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Investigation of progabide absorption from the gastrointestinal tract of the rabbit

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Summary

The oral bioavailability of progabide following its administration as suspensions of varying particle sizes was assessed in the rabbit. This was found to be low (< 5%) with the drug exhibiting a large clearance (13.5 litre/h), large apparent volume of distribution (10.36 litre), and a very short elimination half-life (32 min) in this animal. The concomitant intravenous administration of ranitidine resulted in a drastic reduction of the oral bioavailability of progabide as a result of the inhibition of gastric acid secretion. It therefore appears that the solubility and stability of this drug play significant roles in controlling its oral bioavailability.

Introduction

In previous publications (Farraj et al., 1987a and b), the physicochemical properties of progabide and its related compounds were reported. Based on that, it was anticipated that problems such as the low aqueous solubility and poor stability may limit the oral bioavailability of progabide. It was therefore decided to investigate, in vivo, the effect of gastric pH and gastric residence time together with the effect of the particle size of the drug on the absolute bioavailability.

Progabide in the normal physiological pH range behaves as a weak base for which a pK_a1 of 3.4 can be observed (Farraj et al., 1987b). As a result, any intra- and inter-animal variation in gastric pH in the range 1–5 will in itself alter dramatically the dissolution, degradation, and possibly the absorption characteristics of the formulation. Such uncontrolled pH variations will limit the utility of the animal model and present difficulties in relating changes in the plasma concentration–time profile to any introduced variations in the formulation. In this respect, Altman and Dittmer (1968) quoted the gastric pH of the rabbit, dog, and rat to be 1–1.6, 1–4.5, and 2.0–4.0, respectively. On that basis, the rabbit was chosen as a suitable animal model to investigate progabide oral bioavailability. However, the difficulty in obtaining an empty stomach by traditional fasting methods led Chiou et al. (1969) to conclude that rabbits were unsuitable for oral absorption studies. The strong physiological habit of coprophagy in the rabbit was prevented by introducing modifications such as the muzzle method (Maeda et al., 1977) or the cangue method (Maeda et al., 1979). Neverthe-

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less, other authors (Kaneniwa et al., 1978; Watari and Kaneniwa, 1981) have shown that conventionally fasted rabbits can be used to assess the bioavailability of poorly soluble compounds and that good correlations can be established between the in vivo dissolution rate and absorption rate for the suspension formulations of varying particle size. This is probably related to the fact that the liquid contents of the stomach of the rabbit empty at a much faster rate than the solid components (Pickard and Stevens, 1972; Curt et al., 1980).

On that merit, it was decided to administer suspensions of varying particle size to rabbits in order to assess the oral bioavailability of progabide. The slow gastric emptying and low gastric pH should allow a full examination of their effect on the oral bioavailability.

The pH of the stomach can be altered by the administration of an H_2-receptor antagonist, such as ranitidine or cimetidine, which can block gastric acid secretion and raise the pH in the stomach. Ranitidine was chosen for use in the rabbit because it does not suffer from the limitations and shortcomings of cimetidine; the latter compound was shown to impair the metabolism of some drugs (Bauman and Kimelblatt, 1982) and reduce liver blood flow (Feely et al., 1981). In comparison, ranitidine did not display any effect on liver metabolism (Henry et al., 1980) and did not alter the gastric emptying rate of a test meal (Mignon et al., 1982).

Materials and Methods

Three powder fractions of progabide were prepared as described previously (Farraj et al., 1987c). These were: a 212–300 μm sieve fraction, a 40–63 μm sieve fraction, and a micronised fraction of a geometric volume mean diameter of 11.5 μm with a geometric standard deviation of ± 1.2. Ranitidine hydrochloride was kindly provided by Glaxo Group Research.

Hydroxyethylcellulose (Cellosize QP15000) was purchased from Union Carbide for use as a suspending agent. Heparin sodium injections B.P. of 5000 units per ml (Pularin-mucous) were obtained from Duncan, Flockart and Co., and were diluted to the required strength in sterile normal saline (Phoenix Pharmaceuticals). Glycerol SLR (Evans) and polyethylene glycol 200 SLR (Fisons) were used as supplied.

For i.v. administration and blood sampling, Venisystems Butterfly-23 infusion disposable sets (Abbott Laboratories) were used.

Animals

The male lop-eared rabbits (Hylyne Rabbits) were housed individually in metal cages and maintained on TR2 pellets (L.A. Pilsbury) and water ad libitum unless otherwise specified. They had a mean weight of 3.42 ± 0.5 kg.

Methods

Intravenous administration. Each rabbit was fasted overnight for 22 h prior to the experiment but water was allowed ad libitum. Both food and water were withheld during the experiment. The marginal vein of one ear was cannulated for sample withdrawal and that of the other ear for drug administration, using the infusion sets filled with heparin solution (330 U/ml).

Each rabbit received a 20 mg/kg dose of progabide from a 35 mg/ml solution in PEG 200, as an i.v. bolus injection. At suitable intervals, blood samples (1 or 2 ml) were collected into heparinised tubes placed on crushed ice. The plasma was separated by centrifugation at 3000 r.p.m. for 5 min and then stored at $-20\,^{\circ}$C.

Oral administration. The oral bioavailability of the 3 powder fractions of progabide was assessed by formulating them as 111 mg/g suspensions in 1% w/w hydroxyethylcellulose. The fasting schedule of the rabbits was similar to that in the i.v. experiments. Each rabbit received a 200 mg/kg dose by gastric intubation (Feldman, 1977). The suspension was followed by 1 ml of tap water to flush the tube and ensure the complete delivery of the dose. As with the i.v. administration, plasma samples were obtained and stored frozen.

Co-administration of ranitidine. The effect of ranitidine on the oral bioavailability of the 40–63 μm powder suspension was investigated. Each rabbit received a bolus i.v. dose of 10 mg/kg of ranitidine hydrochloride into the marginal ear vein from a 40 mg/ml solution in normal saline 30 min

prior to the oral administration. The marginal vein of the other ear was then used for blood withdrawal.

Analysis of collected plasma. The method used was described previously (Farraj et al., 1987d). All the samples were analysed within 3 days of their collection. The calibration curves were routinely checked by analysis of standard solutions in the concentration range examined. The presence of ranitidine in some of the plasma samples did not interfere with the analysis as it had a very short retention time.

Results and Discussion

A typical assay chromatogram (Fig. 1) shows that, following the i.v. administration of progabide to the rabbit, the drug is metabolised to yield the acid metabolite, PGA, and further, that SL79.182 is also present. The chemical formulae and structures of these compounds were reported previously (Farraj et al., 1987d). It is worth noting here that, in vitro, progabide transformation by degradation only yielded SL79.182 but not PGA (Farraj et al., 1987a). The plasma concentration–time profiles of progabide, its metabolite PGA, and its degradation product SL79.182 following i.v. administration, are shown in Fig. 2. The data reveal the extensive metabolism of progabide to PGA and confirm that SL79.182 does not play a major role in the overall elimination of progabide from the plasma. It is therefore more likely that the SL79.182 detected is a result of the hydrolytic degradation of progabide outside the liver, e.g. in the plasma.

Ferrandes and colleagues (Ferrandes et al., 1985) investigated the metabolic fate of progabide in 4 animal species in man. They found that the compound was metabolised in the mouse, hamster, rat, baboon, and man via the same pathways: deamidation of the side chain, cleavage of the imine bond, and hydroxylation of the benzophenone moiety at the C_3 or C_5 position. The combination of these routes may lead to 10 metabolites, which are excreted rapidly in urine and bile, mainly as their glucuroconjugate derivatives, although SL79.182 is also excreted in rat and baboon urine

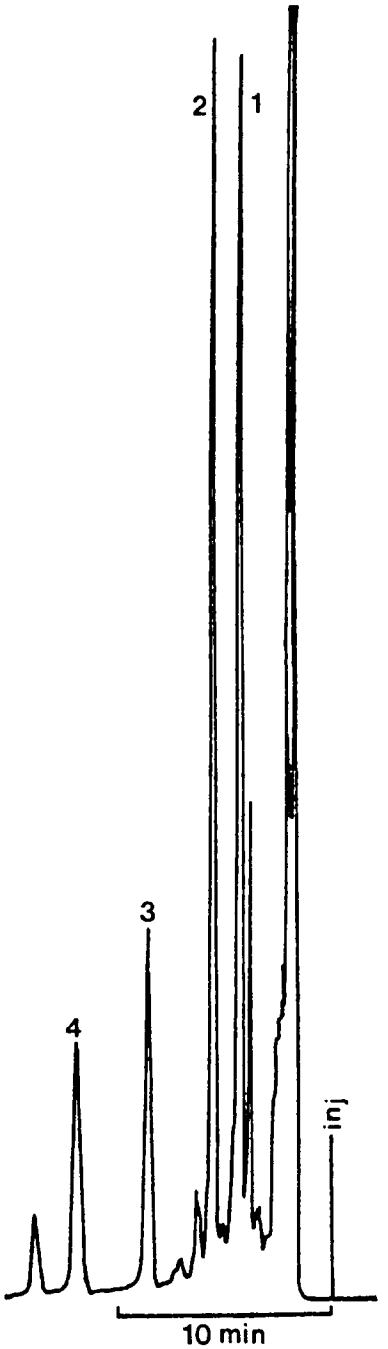

Fig. 1. Typical example of the RP-HPLC chromatogram obtained upon analysis of the plasma of a rabbit given an i.v. bolus injection of progabide; 1, PGA; 2, progabide; 3, SL79.182 and 4, internal standard.

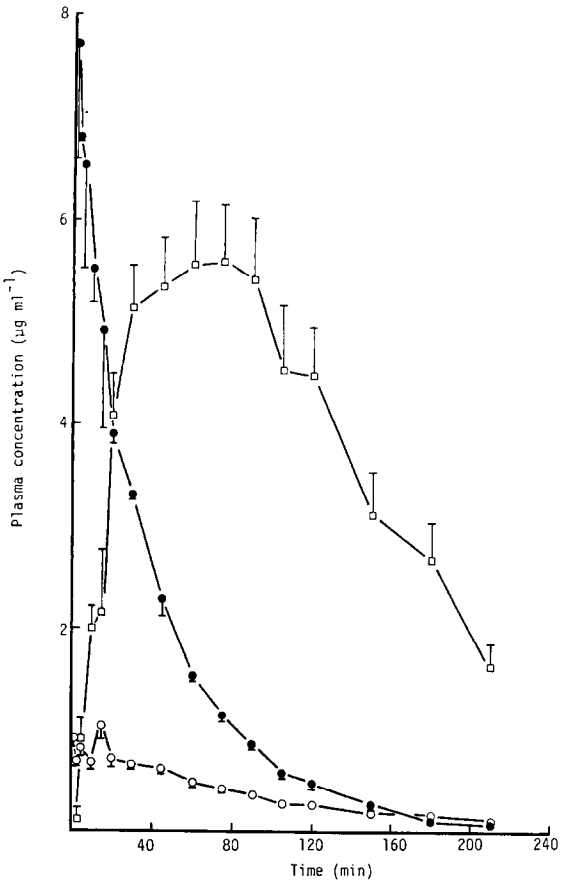

Fig. 2. Plasma concentration-time profiles of progabide (●), SL79.182 (○), and PGA (□) following an i.v. bolus injection of progabide. Each point represents the mean ± S.E.M. for 5 rabbits.

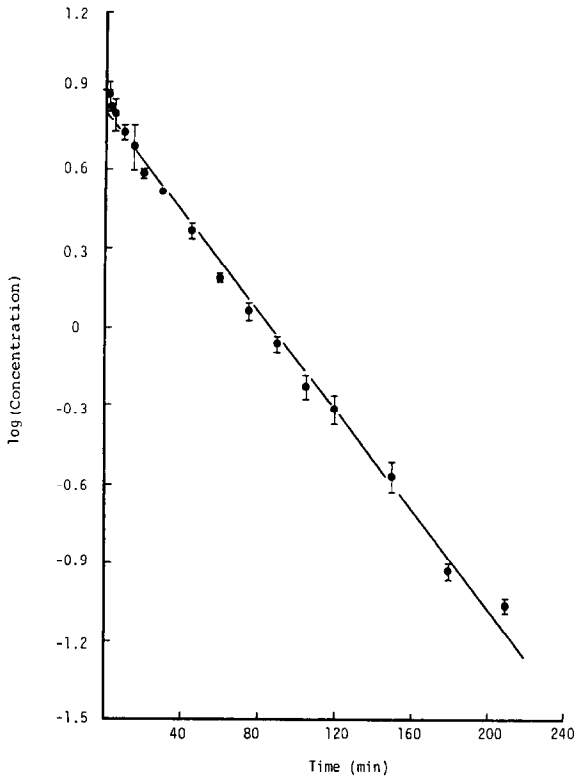

Fig. 3. Semilogarithmic plot of the plasma concentration–time profile of progabide, following an i.v. bolus injection. Each point represents the mean ± S.E.M. for 5 rabbits.

as a sulfoconjugate. Independent of the route of administration, unchanged progabide is only eliminated in trace amounts. SL79.182 resulting from the hydrolysis of the imine bond is more abundant in the glucuro- or sulfoconjugated form after oral administration of progabide than after i.v. treatment. The metabolic pathways of progabide in man are qualitatively and quantitatively similar to those observed in the animal species.

TABLE 1

AUC_t values of the plasma concentration–time profiles following the administration of progabide in rabbits

Formulation and route of administration	$AUC_t \pm$ S.D. ($\mu g \cdot ml^{-1} \cdot min$)		
	Progabide	SL79.182	PGA
Solution in PEG 200; i.v.	291 ± 18	96 ± 36 (0.44)	818 ± 186 (2.80)
Micronised suspension; oral	92 ± 62 ^{ns}	198 ± 114 (2.87) ^{s}	800 ± 585 (8.67) ^{ns}
40–63 μm suspension; oral	143 ± 108	55 ± 24 (0.51)	947 ± 523 (6.60)
212–300 μm suspension; oral	11 ± 12 ^{s}	18 ± 12 (2.18) ^{s}	227 ± 203 (20.58) ^{s}
40–63 μm suspension; oral preceded by i.v. ranitidine	7 ± 3 ^{s}	15 ± 2 (2.86) ^{ss}	108 ± 46 (15.38) ^{ss}

Values in brackets represent the ratio of the mean AUC_t, on a molar basis, to that of progabide. Statistical significance vs 40–63 μm suspension (as control) by Student's t-test: ns, not significant; s, significant ($P < 0.05$); ss, significant ($P < 0.02$).

The GABAmide side chain of progabide is deamidated to yield the active carboxylic acid PGA. Both progabide and PGA are present in plasma in concentrations of the same order of magnitude. The major metabolites found in the urine result from the hydrolysis of the imine bond and account for 55.6 to 75% of the administered dose with SL79.182 representing 43% of the dose. Neither progabide nor PGA is found in urine. A comparison of the kinetics of PGA with that of progabide demonstrates the longer apparent elimination half-life of PGA in all species e.g., in the mouse it is 0.6 h compared with 0.2 h for progabide whereas in man it is 8.8 h compared with 3.1 h for the parent compound. In mouse and rat, PGA is found at greater levels than progabide, but in hamster, baboon, and man, this metabolite is less abundant than the mother drug after a single oral dose.

Levy et al. (1985) investigated progabide pharmacokinetics in the rhesus monkey and in man. Progabide was found to have a large systemic clearance, large distribution volume, and short biological half-life. Oral doses were rapidly absorbed with PGA exhibiting a longer elimination half-life than progabide.

By examining progabide kinetics in the rabbit, it is clear that PGA plays a dominant role over SL79.182 as reflected by its area under the curve (AUC, Table 1) which is 6.4 times that of SL79.182, as calculated by the trapezoidal rule (Niazi, 1979). Both PGA and SL79.182 display longer apparent elimination half-lives than progabide. These can be estimated from semilogarithmic plots of the

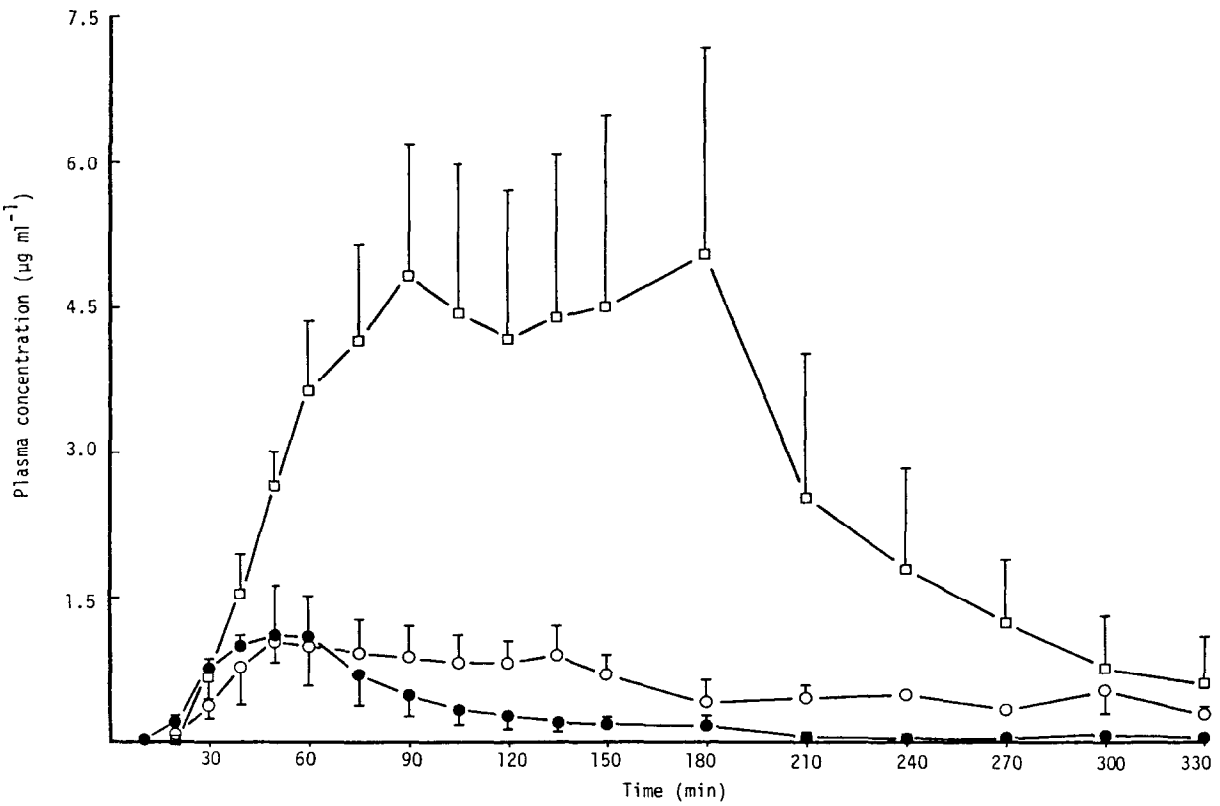

Fig. 4. Plasma concentration–time profiles of progabide (●), SL79.182 (○), and PGA (□) following the oral administration of micronised progabide suspension to 4 rabbits. Each point represents the mean ± S.E.M.

data in Fig. 2 to be 74 and 72 min, respectively. As illustrated in Fig. 3, a one-compartment open pharmacokinetic model could satisfactorily describe the plasma concentration-time profile of progabide following an i.v. bolus injection. The initial concentration achieved, C_0, is given by:

$$C_0 = D/V_d \quad (1)$$

where V_d is the apparent volume of distribution and D is the dose of drug. It therefore follows that the overall elimination rate, β, of progabide in the rabbit is 21.74×10^{-3} min^{-1} with a corresponding half-life of 32 min. The extrapolated initial concentration, C_0, was found to be 6.6 μg/ml and the dose administered, D, for a mean weight of 3.42 kg was 68400 μg. By substitution into the above equation, V_d was calculated to be 10.36 litres. The systemic clearance (Paxton, 1981), Q, was calculated from equation 2 to be 13.5 litre/h.

$$Q = B \cdot V_d \quad (2)$$

Thus, overall, progabide exhibited a large clearance, large apparent volume of distribution, and a very short elimination half-life in the rabbit. Figs. 4–6 show the plasma profiles of progabide, PGA, and SL79.182 following oral administration of three size fractions of progabide powder. These reveal the low absolute oral bioavailability of progabide in the rabbit. The AUC_t values from time 0 to 300 min are shown in Table 1. On the assumption that the AUC_t values represent the whole areas under the curves from time 0 to infinity and that the systemic clearance is independent of the route of administration, the extent of bioavailability, F, for the oral route can be calculated from

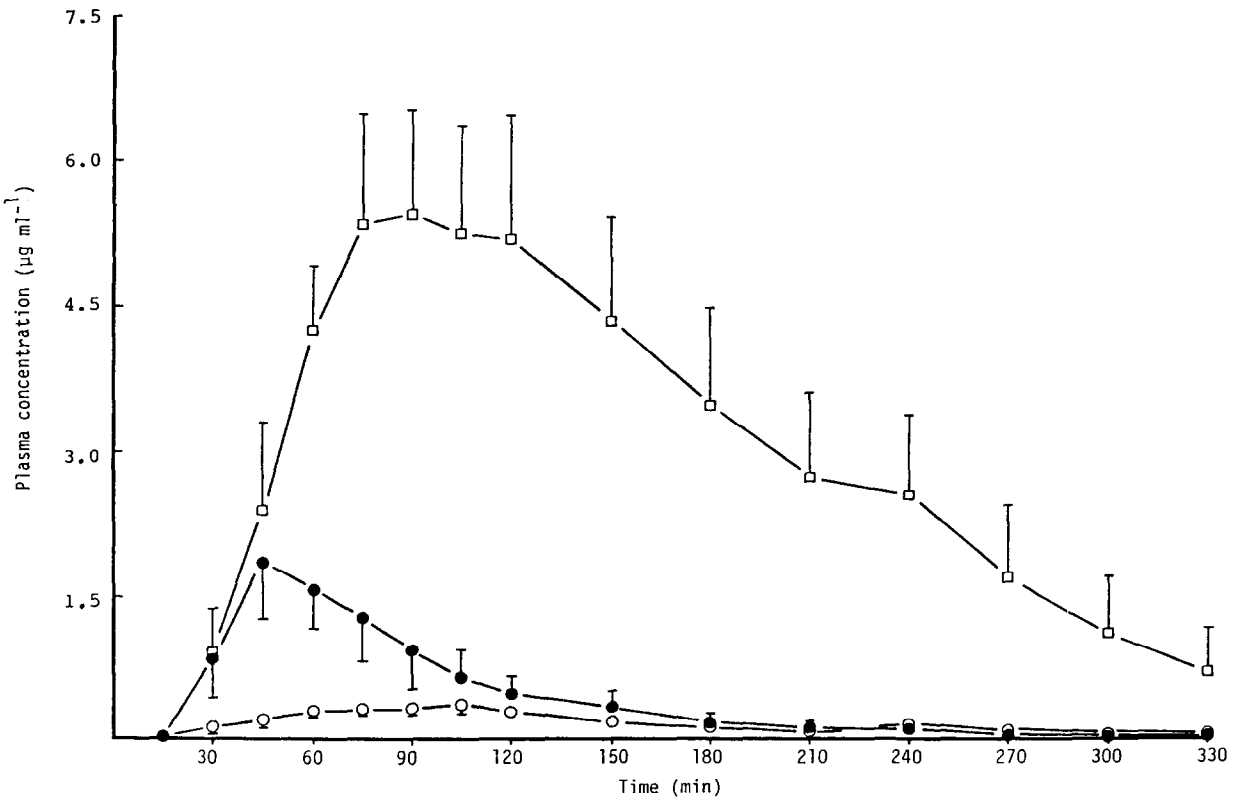

Fig. 5. Plasma concentration-time profiles of progabide (●), SL79.182 (○), and PGA (□) following the oral administration of 40–63μm progabide suspension to 5 rabbits. Each point represents the mean ± S.E.M.

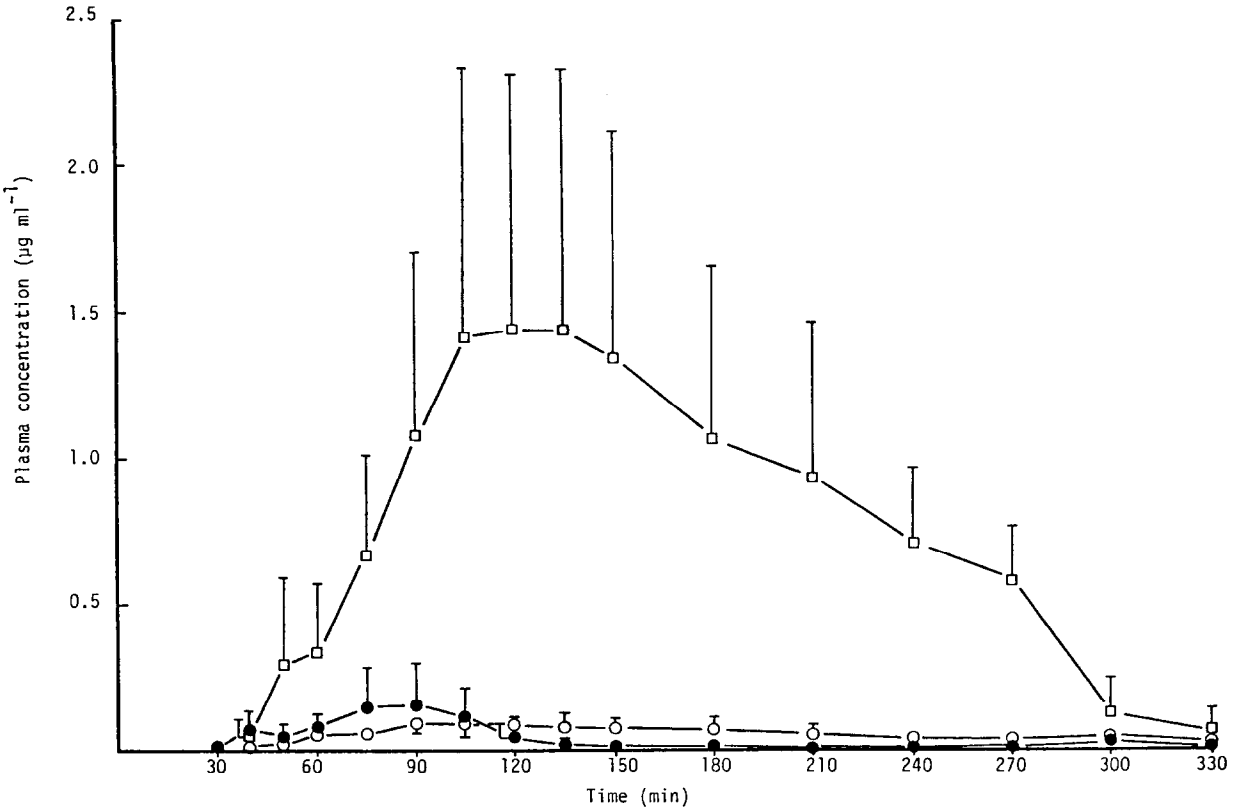

Fig. 6. Plasma concentration-time profiles of progabide (●), SL79.182 (○), and PGA (□) following the oral administration of 212–300 μm progabide suspension to 3 rabbits. Each point represents the mean ± S.E.M.

equation 3 to be 0.032, 0.049, and 0.004 for the micronised, 40–63 μm and 212–300 μm fractions, respectively. Expressed as percentages, the values become 3.2%, 4.9%, and 0.4% in the same order.

$$F = [\text{Dose}_{\text{i.v.}}/\text{Dose}_{\text{oral}}] \cdot [\text{AUC}_{\text{t oral}}/\text{AUC}_{\text{t i.v.}}] \quad (3)$$

Moreover, it appeared that the further particle size reduction to give the micronised suspension did not increase the bioavailability but rather decreased it although the magnitude of change was not statistically significant (Table 1). As expected though, the 212–300 μm suspension gave the least bioavailability.

Examination of the AUC_t values of SL79.182 and PGA is useful in elucidating the possible underlying causes of the low oral bioavailability. Although the high hepatic clearance of the oral formulations, by virtue of the first-pass effect (Gibaldi and Perrier, 1974), accounts for the increased production of PGA with these formulations over the i.v. route it does not primarily account for the low oral bioavailability. This can be best appreciated by summing the AUC_t values of progabide, SL79.182, and PGA profiles for each formulation and comparing them to that of the i.v. formulation. Expressed as percentages, the micronised, 40–63 μm, and 212–300 μm suspensions yielded values of 9.3%, 9.4%, and 2.1%, respectively. By comparing these figures with their respective equivalent *F*-values obtained earlier for progabide alone, i.e. 3.2%, 4.9%, and 0.4%, it becomes clear that the biotransformation of progabide to PGA and/or SL79.182 within the central compartment of the proposed pharmacokinetic model can only account for a small proportion of the oral dose administered. Hence, hepatic

metabolism and plasma conversion of progabide can be ruled out as determinants of the oral bioavailability of this drug in the rabbit. Moreover, the absorption rate can be excluded as a rate-determining step in the absorption of progabide. The plasma profiles of progabide show that it was rapidly absorbed with peak levels of 1.12, 1.86, and 0.16 μg/ml at 60, 45, and 75 min for the micronised, 40–63 μm, and 212–300 μm suspensions, respectively. Since the peak time is inversely proportional to the rate of absorption, it can be concluded that the absorption rate decreases in going from the 40–63 μm suspension to the micronised and 212–300 μm suspensions in that order. The apparent absorption rate constants were estimated, from semilogarithmic plots of the absorption phase, to be 0.0916, 0.1478, and 0.0383 min^{-1} for the micronised, 40–63 μm, and 212–300 μm suspensions, respectively, with corresponding half-lives of 7.6, 4.7, and 17.6 min. The method of data "feathering" was not used in this case to arrive at the absorption rates because absorption continued well into the elimination phase of the plasma profiles.

The pretreatment of the rabbits with ranitidine resulted in a drastic effect on the rate and extent of absorption of the orally administered 40–63 μm progabide suspension as illustrated in Fig. 7. The AUC_t of progabide was reduced to a mere 4.9% of the AUC_t in the absence of ranitidine. Clearly, the inhibition of gastric acid secretion has reflected adversely on the oral bioavailability.

These results are in agreement with those obtained in human volunteers (Arnold et al., 1984) where the bioavailability of a micronised tablet with gastroresistant coating was shown to be less

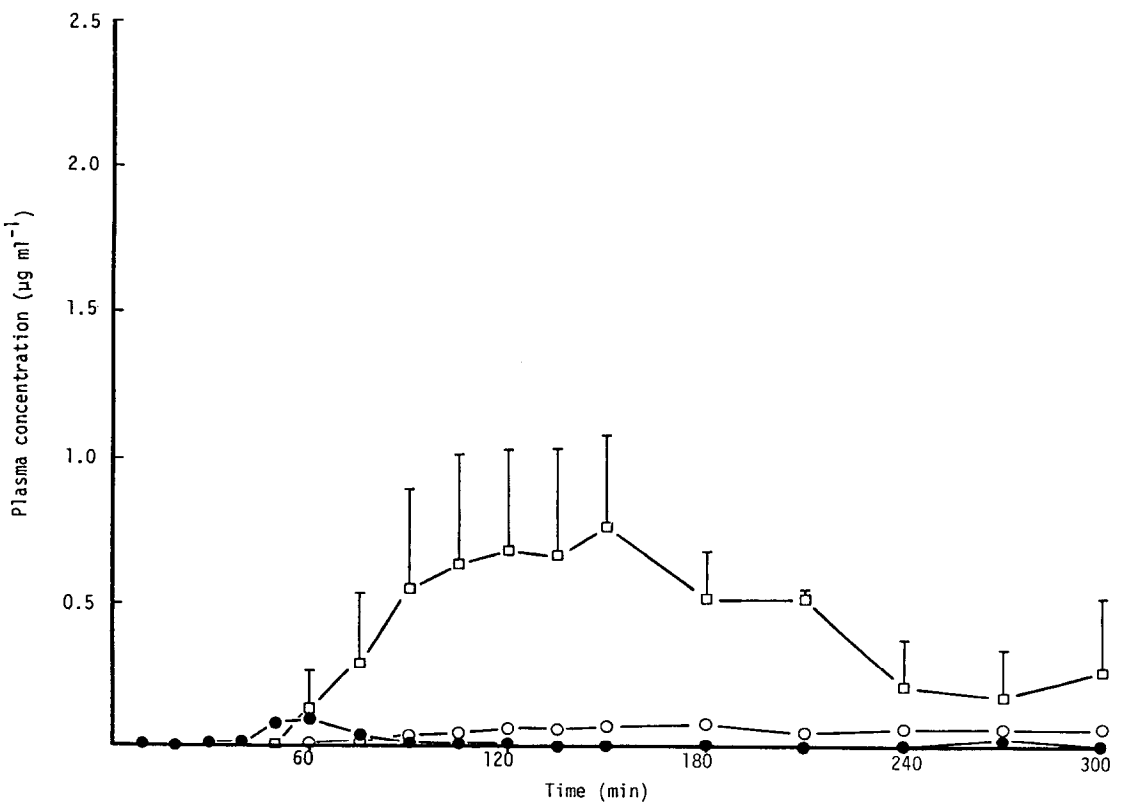

Fig. 7. Plasma concentration-time profiles of progabide (●), SL79.182 (○), and PGA (□) following the oral administration of 40–63 μm progabide suspension to 3 rabbits with i.v. ranitidine 30 min prior to the oral intubation. Each point represents the mean ± S.E.M.

than that of the uncoated tablet, in spite of the instability in acid.

It was shown earlier (Farraj et al., 1987c) that raising the pH of the gastric content would enhance the stability of the administered suspension and simultaneously reduce its rate of dissolution. The results obtained with ranitidine thus infer that gastric dissolution of progabide, under high acidity conditions, is a necessary prerequisite to its oral absorption in the rabbit, yet it is likely that the same conditions in the stomach are responsible for limiting its overall bioavailability through degradative losses.

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