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Biochemical stability in serum of a lipid-soluble probe molecule entrapped in an o/w emulsion as a carrier for passive drug targeting

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Particulates foreign to the body are normally taken up by the reticuloendothelial system from the circulation. This naturally occurring host defense process offers a potentially powerful method for targeting drug substances to the reticuloendothelial system with various microparticulate drug carriers. The term passive (Poste, 1983) or natural (Weinstein, 1983) drug targeting was coined to the concept as applied to lipid vesicles. A fat emulsion for parenteral nutrition is an attractive drug carrier alternative to liposomes possible for the same application (Haynes et al., 1987) in that the pharmaceutical technology has been available in recent years for various fat emulsions (Hansrani et al., 1983).

A high lipophilicity is desirable for exclusive entrapment of a drug substance in the oil droplets of an emulsion system. When a lipophilic derivative of a given substance is chosen for a prodrug in the preparation of an emulsion, in vivo biochemical stability of the derivative becomes an important issue. If the parent compound is relatively polar and conversion of the derivative to the parent compound is fast in vivo, it is conceivable that the parent compound can be released from oil droplets well before they are taken up by the reticuloendothelial system. The present study is concerned with the effect of an o/w emulsion on the rat serum-catalyzed hydrolysis of ethyl ester of flurbiprofen (2-(2-fluoro-4-biphenyl) propionic acid). The compound is extremely lipophilic and readily hydrolyzed to free acid in rat serum. With an emulsion system, the hydrolysis was much slower, but still significant. The rapid initial hydrolysis observed is attributed to the large interfacial area brought about by the submicron oil droplets. Implication of the finding in employing an emulsion system for passive drug targeting is briefly discussed.

The ethyl ester of flurbiprofen was conveniently synthesized by nucleophilic substitution of ethyl iodide with flurbiprofen anion generated by diisopropylethylamine in acetonitrile. Silica gel column chromatography was used for purification. The ester is a clear oil. The partition coefficient of the ester between sesame oil, which was used in the emulsion preparation, and water was measured using a phase volume ratio of one part of oil

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to 34 parts of water containing varying amounts of NaCl. Phases were separated by a high-speed centrifugation (100,000 *g*) overnight at 25 °C. The presence of the salt suppressed unwanted emulsion formation and facilitated phase separation, but also exerted a salting-out effect. Apparent partition coefficients measured were extrapolated to zero salt concentration to obtain a partition coefficient value of 1.71×10^5.

Emulsions containing flurbiprofen ethyl ester were prepared using a Microfluidics Model M-110 emulsifier from a mixture of 10.0 g sesame oil containing the ester, 1.2 g soybean lecithin, 2.0 g glycerin, and sufficient volume of water to make the final volume 100.0 ml. The ester concentration in the final emulsion was in the order of 1.0 mg/ml. The mean diameter of oil droplets was 0.21 ± 0.04 (S.D.) μm as determined by photon correlation spectroscopy (Nicomp Model 200 Laser Particle Sizer). ζ potential (Pen Kem Model 501 Laser Zee Meter) and viscosity at 25 °C were -39 mV and 1.48 cP, respectively. Osmotic pressure was in the range of 250–300 mOsm/kg H_2O.

Sera from various species were obtained by centrifugation of whole blood at 500 *g* for 15 min. Serum-catalyzed hydrolysis of flurbiprofen ethyl ester was carried out at 37 °C in a Tris buffer at 0.15 M ionic strength containing ibuprofen, an HPLC internal standard. The hydrolysis from both emulsion and solution was carried out in the presence of 1.0% ethanol, since the latter utilized a stock solution in ethanol. The emulsion was diluted 500-fold at time zero in a serum-containing buffer. The starting ester concentration was in the range of 2 μg/ml and 1 μg/ml for emulsions and solutions, respectively. At a given time, 1.0 ml aliquots were acidified with 0.25 ml of 1.0 N H_2SO_4 and extracted with 10.0 ml ether. The residue obtained after evaporating ether was taken up in 1.0 ml of 50% CH_3CN, which was subsequently injected to an HPLC column. A gradient system was adopted for a reverse phase HPLC procedure (Fig. 1).

As shown in Table 1, rat serum was extraordinarily active in hydrolyzing the ester. If one assumes that the esterase activity can be extrapolated to 100% serum, its activity is about 50,000 times greater than that of human serum. Interestingly, when human serum or human serum albumin was added to a 1.0% rat serum solution at final concentration 5.0% or 0.2% respectively, the hydrolysis was substantially slowed down. The overall hydrolysis profile was biphasic and very similar both qualitatively and quantitatively to those observed with emulsions in 1.0% rat serum

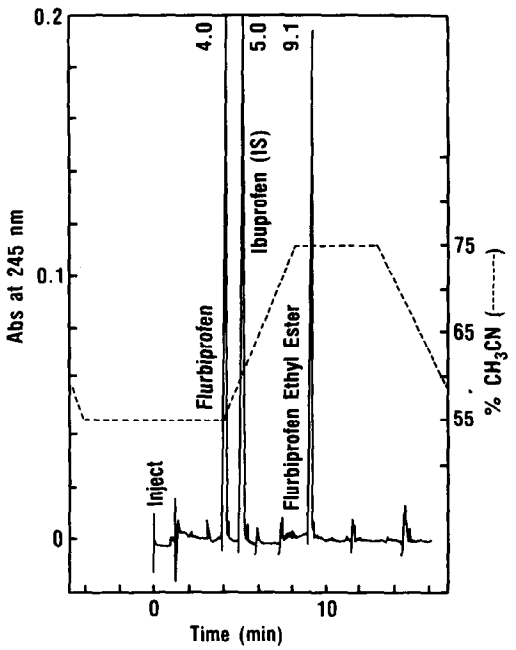

Fig. 1. Reverse-phase HPLC separation of flurbiprofen ethyl ester and flurbiprofen. Ibuprofen served as an internal standard. Dotted lines show the gradient system and numbers on the figure indicate retention time in min. A flow rate of 2.0 ml/min was maintained throughout the gradient. A 0.5 μ ODS column (IBM) was used with a Perkin-Elmer Series 4, Model 600 Autosampler, and a Hewlett Packard Model 3380A Integrator. The detector used was a Spectra-Physics Model 8400.

TABLE 1

Species difference in serum-catalyzed hydrolysis of flurbiprofen ethyl ester at 37 °C

	$k_2 \times 10^5 (s^{-1})$	Ratio
Human	1.83	1
Cow	1.31	0.72
Pig	1.28	0.70
Monkey	0.58	0.32
Rat	(9.3×10^4) *	(5.1×10^5) *

* Values extrapolated from data obtained at 1.0% serum.

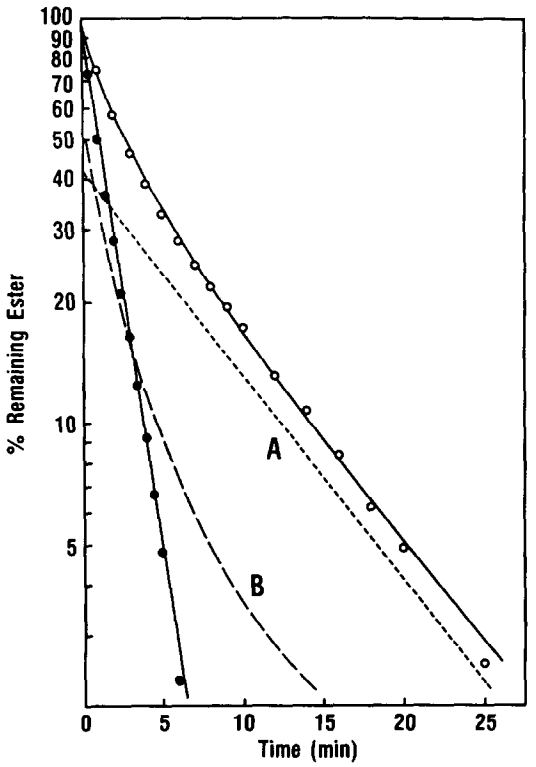

Fig. 2. Hydrolysis of flurbiprofen ethyl ester in 1.0% rat serum at pH 7.4 and 37 °C when the ester was introduced by dilution of an o/w emulsion (○) or an alcoholic solution (●). The solid line for the emulsion system was generated from Eqn. 1 in which $k_2 = 9.26 \times 10^{-3}$ s^{-1}. It was further divided into A (· · · · · ·) and B (- - - - -) components. In both cases of hydrolysis the raw data were normalized. Initial concentrations of the ester were 1.16 mg/ml for the emulsion and 0.42 mg/ml for the alcohol solution. The oil and alcohol concentrations in the system were 0.02% and 1.0% respectively.

(see more later). This finding supports that the rapid hydrolysis in diluted rat serum might be due to lack of binding of the ester to some serum component(s). As shown in Fig. 2, ethyl ester of flurbiprofen underwent hydrolysis following a biphasic profile in 1.0% rat serum when the ester was introduced in an o/w emulsion. This is in contrast to the first-order kinetics observed when the ester in an alcoholic solution was diluted in 1.0% rat serum. The exact cause of the steady decrease in the hydrolysis rate with an emulsion system (open circles in Fig. 2) is not known at the present time. Product inhibition was ruled out by the finding that the hydrolysis profile was clearly identical even when the system contained as much flurbiprofen free acid as its ethyl ester at time zero of the hydrolysis. As described above, a nearly identical hydrolysis profile was observed without any emulsion oil droplets but in the presence of human serum or human serum albumin. If binding of the ester with albumin prevents the ester molecules from the hydrolysis, dissociation of bound molecules should come into the overall hydrolysis kinetics.

In parallel with the ester hydrolysis in the presence of albumin, the observed hydrolysis profile with an emulsion was subject to an A → B → C kinetics model in which A and B are the ester concentrations at a given time in those compartments where hydrolysis cannot and can occur, respectively. Corresponding concentrations at $t = 0$ are designated as A_0 and B_0. Rate constants k_1 and k_2 represent the rates of those two consecutive steps. Mathematical derivation of an equation for total remaining ester is straightforward (Frost and Pearson, 1961). A non-linear estimation technique (SAS, 1985) was adopted in determining values of A_0, B_0, and k_1 for 4 sets of data similar to those shown in Fig. 2. The value of k_2 used in the estimation, 9.26×10^{-3} s^{-1}, was separately determined by monitoring hydrolysis after an alcoholic solution of the ester was diluted in 1.0% serum solution, under an assumption that the B → C step represents normal hydrolysis (see below). Relative values of A_0 and B_0 are listed in Table 2 along with k_1 values thus obtained. According to the model presented above, the majority of the loss in A + B is due to loss from B_0 at an early phase of the hydrolysis. As the hydrolysis con-

TABLE 2

Estimation of A_0, B_0, and k_1 *

	$\%A_0$	$\%B_0$	$K_1 \times 10^3 (s^{-1})$
	43	57	2.30
	43	57	1.95
	39	61	2.23
	48	52	1.35
Average	43	57	1.96

* See text for the definitions of A_0, B_0, k_1 and k_2. The value of k_2, 9.26×10^{-3} s^{-1}, was used in the estimation.

tinues at a rate constant k_2, more ester molecules will be supplied from the oil phase (i.e., A to B). At a later time point, the rate of loss should merely reflect this slow process. Thus, as shown in Fig. 2, the terminal slope becomes parallel to the curve for A. In the absence of other independently obtained experimental data, it is difficult to identify the A → B process. Based on the large partition coefficient observed, one can safely rule out B_0 as the concentration of the ester in the aqueous bulk phase. According to Table 2, on the average, about 57% of total ester is directly exposed to hydrolysis at $t = 0$. It is tempting to speculate that B is the concentration of the ester at the oil/water interface and yet subject to the hydrolysis as if it were in an aqueous environment and that the A → B process is equivalent to bringing the substrate molecules to the interface where enzymatic hydrolysis can occur. In this model the A to B would be reversible (i.e., A ⇌ B) and k_1 would be a composite constant. Rapid initial hydrolysis can then be explained by the large interfacial area; if one assumes a homogeneous size of oil droplets at 0.2 μm diameter, 1.0 ml of 10% o/w emulsion would provide a total of 3×10^4 cm^2 (3 m^2) interfacial area from 2.4×10^{13} droplets.

In conclusion, the present study demonstrates that utilizing a lipophilic prodrug to ensure a high entrapment in oil droplets of an o/w emulsion may not be a sufficient condition in the use of an emulsion system as a drug carrier for passive drug targeting. It is speculated that a large interfacial area can cause a rapid initial loss of a very lipophilic ester compound incorporated in an emulsion in the circulation.

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