Chemical Modification of Triamcinolone Acetonide to Improve Liposomal Encapsulation

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Abstract □ The 21-palmitate of triamcinolone acetonide was synthesized to aid in the liposomal encapsulation of the drug. Encapsulation efficiency of triamcinolone acetonide-21-palmitate was 85%, compared with 5% for the parent drug.

Keyphrases □ Triamcinolone acetonide—synthesis of the 21-palmitate ester, liposomal encapsulation □ Liposomes—encapsulation of triamcinolone acetonide-21-palmitate, synthesis

Liposomes are microscopic vesicles composed of phospholipid bilayers separated by aqueous compartments. Their potential as temporal and spatiotemporal drug delivery systems is being widely investigated (1–3). The applicability of liposomes as selective drug delivery systems for cutaneous administration of drugs has been studied (4, 5) in this laboratory using triamcinolone acetonide as the model drug. Attempts to test various types of liposomes with the same drug revealed a low level of encapsulation of the drug in the multilamellar liposomes.

A relationship between liposomal encapsulation and the structure of the derivatives of another corticosteroid, hydrocortisone, has been reported by Shaw *et al.* (6). The observation that among a variety of derivatives, hydrocortisone-21-palmitate had the maximum encapsulation in the liposomes led us to study a similar approach for enhancing encapsulation of triamcinolone acetonide. The synthesis and purification of triamcinolone acetonide-21-palmitate, based on the principles of the methods reported by Shaw *et al.* (6) and Diamanti and Bianchi (7), is described in this report.

EXPERIMENTAL

9-Fluoro-21-(1-oxohexadecoxy)-11-hydroxy-16,17-[(1-methylethylidene)-bis(oxy)]-pregna-1,4-diene-3,20-dione—Triamcinolone acetonide (435 mg, 1 mmol) was dissolved in 4 mL of *N,N*-dimethylformamide. Pyridine (160 mg, 2 mmol) and palmitoyl chloride (550 mg, 2 mmol) were added, the mixture was stirred magnetically at room temperature for 22 h, and then was poured into 0.5 M sulfuric acid (1600 mL) and stirred vigorously. The product was removed by filtration and was purified by column chromatography on silica gel (toluene–ethyl acetate–acetic acid, 90:10:1). Recrystallization from methanol, gave 471 mg (70% yield) of triamcinolone acetonide-21-palmitate, mp 143–147°C; IR (Nujol): 1760 (ester C=O), 1740 (ketone C=O), 1670 (α,β; $\alpha'\beta'$-unsaturated ketone C=O), 1620 (C=C conjugated with ketone), and 890 cm^{-1} (*cis*-CH of Δ-1,4 system); ^{1}H-NMR ($CDCl_3$): δ 0.88 (t, 3, terminal CH_3), 0.94 (s, 3, 18-CH_3), 1.25 (narrow m, 27, acetonide β-CH_3 and CH_2 chain), 1.42 (s, 3, acetonide α-CH_3), 1.55 (s, 3, 19-CH_3), 1.6–2.5 (m, 4, $CH_2CH_2CO_2$), 4.90 (s, 2, 21-CH_2O), 4.97 (br, 1, 16-CHO), 6.12 (d, 1, $J_{2,4}$ = 0.6 Hz, 4-CH), 6.44 (dd, 1, $J_{2,4}$ = 0.6 Hz, $J_{1,2}$ = 9.5 Hz, 2-CH), and 7.22 ppm (d, 1, $J_{1,2}$ = 9.5 Hz, 1-CH).

Anal.—Calc. for $C_{24}H_{61}FO_7$: C, 71.39; H, 9.44; F, 2.82. Found: C, 71.26; H, 9.25; F, 2.80.

Table I—Comparative Data for Liposomal Encapsulation of Triamcinolone Acetonide and Triamcinolone Acetonide-21-Palmitate.

Procedure	Fraction	Concentration, mg/22 mL: Triamcinolone Acetonide	Concentration, mg/22 mL: Triamcinolone Acetonide-21-Palmitate
Preparation	Crude product	20	20
Filtration	Filtrate	7	19
Centrifugation	Supernatant	6	2
	Pellet (liposomes)	1	17

Preparation of Liposomes—To reliably determine encapsulation efficiency, radioactive triamcinolone acetonide-21-palmitate was prepared as above from [6,7-^{3}H]triamcinolone acetonide. Multilamellar liposomes were prepared as described by Mezei and Gulasekharam (4). Dipalmitoyl phosphatidyl choline (160 mg) and triamcinolone acetonide-21-palmitate (22 mg, 350 μCi) in chloroform–methanol (2:1) were evaporated using a rotary evaporator. The film was then dispersed with 22 mL of aqueous 8 mM $CaCl_2$ solution at 60°C. The preparation was evaluated microscopically and then filtered through a 12-μm polycarbonate filter. The filtrate was then centrifuged at 22,000×*g* for 25 min. Radioactivity in each fraction was measured after each treatment. Triamcinolone acetonide liposomes were prepared in a similar manner.

RESULTS AND DISCUSSION

Results presented in Table I indicate considerable improvement in liposomal encapsulation of triamcinolone acetonide as a result of palmitoylation. In the case of the triamcinolone acetonide liposomal preparation the first major loss occurred after filtration through a 12-μm polycarbonate filter. This filtration step is necessary with the liposomal encapsulation of a lipophilic drug. Since the drug is insoluble or very slightly soluble in the aqueous medium, only that portion is encapsulated that is in solution and intimately associated with the lipid bilayers; the remaining portion is in solid form which, although it is unencapsulated, would be present in the liposomal fraction after centrifugation. The loss of 13 mg (*i.e.*, 65%) of triamcinolone acetonide by the filtration process was due mainly to unencapsulated crystals observed in the crude preparation. Such crystals were absent in the filtrate. No crystals were seen even in the unfiltered triamcinolone acetonide-21-palmitate liposomal fraction; consequently, only 1 mg (*i.e.*, 5%) was lost by the filtration. This suggests that the palmitate form has a stronger association with the lipid layers and that almost complete encapsulation is achieved.

The second major loss in the case of triamcinolone acetonide liposomes was in the supernatant after centrifugation of the filtrate. This is due mainly to the drug in solution but not associated with liposomes. Evidence for this was demonstrated by gel filtration chromatography. Thus, the overall encapsulation of triamcinolone acetonide, determined in the final purified liposomal fraction, was only 5% while that of its palmitate derivative was found to be 85%. The encapsulation of the palmitate derivative could be close to 100%, if one considers the unavoidable loss due to the filtration and centrifugation procedures, *i.e.*, by adsorption to the filter and glassware. Another minor but inherent loss of liposomes could be due to the presence of small (<0.5-μm) liposomes, which are not completely sedimented by centrifugation. However, for comparison purposes, both preparations were analyzed by the same procedures, and consequently, the same considerations should be applied.

A possible reason for increased encapsulation of the palmitate derivative could be the change in the partition coefficient. For lipid-soluble compounds a logarithmic partition coefficient (log P) between 1.7 and 4 is unfavorable for liposomal encapsulation (8). Triamcinolone acetonide has a log P of 2.53 (9). Palmitoylation would increase this to ~11 as calculated using substituent constants (9).

The mechanism for increased encapsulation could be predicted to be analogous to that of hydrocortisone-21-palmitate (10). The palmitoyl chain may act as "hydrophobic anchor" holding the steroid head group on the surface of the lipid bilayer.

One of the drawbacks in liposomal drug delivery systems is the poor encapsulation of the drug in the liposomes. Chemical modification is likely to be a powerful approach to overcome such an obstacle, as evidenced here and elsewhere (6). Although only a small amount of the drug was associated with the liposomes, the drug disposition was altered favorably on dermal application of a triamcinolone acetonide liposomal preparation (4, 5). In light of this, the potential usefulness of this highly concentrated liposomal triamcinolone

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acetonide-21-palmitate preparation as a selective drug delivery system for cutaneous administration may be optimal.

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Quantitation of Amphotericins by Reverse-Phase High-Performance Liquid Chromatography

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Abstract □ A reverse-phase high-performance liquid chromatographic (HPLC) method was developed for simultaneous potency determination of amphotericin A and amphotericin B in bulk amphotericin B preparations. This single, rapid, specific, and simple method can be used for qualitative and quantitative analyses and is proposed to replace a combination of cumbersome official tests presently in use. This new HPLC method employs an isocratic acidic phosphate buffer–methanol mixture in a reverse mode on an octadecylsilane-bonded silica column at ambient temperature, using UV detection at 313 nm. Results obtained by HPLC compare favorably with the official assay results. During these studies, an apparent heptaenic component, not previously described, was detected in commercial amphotericin B preparations at concentrations ranging from ~6 to 14%.

Keyphrases □ Quantitation—amphotericin B, amphotericin A, reverse-phase HPLC □ Amphotericin B—quantitation with amphotericin A, reverse-phase HPLC

Amphotericin B (1) (CAS registry 1397-89-3), produced by a strain of *Streptomycetes nodusus*, is an amphoteric macrocyclic polyene antibiotic that has become a valuable therapeutic agent in the treatment of fungal and monilial infections in humans (1). Polyene antibiotics are characterized chemically by strong absorption in the UV and visible regions of the spectrum, ascribed mainly to conjugated unsaturation with four (tetraenes) to seven (heptaenes) double bonds (1, 2). These substances are relatively unstable, and the heptaenes, in particular, are insoluble in most solvents.

Bulk amphotericin B is commercially available in two forms—one is purified for use in parenteral products (type I) and the other is a cruder grade for topical applications (type II). According to the official monograph (3), the potency of each form must be ≥750 μg/mg on the anhydrous basis. Both types may also contain amphotericin A, a cofermented tetraene very similar to nystatin; however, type I may contain ≤5% amphotericin A, whereas type II may contain ≤15% amphotericin A. Characteristics of amphotericin B are extensively discussed and reviewed with respect to physicochemical and analytical properties by Asher *et al.* (1) and Thomas (2).

Official requirements for the premarketing certification of amphotericin B (3) include a microbiological agar diffusion assay, a quantitative differential UV spectrophotometric procedure for the determination of the amphotericin A content, and a qualitative UV spectrophotometric identity test for amphotericin B. The microbiological assay measures the total activity of substances responding to a particular organism in comparison with an established standard of defined activity. Selectivity, precision, and reliability of this microbiological assay are inadequate, especially because of the deleterious effect of the high pH (4, 5) needed to solubilize the substance during this assay.

Reverse-phase high-performance liquid chromatography (HPLC) was demonstrated by Mechlinski and Schaffner (6) to be well suited to the qualitative analysis of such intractable