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Demonstration of the importance of biphasic oleic acid delivery for enhancing the bioavailability of propranolol in healthy volunteers

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Abstract

Two studies involving six healthy volunteers were carried out to assess the importance of biphasic rapid and sustained release of oleic acid in enhancing the bioavailability of propranolol using the HALO™ drug delivery system. The results of the first study showed that mean Cmax and AUC values for propranolol were increased by at least 300% using biphasic HALO™-propranolol capsules compared with Half Inderal® LA, both containing an 80mg dose of propranolol. In the second study no significant differences in propranolol bioavailability were observed between monophasic enteric-coated capsules containing only the sustained release component of the HALO™ delivery system and Half Inderal® LA, when an equivalent 80mg dose of propranolol was administered. The use of improved enteric-coating procedures increased propranolol bioavailability compared with previous studies using biphasic HALO™-propranolol capsules. The possible explanations for the effectiveness of biphasic rapid and sustained delivery of oleic acid for enhancing the bioavailability of propranolol are discussed.

Keywords: Propranolol; HALO™ drug delivery system; Half Inderal® LA; Drug delivery; Enhanced bioavailability

1. Introduction

Commonly used lipophilic drugs, exemplified by calcium channel antagonists, certain beta blockers and many psychotropic agents, often demonstrate wide variations in patient response. This is because of poor systemic bioavailability after oral administration resulting from incomplete absorption and/or extensive presystemic extraction, usually by the liver. Hepatic first-pass metabolism of lipophilic drugs is frequently exacerbated by the use of sustained-release preparations, introduced to improve patient compliance, because the slower rate of delivery ensures that drug metabolising enzymes are unlikely to be saturated (Pond and Tozer, 1984; Greenblatt, 1993). One possible way of avoiding hepatic first-

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pass metabolism is to redirect drug absorption into the lymphatic system. Considerable debate has taken place concerning the capacity of the lymphatic system to carry a therapeutically relevant dose of a lipophilic drug, and deliver it intact to the systemic circulation, (Muranishi, 1991; Charman and Stella, 1986). Less controversial, however, is the general understanding that the lymphatic system is responsible for the almost complete absorption of large quantities of dietary long-chain fatty acids, cholesterol and fat-soluble vitamins such as vitamin E and vitamin A (Bloom et al., 1951; MacMahon et al., 1970, 1971). It is therefore probably not unreasonable to expect other lipophilic molecules including drugs to also be extensively absorbed via the lymph (Charman and Stella, 1991; Muranishi, 1991).

Studies in animals and using cell culture techniques have demonstrated that fatty acids, particularly oleic acid, can stimulate lymph flow and/or lymph component secretion (Davidson et al., 1986; Davidson et al., 1988; Renner et al., 1986; Field et al., 1988; Dashti et al., 1990; Moberley et al., 1990). Indeed the possibility that oleic acid may stimulate lymphatic drug delivery has been explored by a number of investigators with varied degrees of success (Stella et al., 1978; Tokumura et al., 1987; Ichihashi et al., 1991a; Ichihashi et al., 1991b; Ichihashi et al., 1992). In recent preliminary studies however, Barnwell et al. (1993) and Barnwell et al. (1994) were able to demonstrate that a biphasic delivery system which achieved an initial rapid-release and subsequent sustained-release of oleic acid, increased the bioavailability of propranolol in healthy volunteers by at least 2-fold compared with a standard commercial sustained-release preparation. In another study, Aungst and Hussain (1992) showed that greatly improved bioavailability resulted from the biphasic delivery of propranolol laurate in dogs.

In the present investigation, two volunteer studies were performed, the first using biphasic HALO™-propranolol capsules, the second using capsules containing only the sustained-release component of the formulation, with the aim of demonstrating the importance of the biphasic oleic acid delivery concept for improving the bioavailability of lipophilic drugs. The possible mechanisms by which a biphasic delivery system containing oleic acid may improve drug bioavailability are discussed.

2. Materials and methods

2.1. Manufacture of HALO™-propranolol capsules

The manufacture of monophasic and biphasic HALO™-propranolol capsules was carried out by the use of conventional hard gelatin capsule liquid-filling and sealing technology (M.W. Encap Limited, Livingstone, West Lothian, UK) followed by enteric-coating (Pharma-Vinci A/S, Denmark), in accordance with the findings of Burns et al. (1994).

2.2. Formulations

Biphasic HALO™-propranolol capsules were formulated to give an initial rapid-release and subsequent sustained-release of propranolol. Propranolol base dissolved in oleic acid provided an initial rapid release; subsequent sustained-release of drug was from an erodible matrix containing polyglycolysed glycerides (Gelucire®), polyoxyl-40-hydrogenated castor oil (Cremophor® RH40) and colloidal silicon dioxide (Aerosil® 200). The final potency was 80 mg of propranolol per capsule. Monophasic sustained-release propranolol capsules contained only the sustained-release component of the HALO™-propranolol formulation, requiring the administration of two capsules to provide the equivalent 80 mg dose of propranolol present in the comparitor product, Half-Inderal® LA (ICI Batch No LU80A). The dissolution performance of the capsules was assessed using the method described by Burns et al. (1995).

2.3. Sample preparation and analysis

Plasma samples were prepared by centrifugation and stored at −20°C until analysed for propranolol content by validated HPLC procedures (and taking the precautions on sample collection outlined by Cotham and Shand, 1975). In

the case of Study 1 samples were analysed by a method based on that described by Harrison et al. (1985), while in the case of Study 2 the method was similar to that employed by Lefebvre et al. (1981). Pharmacodynamic effects were evaluated by comparing mean resting heart rate, systolic and diastolic blood pressure between treatments.

2.4. Volunteer studies

Two open, randomised, single-dose, cross-over studies, were performed each using six healthy male volunteers aged between 18 and 45, who had given their informed consent. The protocols were approved by ethics committees. All volunteers were shown to be in good health by medical examination and a series of standard laboratory tests. The subjects were also free of all other medication for at least 14 days before the start of the study. A 14-day wash-out period was used between each treatment. Pharmacodynamic effects were evaluated by comparing mean resting heart rate, systolic and diastolic blood pressure between treatments. Study 1 was carried out at BIOS (Consultancy and Contract Research) Limited, Pinewood, College Ride, Bagshot, Surrey, UK, while Study 2 was performed by the Guildford Clinical Pharmacology Unit, Guildford, Surrey, UK.

2.5. Dosage regimen

2.5.1. Study 1

The first study was to compare biphasic HALO™-propranolol capsules with the comparitor product, Half-Inderal® LA. On each study day, the volunteers received their medication after an overnight fast. Standard meals were subsequently provided at 13:00 and 18:00 h. Blood samples were obtained from each subject at the following times: 0 (pre-dose), 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0, 12.0, 18.0 and 24.0 h following drug administration.

2.5.2. Study 2

A second study was performed to compare the bioavailability of the sustained-release component of the HALO™-propranolol formulation with Half-Inderal® LA. The subjects were fasted overnight and given a standard breakfast on each study day at 08:30 h before administration of the medication at 09:00 h. Blood samples were obtained from each subject at the following times post dose on each study day: 0 (pre-dose), 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0, 11.0 and 12.0 h. A further standard meal was administered 4 h post-dose.

The results obtained from the studies were evaluated for C_{max} and T_{max} and the AUC to 24 h was calculated by the linear trapezoidal rule. Differences were tested for significance by using Student's t-test and the Wilcoxon two-sample test. A standard crossover analysis of variance was used to test for subjects, treatment and period effects.

3. Results

3.1. Study 1

Individual pharmacokinetic data obtained when the subjects received either biphasic HALO™-propranolol or Half-Inderal® LA are shown in Table 1. The propranolol concentration-time curves are shown in Fig. 1a–f. The C_{max} and AUC were increased 3.7- and 3.1-fold, respectively, with HALO™-propranolol relative to the comparitor product Half-Inderal® LA. A mean plasma half-life of 7.03 $\pm$ 2.43 h (SD) was calculated for HALO™-propranolol, however the erratic characteristics of the terminal concentration-time data prevented calculation of meaningful half-life values in the case of Half-Inderal® LA in several instances. The time to peak plasma propranolol concentration (T_{max}) ranged from 3 to 10 h for Half-Inderal® LA compared with 3 to 6 h for HALO™-propranolol. These differences were not significant. Analysis of variance of logarithmically transformed C_{max} data from five subjects showed significant ($p = 0.02$) differences indicating enhanced bioavailability of propranolol with the HALO™ formulation. The AUC 0–24-h values were larger for HALO™-propranolol in five of the six subjects but were not significantly ($p > 0.05$) different between products, probably because of the small number of

Table 1
Comparison of pharmacokinetic parameters for Half-Inderal® LA and biphasic HALO™-propranolol capsules

Subject	Half-Inderal® LA			Biphasic HALO™-propranolol			C_{max} Ratio	AUC ratio
	C_{max} (ng/ml)	AUC (h·ng/ml)	T_{max} (h)	C_{max} (ng/ml)	AUC (h·ng/ml)	T_{max} (h)		
1	9.7	29.9	5	24.3	261.4	4	2.5	9.0
2	7.9	37.3	5	41.7	320.9	6	5.3	8.6
3	40.9	548.2	5	197.7	2039.8	5	4.8	3.7
4	17.2	161.5	3	25.4	119.2	3	1.5	0.7
5	<0.5	NC	NC	24.2	154.8	3	NC	NC
6	16.9	263.0	10	47.1	351.8	5	2.8	1.3
Mean	16.3	173.3	5	60.1	541.3	5	3.7	3.1
S.D.	13.0	208.7	(3–10)	68.1	739.7	(3–6)		

The dose of propranolol administered to the healthy volunteers was 80 mg for each treatment. T_{max} values are medians with range. NC denotes not calculated.

subjects used. There was no evidence of any sequence effects. Resting heart rate, systolic and diastolic blood pressure were measured 5 min prior to each blood sampling time, a decrease from baseline (pre-dose) values being observed following the administration of both products. The maximum decrease in these parameters in each subject was not statistically different for the two products although in each of the six subjects the decrease was larger for HALO™-propranolol.

3.2. Study 2

Table 2 summarises the pharmacokinetic data from Study 2 when the subjects received either Half-Inderal® LA or the sustained-release component of the HALO™-propranolol formulation after a standard breakfast. Statistical analysis of the data found no significant treatment related differences in terms of AUC and C_{max}. The absorption of propranolol following administration of the sustained-release component of the HALO™-propranolol formulation was delayed compared with Half-Inderal® LA as indicated by the significant difference ($p < 0.05$) in median T_{max} values, 11 (range 8–12 h) and 7 h (range 5–10), respectively. The delay in the appearance of propranolol in plasma with the sustained-release component of the HALO™-propranolol compared to Half Inderal® LA was probably the result of the time taken for the enteric-coated capsules to pass from the stomach to the duodenum. In the case of subject 5, no propranolol was detectable in the plasma samples after receiving the sustained-release component of the HALO™-propranolol formulation. A decrease from baseline (pre-dose) values was observed for heart rate, systolic and diastolic blood pressure following administration of both products. The maximum decrease in these parameters in each subject was not significantly different for the two products, however in the case of subject 4 an adverse reaction, bradycardia with 45 beats per min was attributed to the administration of the sustained-release component of the HALO™-propranolol formulation 24 h after dosing.

4. Discussion

The present investigation demonstrates that enteric-coated biphasic HALO™-propranolol capsules, containing propranolol formulated with oleic acid in a rapid and sustained-release format, is able to increase the bioavailability of the propranolol formulated therein by at least 300% when compared with a standard commercial sustained-release preparation in healthy volunteers (Study 1). The importance of biphasic release dynamics in conferring increased bioavailability is defined when it is seen that the sustained-release component of the HALO™-propranolol formulation when used alone but at a similar dose level, is unable to increase propranolol bioavailability

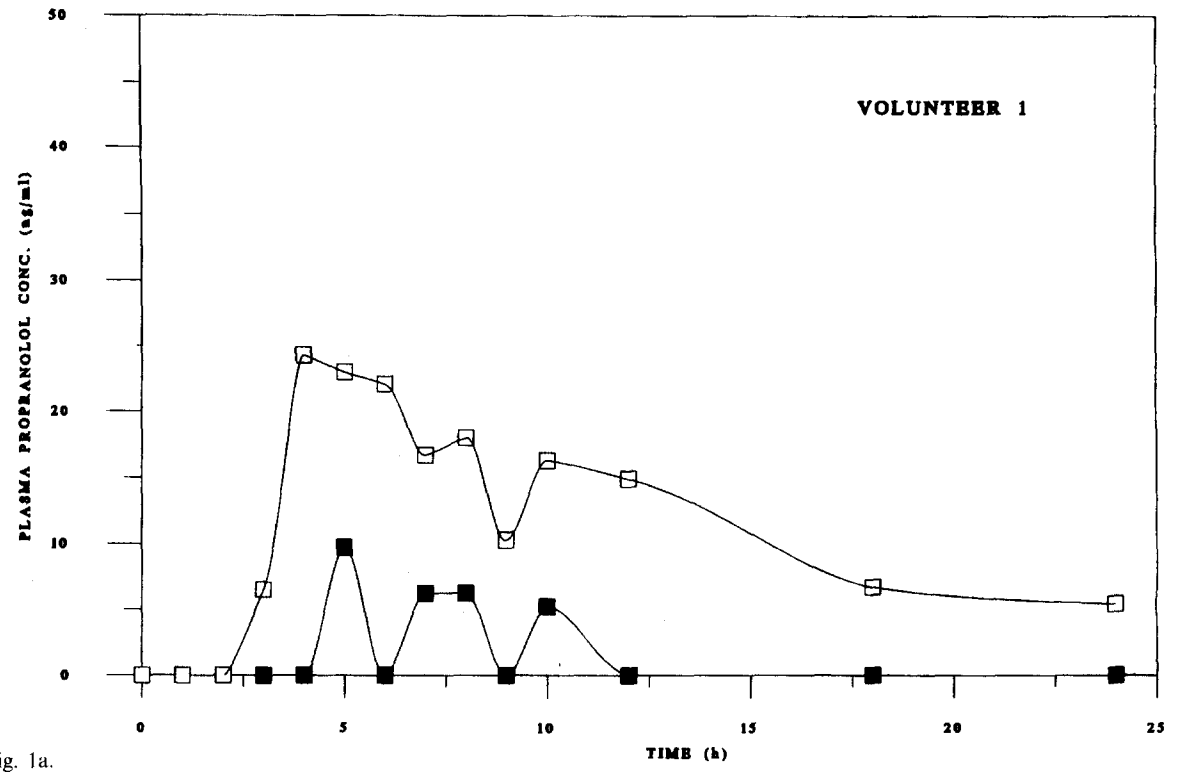


Fig. 1a.

Fig. 1(a–f). Plasma propranolol concentrations are expressed as a function of time for each subject receiving Half-Inderal® LA (■) or HALO™-propranolol (□) at a dose of 80 mg. Values are expressed as means of duplicate or triplicate determinations.

(Study 2). It has also previously been shown by Barnwell et al. (1992) that propranolol formulated with oleic acid for rapid-release only is unable to increase substantially propranolol bioavailability or prolong the appearance of propranolol in plasma. The results of Study 1 show a further increase in propranolol bioavailability using the HALO™ delivery system compared with the studies of Barnwell et al. (1993), and Barnwell et al. (1994) in which it was demonstrated that an enteric-coated biphasic HALO™-propranolol formulation could increase the bioavailability of propranolol by 200%, compared with Half Inderal® LA, in healthy volunteers. The improved results in the present study are probably explained by the increased levels of enteric-coating applied to the capsules as described by Burns et al. (1994).

It has been suggested that co-administration of oleic acid may increase bioavailability of certain drugs by promoting their lymphatic absorption, thereby avoiding hepatic first-pass metabolism. Therefore one possible explanation for the differences in performance observed between the biphasic HALO™-propranolol formulation and the sustained-release only preparation is that an initial bolus release of oleic acid is required to activate lymph production and therefore enhance propranolol bioavailability. This hypothesis is supported by cell culture studies which demonstrate that intestinal cells require a threshold concentration of oleic acid to be surpassed before the secretion of lymphatic lipoproteins, chylomicrons and VLDL, can take place (Dashti et al., 1990). The importance of the subsequent sustained-release of oleic acid-propranolol in maintaining improved bioavailability of propranolol may be explained by the need to avoid saturating the absorptive capacity of the lymphatic system for lipophilic drugs, which is generally believed to be limited (Charman and Stella, 1986).

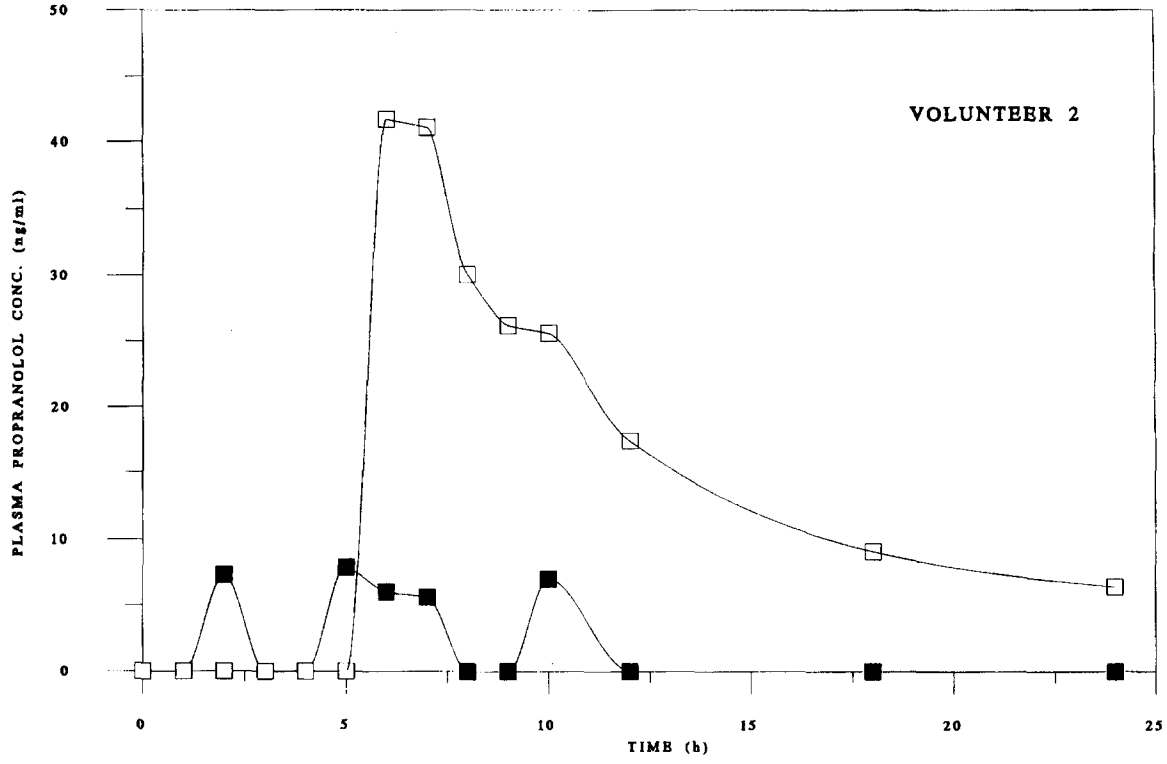
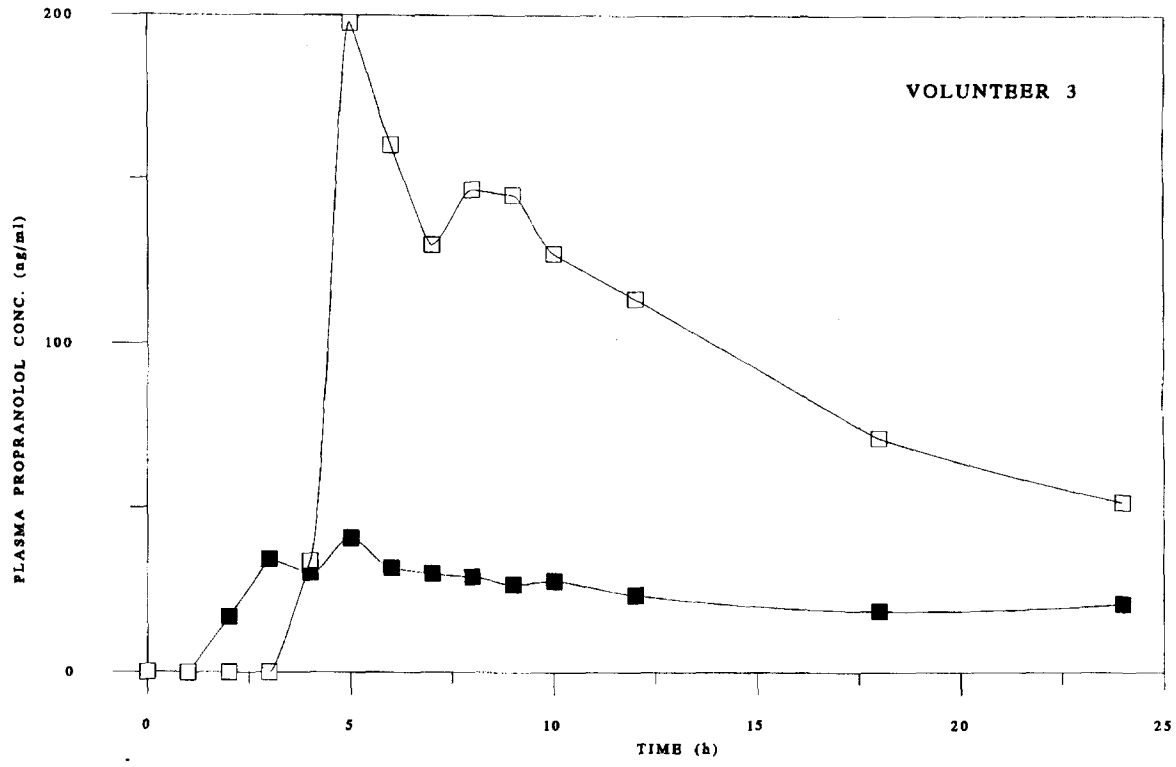


Fig. 1b.

An alternative explanation for the improved bioavailability of propranolol observed with HALO™-propranolol capsules, forwarded by Tucker (1993) was that oleic acid inhibits hepatic metabolism of propranolol. Arguing against this hypothesis however is the evidence of relative ineffectiveness of the rapid-release (Barnwell et al., 1992) and sustained-release component (Study 2) of the HALO™-propranolol formulation used independently, compared with the much greater effectiveness of using the two parts of the formulation together. For the inhibition of metabolism hypothesis to be correct, the bioavailability of propranolol would be expected to be greatly increased when either the rapid or sustained-release component was used independently, as each contained an excess of oleic acid, however, enzyme inhibition may have a relevance to the extent of lymphatic delivery of drugs. Propranolol (Sozzani et al., 1992) and many other lipophilic drugs which undergo hepatic first-pass metabolism such as chlorpromazine, fluphenazine, haloperidol, imipramine, phentolamine, diltiazem, dibucaine, tetracaine, trifluoperazine (Blest et al., 1992; Mori et al., 1980; Uratsuji et al., 1985) inhibit protein kinase C, a key enzyme involved in cellular signal transduction (Nishizuka, 1984a; Nishizuka, 1984b) which controls secretory events including lymph production. It is therefore possible that many lipophilic drugs inhibit lymph production, preventing their extensive lymphatic absorption as would be predicted on the basis of their physicochemical properties. Inhibition of lymph production by lipophilic drugs is likely to result in spill-over of drugs into the hepatic portal circulation and therefore first-pass metabolism. Evidence exists that oleic acid reverses inhibition of protein kinase C by propranolol (Diaz-Guerra et al., 1991; Khan et al., 1992; Khan et al., 1993; Sidiqui and Exton, 1992; Sozzani et al., 1992). Therefore,


VOLUNTEER 3
PLASMA PROPRANOLOL CONC. (ng/ml)
200
100
0
0
5
10
15
20
25
TIME (h)

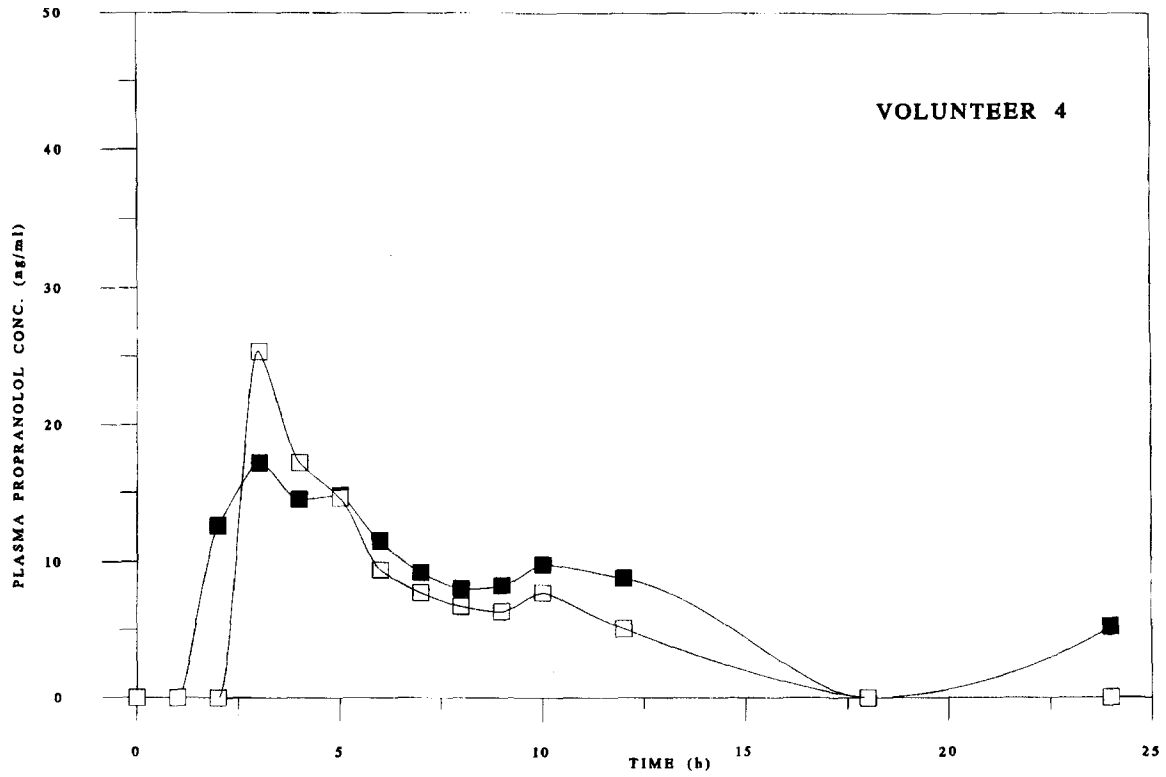
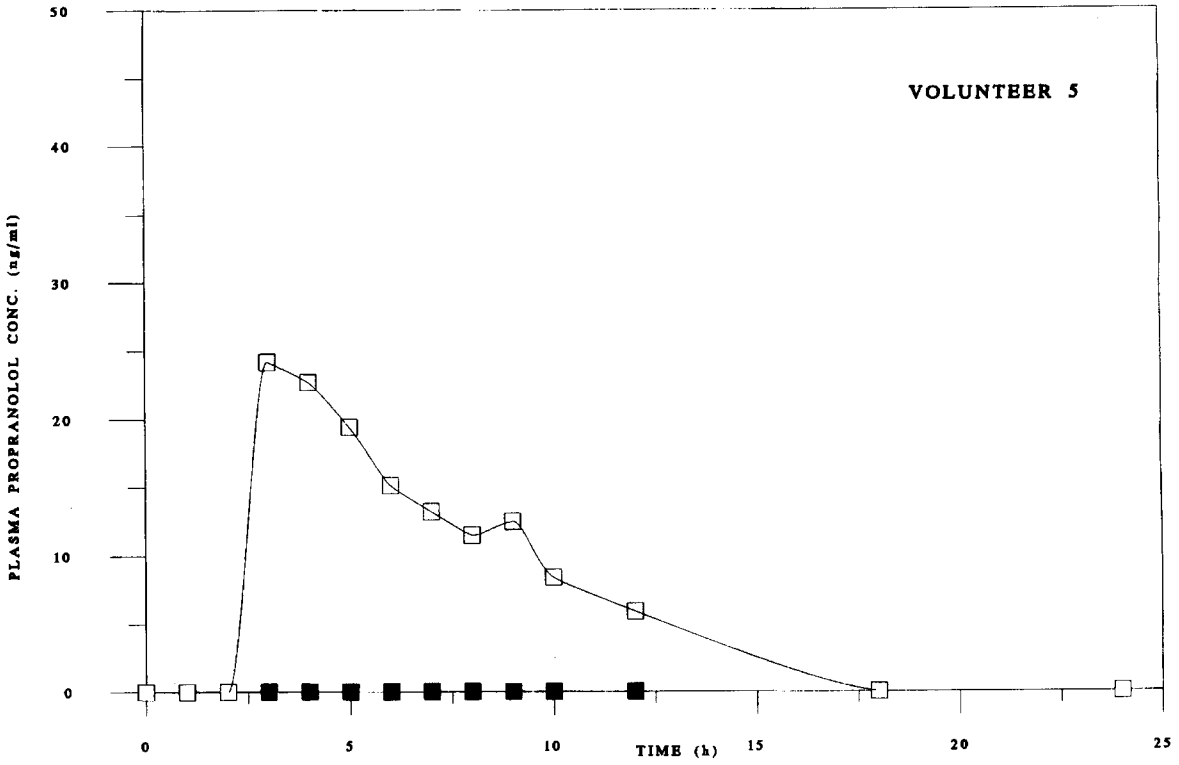

VOLUNTEER 4
PLASMA PROPRANOLOL CONC. (ng/ml)
50
40
30
20
10
0
0
5
10
15
20
25
TIME (h)

Fig. 1c and 1d.


VOLUNTEER 5
PLASMA PROPRANOLOL CONC. (ng/ml)
50
40
30
20
10
0
0
5
10
15
20
25
TIME (h)

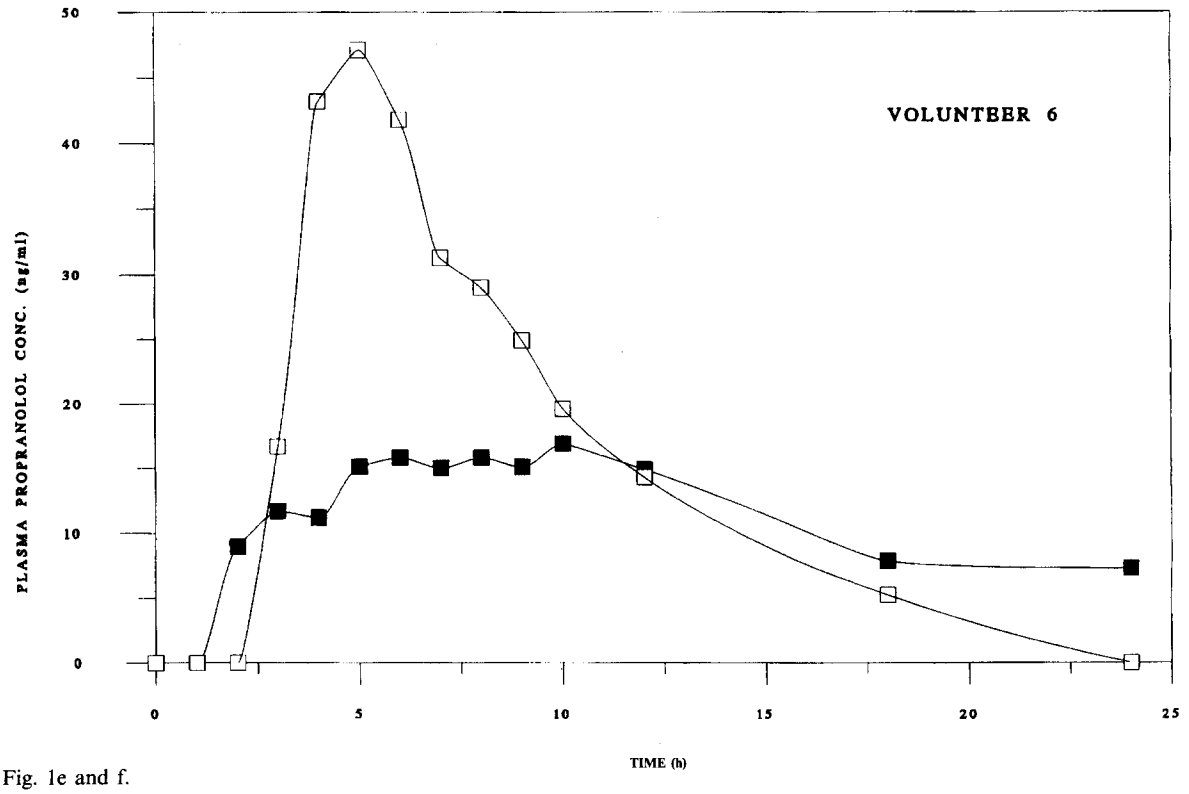

VOLUNTEER 6
PLASMA PROPRANOLOL CONC. (ng/ml)
50
40
30
20
10
0
0
5
10
15
20
25
TIME (h)

Fig. 1e and f.

Table 2
Comparison of pharmacokinetic parameters for the sustained-release component of the HALO™-propranolol formulation with Half-Inderal® LA

Subject	Half-Inderal® LA			Sustained-release only HALO™-propranolol		
	C_{max} (ng/ml)	AUC (h·ng/ml)	T_{max} (h)	C_{max} (ng/ml)	AUC (h·ng/ml)	T_{max} (h)
1	7.8	34.1	10	14.9	52.0	12
2	18.6	147.3	9	4.0	12.0	12
3	24.5	163.9	5	64.9	173.6	10
4	5.1	40.8	5	6.4	13.4	11
5	4.2	32.4	5	<0.5	NC	NC
6	7.4	52.5	10	20.1	126.8	8
Mean	11.3	78.70	7	18.4	63.6	11
S.D.	8.3	60.3	(5–10)	24.0	24.0	(8–12)

The dose of propranolol administered to the healthy volunteers was 80 mg for each treatment. T_{max} values are medians with range. NC denotes not calculated.

in the case of the HALO™ delivery system the co-administration of oleic acid in close association with a lipophilic drug in a biphasic format may allow restoration and maintenance of lymph production enabling extensive lymphatic drug delivery of propranolol to take place.

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