellulose and xanthan gum in a two-way cross-over test in 8 rabbits substantiate those obtained with the aspirin suspensions and indicate that the effect of viscosity on the hydrolysis of aspirin in the gastro-intestinal tract is not important from a bioavailability points of view.

The unsuitability of traditional flask-stirrer and dialysis techniques as methods capable of providing results for satisfactory correlation with the in vivo bioavailability parameters obtained in this study is discussed.

INTRODUCTION

Numerous studies on the effects of formulation on the bioavailability of drugs from solid dosage forms have been reported, but few have been devoted to a consideration of suspension formulations. In addition, some of the few have been concerned with commercial products and the formulation ingredients are not specified (Bates et al., 1969; Tom Bergan et al., 1973; Antal et al., 1975; Marty and Hersey, 1975), and in another (Seager, 1968) the mechanism of the effect of the suspending agent is not clarified. It is possible that the main reason why suspensions have received little attention arises from the fact that they appear immediately after solutions in a general ranking order of dosage forms with respect to their inherent bioavailability problems (Gibaldi, 1971). However, exceptions to this general order have been reported (Riegelman, 1969;

Berlin et al., 1972), and these indicate that the bioavailability of drugs from suspensions may, occasionally, be inferior to that from other solid dosage forms.

In view of the above comments the present investigation was carried out in an attempt to elucidate the effects of various macromolecular suspending agents on the bioavailability of a given drug. Rabbits were used in the investigation and aspirin was chosen as the drug so that the results could be compared with those obtained from previous rheological, complexation and in vitro release studies (Barzegar-Jalali and Richards, 1979). Additional in vivo experiments involving salicylic acid suspensions were included in the present study in order to obtain information on the possible significance, from a bioavailability point of view, of the effects of suspending agents on the hydrolysis of aspirin in the gastrointestinal tract.

MATERIALS AND METHODS

Aspirin Powder B.P. (Thornton and Ross) was sieved and the 60/100 portion (mesh size 150–250 μm) was used to prepare suspensions containing 4% w/v of the drug. In addition to distilled water (A) the following dispersions were used as the suspension media: 1.15% w/v sodium alginate (Manucol DM) (B), 1.5% w/v methylcellulose (low substitution) (C), 1.0% w/v sodium carboxymethylcellulose (Edifas 50) (D), 7.0% w/w polyvinylpyrrolidone (M.W. 700,000) (E), 1.0% w/v xanthan gum (F), 1.0% w/v Tragacanth Powder B.P. (G) and 4.0% w/v Compound Tragacanth Powder B.P. (H). Details of the sources of the above materials and the methods of preparation of the suspension media have been reported previously together with the results of rheological, complexation and in vitro release rate studies on the same systems (Barzegar-Jalali and Richards, 1979). Aspirin powder was dispersed in 24 h aged media and the resultant suspensions were stored over night in a cool place (about 10°C). On the following morning the suspensions were allowed to reach room temperature and shaken vigorously before use.

Adult male New Zealand White rabbits, fed previously with a standard diet, were fasted for 20 h before each experiment although water was available ad libitum. During the experimental period water was withdrawn. Calculated volumes of suspensions containing doses of aspirin equivalent to 120 mg per kg body weight were introduced directly into the rabbits' stomachs by means of a catheter and syringe. The dose was based on the work of Lessel and Cliffe (1964). The catheter was flushed out with water (one-third of the dose volume) before removal. The order of administration of the suspensions was based on an 8 X 8 latin square pattern designed in such a way that every formulation was preceded and succeeded by each of the other formulations an equal number of times (Westlake, 1973). A minimum wash-out period of 10 days was allowed between successive experiments.

Immediately before administration of a suspension 0.7 cm^3 of blood was removed from the marginal ear vein into a heparinized 1 cm^3 syringe. Further samples were obtained at specified times during the 9 h that followed administration. The samples were maintained at 25°C for at least 2 h in order to ensure complete hydrolysis of aspirin (Cotty et al., 1965) and the total salicylate in the blood was determined using Trinder's method (Trinder, 1954). The concentration of salicylate in each sample was calculated

from a calibration curve obtained by measuring the absorbances of a series of aqueous solutions containing known concentrations of salicylic acid after treatment with Trinder's colour reagent. The reliability of the method was checked by using samples of heparinized blood containing known concentrations of aspirin. The observed results were within 99.5–101.0% of the expected values.

Salicylic Acid Powder B.P. (Evans Medical Ltd.) was sieved and the same particle size range as for aspirin was used to prepare suspensions containing 4% w/v of the drug in the low viscosity dispersion medium D and the high viscosity dispersion medium F. The formulations were compared by using a two-way crossover test in 8 rabbits (Wagner, 1975). The methodology used in this test was the same as that used in the aspirin studies.

RESULTS AND DISCUSSION

Blood concentrations of salicylate versus time curves were plotted for each set of experimental data. Values of the three important bioavailability parameters (Rischel, 1976), i.e. peak time (PT), peak concentration (PC) and area under the curve (AUC) were derived from these plots, the AUCs for the period 0–9 h post-administration being calculated by the trapezoidal method (Notari, 1975).

Plots of the mean concentrations of salicylate versus time following the administration of aspirin suspensions in water (A) and in the most viscous dispersion medium i.e. 1% w/v xanthan gum (F) are shown in Fig. 1. The lines obtained for the other aspirin suspensions lie between these two curves but have been omitted from the figure for the sake of clarity. Analyses of variance and Duncan's multiple range test (Duncan, 1955) were applied to the values obtained for the three bioavailability parameters from the experiments involving aspirin suspensions and the results of the latter test are summarized in Table 1.

Since no appreciable complexation between aspirin and the suspending agents could be detected, it is suggested that the changes in the amount of salicylate appearing in the

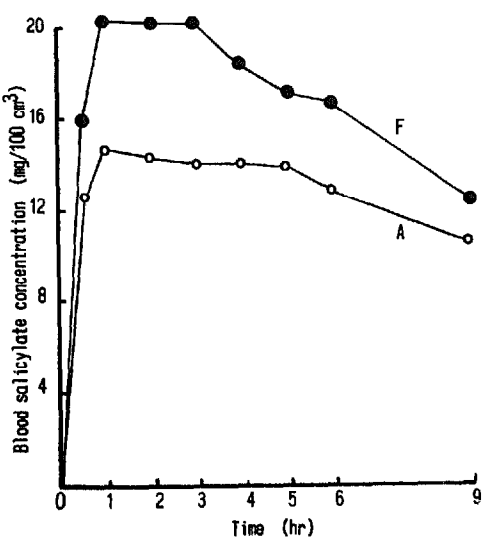

Fig. 1. Mean blood concentrations of salicylate versus time after administration of 4% w/v suspensions of aspirin in water (A) and 1% w/v xanthan gum (F) to 8 rabbits.

TABLE 1

RESULTS OF DUNCAN'S MULTIPLE RANGE TEST ON THE VALUES OBTAINED FOR PEAK TIME (PT), PEAK CONCENTRATION (PC) AND AREA UNDER THE CURVE (AUC_0^9) AFTER ADMINISTRATION OF ASPIRIN SUSPENSIONS (4% W/V) TO RABBITS IN AN 8 × 8 LATIN SQUARE DESIGN [a]

PT (h)	Suspension medium [b]	C	E	G	H	D	A	B	F
	mean	1.50	1.63	1.75	1.94	2.06	2.44	2.50	2.50
PC (mg/100 cm^3)	Suspension medium	A	B	D	C	H	E	G	F
	mean	15.94	17.56	18.41	21.03	21.53	21.75	22.00	23.25
AUC_0^9 (mg · h/ 100 cm^3)	Suspension medium	A	B	D	C	E	H	G	F
	mean	110.74	122.03	122.95	136.16	136.32	141.09	148.28	149.42

[a] Any two means *not* underscored by the same line are significantly different ($P < 0.05$). Any two means underscored by the same line are not significantly different.

[b] See text for key to suspension media.

blood, after administration of the different aspirin suspensions, may be caused by variation in the viscosity of the gastrointestinal (GI) contents brought about by the suspending agents. This suggestion arises from the linear relationships between the AUCs or peak concentrations and the logarithm of the apparent viscosities (η_{app}) of the dispersions of the suspending agents at pH 1.2 and a shear rate of 100 s^{-1} that are shown in Figs. 2 and 3, respectively. These relationships indicate that AUC_0^9 and PC increase with increase in viscosity of the suspension medium.

An increase in viscosity of the GI contents may lead to the following general effects: (a) a decrease in gastric emptying rate, i.e. an increase in the gastric residence time; (b) a decrease in intestinal motility; (c) a decrease in dissolution rate of the drug; and (d) a decrease in the rate of movement of drug molecules to the absorbing membranes. In addition, more specific effects may be superimposed in the case of particular combinations of suspending agents and drugs. Levy and Jusko (1965) demonstrated the occurrence of effect (d) by comparing the rates of gastric absorption of salicylic acid from viscous and non-viscous solutions inserted into the ligated stomachs of anaesthetized rats. They also verified the occurrence of effects (a) and (b) in rats by measuring the distribution of the poorly absorbed agent phenol red after peroral administration in viscous solutions. These latter results showed that the major effect of viscosity on GI transit could be attributed to a delay in gastric emptying. Levy and Jusko concluded that, in the case of viscous solutions of drugs, where effect (c) would not be involved, effects (a), (b) and (d) lead to a decrease in the rate of absorption of drugs from the GI tract. This conclusion agrees with observations made on the effects of sucrose solutions on the induction time of phenobarbitone sodium (Malone et al., 1960), methylcellulose on the absorption from solutions of sodium salicylate (Davison et al., 1961), different gums on the urinary excretion rate of sodium salicylate solutions (Bachynsky et al., 1976) and sodium alginate on the bioavailability of phenolsulphonphtalein solutions (Ashley and Levy, 1973). The effect of

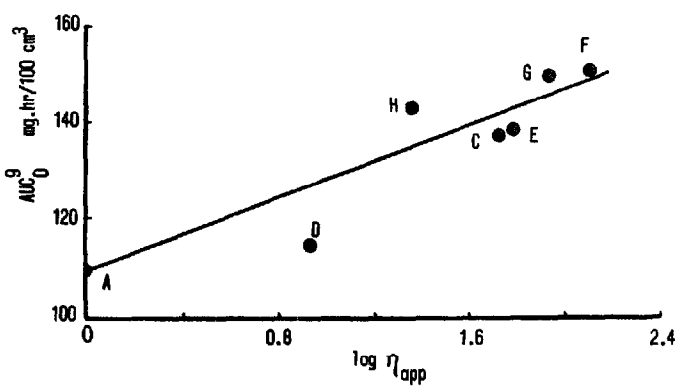

Fig. 2. Correlation between the mean area under the blood salicylate concentration versus time curve from 0 to 9 h (AUC_0^9) after administration of 4% w/v aspirin suspensions to 8 rabbits and the apparent viscosity of the suspension medium. η_{app} is the apparent viscosity of the dispersion medium at pH 1.2 and shear rate 100 s^{-1}. The values for the different media are A 1, D 9, H 23, C 55, E 60, G 89 and F 142 nM s m^{-2}. A value for medium B was not calculated because of the complex nature of this system at pH 1.2 (see Barzegar-Jalali and Richards, 1979). See text for key to suspension media. From the data $AUC_0^9 = 17.68 \log \eta_{app} + 109.98$ ($r = 0.9440$, $P < 0.001$).

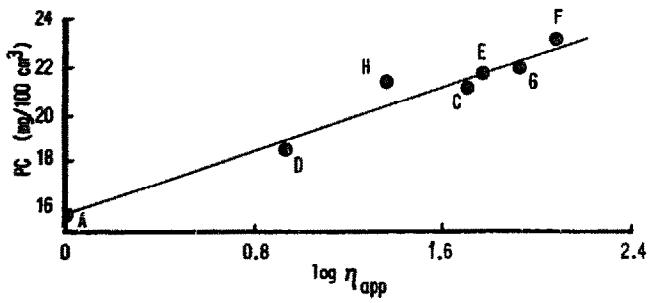

Fig. 3. Correlation between the mean peak salicylate concentration in blood (PC) after administration of 4% w/v aspirin suspensions to 8 rabbits and the apparent viscosity of the suspension medium. See legend to Fig. 2 for definition of η_{app} and reason for omission of formulation B. See text for key to suspension media. From the data PC = 3.291 η_{app} + 15.91 (r = 0.9800, $P < 0.001$).

methylcellulose on the absorption of nitrofurantoin from aqueous suspensions of this drug (Seager, 1968) could also be explained by the factors (a)–(d), although it has been suggested that nitrofurantoin may form complexes with methylcellulose (Shah and Sheth, 1976). The viscosity-linked factors that may lead to a decrease in the rate of absorption of drugs did not appear to have a marked effect in this investigation because the peak times did not differ to a significant extent.

The effect of viscosity on the amount of drug absorbed from suspension or solution dosage forms does not appear to have received any great attention. If such an effect does occur it may be more important for suspensions containing drugs with bioavailabilities that are dissolution rate dependent. In these cases an increase in viscosity could lead to (i) a decrease in the amount of bioavailability because of a decrease in the dissolution rate or (ii) an increase in the amount of bioavailability because an extended gastric residence time, or a slower intestinal transit, would allow a longer period in which drug dissolution could occur. The increase in gastric residence time caused by an increase in viscosity could also enhance the amount of absorption of drugs with pK_a values that permit absorption of those drugs from the stomach. Similarly, an increase in intestinal transit time would favour an increase in the amount of absorption of the majority of drugs since the intestine is the optimal site of absorption of most drugs. Evidence from the literature suggests that factors which affect gastric emptying rate are likely to have significant effects on the absorption of drugs. For example, Rosenberg and Bates (1976) have shown that the bioavailability of nitrofurantoin in humans is enhanced by the presence of food in the stomach. They ascribe the effect of the food to its viscosity-increasing properties or lipid content, which both increase the residence time of solids in the stomach. Thus, a greater proportion of drug is dissolved before it passes into the duodenum, from where nitrofuration is optimally absorbed. In addition, Bates and Seuqueira (1975) reported that the bioavailability of griseofulvin from an oily product is enhanced because of the delaying effect of the oil on the gastric emptying rate, and Cooke and Hunt (1970) have shown that the absorption of aspirin from solid dosage forms is decreased when the gastric emptying rate is increased.

The present study on aspirin also suggests that the absorption-enhancing effects of increased viscosity operate to a greater extent than the absorption-reducing effects. How-

ever, it is possible that the viscosity of the environment could affect the rate of hydrolysis of the aspirin in the gut. Since the ease of absorption of salicylic acid appears to differ from that of aspirin (Garner, 1975), then the effect of viscosity on the hydrolysis process in the gut may affect the apparent bioavailability of aspirin as determined by the appearance of total salicylate concentration in the blood.

The mean concentrations of salicylate in the blood samples taken at various times during the two-way crossover test performed on the salicylic acid suspensions used in this investigation are shown in Fig. 4. The bioavailability parameters derived from plots of similar data provided by the individual experiments in the test were analysed by the method recommended by Wagner (1975). The mean values of the bioavailability parameters and the results of the test on the significance of the differences between these values are shown in Table 2. It can be seen that, as with the aspirin suspensions, there is a significant difference ($P < 0.05$) between the peak concentrations but not between the peak times. Unlike the aspirin suspensions the difference between the AUC_0^9 values for the salicylic acid suspensions is not statistically significant at a probability of 0.05. However, the relative bioavailability, calculated from $100 \times AUC_D/AUC_F$ was 77.6% for the salicylic acid suspensions, and this value is reasonably similar to that of 82.0% obtained for the aspirin suspensions in the same dispersion media D and F. It is concluded from these points that the effects of the different suspending agents on the hydrolysis rate of aspirin in the GI tract do not seem to have a marked effect on the relative bioavailability of this drug as measured by the appearance of total salicylate in the blood, i.e. the effect of viscosity is not one that is specific for aspirin suspensions.

A rank order relation between the apparent viscosity of the suspension medium and the apparent dialysis rate constant of aspirin from suspensions was established in a previous in vitro study (Barzegar-Jalali and Richards, 1979), the higher the viscosity the lower the rate constant. It is obvious that the results of the present in vivo investigations cannot

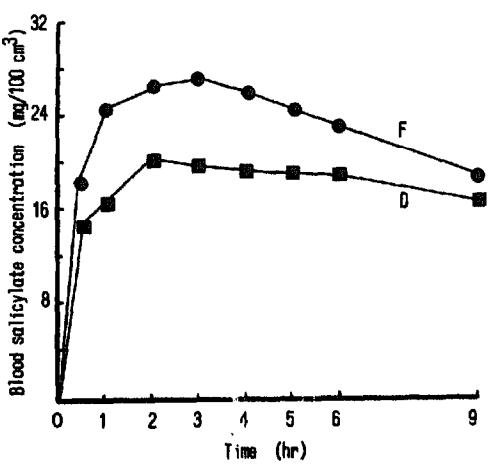

Fig. 4. Mean blood concentrations of salicylate versus time after administration of 4% w/v suspensions of salicylic acid in 1% w/v sodium carboxymethylcellulose (D) and in 1% w/v xanthan gum (F) to 8 rabbits.

TABLE 2

MEAN VALUES OF PEAK TIME (PT), PEAK CONCENTRATION (PC) AND AREA UNDER THE CURVE (AUC_0^9) AFTER ADMINISTRATION OF SALICYCLIC ACID SUSPENSIONS (4% W/V) IN A TWO-WAY CROSSOVER TEST [a] TO 8 RABBITS

	Suspension medium	
	D [b]	F
PT (h)	3.75	3.65
PC (mg/100 cm^3)	22.37	30.37
AUC_0^9 (mg · h/100 cm^3)	159.25	205.22

[a] Any two means *not* underscored by the same line are significantly different ($P < 0.05$). Any two means underscored by the same line are not significantly different.
[b] Suspension media D and F are 1% w/v sodium carboxymethylcellulose and 1% w/v xanthan gum, respectively.

be correlated with those from that study because, on the one hand viscosity appears to enhance the amount of drug absorbed, but on the other hand it decreases the rate of release in vitro. It is important to realize that this lack of correlation is likely to occur whenever traditional in vitro methods are used since, although they may mimic the effects of viscosity on dissolution rates and transport rates of solute molecules and undissolved particles, they do not mimic its effects on gastric and intestinal residence times.

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