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RESEARCH PAPER

Effects of Oils and Pharmaceutical Excipients on the Bioavailability of Ampicillin Orally Administered, Different Oily and Aqueous Suspensions in Rabbit

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ABSTRACT

The in vivo bioavailability and in vitro drug-release studies of ampicillin trihydrate in different oily and aqueous suspensions have been investigated. In addition, partition, solubility, and rheological measurements have also been carried out. The in vivo experimental design was based on a 6×6 latin square using the rabbit as the test animal. The bioavailability of ampicillin was determined using the plasma levels, which were measured microbiologically. Results of the study showed that oily and sucrose-containing aqueous formulations enhanced the extent of ampicillin absorption, although not statistically significantly, but was close to the borderline of significance. Ampicillin appears to be absorbed at essentially the same rate from both aqueous and oily formulations. The latter showed plasma-level time curves with biphasic absorption and are likely to produce prolonged plasma concentrations of ampicillin because of the effects of enterohepatic recycling. Viscosity appears to play an insignificant role in the results obtained since the bioavailability parameters correlate poorly with the viscosity except C_{max}. It is suggested that enhancement in the bioavailability of ampicillin is due to the decrease in the gut transit rate brought about by the oil which predominates and masks the other effects of viscosity and osmotic effects of sucrose. The existence of a correlation between the in vitro drug-release rate ($t_{50\%}$) and viscosity and the lack of a correlation between in vivo and in vitro parameters support the above suggestion and indicate that traditional dissolution rate tests, such as flask-stirrer method, are unsatisfactory as bioavailability indicators when applied to dosage forms that caused marked changes in physiological factors like GER and biliary excretion.

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INTRODUCTION

Several reports indicate that the bioavailabilities of drugs may be altered by their administration in oil-containing dosage forms, for example, salicylates,[1,2] phenytoin,[3] greseofulvin,[4] nitrofurantoin,[5] and ampicillin.[6–8] The observed changes in bioavailability were attributed to the physiological effects of the oil on gastric-emptying rate (GER) and GI residence time. The effects of reduced GER on the bioavailability of ampicillin brought about by food or fatty meals[9–12] or by propantheline[13] have been extensively reported and reviewed. The effects of formulation[14,15] and route of administration[16] on the bioavailability of ampicillin have also received attention. However, most of these investigations were done on solid-dosage forms (tablets and capsules) and the bioavailability studies of ampicillin administered in liquid-dosage forms in a nonaqueous vehicles has received little attention.[6–8] The latter investigations were restricted to s.c. and i.m. administration compared with oral tablets or capsules in pigeon,[6] i.m. administration of amoxicillin trihydrate in oil base in pigs[7] and the effects of medium-chain triglycerides on enternal and rectal absorption of ampicillin.[8] The bioavailability studies of ampicillin administered orally as suspension in oily vehicles appears to be virtually nonexistent, even though this type of dosage form may, in some circumstances, offer advantages over the use of more conventional ones, e.g., provides good flow properties, is highly resistant to settling and caking of suspended materials, and is useful for preparing ready-to-use pharmaceutical suspensions of water-degradable physiologically active agents.

In view of this deficiency and because oily vehicles for pharmaceutical formulations may provide the above-mentioned advantages, the present work was undertaken in order to investigate the effects of oil and various pharmaceutical excipients, either alone or in combination, on the bioavailability of ampicillin from a suspension of this compound in fractionated coconut oil (FCO). The in vivo study was carried out using the rabbit as the test animal. It was complemented by in vitro drug-release studies and by partition and rheological measurements.

MATERIAL AND METHODS

Materials

Ampicillin was obtained from Beecham Pharmaceuticals (London, UK). Antibiotic medium No. 1CM 327, adjusted to pH 7.9, and nutrient broth CM1 were obtained from Oxoid Ltd (Basingstoke, Hampshire, England). Bacillus subtilis (No. 8236) was obtained from the N.C.T.C. Fractionated coconut oil (FCO) BPC (Alembic Products Ltd., Chester, Cheshire, England), aluminium mono- and distearates (Witco Chemicals Ltd., USA), colloidal silica (Cab-o-sil) and lecithin 90% (Refined grade) (BDH Chemicals Ltd., Leicester, England), and hydrogenated castor oil (Akzo Chemie Ltd., UK) were used as obtained from the suppliers. Powdered sucrose previously seived (63–75 μm) was obtained from The British Sugar Corporation Ltd. (Leicester, England).

Preparation of Vehicles

The vehicles used in the preparation of suspensions containing 2% w/v of ampicillin trihydrate are listed together with their coding letters in Table 1.

Vehicle (formulation) F was prepared according to the patent of Stephens and Su[17] by dissolving the lecithin in a portion of the FCO. Dissolution was facilitated by heating the FCO to about 90°C–100°C and agitating the mixture thoroughly until all the solids dissolved. The aluminium stearate and hydrogenated castor oil were added to this solution. With the heat maintained, the resulting mixture was shaken until the latter two ingredients were thoroughly dispersed. The sucrose, previously sieved to a mesh size of 63–75 μm, was added and the resulting dispersion was mixed thoroughly at 90°C–100°C for 3 hr then cooled to room temperature with continued mixing. Finally, the remainder of the FCO was added to bring the dispersion up to the volume. Care was taken to avoid entry of any moisture into the vehicle, since preliminary studies showed that water affects the structure of the gel.

Vehicle E was prepared according to the patent of Lin and Pramoda[18] by adding the sucrose in successive portions to a portion of the FCO in a suitable

Table 1. Experimental design.

	Time period					
Rabbit no.	1	2	3	4	5	6
1	A	B	C	D	E	F
2	B	C	F	A	D	E
3	C	F	B	E	A	D
4	D	A	E	B	F	C
5	E	D	A	F	C	B
6	F	E	D	C	B	A

The suspension used contained ampicillin trihydrate 2% w/v in:
A = Fractionated coconut oil (FCO).
B = 30% w/v sucrose in FCO.
C = distilled water.
D = 30% w/v sucrose in distilled water.
E = 30% w/v sucrose + 1.25% w/v Cab-o-sil in FCO.
F = 0.5% w/v aluminium stearate (50:50 mixture of mono- and distearate) + 0.7% w/v lecithin + 0.35% w/v hydrogenated castor oil + 30% w/v sucrose in FCO.

container and stirring until the system was suitably dispersed and suspended. The Cab-o-sil was then added and stirred until dispersed. Sufficient additional oil was added and stirred to obtain a uniform dispersion. Although it was not specified in this patent, the sucrose was sieved and the portion corresponding to a mesh size of 63–75 μm was used in the preparation of the vehicle. Vehicle B was prepared in a similar manner. Precautions were taken to avoid entry of moisture into the vehicles for the same reason mentioned above.

Determination of Apparent Partition Coefficient and Solubility

Fifty milliliters of solution containing 100 mg of ampicillin in 100 mL of 0.1 M HCL was equilibrated with 50 mL of fractionated coconut oil (FCO) for 24 hr in a 250 mL glass-stoppered conical flask kept at 37°C in a shaking water bath and agitated at 100 oscillations per minute. The drug concentrations in the HCL were determined by the method of Smith et al.[21] Preliminary studies showed that equilibrium was attained within 5 hr. The apparent partition coefficient of ampicillin was calculated by means of the following equation:

$$\text{Apparent partition coefficient} = \frac{C_1 - C_2}{C_2}$$

where C_1 is the original concentration of the drug in the HCL and C_2 is the equilibrium concentration in the HCL.

The solubility of ampicillin in FCO was carried out using the same procedure described previously.[5]

Rheological Measurements

Rheological measurements of the vehicles were carried out using the same procedures described previously.[2] A Haake Rotovisco viscometer fitted with concentric cylinder sensors, measuring head 500, and a temperature-controlled water jacket at 37°C was used.

In Vitro Drug-Release Studies

The flask-stirrer method used in this drug-release study was based on the apparatus described by Poole[19] and used for in vitro release studies of nitrofurantoin,[5] except for the volume of the dissolution medium,which was 200 mL 0.1 M HCL, and the speed of the stirrer was 60 rpm. Ten milliliter of 2% w/v freshly prepared suspensions of ampicillin trihydrate were injected through the side neck of the dissolution flask from a 10-mL graduated syringe. The latter was washed out with 5 mL of 0.1 M HCL and the washings were added to the flask. Sink conditions prevailed because the solubility of ampicillin in 0.1 M HCL at 37°C is more than 2% w/v.[20] Two-milliliter samples were removed at specified time intervals and each sample was replaced immediately by 2 mL of 0.1 M HCL. The samples were filtered through a 0.45 Millipore filter and the ampicillin content of each was determined by the method of Smith et al.[21]

In Vivo Bioavailability Studies

These were carried out using the method described previously,[1] except that a 6×6 latin-square pattern of experimental design was employed and the sampling times of the blood were 0, 1/2, 1, 1.5, 2, 3, 4, 6, and 8 hr after drug administration. The experimental design is shown in Table 1 and the six formulations are represented by the letters A–F.

The specified amount of the drug was added to the particular vehicle, which had been prepared and left overnight at room temperature, just before dosing

the rabbit. Doses of 50 mg/kg body weight were given to adult male New Zealand white rabbits weighing 2.15–3.8 kg in a dose size of 2.5 mL/kg body weight.

After collection, the blood samples were centrifuged at 2000 *g* for 15 min and the plasma was stored in a refrigerator until required for the microbiological assay of ampicillin. This assay was commenced on the same day as the bioavailability test, immediately after the last blood sample had been obtained.

A cup-plate assay method was used. This method was very similar to that described by Bennett et al.[22] and the medium, bacterial suspension, and other conditions were as described by Arret et al.[23] and summarized as follows.

A few drops of nutrient broth were added to the freeze-dried sample of B. subtilis and this mixture was used to inoculate a slope of the solid antibiotic medium. After incubation at 37°C for 24 hr, the slope was washed with sterile distilled water and the washings were used to inoculate a larger slope. This was incubated at 37°C for seven days. The sporing organisms were then washed off and the resulting suspension was standardized by adjusting its density to 1/5 of Brown's tube No. 1, so that the number of organisms was in the range $10–100 \times 10^6$ organisms/mL. The suspension was then heated to 80°C for 10 min in order to kill vegetative organisms. The spore-containing suspension was finally stored at 4°C until required for the assay. Bennett et al.[22] reported that the suspension is stable for four weeks when stored under such conditions. Three suspensions were prepared during the course of this study.

When an assay was to be performed, molten agar medium, whilst at approximately 50°C, was seeded with the standardized B. sabtilis suspension (2.5 mL of suspension in 250 mL of agar). The seeded agar was allowed to set at room temperature for about 1 hr. Thirty-six holes of 10 mm diameter and 10 mm depth were then made in each plate. Twenty of these holes were to be filled with four replicates of five different standard solutions of ampicillin in plasma and the remaining 16 holes were to be filled with the test samples and their replicates. (Single specimens were used for the zero and 8 hr time samples and duplicate specimens were used for each of the seven intermediate time samples.)

Although ampicillin is known to have a low affinity for protein binding,[24] the standard solutions were prepared with pooled rabbit's plasma in order to eliminate possible variations in antibacterial activity due to this effect.[22] A constant volume (0.3 mL) of pooled plasma was added to 0.2 mL amounts of different concentrations of ampicillin solution in phosphate buffer (0.1 mol/L and pH 7.9 ± 0.1 to produce 0.5 mL quantities of five standard solutions, which contained 0.5, 1, 2, 4, and 8 μg/mL, respectively.

The standard solutions were made on each day of the study after the first or second hourly blood sample had been obtained during the bioavailability test. The standard solutions were then stored along with the unknown plasma samples in a refrigerator so that they were subjected to the same conditions.

The plasma samples and standard solutions were coded and a constant volume of each (50 μL) was put into the appropriate hole in an agar plate. Even though the level of each plate was adjusted by means of a spirit-level, the distribution of test and standard samples was arranged to compensate for any variation in agar thickness as well as for the time factor involved in adding the samples to the plate. The plates were then allowed to stand undisturbed for 2 hr at room temperature to allow diffusion of the antibiotic to occur. At the end of this period, they were transferred to an incubator and maintained at 37°C for 16 hr.

The diameter of the zones of inhibition around each cup were measured, with the aid of callipers, after the incubation period. The regression coefficient (b) of a plot of the mean diameters, given by the four replicates of each of the five standard solutions, vs. the logarithm of the ampicillin concentration in those solutions was calculated for each plate. The unknown concentrations of ampicillin in the plasma samples used on that plate were then determined by means of Eq. (1),

$$X = \frac{(Y - \bar{Y}') + b\bar{X}'}{b} \tag{1}$$

where X = log concentration, Y = zone diameter, and $\bar{X}'$ and $\bar{Y}'$ are the mean values of these parameters.

RESULTS AND DISCUSSION

In Vitro Studies

Apparent Viscosity of Vehicles (η_{app}), Solubility, and Partition Coefficient

The solubility of ampicillin in FCO and its partition coefficient between the oil and 0.1 M HCL were <3 mg/100 mL and 0.052, respectively.

Table 2. $t_{50\%}$ and percent ampicillin dissolved at various times from different formulations using the flask-stirrer method. Each value is represented as mean with SD from three experiments.

	Time (min)							
Formulation[a]	5	10	20	30	45	60	$t_{50\%}$ (min)	η_{app}[b] mN s m^{-2}
A	67.6±2.9	80.5±2.2	97.3±2.3	99.0±2.5	98.1±2.1	98.6±1.9	3.6±1.8	17.5
B	35.5±4.7	47.6±5.8	63.4±4.6	69.5±6.2	82.3±5.4	92.1±4.5	12.3±4.1	64
C	80.6±1.8	99.9±1.6	101.2±1.6				3.0±1.4	0.695[c]
D	81.3±2.1	99.4±1.5	100.9±1.7				3.0±1.5	2.32
E	13.2±4.6	20.3±4.1	29.6±5.1	35.3±4.9	46.3±3.8	55.3±5.2	51.0±3.9	150
F	15.1±4.2	28.6±6.1	43.0±4.5	55.2±5.1	69.8±4.3	79.9±4.8	25.7±4.0	140

[a]See Table 1 for formulation (vehicle) codes.
[b]η_{app}, apparent viscosity, at shear rate of 100 s^{-1} and temperature of 37°C.
[c]Lilley et al. (1963) (From Ref. 27).

The results presented are mean values of three determinations.

Rheogram of the measurements obtained in this study indicate the differences in the flow properties of the vehicles. These range from Newtonian flow for FCO and distilled water, through varying degrees of pseudoplastic behavior for the rest of the vehicles except vehicle F, which showed pseudoplastic behavior with slight thixotropy. The apparent viscosities at an arbitrarily chosen low-shear rate of s^{-1} are shown in Table 2.

Drug-Release Studies

Table 2 shows the mean percentage of ampicillin dissolved at different times in the flask-stirrer method for each formulation. Plots of these percentages against sampling times, to give the dissolution-rate curve for each formulation, is given in Fig. 1. The time required for 50% of the drug to appear in solution, i.e., $t_{50\%}$, calculated from individual dissolution-rate curves for each formulation, was used as an index of the dissolution rate of ampicillin. Analysis of variance and multiple-range test[25] were carried out to distinguish the significance or otherwise of the differences between the mean $t_{50\%}$ values. The results are summarized as follows:

Mean values of	C	D	A	B	F	E
$t_{50\%}$ in rank order	3.0	3.0	3.6	12.3	25.7	51.0
5% level						

Any two means not underlined by the same line are significantly different. Any two means underlined by the same line are not significantly different.

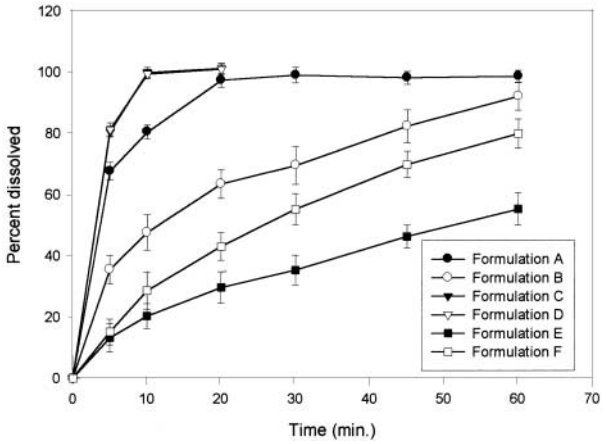

Figure 1. Percent ampicillin dissolved at various times from different aqueous and oily formulations using the flask-stirrer method.

The results obtained in this study (Table 2) parallel those obtained with the nitrofurantoin ones.[5] This is not surprising because both drugs possess low oil: 0.1 M HCL partition coefficients—0.052 and 0.48[5] for ampicillin and nitrofurantoin, respectively. Thus, the aqueous suspensions (C and D) gave the lowest $t_{50\%}$ values and the inclusion of 30% sucrose in D did not cause any apparent effect. The $t_{50\%}$ value for the suspension in FCO alone could not be distinguished statistically from those of the aqueous formulations, $p > 0.05$, and neither could that for the oily suspension B, which contained 30% sucrose. The low lipophilicity of ampicillin trihydrate was visually apparent in these systems because the solid particles could be seen to be released from the FCO in formulation A and fall through the dissolution medium in the

same way as they did with the aqueous suspensions. It is, therefore, not surprising that the $t_{50\%}$ value for this formulation should be very similar to those of the aqueous suspensions C and D.

The formulation F gave significantly a longer $t_{50\%}$ value than the simple oily suspension A and formulation B (30% w/v sucrose in FCO), $p < 0.05$. It should be pointed out that the sucrose content of the appropriate ampicillin formulations was 30% rather than 20%, as in the nitrofurantoin suspensions.[5] Finally, the longest $t_{50\%}$ value was given by formulation E, which contained 30% sucrose plus 1.25% Cab-o-sil, so that not only was the sucrose content higher than that in the nitrofurantoin suspensions, but also the Cab-o-sil content was increased. This formulation had a very high apparent viscosity (η_{app}), as shown in Table 2. The values given in this table also show that there is an approximate rank order relationship between $t_{50\%}$ and $\eta_{app.}$

Like the nitrofurantoin formulation[5] a the ampicillin suspensions B, F, and E formed large pear-shaped globules and the lifetimes of these appeared to be a major factor in determining the release of ampicillin. In addition, the dispersion of the vehicles into these globules and the formation of oily layers on the surface of the dissolution medium in an uncontrollable manner was probably responsible for the poor reproducibility of the results. Although this poor reproducibility may be a criticism of the method used to determine the release of drug, it is likely that a similar phenomenon will occur in vivo in the gastric fluids.

In Vivo Bioavailability Studies

All zero-time plasma samples yielded, without any exception, no detectable activity against the test organism. This is in agreement with the finding of Macleod et al.[26] The mean concentrations of ampicillin in the plasma samples that were taken from the six rabbits at various times after oral administration of ampicillin trihydrate suspensions are ploted vs. time and shown in Fig. 2.

The values of the three bioavailability parameters, i.e., area under the curve (AUC_0^8), peak plasma concentration (C_{max}), and time at which that peak was reached (T_{max}), were determined (Table 3). The AUC_0^8 was calculated by using the trapezoidal rule. Analysis of variance were carried out on the experimental data followed by the application of the multiple-range test.[25] The results of these

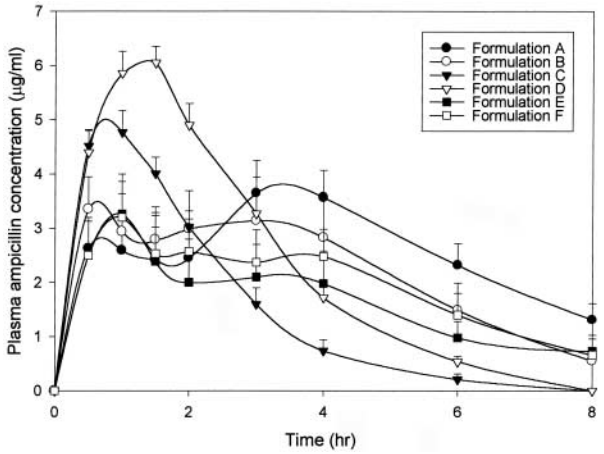

Figure 2. Mean plasma ampicillin concentration vs. time after oral administration of 2% w/v ampicillin trihydrate suspension in different formulations as a single dose of 50 mg/kg body weight. Each value is the average of results obtained in six rabbits with SD as bars.

analyses, which are summarized below, show that there are no significant differences between the AUC_0^8 values or the T_{max} values ($p > 0.05$), and that only the C_{max} for formulation D differs significantly from those of B, E, and F ($p < 0.05$).

			T_{max} (hr)			
Formulation	C	D	B	F	E	A
Mean[a]	1.0	1.2	1.3	1.8	1.9	2.1
			C_{max} (µg/mL)			
Formulation	E	F	B	A	C	D
Mean[a]	3.5	3.7	3.8	4.9	5.2	6.4
			AUC_0^8 (µg·hr/mL)			
Formulation	C	E	F	B	D	A
Mean[a]	12.0	13.2	15.3	17.4	18.6	20.1

[a]Any two means not underlined by the same line are significantly different ($p < 0.05$). Any two means underlined by the same line are not significantly different ($p > 0.05$).

The results obtained in this study showed that the AUC_0^8 of ampicillin administered as a simple aqueous suspension (C) was smaller than that obtained with other formulations, i.e., AUC_0^8 of formulation C was 60%, 64%, 68%, 78%, and 90% of A, D, B, F, and E respectively. In other words, if it is considered that the simple oily suspension (A) is 100% bioavailable, then the bioavailability of (C) is 60% of (A). Inclusion of 30% w/v sucrose in the aqueous suspension (D)

Table 3. Peak plasma concentrations (C_{max}), peak times (T_{max}), and area under the curve (AUC_0^8) of ampicillin following oral administration of 50 mg/kg body weight of ampicillin trihydrate, as a single dose in different formulations, to six rabbits. Each value is represented as mean ± SD from six experiments.

	Formulation[a]					
Parameter	A	B	C	D	E	F
C_{max} (μg/mL)	4.9 ± 1.4	3.8 ± 2.2	5.2 ± 1.1	6.4 ± 1.3	3.5 ± 1.6	3.7 ± 1.6
T_{max} (hr)	2.1 ± 0.5	1.3 ± 0.8	1.0 ± 0.5	1.2 ± 0.6	1.9 ± 0.7	1.8 ± 0.8
AUC_0^8 (μg hr/mL)	20.1 ± 3.1	17.4 ± 3.6	12.0 ± 2.5	18.6 ± 2.7	13.2 ± 3.2	15.3 ± 3.6

[a]See Table 1 for formulation (vehicle) codes.

enhanced the extent of absorption to a degree which is close to that of formulation A. Even though the difference is statistically insignificant, $p > 0.05$, translated into clinical practice the differences may have considerable impact on the bioavailability of ampicillin.

A variety of factors could be responsible for the slight enhancements in the extent of absorption of ampicillin. The most likely explanation is, in fact, decrease in the GER brought about by oil[28] or osmotic pressure,[29] because the inclusion of 30% sucrose in the aqueous suspension (D) enhanced the extent of absorption of ampicillin, as reflected by the AUC_0^8, from 12 to 18.6 μg hr/mL, which is close to the AUC_0^8 of 20.1 μg hr/mL for formulation A. It should pointed out that the inclusion of 30% w/v sucrose in the oil, i.e., formulation B, did not enhance the extent of ampicillin absorption but it did enhance the absorption from the aqueous formulation D. It is suggested, therefore, that sucrose enhances the absorption of ampicillin from the aqueous vehicle by virtue of its delaying effect on the GER and has no additional effect on the GER over that caused by the oil itself. This suggestion is supported by the fact that AUC values of formulations A, B, and D are close to each other.

Since ampicillin is an amphoteric compound with an isoelectric point of 4.95, it follows that its solubility will be greater in the gastric fluid than in the mildly acidic intestinal fluid, which has a pH closer to the isoelectric point. Thus, a longer gastric residence time of ampicillin would improve dissolution and enhance bioavailability. In addition, Kirby and Kind[30] indicated that appreciable absorption of ampicillin occurred from the stomach and Swahn[31] reported that the absorption of radiolabelled ampicillin from the stomach is up to 30% of the total amount absorbed. Therefore, if GER is decreased, the degree of absorption from the stomach might be increased, so enhancing the overall extent of absorption of ampicillin.

If the extent of absorption of ampicillin is increased by a reduction in the GER, then one would expect this extent to be reduced by an increase in GER as found by Ali and Farouk,[12] who studied the effect of Sudanese diet on the bioavailability of ampicillin. They indicated that the reduced extent of absorption and peak concentration (C_{max}) could be attributed to increase in the GER and the total GI motility caused by the Sudanese diet, which is rich in bran and fibrous substances that are known to accelerate gastric emptying and might also increase the GI motility. They suggested that when gastric emptying is delayed, ampicillin will stay in the GI tract longer, and hence, more complete absorption will occur. In fact, Haruta et al.[13] reported that extent of bioavailability of ampicillin was increased in rats pretreated with propantheline (a drug which delays GER), and Kund et al.[9] found that absorption of ampicillin was improved when the drug is given with food. The results obtained in the present study are in good agreement with these findings and suggestions.

If the enhancement in the extent of ampicillin absorption from formulations A and D compared to that from C can be explained solely on the basis of the decrease in GER brought about by oil or by sucrose, it leads to the conclusion that the effects of oil on gastric secretion, formation of bile salt-mixed micelles, stimulation of the lymph flow, and the effect of viscosity of the formulation (Table 2) are unlikely explanations of the results obtained in this study. It is suggested, therefore, that delay in the GER is the most likely explanation of the enhancement obtained in the extent of ampicillin absorption when the drug is administered in oily formulations and formulations possessing high osmotic pressure.

The oily formulations E and F allowed a greater extent of ampicillin absorption than the simple aqueous suspension (C), but provided a lower extent than A, D, or B. This latter rank-order relationship, i.e., extent of absorption from A, D, and B > extent of absorption from F and E, is paralleled by the results obtained in the in vitro dissolution-rate studies (Table 2). It is possible, therefore, that the in vivo results can be explained to some extent on the basis of differences in the rates of release of drug from various formulations.

In the case of the oily formulation E, it may be suggested that the possible adsorption of ampicillin on to the Cab-o-sil may interfere with the release of the drug, and so reduce the bioavailability. However, this suggestion is unlikely because Poole et al.[32] detected no change in the bioavailability of ampicillin when 0.99% Cab-o-sil was included in their aqueous suspension.

Comparison of the T_{max} values of the oily and aqueous formulations of ampicillin shows that no significant difference was detected in the T_{max} values for all the formulations ($p > 0.05$), This suggests that ampicillin is absorbed essentially at the same rate from the oily and aqueous formulations. Unlike salicylate,[2] ampicillin is more hydrophilic, and hence, one does not expect the oil to be a reservoir for this drug. However, the trend toward a slower absorption from the oily formulations is likely because of the delay in the GER by the oil, which is more pronounced than that caused by the osmotic pressure exerted by aqueous formulation D.

Comparison of the C_{max} values of the aqueous suspensions (C and D) with those of the oily ones (Fig. 2) suggests that higher concentrations are provided by the former systems. However, only the aqueous one containing sucrose (D) gave significantly a different C_{max} value from those obtained with the oily B, E, and F formulations ($p < 0.05$), and the C_{max} provided by formulation A, the simple oily suspension, did not differ significantly from either of the aqueous formulations C and D ($p > 0.05$).

It should be pointed out that when comparison between the plasma concentration vs. time curves of ampicillin given by the oily and aqueous systems is made (Fig. 2), it is quite clear that all the oily formulations still maintain a measurable plasma concentration at 8 hr post-administration whilst the aqueous ones gave zero concentration at that time. In fact, some individual rabbits gave zero plasma concentration as early as the 4–6 hr samples. The prolonged plasma concentrations of ampicillin, that are obtained when oily vehicles are used, may arise because oil stimulates the evacuation of bile from the gall bladder[33] and it is well known that ampicillin is rapidly excreted in the bile in an active form[34] with a low susceptibility to the inactivating mechanism within the liver.[30] Enhancement of biliary recycling by the oil would therefore lead to reabsorption of ampicillin and a prolongation of blood levels. In fact, Ritschel[35] stated that, "drugs entering the bile must be considered as drugs administered perorally. Upon emptying of the bile into the duodenum, the drugs may be reabsorbed by one of the absorption mechanisms into the portal circulation and returned to the liver from whence they are re-excreted into bile." Therefore, enterohepatic recycling is the most likely explanation of the occurrence of the multiphasic blood-level curves, with two peaks that were given by the oily formulations. In some cases the second peak was regarded as the peak plasma concentration (C_{max}), as in formulation A, since it is higher than the first one. An alternative explanation of this periodicity is that it could be attributed to the sequential absorption of the drug, first from the stomach and then from the small intestine after gastric emptying had occurred. However, this latter explanation seems to be unlikely since the aqueous formulation D, which should also delay GER because of its sucrose content, did not show this phenomena (i.e., a multiphasic blood-level curve). Biphasic absorption was also reported[6] when suspensions of ampicillin in oily vehicles were given by s.c. or i.m. to homing pigeon. They suggested that the initial peak is representing an absorption and distribution phase and the second peak is reflecting the depot nature of the drug. The partition coefficient of ampicillin between oil and 0.1 M HCL, 0.052, would suggest that this latter explanation seems to be unlikely. Thus, enterohepatic recycling seems to be the most likely explanation of this effect.

Relation Between In Vivo and In Vitro

The AUC_0^8 and T_{max} values correlate poorly with viscosity, as indicated by Eqs. (2) and (3). However, the existence of a reasonable correlation between C_{max} and viscosity (Eq. (4)) is probably due to the gastric absorption of ampicillin, so that the higher the viscosity the slower absorption of the drug. The effect of viscosity on the in vitro dissolution and release of

ampicillin is shown by the existence of correlation between $t_{50\%}$ and the viscosity (Eq. (5)).

$$\mathrm{AUC}_0^8 = 17.155 - 0.0169\eta_{\mathrm{app}} \quad r = -0.3462, \quad p > 0.1 \tag{2}$$

$$T_{\max} = 1.344 + 0.003456\eta_{\mathrm{app}} \quad r = 0.5322, \quad p > 0.1 \tag{3}$$

$$C_{\max} = 5.463 - 0.0141\eta_{\mathrm{app}} \quad r = -0.851, \quad p < 0.05 \tag{4}$$

$$t_{50\%} = 0.373 + 0.2573\eta_{\mathrm{app}} \quad r = 0.917, \quad p < 0.01 \tag{5}$$

Analysis of the results also showed that the three in vivo parameters did not correlate with the in vitro parameter $t_{50\%}$ ($p > 0.1$ for AUC_0^8 and $T_{\max}$ and $p > 0.05$ for $C_{\max}$).

Although there is a poor correlation between $t_{50\%}$ and the in vivo parameters, the slower release rates of ampicillin from the oily formulations E and F are paralleled by the lower bioavailabilities of this drug from these two in vivo formulations.

CONCLUSION

Delay in the GER, brought about by the oil or by the osmotic effect of sucrose, produced a slight enhancement in the extent of ampicillin absorption. Although this enhancement was not statistically significant ($p > 0.05$), but was close to the borderline of significant, the differences translated into clinical practice may have considerable impact on the bioavailability of ampicillin. In addition, the in vivo–in vitro correlation studies indicate and confirm the suggestion that the viscosity-enhancing agents have no significant effect on the bioavailability of ampicillin when this compound is administered in an oily vehicle, since the major effect of the oil in decreasing GER predominates and masks the other effects of viscosity. However, viscosity did affect, and correlate with, the in vitro dissolution and release rates of ampicillin. Furthermore, it is suggested that the reasonable correlation found between $C_{\max}$ and viscosity is related to the appreciable gastric absorption because its rate of absorption is likely to be affected by the rate at which it arrives at the gastric mucosa.

Finally, the lack of correlation between in vivo and in vitro parameters illustrate the problem that arises when attempts are made to mimic the in vivo bioavailability of a drug by in vitro methodology, if such bioavailability is influenced substantially by physiological responses to formulation ingredients.

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