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Improved oral bioavailability of propranolol in healthy human volunteers using a liver bypass drug delivery system containing oleic acid

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Summary

Propranolol, a clinically proven lipophilic β-adrenergic blocking agent, undergoes extensive and unpredictable first-pass hepatic metabolism when administered as a conventional oral formulation. A nine-subject three-way cross-over study was performed in healthy human volunteers to assess the relative bioavailability of two novel oral formulations of propranolol, designed to bypass hepatic first-pass metabolism. These formulations contained a mixture of unsaturated fatty acids, mainly oleic acid, and surfactants in enteric-coated liquid-filled hard gelatin capsules. Using these formulations selective increases of up to more than 6-fold in AUC and 4-fold in C_{max} were achieved in subjects who responded poorly to Inderal®. The increased propranolol bioavailability achieved using the liver bypass formulations was associated with a reduction in the coefficient of variance for both C_{max} and AUC of up to 44%, when compared to Inderal® The results of the present study suggest the possibility of developing a predictable reduced dose delivery system for basic lipophilic drugs which undergo extensive hepatic first-pass metabolism.

Introduction

Hepatic first-pass metabolism reduces the reliability and effectiveness of many commonly used lipophilic cardiovascular bronchopulmonary and psychoactive drugs when administered orally (Jack et al., 1982; Pond and Tozer, 1984). Propranolol, a well studied and clinically proven β-adrenergic blocking agent is almost completely absorbed from the gastrointestinal tract and is then subject to an extensive and variable metabolism by the liver. Bioavailability estimates of 30% have been obtained using conventional formulations of propranolol (Routledge and Shand, 1979), however, a number of studies have shown that propranolol demonstrates non-linear kinetics, requiring a dose

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of 20–30 mg to saturate liver metabolism (Shand and Rangno, 1972; Dvornic et al., 1983; Dey et al., 1986). Failure to saturate liver metabolism is thought to result in the poor performance of sustained release formulations of propranolol (Wagner, 1985; Nace and Wood, 1987). Bioavailability of propranolol using sustained release formulations has been estimated at only 40–70% of that observed using conventional formulations (Leahey et al., 1980; Serlin et al., 1983; Perucca et al., 1984).

Interestingly, studies using rectal administration have demonstrated improved delivery of propranolol in animals (DeBoer et al., 1981; Iwamato and Watanabe, 1985) and humans (Cid et al., 1986). Rectal administration results in liver bypass as drugs absorbed into the haemorrhoidal veins do not enter the hepatic portal blood supply.

Another potential strategy for avoiding hepatic first-pass metabolism of orally administered drugs is to redistribute absorption into the lymphatic system, which has direct access to the systemic circulation (Charman and Stella, 1991; Muranishi, 1991). Previous studies have demonstrated the appearance of drugs in lymph following oral administration (Sieber et al., 1974). These compounds are lipophilic (Sieber, 1976) and include propranolol (White et al., 1991). The extent of the absorption of drugs into the lymph has been shown to be increased by the concomitant administration of lipids and surfactants (Yoshikawa et al., 1981; Palin and Wilson, 1984; Takada et al., 1985; Charman and Stella, 1986a; Charman et al., 1986; Omotosho et al., 1990). Lymphatic absorption of drugs has also been shown to result in a corresponding increase in systemic drug bioavailability (Palin et al.,1982; Caldwell et al., 1982; Grimus and Schuster., 1984; Palin and Wilson, 1984; Gowan and Stavchansky, 1986; Ichihashi et al., 1991).

The present study reports the effects of two formulations of propranolol, designed to avoid hepatic first-pass metabolism, in healthy human volunteers. A comparison is made with a standard tablet formulation of propranolol, Inderal®, and the implications for lymphatic drug delivery discussed.

Materials and Methods

Chemicals

Propranolol was obtained from Alfa Chemicals Ltd (U.K.) for formulation and bioavailability studies. Oleic acid B.P. was supplied by Sherman Chemicals Ltd. Tween 80 (polysorbate 80) was obtained from ICI Speciality Chemicals. Glycerol B.P. was supplied by William Rawson Ltd. Hydroxypropylmethylcellulose phthalate, B.P or USP.NF grade, was obtained from Stancourt, Sons and Muir Ltd and hard gelatin capsules, Licaps®, transparent size 1, from Capsugel (Pontypool, U.K.). Inderal®, 80 mg (ICI) was obtained from commercial sources and bile acids supplied by Consolidated Chemicals Ltd, Wrexham (U.K.). All other chemicals and solvents used were of an appropriate grade and obtained from Sigma (Poole, Dorset, U.K.), BDH (Speke, Liverpool, U.K.) or Metlab (Hawarden, Clwyd, U.K.).

Manufacture of dosage forms

Two formulations designed to by-pass hepatic drug metabolism using lymphatic delivery were manufactured containing propranolol, at a potency of 80 mg per capsule, and oleic acid. Formulation A, containing bile acids, was based on that previously tested in pigs (White et al., 1991). In formulation B the bile acids were replaced by the non-ionic surfactant Tween 80. To manufacture formulation A, propranolol was first dissolved in oleic acid by heating at 45°C, with mixing, followed by addition of an alcoholic solution containing bile acids. Alcohol was subsequently removed from the formulation under vacuum and the resulting material filled into size 1 hard gelatin capsules, which were sealed using the Licaps® process (Cade et al., 1986).

Formulation B was manufactured using oleic acid, containing 1% w/w d-α-tocopherol, which was heated to 45°C and propranolol added with mixing until completely dissolved. After cooling, the Tween 80, glycerol and water were added, also with mixing, and the resulting formulation filled into size 1 hard gelatin capsules, which were sealed using the Licaps® procedure (Cade et al., 1986). The glycerol and water were added

to the formulation to prevent capsule embrittlement caused by surfactants (Cole et al., 1992).

Capsules containing formulations A and B were subsequently enteric-coated, by top spraying, in a Uni-Glatt® fluidised bed (air suspension system) with an alcoholic solution containing HPMC-phthalate. The temperature and air flow in the Uni-Glatt® system, were sufficient to efficiently evaporate the solvent used but remained below that at which damage to the hard gelatin capsules would occur. The capsules were finally assayed for propranolol content by the high-performance liquid chromatography (HPLC) method described below.

HPLC of propranolol formulations

The chromatographic procedure, based on the B.P. test for propranolol, was carried out using a 20×0.46 cm Spherisorb® S5ODS2 HPLC column (Phase Separations Ltd, Deeside, Clwyd) with a mobile phase flow rate of 1.8 ml min^{-1}. The mobile phase consisted of 1.15 g of sodium dodecyl sulphate, 10 ml of a mixture of 1 volume of concentrated sulphuric acid and 9 volumes of water, 20 ml of a 1.7% (w/v) solution of tetrabutylammonium dihydrogen orthophosphate, 370 ml water and 600 ml acetonitrile with the mixture adjusted to pH 3.3 using 2 M sodium hydroxide. Detection was by ultraviolet spectrophotometry at 292 nm. The retention time of propranolol in this system was approx. 2.5 min.

Single capsules containing propranolol, or drug-free placebo formulations, were weighed into 50 ml volumetric flasks and 25 ml of the mobile phase added. The flasks were incubated at 55°C for 5 min in a water bath and subsequently shaken for 10 min on a wrist-action shaker. The flasks were allowed to cool to ambient temperature and then made up to a final volume of 50 ml with mobile phase and left to stand for 30 min. An aliquot of the extract was centrifuged at 4500 rpm, at ambient temperature for 10 min, and a 1 ml sample subsequently diluted with an equal volume of mobile phase before being analysed by HPLC. The injection volume used was in each case 10 μl. Determinations were performed in duplicate or triplicate with a coefficient of variance of less than 2%. A single point calibration using a 0.75 mg ml^{-1} high purity standard of propranolol prepared in mobile phase was used throughout.

Dissolution testing

The dissolution characteristics of the enteric-coated liquid-filled hard gelatin capsules containing formulation A or B were assessed using the dissolution apparatus as specified by USP XXII (apparatus 2). Dissolution testing was carried out using 0.1 M HCl or Sorensens phosphate buffer, pH 6.8, containing 0.2% (w/v) sodium cholate and 0.1% (w/v) sodium deoxycholate at 37°C. The total volume of the dissolution vessel was 900 ml and paddle rotation speed was set at 70 rpm. Capsules containing formulation A or B were found to float at the surface of the dissolution vessel, therefore, to provide sufficient mixing the paddle height was set such that the blades were positioned at the surface of the dissolution fluid. Using this system the capsules were shown to remain intact for at least 1.5 h in 0.1 M HCl. Dissolution testing at pH 6.8 resulted in a rapid release of the capsule contents within 5 min. No decline in the performance of the enteric-coating was observed during the period of the study.

Stability studies

Capsules containing formulations A and B were stored in the dark at some or all of the following temperatures; ambient temperature, constant 4°C, constant 30°C and constant 37°C. The capsules were stored in securely fastened white polypropylene screw-cap bottles for up to 1 year following manufacture. Capsules were removed at specified intervals and analysed for their propranolol content using the HPLC method described above.

Determination of propranolol in human plasma

Determination of propranolol in human plasma was carried out using the HPLC method described by White et al., (1991). This method combines efficient solid-phase extraction of propranolol and its metabolites from plasma with HPLC analysis using fluorescence detection and is based on that reported by Harrison et al., (1985).

Clinical study

Nine healthy male and female volunteer subjects, who were employees or students of the Academy of Medicine, Bialystok, Poland, aged between 21 and 44 years and within $\pm 10\%$ of ideal body weight, participated in the study. The subjects were shown to be in good health by a physical examination and a series of hospital laboratory tests. To comply with ethical approval conditions, the protocol for the study was approved by independent researchers at the Academy of Medicine, Bialystok, and the subjects gave their informed consent.

The subjects were free of other medication for 2 weeks before the start of the study and until the collection of the final blood sample. Food was withdrawn for 12 h over the night preceding each leg of the study. A light breakfast was allowed 3 h post-dose after which time the subjects were allowed to follow their normal daily diet.

The study was a three-way cross-over design with the subjects receiving formulation A, formulation B or Inderal® containing an equivalent 80 mg dose of propranolol. The medication was taken with 250 ml of boiled tap water. The zero time blood sample was taken within a 5 min period preceding the administration of the medication. Subsequent blood samples were taken at 1, 2, 3, 4, 5, 6, 8 and 12 h. The blood samples were drawn into chilled EDTA vacutainers and the stoppers discarded immediately after sample collection, but before sample mixing, to avoid the spuriously low plasma propranolol concentrations reported following contact with the stoppers (Cotham and Shand, 1975). Plasma was separated by centrifugation at 2.0×10^4 $g \cdot \min$ within 15 min of collection, transferred into clean tubes and stored at -20°C until analysed. After 1 week the patients received one of the alternative treatments followed by the remaining treatment after a further 1 week period. Plasma samples were analysed by the HPLC method described above. No degradation of propranolol was observed during the period of storage at -20°C.

The results of the clinical study were evaluated using the observed values of maximum plasma concentration of propranolol (C_{max}) and time to C_{max} (t_{max}). The areas under the plasma concentration curves were calculated using the trapezoidal rule.

TABLE 1

Effect of storage conditions on the potency of enteric-coated liquid-filled hard gelatin capsules containing propranolol

Sample date	Storage conditions	Formulation A	Formulation B
0 weeks	zero time	80 1	79 9
+4 weeks	4°C		79.9
	ambient	79 9	77 6
	30°C		78 1
	37°C		75.8
+8 weeks	4°C		79 2
	ambient	N D.	78 3
	30°C		79 8
	37°C		79 4
+12 weeks	4°C		80 9
	ambient	79 8	79 5
	30°C		78 3
	37°C		76 3
+50 weeks	ambient	N.D.	80 2

Values are in mg per capsule and represent means of 10 individual determinations made in duplicate or triplicate The coefficient of variance was in each case less than 2%. Zero time determinations of capsule potency were carried out 3 days post-manufacture. Potency based on input amount of propranol was 80.1 and 79.9 mg per capsule for formulation A and B, respectively.

Results

Stability testing

The effect of storage conditions and time on the propranolol content of the capsules containing formulation A or B is shown in Table 1. The results show that under conditions of storage at ambient temperature, there was no measurable decline in propranolol potency of the capsules containing formulation A or B for the 8 week period from manufacture to completion of the clinical study. More detailed analysis of formulation B shows that the propranolol content of the capsules did not decline after more than 1 year of storage at ambient temperature. The only noticeable decline of propranolol potency was at the

TABLE 2

Pharmacokinetic parameters for propranolol· C_{max} (ng ml^{-1})

Subject	Formulation			Ratio	
	A	B	Inderal	A/Inderal	B/Inderal
1	27	56	29	0 9	1.9
2	104	60	47	2.2	1.3
3	102	114	114	0 9	1.0
4	29	111	27	1 1	4 1
5	99	65	39	2 5	1 7
6	58	47	68	0 9	0 7
7	54	81	49	1.1	1 7
8	30	52	20	1 5	2 6
9	46	84	114	0 4	0 7
Mean	62	74	57		
SD	31	25	35		
CV%	50	34	61		

TABLE 4

Pharmacokinetic parameters for propranolol· t_{max} (h)

Subject	Treatment		
	A	B	Inderal
1	3	4	1
2	5	4	4
3	3	4	3
4	3	2	2
5	5	3	4
6	3	2	4
7	4	2	2
8	4	3	3
9	2	3	1
Mean	3.6	3 0	2 6
SD	1 0	0 9	1 2
CV%	28	30	46

highest storage temperature of 37°C after 3 months.

Clinical study

The pharmacokinetic data for each of the formulations are listed in Tables 2–4. The concentration-time curves for each subject receiving the three separate formulations are shown in Fig. 1. The AUC, C_{max} and t_{max} values obtained for Inderal® 80 mg tablets were similar in terms of extent and variability to those reported previously (e.g., Cid et al., 1986; Walle et al., 1986).

TABLE 3

Pharmacokinetic parameters for propranolol AUC(ng ml^{-1} h^{-1})

Subject	Formulation			Ratio	
	A	B	Inderal	A/Inderal	B/Inderal
1	183	448	199	1.0	2 3
2	875	489	358	2 4	1 4
3	597	596	824	0 7	0.7
4	191	701	151	1.3	4.6
5	668	426	196	3 4	2.2
6	574	167	518	1 1	2.5
7	228	540	213	1.1	2.5
8	410	185	60	6 8	3.1
9	292	400	592	0 5	0.7
Mean	446	439	346		
SD	245	175	250		
CV%	55	40	72		

Using formulation A containing bile acids, C_{max} was increased by up to 2.5-fold and AUC by up to 6.8-fold in three out of nine subjects (see Fig. 1; subject nos 2, 5 and 8). The overall increase in AUC with formulation A of 30% was associated with a decline in the coefficient of variance from 72 to 55% compared to Inderal® (Table 3). Using formulation B, containing oleic acid and non-ionic surfactant instead of bile acids, a more obvious increase in C_{max} and AUC was achieved compared to Inderal®. In this case, C_{max} values increased by up to 4-fold and AUC values by up to 4.6-fold in six out of nine subjects (Fig. 1; subject nos 1, 2, 4, 5, 7 and 8). The overall increase in C_{max} of 30% obtained with formulation B was associated with a reduction in the coefficient of variance of nearly 45% (Table 2) compared to Inderal. Similarly, the 30% overall increase in AUC observed with formulation B, compared to Inderal, was associated with a reduction in the coefficient of variance from 72 to 40% (Table 3). Interestingly, with both formulations A and B there appeared to be a tendency to increase propranolol bioavailability in subjects with a poor response to Inderal® (Fig. 2).

In Table 4 the t_{max} values observed for each of the formulations have been recorded. The mean

t_{max} values were 3.0, 3.5 and 2.6 h for formulation A, formulation B and Inderal®, respectively. The delay in t_{max} with formulations A and B compared to Inderal® was probably due to the time taken for the enteric-coat to be removed from the liquid-filled hard gelatin capsules.

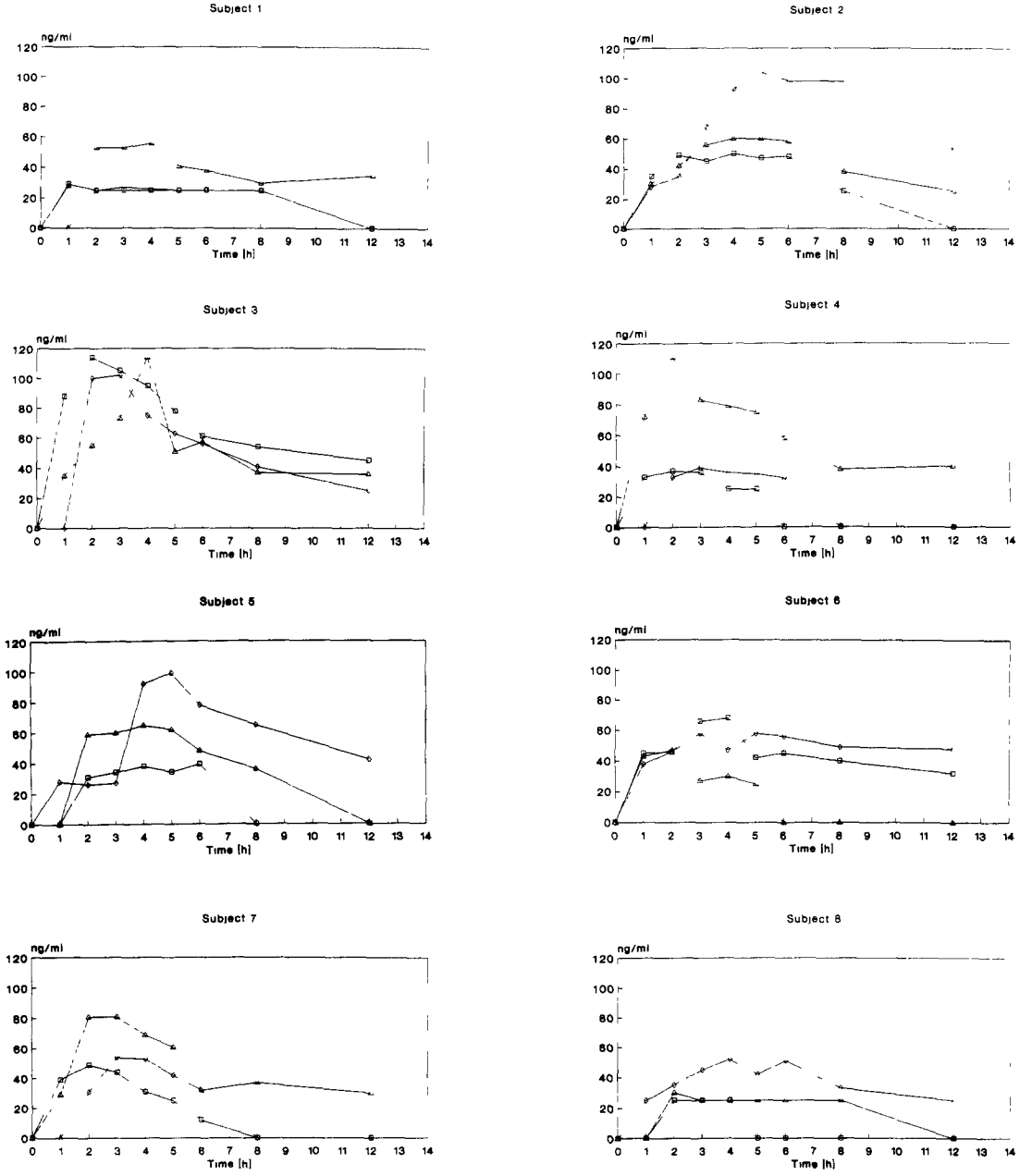

Fig 1 Individual plasma propranolol concentrations (ng ml^{-1}) in subjects receiving formulation A (◇), formulation B (△), and Inderal® (□)

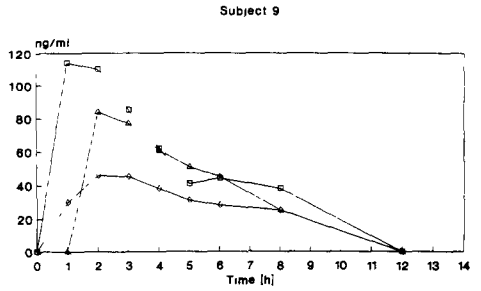

Fig. 1 (continued)

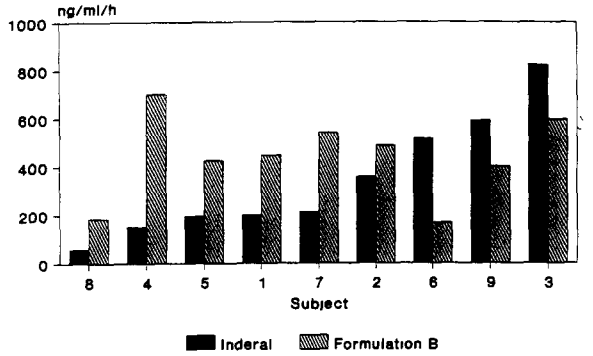

Fig. 2. Comparison of Inderal and formulation B AUCs Individual subjects ranked in order of increasing AUC response to Inderal together with corresponding AUC response to formulation B.

Discussion

The results of the present study have demonstrated that formulations containing oleic acid increase the bioavailability of propranolol in subjects who respond poorly to Inderal. The results in Tables 2 and 3 show that formulation B produced a greater overall increase in AUC and C_{max} with a reduced coefficient of variance compared to both formulation A and Inderal. These observations suggest that bile acids are not an essential component of the formulations resulting in the improved delivery of propranolol. The relationship between the improved response of subjects receiving formulation B in poor responders to Inderal is further illustrated in Fig. 2.

Previous studies using oleic acid containing a dissolved lipophilic basic drug have demonstrated a beneficial effect on drug bioavailability, an improvement ascribed to enhanced drug dissolution characteristics (Stella et al., 1978). Interestingly, however, the appearance of lipophilic drugs in lymph has been observed and shown to be influenced by the presence of a number of excipients such as surfactants and oils, including oleic acid (Sieber et al., 1974; Yoshikawa et al., 1981; Palin and Wilson, 1984; Charman and Stella., 1986a; Charman et al., 1986). Previous studies in pigs have shown the appearance of high levels of propranolol in lymph which were further increased using a formulation similar to formulation A described in the present study (White et al., 1991). Furthermore, studies using cell lines derived from liver (HepG2 cells) and intestine (CaCo2 cells) have demonstrated the hormone-like effects of oleic acid and other C_{18} unsaturated fatty acids on promoting the increased secretion of chylomicrons and VLDL (Davidson et al., 1988; Pullinger et al., 1989; Dashti et al., 1990; Moberly et al., 1990; Homan et al., 1991). A similar effect of oleic acid upon the increased lipoprotein composition of lymph in animals has also been observed (Renner et al., 1986; Cheema et al., 1987; Tso et al., 1987). The major source of oleic acid in vivo is probably biliary lecithin (Simmonds et al., 1968; Tso et al., 1977; Barnwell et al., 1987).

It is believed that in the present study the results obtained depended upon the lipophilic nature of propranolol, which predisposes its selection for lymphatic absorption. It is also believed that lymphatic absorption may be further facilitated by a strong ion pair effect, at the gastrointestinal membrane, between propranolol and oleic acid, as discussed by Green and Hadgraft (1987). Furthermore, once inside the enterocytes, oleic acid is known to participate in the initiation of a number of intracellular second messenger events, such as activation of protein kinase C (Diaz-Guerra et al., 1991; Khan et al., 1992). Oleic acid also promotes the acidification of intracellular compartments, made possible by its ability to function as a hydrogen ion ionophore (Simpson et al., 1988; Wrigglesworth et al., 1990), a process associated with the budding of secretory vesicles from the trans Golgi network. In addition to stimulating the production of chylomicrons, oleic acid provides one of the essential

building blocks for triglyceride, a major component of this lipoprotein sub-class (Thomson and Dietschy, 1981).

Tight association of drugs with chylomicrons would provide the means of compartmentalising drugs and therefore maintaining a high drug concentration within the lymph compared to other tissue compartments (Charman and Stella, 1986b). Delivery of drugs carried to the lymph in this way would result in their direct access to the systemic circulation without first passing through the liver and explain the increased levels of drugs observed in the systemic circulation following enhanced lymphatic absorption (Caldwell et al., 1982; Palin et al., 1982; Grimus and Schuster, 1984; Palin and Wilson., 1984; Gowan and Stavchansky, 1986; Ichihashi et al., 1991). It is believed that the formulation system described in the present study may provide a means of achieving reliable delivery of many drugs subject to extensive liver metabolism when administered via the oral route.

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