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Use of a lipid emulsion as a novel carrier for corticosteroids

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Liposomes are easily taken up by reticuloendothelial systems (RES) and some inflammatory cells and because of this corticosteroids have been incorporated into them and have been found to inhibit inflammation, for example arthritis in animals (Dingle et al 1978) and man (De Silva et al 1979), more intensively than a corresponding amount of free drug. Problems with liposomal therapy are instability of the liposomes and lack in clinical experience. We incorporated dexamethasone palmitate in a lipid emulsion (intralipid) that is taken up readily by phagocytic cells (Hallberg 1965) and is very stable and in widespread use for parenteral nutrition in man. We now report the tissue distribution patterns of dexamethasone-lipid emulsion and its anti-inflammatory activity in rats.

Dexamethasone (Merk) and [6,7-^{3}H(N)]dexamethasone (NEN) were used. [^{3}H]Dexamethasone palmitate and [^{3}H]dexamethasone disodium phosphate were synthesized from the [^{3}H]dexamethasone. Unlabelled salts were prepared from dexamethasone in the same way. They were identified by t.l.c., i.r. and n.m.r. The [^{3}H]dexamethasone were mixed with the unlabelled drug in an appropriate ratio before use. The synthesis of [6,7-^{3}H]dexamethasone palmitate was carried out as follows: [6,7-^{3}H]dexamethasone 0·107 μmol, in 5 ml of pyridine and 0·2 μmol of palmitoyl chloride in 0·5 ml of ether were mixed at 0 to 4 °C and then, reacted at 17 °C for 24 h. The solvents were evaporated and the residual dissolved in a small volume of ethanol applied to a silica gel column (2·5 cm × 30 cm) and the dexamethasone palmitate eluted with butyl acetate–benzene (1:1 by volume).

[6,7-^{3}H]Dexamethasone palmitate was obtained by completely evaporating the solvent. The chemical and radiochemical purities were 99·8% and higher than 99·9%, respectively t.l.c.: butyl acetate–benzene 1:1 v/v.

The [6,7-^{3}H]dexamethasone disodium phosphate was synthesized by the method of Chemerda et al (1960) using [6,7-^{3}H]dexamethasone derived from [6,7-^{3}H]dexamethasone iodide via methyl sulphonyl dexamethasone, and the compound obtained reacted with phosphoric acid in acetonitrile in the presence of silver phosphate. The resultant [6,7-^{3}H]dexamethasone phosphate, was purified by cation exchange column (Amberlite 120B, H type) and converted to the disodium phosphate by neutralizing with sodium hydroxide. The chemical and radiochemical purities were 99·5% and 99·9%, respectively (t.l.c.: chloroform–ethanol 9:1 v/v).

The lipid emulsion containing dexamethasone palmitate was prepared as follows: Dexamethasone palmitate (482 mg) was dissolved in 22·4 g of soybean oil containing 2·4 g of yolk phospholipids which consisted of 79% phosphatidylcholine, 17% phosphatidylethanolamine and 4% other phospholipids, such as sphingomyelin, lysolecithin. The mixture was emulsified with a Manton Goulin homogenizer at a pressure of 100 kg cm^{-2} under nitrogen until no particles larger than 1 μm were detected by light microscopy. The solution was poured into 90 ml of water.

The average particle size of the lipid emulsion was 0·25 μm all particles being less than 1 μm in diameter. One hundred ml of the lipid emulsion contained 4·82 mg dexamethasone palmitate (3 mg as dexamethasone), 10 g of soybean oil, 1·2 g yolk phospholipids and 2·25 g of glycerol, and the labelled preparation had a radioactivity of 3 μCi ml^{-1}.

Male rats of Wistar strain, 140 to 160 g were injected with 0·1 ml of 1% solution of λ-carrageenan (Sigma) into the hind paw. One ml of either the lipid emulsion containing [^{3}H]dexamethasone palmitate or the solution of

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Table 1. Tissue distribution of [^{3}H]dexamethasone palmitate given as a lipid emulsion and [^{3}H]dexamethasone phosphate given as an aqueous solution in rats (n = 3–4).

		Percent of administered radioactivity (mean ± s.e.)						
		Hours after injection of dexamethasone						
Tissues	Dexamethasone	0*	1/4	3/4	2·5	4·5	7	24
Plasma	palmitate	69·2 ± 4·5	51·0 ± 8·0	13·3 ± 0·4	3·2 ± 0·1	2·0 ± 0·1	1·1 ± 0·1	0·4 ± 0·1
	phosphate	14·9 ± 1·8	4·2 ± 0·2	3·8 ± 0·1	2·2 ± 0·1	1·5 ± 0·1	1·3 ± 0·1	0·7 ± 0·1
Muscle	palmitate	8·0 ± 0·4	17·3 ± 1·4	19·0 ± 0·7	20·5 ± 0·7	14·7 ± 0·7	7·6 ± 1·1	4·1 ± 1·8
	phosphate	35·5 ± 5·0	28·5 ± 1·4	27·3 ± 0·9	18·0 ± 0·3	12·7 ± 3·1	10·4 ± 1·4	6·2 ± 1·1
Spleen	palmitate	0·21 ± 0·10	2·24 ± 0·20	1·60 ± 0·14	0·25 ± 0·04	0·14 ± 0·04	0·09 ± 0·03	0·03 ± 0·01
	phosphate	0·16 ± 0·04	0·17 ± 0·04	0·17 ± 0·02	0·17 ± 0·04	0·09 ± 0·01	0·07 ± 0·003	0·05 ± 0·01
Inflamed paw	palmitate	0·13 ± 0·01	0·34 ± 0·06	0·38 ± 0·10	0·30 ± 0·03	0·14 ± 0·01	0·12 ± 0·04	0·05 ± 0·003
	phosphate	0·20 ± 0·01	0·20 ± 0·03	0·26 ± 0·05	0·14 ± 0·01	0·11 ± 0·02	0·08 ± 0·01	0·05 ± 0·01
Non-inflamed paw	palmitate	0·10 ± 0·01	0·13 ± 0·02	0·17 ± 0·03	0·17 ± 0·001	0·11 ± 0·01	0·08 ± 0·01	0·04 ± 0·01
	phosphate	0·19 ± 0·03	0·18 ± 0·09	0·20 ± 0·03	0·12 ± 0·004	0·09 ± 0·003	0·07 ± 0·004	0·05 ± 0·01
Inflamed/Non-inflamed	palmitate	1·3	2·7	2·3	1·8	1·3	1·5	1·2
	phosphate	1·0	1·3	1·3	1·2	1·2	1·2	1·1

* Immediately after the injection.

[^{3}H]dexamethasone disodium phosphate (3 μCi) was injected via the tail vein 30 min after the carrageenan injection. The animals were killed at various intervals, the blood obtained from the heart, and then several organs and paws were removed. About 200 mg of tissues or 0·2 ml of plasma were accurately weighed to a plastic vial for liquid scintillation counter (Packard Instrument Company). One ml of Soluen 350 (Packard Instrument Company) was added into the vial and incubated at 37 °C for 24 h to solubilize the tissues or plasma. After solubilizing the tissues completely, 10 ml of Dimilume (Scintillator, Packard Instrument Company) was added to the vial and the radioactivity was counted with a liquid scintillation counter (Packard Instrument Company).

The concentrations of [^{3}H]dexamethasone palmitate and [^{3}H]dexamethasone phosphate in some tissues are shown in Table 1. They were markedly different between the two preparations up to 2·5 h. Dexamethasone palmitate given as a lipid emulsion showed a much higher concentration in the blood, spleen (a RES-rich organ) and inflamed tissues than dexamethasone phosphate, which had a high concentration in the muscle, a water-rich tissue. The weights of whole plasma and muscles were regarded as 4 and 45·5% of body weight, respectively.

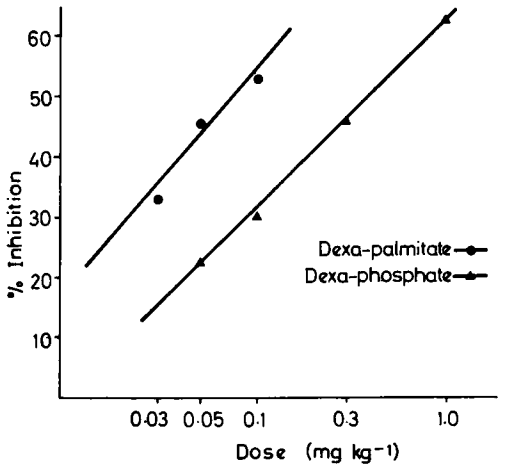

FIG. 1. Dose response curve (as dexamethasone) of inhibitory effects on carrageenan granuloma pouch of dexamethasone palmitate incorporated in a lipid emulsion and dexamethasone disodium phosphate in an aqueous solution. Each point refers to an average value of 10 animals.

Male rats of Wistar strain, 110 to 130 g were injected in the back with 4 ml of 2% solution of carrageenan on day 0. Animals with pouches of uniform size were selected on day 5. Either dexamethasone palmitate incorporated in a lipid emulsion, dexamethasone disodium phosphate in 0·9% NaCl (saline), the lipid emulsion alone, or saline alone was injected intravenously on days 5, 6 and 7. The animals were killed on day 8 and the wet weights of granulomas were measured. The inhibitory effects of the two preparations are shown in Fig. 1. ED50 calculated from the dose response curve shown in Fig. 1 was 0·45 mg kg^{-1} for free dexamethasone phosphate and 0·08 mg kg^{-1} for the dexamethasone palmitate–lipid emulsion. Therefore, the anti-inflammatory activity (ED50) of the latter was 5·6 times as potent as the former. The lipid emulsion alone showed no inhibition.

This study suggests that corticosteroids incorporated in lipid emulsions are taken up by RES and some inflammatory cells much more than free corticosteroids, resulting in a stronger anti-inflammatory activity. Therefore, corticosteroids in lipid emulsion may be of value in the treatment of certain diseases such as rheumatoid arthritis where phagocytic inflammatory cells have an important role in the pathogenesis, and auto-immune haemolytic anaemia and idiopathic thrombocytopenic purpura in which corticosteroids are effective at least partly by blocking RES.

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