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The in vivo release of cortisone esters from liposomes and the intramuscular clearance of liposomes

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Summary

Studies are reported in which multilamellar liposomes (MLVs) are used as a vehicle to administer cortisone esters by the intramuscular route. The following conclusions were reached.

(1) The clearance half-life of dipalmitoylphosphatidylcholine (DPPC) cortisone palmitate MLVs, containing less than 1 mole % partially iodinated egg lecithin is approximately 8.5 days as assessed by γ-scintigraphy.

(2) No detectable removal of intact MLVs from the injection site occurs.

(3) No spreading of the MLVs at the injection site occurs over the period of 1 h to 7 days post-injection.

(4) The [^{131}I]lecithin complex is stable in vivo.

(5) Encapsulation in DPPC liposomes has been shown to significantly prolong the absorption of cortisone hexadecanoate from the i.m. site.

(6) In vitro data cannot give precise information about the in vivo performance of liposome formulations.

(7) It is possible that the primary release mechanism of cortisone prodrug clearance is diffusion from liposomal material at the i.m. site. However, the presence of secondary depots cannot be completely discounted.

Introduction

Several workers have previously described the clearance of liposomes administered via the intramuscular route (Dapergolas et al., 1976; Zierenberg and Betzing,

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1979; Kirby et al., 1980). However, their objectives differ from the current objective of assessment of liposomes as a vehicle for sustained systemic therapy. Zierenberg and Betzing (1979) injected a sonicated suspension of the highly unsaturated soya lecithin, a formulation whose biological stability was known to be low, and hence is ideal for studying the metabolic fate of liposomes administered by the i.m. route. The reported clearance half-life of 8.6 h is unsuitable for sustained drug delivery.

Other workers have viewed the i.m. route as a means of delivering liposomes to the systemic circulation (Dapergolas et al., 1976). In order to accomplish this, highly sonicated vesicular suspensions were administered whose consequent latency is poor. However, it was reported that, in the short-term, multilamellar vesicles (MLV) remained close to the i.m. injection site. Should clearance and/or degradation of the liposomes from such a site be significantly slower than drug permeation from the liposomes, then ensuing plasma drug levels will be controlled by the latter process and probably predictable from in vitro liposome efflux determinations (Arrowsmith et al., 1983).

It has been reported that the efflux of liposome encapsulated materials in vivo differs from that predicted from in vitro experiments. Arakawa et al. (1975) found that the stability of liposomes was unaffected by the presence of a rat muscle homogenate. However, i.m. injection into rats led to a 5-fold increase in the rate of efflux of cefazolin sodium compared with in vitro rates. Hence, it is vital to test prospective liposome formulations in an animal model.

Sund and Schou (1964) first reported methods for evaluating the kinetics of drug absorption from the rat i.m. site. Blunt dissection of the rat thigh musculature ensures injection to the centre of the muscle mass. Periodic sacrifice and removal of the injected muscle allows assay of the material remaining at the injection site. However, during the entire process the rat is anaesthised, a condition which is known to affect drug absorption processes (Woolfrey et al., 1983). Also, the anaesthetized condition severely limits the time over which absorption kinetics may be studied. The advantage of this method is that a more direct measurement of absorbed drug is made, rather than through pharmacokinetic means.

It has been reported that absorption rates from sustained release vehicles in man are slower than those found in rats but similar to those of larger mammals (Jorgensen and Gottfries, 1972). However, it is not feasible to sacrifice large numbers of the latter animal type to directly measure absorption rates and therefore absorption data are obtained by an analysis of plasma/time and excretion/time profiles (Frantz et al., 1961).

Gamma-scintigraphy has been previously used to study the fate of liposomes injected by several routes (McDougall et al., 1975). However, an aqueously entrapped gamma marker was used which, following leakage or liposomal breakdown, would not have the same distribution as liposomal material. A marker of high bilayer specificity, which may also be used to follow the metabolic fate of liposomal material would be ideal. Preformed liposomes have been labelled with ^{99m}technetium by reacting the pertechnetate ion with the external polar head groups in the presence of stannous chloride (Richardson et al., 1976; Farr et al., 1983). Such labelling is satisfactory for short-term diagnostic applications. The short half-life of ^{99m}tech-

netium (6 h), the presence of label on the external monolayer only and possible effects upon bilayer stability, render this labelling technique unsuitable for the monitoring of the i.m. clearance of MLVs.

Recently the labelling of unsaturated egg lecithin with ^{131}iodine in the presence of chloramine-T in an aqueous/ethanol environment has been reported (Hardy et al., 1980). The advantages of such a method are that ^{131}I has a long radioactive half-life (8.04 days) and the label is covalently bound to the hydrocarbon chains of the lecithin. However, the energy of the ^{131}I main emission (82%) is high (0.364 MeV) and this leads to a slight loss in image definition.

Lubran and Pearson (1958) have reported a technique utilizing iodine monochloride for the labelling of triglycerides. This method uses a low boiling organic solvent and would therefore be suitable for use with heat-labile lecithins.

The aim of this study is to determine the fate of cortisone ester loaded DPPC MLVs following injection by the i.m. route. Female New Zealand white (NZW) rabbits were chosen as subjects as they are of a suitable size for whole body imaging; have a fairly constant body temperature of 37 °C; possess a substantial and easily located hind leg musculature and are of a docile nature and will sit, static, for long periods. The rate of degradation and/or clearance of the formulation will be fundamental to its success as a sustained release device.

Monitoring of plasma levels for steroidal activity will permit calculation of the kinetics of drug clearance from the injection site. Comparison of colloid and drug clearance kinetics can therefore be made.

Materials and Methods

Lipid and steroid sources and the synthesis of cortisone 21-esters are detailed in Arrowsmith et al. (1983a). Sodium ^{131}iodide (20 mCi · μg^{-1}) was obtained from Amersham International, U.K. and Patent Blue V 2.5% solution for injection, from Gourbet, France. All other solvents were of analar or reagent grade. NZW rabbits were 3.0 kg.

Procedure for labelling of egg lecithin with ^{131}iodine

10 mCi of sodium ^{131}iodide were diluted to 1 ml with distilled water in a 10 ml stoppered test tube and 1 ml of potassium iodide solution (0.1 mg · ml^{-1}) added. This was followed by the addition of 0.5 ml of potassium iodate solution (2 mg · ml^{-1}) and acidification with 0.2 ml sulphuric acid (2 N). The resulting iodine was extracted into 2 ml of chloroform and the aqueous phase discarded.

A saturated solution of chlorine in chloroform was prepared by bubbling the gas, liberated by the action of hydrochloric acid (36%) upon crystalline potassium permanganate, into the solvent for 15 min. The chlorine solution was added dropwise to the violet coloured solution of ^{131}iodine in chloroform until decolourized (2 drops); indicating the formation of ^{131}iodine monochloride. This solution was added to 2 ml of a solution of egg lecithin in chloroform (21.5 mg · ml^{-1}) in a 15 ml stoppered test tube and left to stand for 90 min at room temperature to ensure

complete labelling. After this period the solution of iodinated lecithin was washed twice with 5 ml of an aqueous solution of potassium iodide and sodium thiosulphate (5% w/v) and finally twice with 5 ml of distilled water. Between each washing the phases were separated by 10 min centrifugation at $1000 \times g$.

Labelling efficiency was assessed in a well counter and was found to be an overall 77%, giving a specific activity of 1.18 $mCi \cdot \mu mol^{-1}$.

Preparation of the liposome injection

A film of 8.25 mg of DPPC and 6.56 mg of cortisone hexadecanoate and 0.912 μCi of [^{131}I]lecithin (0.65 mg) was prepared by rotary evaporation of a chloroform solution at 40 °C for 15 min. The film was distributed in 3 ml of saline for injection by handshaking in the presence of glass beads, following hydration at 60 °C for 2 h. The liposome suspension was left to stand overnight, followed by centrifugation at $3000 \times g$ for 30 min. The gamma-activity of the supernatant was assessed before discarding. The weight of the tube contents was adjusted to 0.65 g with saline and the soft liposome pellet resuspended to produce a final suspension of 12.5% w/w steroid/lipid complex of which 9 mole % is cortisone hexadecanoate.

Injection and imaging procedure

The rear thigh of each rabbit was shaved and 0.125 ml of the liposome suspension, equivalent to 175 μCi ^{131}I, was injected unilaterally into the tensor fascia lata muscle of three rabbits using a 1 ml syringe (Gillette, U.K.) equipped with 1 cm 25-gauge hypodermic needle (Gillette, U.K.). Scintiscans were recorded on floppy discs using a computer (Link Systems, U.K.) from a Nuclear Maxicamera II (G.E.C., U.S.A.) with the rabbit seated in a perspex restrainer, placed on the camera face, equipped with a high energy collimator. A dynamic study of 30×1 min frames was undertaken, at a window setting optimised at 93, immediately after injection.

Further, static scintiscans were recorded periodically over the next 14 days. These were initially of 60 s duration but were lengthened to a maximum of 180 s to accomodate radioactive decay. The computer produced images of the intensity of the radiation for display on a television monitor, colours varying through blue (low intensity) to white (high intensity). The user is able to determine the lower and upper levels of such gradation and the images are displayed on a 128 by 128 cell scale for static views (64 by 64 for dynamic).

Standard computer programs allowed the designation of regions of interest (ROI). In addition to the injection site, other regions used were the liver/stomach area and the thyroid/neck region. The number of counts and also the number of cells within each ROI were determined for each view. These were corrected for radioactive decay and background, before standardization to a 60 s count.

The activity remaining at the injection site was corrected for background count and circulating activity by subtraction of the counts for equivalent parameters and the same area over the contralateral site. The corrected counts per min (cpm) for the injection site ROI were expressed as a percentage of the initial cpm for the same ROI, immediately after completion of the dynamic study.

Suitability of the injection site

The diameter of the rabbit tensor fascia lata muscle was determined in situ using calipers with the rear leg extended and the animal restrained as for injection. 0.1 ml injections of the 'Patent Blue V' were made bilaterally 2–3 cm above the near knee, using a 1 ml syringe (Gillette, U.K.) equipped with a 26-gauge, 1.5 cm hypodermic needle. This was fitted with a baffle of such length that the injection would be made to the centre of the muscle mass. The animal was immediately sacrificed and the injected muscle dissected and examined to assess the spread of the injection.

Preparation of DPPC liposome injections

DPPC was weighed accurately into a 25 ml round-bottom flask with 9 mole % of [^{3}H]cortisone-hexadecanoate (24 μCi · μmol^{-1}). A film was produced in a 50 ml round-bottom flask by rotary evaporation at 45 °C for 15 min, from ethanolic solution. 3 ml saline for injection was added and the flask sealed and placed in a shaking water bath at 60 °C for 1 h. Distribution was completed by two 30-s periods of vigorous mechanical shaking. The suspension was washed (2 × 1 ml saline) into a tared 10 ml polycarbonate centrifuge tube (M.S.E., U.K.) previously wet heat sterilized, and centrifuged at 50,000 × *g* for 1 h. Duplicate 10 μl supernatant samples were taken for scintillation counting, and sufficient supernatant removed to adjust the preparation to a content of 12.5% w/w DPPC. The liposomes were redistributed by mechanical shaking (2 × 1 min). A suspension of an equivalent amount of cortisone hexadecanoate as was liposomally entrapped (0.1 mg · ml^{-1}) was prepared. [^{3}H]Cortisone hexadecanoate (24 μCi · μmol^{-1}) was accurately weighed into a 10 ml Erlenmeyer flask and dissolved in ethanol. Sufficient sterile normal saline was added to produce 90% of the vehicle by volume and the suspension mechanically shaken for 5 min. 0.125 ml (50 μCi) was injected into the tensor fascia lata muscle of three NZW rabbits.

It has previously been reported that labelled compounds, prepared by exchange tritiation with the hydrogen atoms of the cold compound, have lost their label to body water after injection (Aaes-Jorgensen et al., 1977). Cortisone used in these experiments is labelled in the 1,2-position by reduction of prednisone with tritiated gas. It is likely that labelling performed under such conditions will be stable in biological conditions.

Intramuscular (i.m.) injection procedure and measurement of plasma / time profile

0.125 ml aliquots containing approximately 50 μCi ^{3}H, were injected intramuscularly into rabbits as previously described and blood samples periodically removed from the marginal ear vein by venipuncture. Immediately after collection, 2 ml blood samples were centrifuged at 1000 × *g* for 20 min and the plasma assayed for tritium content. The remainder was immediately frozen and stored at −17 °C. After two weeks the rabbits were sacrificed and the injected muscle mass removed, weighed and divided before homogenization (Top Drive Macerator, Townson and Mercer, U.K.) with sufficient distilled water to produce a total weight of 200 g. Four 1 ml portions were assayed by scintillation counting. Counts of similar samples prepared from the contralateral site were deducted as background. Compensation for any

circulating activity is also achieved by this latter procedure.

Pooled samples of plasma were vortexed with 2 × 10 ml of ethyl acetate, the two phases being separated by brief centrifugation (1000 rpm for 10 min). The ethyl acetate was rotary evaporated to dryness and the residue dissolved in the minimum of chloroform. The relative amounts of cortisone and 21-ester were estimated.

The hydrolysis of cortisone hexadecanoate in a 5% w/v liver homogenate during 20 and 45 min was determined using the techniques described by Arrowsmith et al. (1983b).

Urine samples (0–72 h) were analyzed for the presence of cortisone-21-esters using the extraction procedure reported by Frantz et al. (1961). Excreted material upon sawdust from the floor of the rabbit cage, was partitioned between 20% sodium sulphate in water (150 ml) and ethyl acetate (100 ml). The ethyl acetate was collected and washed successively with 5 ml of 1 N sodium hydroxide solution containing 15% disodium sulphate, 5 ml of 0.5% acetic acid containing 15% disodium sulphate and finally 2 ml of distilled water. The ethyl acetate was evaporated to dryness (30 °C) and the residue redissolved in chloroform/methanol (1 : 1). The relative proportions of esterified and non-esterified steroids were established by separation upon silica gel TLC plates eluted with toluene/ethylacetate (13 : 7). The spots identified under UV light, were scraped into 10 ml of scintillant and shaken with 1 ml of water for 5 min. The vials were stood in darkness overnight to allow chemiluminescence to subside before counting.

The i.v. administration of [^{3}H]cortisone

150 μg of [^{3}H]cortisone (360 $\mu Ci \cdot \mu mol^{-1}$) was rotary-evaporated from ethanolic solution to a film and 1.5 ml of injectable saline added. The flask was sealed and shaken overnight to aid dissolution. 25 $\mu Ci \cdot kg^{-1}$ was injected into the marginal ear vein of the rabbits. Blood samples were collected from the contralateral vein after nicking with a scalpel blade and plasma tritium content assessed.

Calculations

The amount of drug remaining at the muscle site was calculated from the plasma data by employing the Wagner-Nelson equation (Wagner and Nelson, 1963) The areas under the plasma time profiles were calculated using the method of trapezoids.

Results and Discussion

Evaluation of the injection technique

The rabbit tensor fascia lata has been reported as an easily locatable muscle mass which has been previously used successfully for intramuscular studies. The ease of the 'Patent Blue V' injection into this muscle verified the former conclusion and subsequent dissection showed that little leakage of drug from the injection site occurred.

Gamma-scintigraphy studies

Following the i.m. administration of [^{131}I]labelled liposomes into the right hind

leg of the rabbit, an intense spot was evident at the injection site. No other sites of a significant accumulation of activity were seen throughout the study and the gamma-camera was used essentially as a counter to determine the activity remaining at the injection site over a 14-day period. The data are expressed as a percentage of those recorded for the first static views (1 h post-injection) as a 5% discrepancy was found between the activity recorded on dynamic and static studies. Should an initial rapid removal of injected liposomes have occurred such a procedure would lead to an inaccurate clearance profile. The dynamic study (0–30 min) showed there was no significant removal of material from the injection site during this period. Other workers have reported that intact MLVs entering the circulation accumulate essentially in the liver and spleen (Hinkle et al., 1978). Very little accumulation of activity in the liver occurred demonstrating that rapid removal of intact liposomes from the injection site did not occur.

Throughout the study labelled material lost from the injection site was spread diffusely throughout the body. The liver region showed a very slight accumulation of ^{131}I which may be due to its high vascularity. However, Zierenberg and Belzing (1979) have reported that phospholipid metabolism is hepatic based and this small level of liver [^{131}I]activity could be due to the accumulation of liposomal degradation products.

The concentration of ^{131}I in the neck region of rabbits has been reported following i.v. administration of liposomally entrapped sodium ^{131}iodide and liposomes composed of [^{131}I]phospholipid (Hardy et al., 1980). However, the thyroid accumulation of radioactivity in this study was never significantly in excess of background and circulating activity. This demonstrates that the marker is strongly bound to the phosphatidylcholine as only free iodide accumulates in the thyroid.

A standard computer programme enables the profile of radioactive concentration to be depicted either in a vertical or horizontal axis through the point of highest activity for each ROI. It was thought likely that spreading of the liposome formulation post-injection would be most significant along the planes of the muscle, i.e. along the vertical axis. However, measurement of the half-height width of the peaks for times 1 h and 7 days showed no significant difference. It would appear that the spreading of liposome vehicles following i.m. injection is similar to that reported for oils, and that removal of phospholipid from the site is not preceded by spreading within the local musculature.

The zero-order clearance of 131-iodinated material from the i.m. injection site is depicted in Fig. 1. This profile, with a half-life of 8.5 days indicates the suitability of DPPC liposomes as a vehicle for the provision of sustained therapy over a 14-day period. The clearance of polyunsaturated lecithin, sonicated liposomes, reported by Zierenberg and Betzing (1979) was considerably faster than the figures presented in Fig. 1. However, the clearance profile is reported as biphasic. The initial fast phase was attributed to the rapid removal of vesicular lipids, and the second slower removal phase was attributed to the clearance of small MLVs. Coulter Counter analysis has established that 'cold' liposomes prepared by an analogous procedure had a mean particle size of 6–7 μm, which is a very large liposome.

The clearance data support the conclusion drawn from the work of Dapergolas et

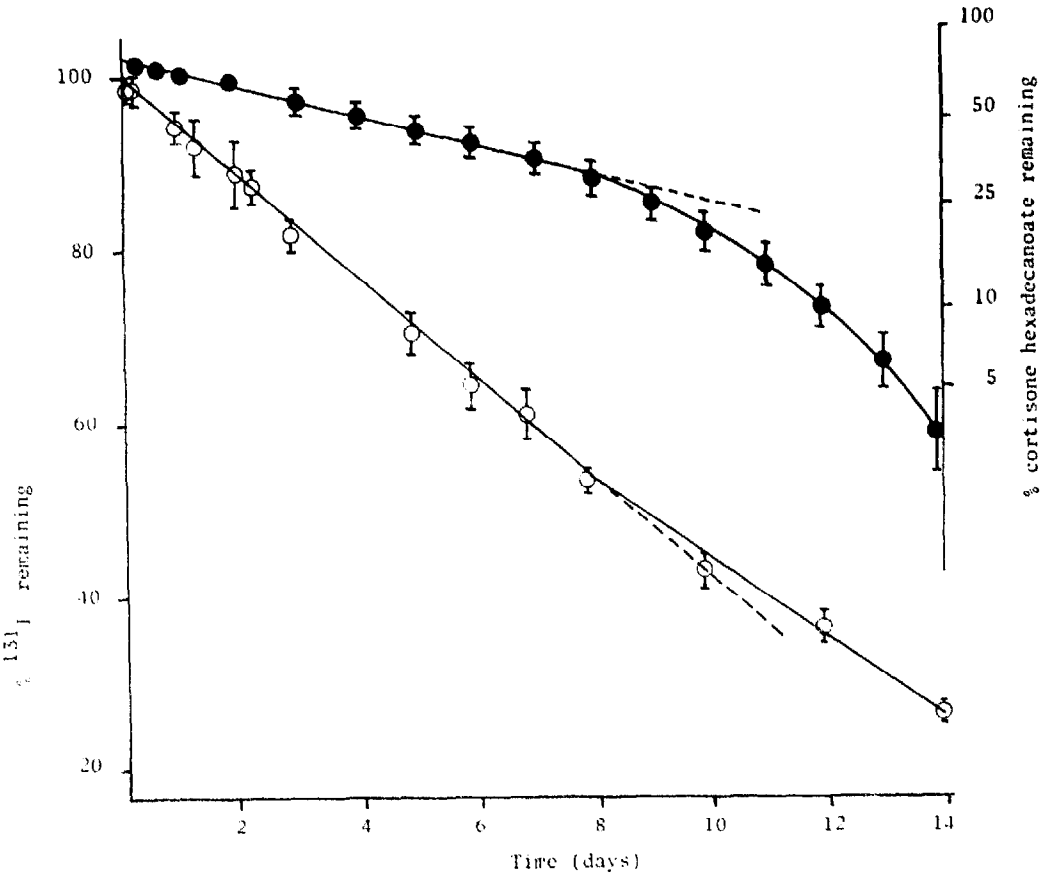

Fig. 1. A comparison of the clearance of [^{131}I]labelled liposomes and cortisone hexadecanoate entrapped in liposomes from the rabbit i.m. site. ●, cortisone hexadecanoate; ○, [^{131}I]liposomes. Bars represent S.E.M. for 3 rabbits.

al. (1976) and Kellaway and Chawla (1981) that MLVs injected by an extravascular route remain at the injection site for long periods and are probably degraded in situ.

It is impossible to determine whether the iodinated phospholipid exists at the injection site incorporated into intact liposomes or associated with cellular material following liposomal degradation. However, the use of iodinated egg lecithin appears to be suitable to trace the fate of liposomal material on a macroscopic level. The incorporation of the 0.8 mole % of this material used, is unlikely to greatly effect the properties of the DPPC/cortisone hexadecanoate bilayer. Also, being a lecithin, the marker is unlikely to be removed from the liposome to any greater extent than DPPC molecules.

It may be concluded that such a formulation has a maximum 'life' of approximately 14 days since it is largely cleared from the i.m. site during this time. However, it is possible that in situ liposome degradation occurs at a faster rate than marker clearance from the i.m. site. The apparent slowing in clearance rate evident over 8–14 days may be due to the incorporation of [^{131}I]lecithin into membranes at the injection site.

The formulation of the injection

A 0.125 ml i.m. dose volume injected into a rabbit is approximately equivalent to the maximum advisable i.m. dose in man, i.e. 5 ml. However, the rabbit leg musculature is relatively more developed than man's and the rabbit did not show any great discomfort during, or subsequent to, the injection procedure.

Injections containing 35, 20, 15 and 10% w/w of DPPC, bearing 9 mole % of cortisone hexadecanoate were suspended in saline. Only the preparations containing the lowest concentration of DPPC were sufficiently fluid to allow uptake into a syringe and subsequent removal of incorporated air bubbles. A lipid concentration of 12.5% w/w was used for all injections. Ladbrooke and Chapman (1969) have reported that water mixed with lecithin in excess of 25% w/w shows an ice transition and may be regarded as solvent water. We have demonstrated that, in the presence of 9 mole % cortisone hexadecanoate the suspension's saline content must be raised to 85% w/w to produce a suitable fluid injection.

The plasma / time profiles following the i.m. administration of [^{3}H]cortisone-21-derivatives

Quenching was found to be low for plasma samples provided no blood lysis occurred. Hence, all blood samples were centrifuged immediately after collection and the plasma solubilized for scintillation counting. Extraction of the remaining red blood cells with 10 times the volume of dichloromethane or ethyl acetate led to the conclusion that very little tritiated material was associated with this blood fraction. Similar results have been found for cortisone binding to mammalian red blood cells in vitro (Ohtsuka and Koide, 1969).

The plasma tritium-level/time profiles following the administration of DPPC liposome-encapsulated cortisone hexadecanoate, cortisone octanoate and a suspension of cortisone hexadecanoate are shown in Fig. 2. The plasma profile following [^{3}H]cortisone hexadecanoate loaded in DPPC liposomes shows a sharp peak after 6 h and following the 12 h sample blood levels slowly decrease over the next 14 days. Variation is greatest during the initial phase but after 24 h plasma levels became more reproducible. A rapid entry of drug into the circulation occurred during the first 3–6 h in two of the three rabbits injected with cortisone hexadecanoate loaded liposomes.

This effect cannot be explained by consideration of the clearance data of liposomes administered via the i.m. site, as a rapid initial clearance phase did not occur (Fig. 1). The [^{131}I]labelled liposomes were prepared using much lower centrifugal forces than those employed in this part of the study. Assay of the supernatant of the iodinated material indicated the presence of only a small amount of material (0.7%) which is insufficient to cause such an effect. However, Coulter analysis (Table 1) shows the iodinated liposomes to have a generally larger particle size, but it is thought such differences are not great enough to cause a relatively rapid efflux from the i.m. site of the liposomes bearing tritiated material.

Plasma levels plateau over the 30 h to 4 day period. This phase may represent plasma activity achieved through diffusion of material from the injection site, without carrier loss. The plasma/time profiles following the i.m. injection of an oily

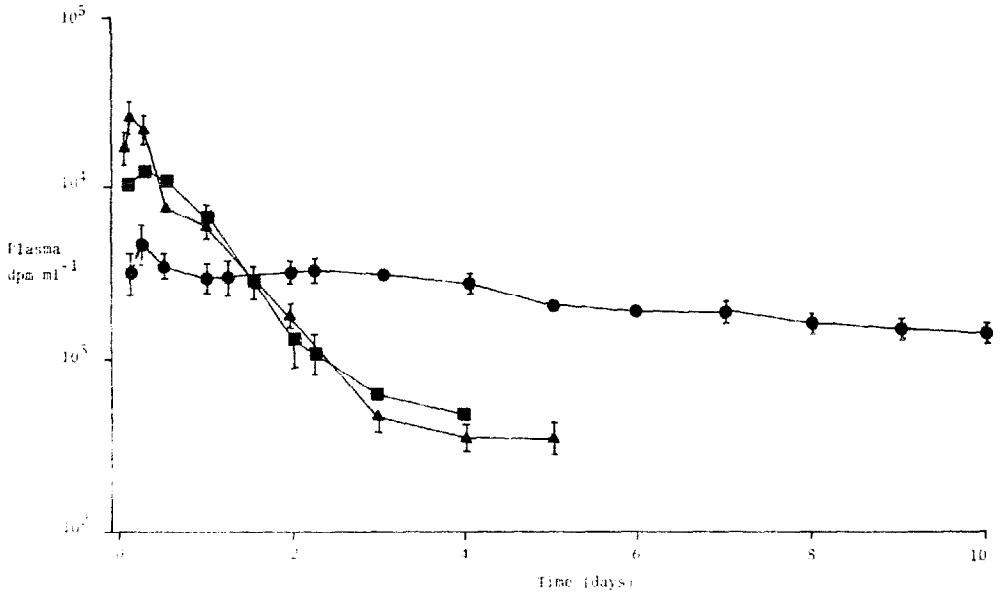

Fig. 2. In plasma radioactivity/time profiles following the administration of cortisone derivatives to the rabbit i.m. site. ●, 12.5% w/w DPPC suspension loaded with 9 mole % [^{3}H]cortisone hexadecanoate; ■, 12.5% w/w DPPC suspension loaded with 9 mole % [^{3}H]cortisone octanoate; ▲, 0.1 mg·ml^{-1} cortisone hexadecanoate suspension.

depot of fluphenazine aliphatic esters have shown a slow steady ascent to peak levels over a 5–11-day period (Dreyfuss et al., 1976). The clearance of oils from the i.m. site is slow in comparison with the clearance of the dissolved drug (Armstrong and James, 1980), hence a slow ascent to peak drug blood levels will occur. The slow decline in plasma levels over the following 10 days is indicative of a first-order release from the depot.

Non-encapsulated cortisone hexadecanoate was administered to the i.m. site in order to obtain a qualitative assessment of the latency incurred by liposome

TABLE I

THE SIZE DISTRIBUTION OF INJECTED MATERIAL AS MEASURED BY COULTER COUNTER (±S.D. OF ALL COUNTED PARTICLES)

	Cortisone hexadecanoate loaded DPPC liposomes (n = 4)	Cortisone octanoate loaded DPPC liposomes (n = 3)	[^{131}I]-labelled liposomes
Mean particle size (μm)	5.5 ± 1.7	7.1 ± 0.9	6–7
% < 1 μm	1.9 ± 1.3	2.0 ± 0.7	1.0
% > 10 μm	12.5 ± 5.4	30 ± 1.4	25

incorporation of the drug. It was found impossible to produce this cortisone derivative with a sufficiently high specific activity so as to allow the dissolution of material equivalent to 50 μCi of ^{3}H in 0.125 ml of saline. Hence, a suspension of the material, equivalent in drug concentration to that administered in liposomes, was used. The size of the suspension particles was large (20–50 μm) in comparison with the liposomes (Table 1). Such a large particle size and the low aqueous solubility of cortisone hexadecanoate should result in a slow leaching from the i.m. site. However, as is shown in Fig. 2, the [^{3}H]plasma/time profile shows a transient response in comparison with the encapsulated material. This illustrates that liposome encapsulation significantly retards the clearance of cortisone hexadecanoate from the i.m. site. The [^{3}H]plasma level is higher than expected 24 h after injection. These extended levels may be due to the slow dissolution of a few extremely large particles.

The plasma/time profile following the injection of [^{3}H]cortisone octanoate encapsulated in DPPC liposomes is also shown in Fig. 2. Peak plasma levels were achieved after 6 h but the general pattern of release is much faster than that seen with the longer ester.

Encapsulated cortisone hexadecanoate is therefore the optimum formulation tested, providing prolonged therapy over a 10–14-day period. However, the use of log plasma levels confers an appearance of sustained therapy which is probably exaggerated.

The pharmacological activity of corticosteroids has been reported as being longer than their plasma/time profile would indicate (Robinson, 1978). Hence testing of the therapeutic efficacy of this preparation would be best determined by measuring its anti-inflammatory activity. However, the aim of these in vivo experiments was to examine how the biological environment affected the release profiles of liposome encapsulated prodrugs. Such indirect evidence as that reported by Porter and Silber (1953) would not be sufficiently precise to allow conclusions to be drawn.

The plasma clearance of formulations of cortisone and its 21-derivatives

The clearance of [^{3}H]cortisone from the rabbit plasma following bolus i.v. injection of 50 $\mu Ci \cdot kg^{-1}$ is shown in Fig. 3. The distribution, or α-phase, is complete within 15 min and therefore the pharmacokinetic model may be regarded as one compartment. Hence the elimination rate constant (β) is equal to the slope of the clearance plot. Clearance was determined for five NZW rabbits and the mean value of $\beta = 0.271\ h^{-1}$ (± 0.020 S.D.). This is equivalent to a plasma half-life of 2.56 h; a value which compares with a range from 0.75 to 1.6 h in man (Peterson et al., 1957).

The Wagner-Nelson equation allows the calculation of k_1 in the model below, provided β is known.

$$\text{Depot} \xrightarrow{k_1} \text{Plasma} \xrightarrow{\beta}$$

where k_1 is the rate constant of absorption, or release of drug from the depot into the plasma.

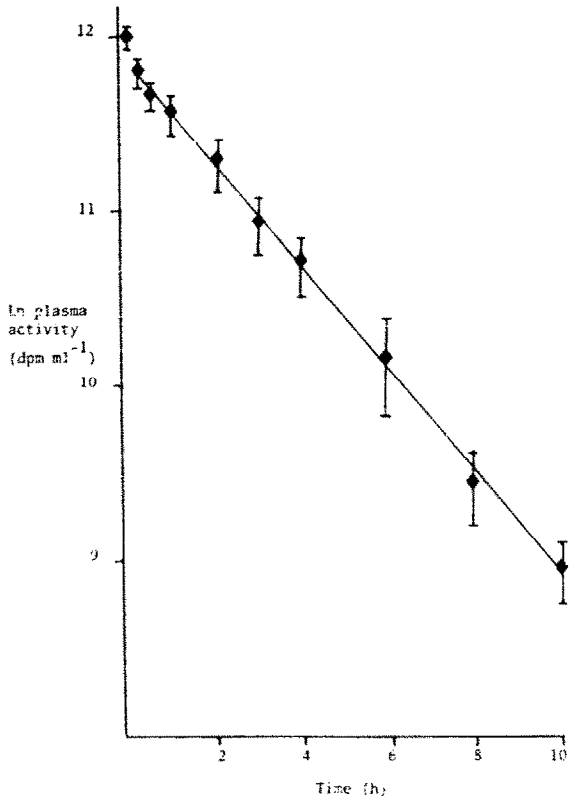

Fig. 3. The clearance of [^{3}H]cortisone following bolus i.v. injection into NZW rabbits. S.D. bars n = 5.

It was not possible to synthesize cortisone esters of sufficiently high specific activity to allow their bolus i.v. injection. Hence, i.v. data for the parent drug, cortisone, has been used. There is some evidence that the elimination rate of the parent drug does not differ significantly from that of the prodrugs.

(1) Extraction of the urine and occasional faeces samples of the rabbits showed no elimination of non-hydrolyzed prodrug.

(2) The areas under the [^{3}H]plasma time profiles for both i.m. administration of prodrugs and i.v. administration of cortisone are similar (see Table 2).

TABLE 2

THE AREAS UNDER THE PLASMA/TIME PROFILES OF DIFFERENT FORMULATIONS OF CORTISONE (dpm·h) (MEAN OF 3 ± S.E.M.)

Cortisone hexadecanoate suspension (i.m.) 483,607 ± 20,354	Cortisone hexadecanoate, liposome-encapsulated (i.m.) 469,335 ± 45,001
Cortisone octanoate, liposome-encapsulated (i.m.) 433,046 ± 27,002	Cortisone solution (i.v.) 448,026 ± 40,219

TABLE 3

THE HYDROLYSIS OF CORTISONE HEXADECANOATE IN A 5% RABBIT LIVER HOMOGENATE

Time	20 min	45 min
% Hydrolyzed	72%	87%

(3) The presence of plasma prodrug was not detectable at any time after i.m. administration using the TLC assay procedure described in the methodology section.

(4) Cortisone octanoate and hexadecanoate are both plasma hydrolyzed (Arrowsmith et al., 1983b) and cortisone hexadecanoate is readily hydrolyzed by tissue homogenates (Table 3).

(5) Other workers have reported that i.v. clearance of aliphatic esters was very similar to that of the parent drug (Dreyfuss et al., 1976).

The evidence in (1) indicates that unless the prodrug hydrolysis rate in the body is slower than the elimination rate of the parent drug, this elimination rate may be used in calculation of the prodrug absorption rate. The evidence presented in (3) and (4) indicate that for cortisone octanoate the hydrolysis rate in the body is probably faster than the elimination rate. However, no firm conclusions may be drawn for hydrolysis of cortisone hexadecanoate from this evidence.

Notari (1980) has shown, using computer models, that if only the rate of absorption is altered (i.e. k_1) then the area under the plasma/time profile is constant. Alterations in the elimination rate cause differences in this parameter. As the area under the curves for the different formulations, but equivalent doses (50 μCi), are similar (Table 2), the conclusion may be drawn that the elimination rate (β) is also similar.

Fig. 4 shows the clearance profiles of the three i.m.-administered formulations as

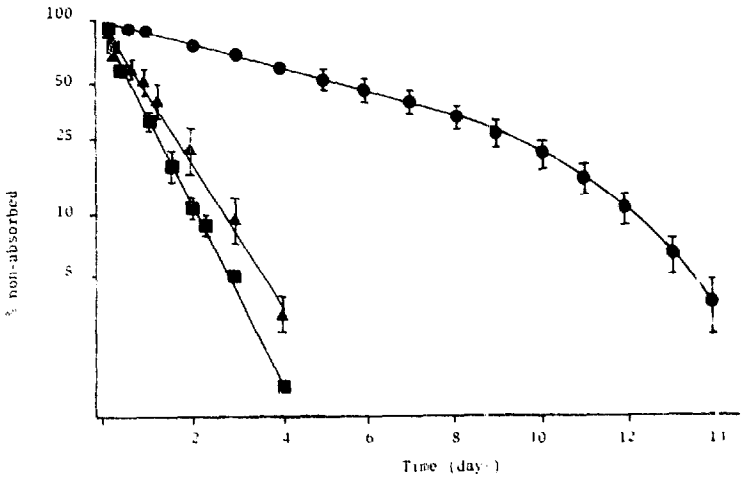

Fig. 4. The clearance of cortisone derivatives from the i.m. site, calculated from plasma level data. ●, liposome-entrapped cortisone hexadecanoate; ■, liposome-entrapped cortisone octanoate; ▲, cortisone hexadecanoate suspension.

calculated from Wagner-Nelson analysis of the plasma level/time profile. As expected the clearance of the cortisone hexadecanoate suspension and liposome-entrapped cortisone octanoate was much faster than the liposome entrapped hexadecanoate. It is perhaps surprising in light of the plasma/time profiles, that the overall rate of absorption of the suspension is found to be slower than the rate for cortisone octanoate. However, the important initial absorption rate is seen to be slightly faster.

The absorption kinetics of encapsulated cortisone hexadecanoate appear to start as first-order, but the rate of appearance in the plasma increases after 8 days. It is evident that liposomal encapsulation significantly retards clearance of cortisone hexadecanoate. Since there are several sources of error in the calculation of absorption rates using the Wagner-Nelson equation, the clearance profiles illustrated in Fig. 4 should be regarded as only an indication of the true rates.

Fig. 1 permits the comparison of the clearance of [^{131}I]lecithin and cortisone hexadecanoate from the i.m. site. The increase in rate of clearance of the drug 8 days post-injection cannot be explained by increasing liposomal degradation during this period. The clearance of the lecithin is slightly slower over the 8–14-day interval. It is possible that drug release is occurring through a different dominant process in this second phase. Such a process might result in the greater incorporation of [^{131}I]lecithin into biological membranes so retarding its clearance from the site.

The differences in both half-life and order of liposome and drug clearance from the i.m. site (Table 4) indicate that these processes occur through independent mechanisms. It is inferred that the primary drug release process is diffusion from liposomes at the i.m. site.

Comparison of in vitro and in vivo release

Comparison of the in vitro and muscular half-lives of liposome encapsulated cortisone octanoate and hexadecanoate shows no trend (Table 4). The release of cortisone hexadecanoate is slower in vivo than in vitro but the opposite is true for the short-chain ester. It is generally expected that the association of encapsulated and liposomal material will be more tenuous in vivo than in vitro. Hence the slower muscular efflux of the longer ester is not expected.

TABLE 4

COMPARISON OF IN VITRO AND IN VIVO KINETICS

Preparation	$t_{1/2}$ in vitro *	$t_{1/2}$ muscular * clearance
[^{3}H]cortisone hexadecanoate in DPPC liposomes	2.92	4.70
[^{3}H]cortisone octanoate in DPPC liposomes	1.21	0.68
[^{3}H]cortisone hexadecanoate suspension	..	0.79
[^{131}I]liposomes	–	8.48 †

* All kinetics first order except † zero-order.

It is possible that the greater biological stability of liposomes containing cortisone hexadecanoate compared to the steroid-free liposomes (Arrowsmith et al., 1983b) may cause discrepancies in their in vitro and in vivo efflux rates. However, it does not appear feasible that such small differences can account for the very great differences illustrated in Table 4.

It has recently been postulated (Armstrong and James, 1980) that discrepancies in the plasma half-lives and whole-body half-lives of neuroleptic prodrugs (Dreyfuss et al., 1976; Aaes-Jorgensen et al., 1977) may be explained by the presence of secondary depots in lipid rich regions of the body. Such effects may explain the great differences in the behaviour of the two cortisone esters in vivo.

Comparison with reported data for the bioavailability of other steroid / liposome formulations

The 24-h plasma levels following i.m. administration of liposome-encapsulated corticosteroids have been previously reported (Shinozawa et al., 1979). The large injection volume (0.6 ml) of liposomally encapsulated [^{3}H]prednisolone administered to the 'hip' muscle of rats led to far higher plasma and tissue levels than the injection of drug in a similar volume of propylene glycol. The fast efflux of steroids from DPPC liposomes was reported by Arrowsmith et al. (1983a). Hence, it is unlikely that prednisolone remains associated with liposomes in vivo for a significant length of time. The greater tissue and plasma levels following injection of the liposome formulation may reflect a poor bioavailability from the propylene glycol preparation.

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