J. Pharm. Pharmacol. 1995, 47: 812–817
Received December 1, 1994
Accepted February 15, 1995

Liposome-incorporated Dexamethasone Palmitate Inhibits In-vitro Lymphocyte Response to Mitogen

H. BENAMEUR, N. LATOUR, L. SCHANDENE*, J. P. VAN VOOREN†, B. FLAMION AND F. J. LEGROS

*Laboratory of Pathophysiology, Medical School, Free University of Brussels, Campus Erasme Bldg E, 808, Route de Lennik, 1070 Brussels, *Laboratory of Immunology Erasme Hospital, Brussels, and †Chest Department, Erasme Hospital Brussels, Belgium*

Abstract

The use of liposomes for the pulmonary delivery of corticosteroid is an area that is under active investigation. We have recently developed a novel liposomal corticosteroid preparation based on the incorporation of dexamethasone palmitate (DMP) within the bilayer of small unilamellar vesicles (SUVs) made of egg yolk phosphatidylcholine (EPC) and cholesterol; molar ratio EPCC : cholesterol : DMP, 4 : 3 : 0·3.

In the present study, the biological activity of DMP-SUVs was evaluated using the lymphocyte transformation test with peripheral blood mononuclear cells (PBMCs) and a γ-interferon production assay. Results showed that DMP-SUVs (but not empty SUVs) inhibited [^{3}H]thymidine uptake and γ-interferon production by concanavalin A-stimulated PBMCs by 94 and 96%, respectively, at a concentration corresponding to 10^{-6} M dexamethasone. The inhibition by DMP-SUVs was found to require a 24-h pre-incubation with unstimulated PBMCs, suggesting that interaction of SUVs with lymphocytes may be altered by mitogen stimulation.

We conclude that our DMP liposomal preparation is biologically active and may be considered a promising alternative to conventional local glucocorticoid therapy.

Attempts to control the pharmacokinetics and disposition of drugs have involved a variety of drug delivery systems. Liposomes (phospholipid vesicles) in particular, when used as carriers for drugs, can be delivered to a designated body cavity where they may release their content in a sustained or controlled way, while reducing drug toxicity and side-effects (Gregoriadis 1976; Szoka & Paphadjopoulos 1980; Mayer et al 1986). Among the various categories of drugs that should benefit from liposomal entrapment are immunosuppressive anti-inflammatory agents, and especially glucocorticoids, because even when used locally, these potent effectors may produce serious systemic side-effects. Although some successful endeavours to incorporate corticosteroids into phospholipid preparations have been reported, the vast therapeutic potential for liposome-incorporated corticosteroids has been hampered by difficulties in controlling the efficiency of drug incorporation and retention (Shaw et al 1976). For instance, unesterified cortisol escapes readily from liposomes (Shaw et al 1976; Cleland et al 1982), while more lipid-soluble esters of cortisol are retained more efficiently (Shaw et al 1976; Fildes & Oliver 1978; Phillips et al 1979) and may be clinically useful (Dingle et al 1978; De Silva et al 1979; Cleland et al 1982).

Dexamethasone, the most powerful glucocorticoid, has found application either as a lipid emulsion (Mizushima et al 1982), as a multilamellar vesicle preparation for the local treatment of articular inflammation in rabbits (Bonanomi et al 1987), or as an aerosol for delivery to the lungs (Tremblay et al 1993). The latter route is particularly interesting, because inflammatory diseases involving the respiratory tract (asthma, chronic obstructive pulmonary disease, hypersensitivity pneumonitis) are primary targets for local corticosteroid treatment, and liposomes may improve the efficiency of conventional aerosol delivery of drugs by promoting pulmonary drug retention (Schreier et al 1993). Liposomes may also direct their content to alveolar macrophages, a specific advantage for corticoid therapy (Fidler et al 1989). However, liposomal dexamethasone preparations for aerosolized delivery should be designed carefully. It is notable that in one recent study using native dexamethasone entrapped in small unilamellar vesicles (SUVs), the levels of drug incorporation varied from 57 to 75% (Tremblay et al 1993). Based on these premises we have reinvestigated the incorporation of dexamethasone in liposomes that could eventually be delivered to the lungs.

According to a recent study, retention of a fluorescent marker during aerosolization is highest for lipid vesicles containing ~30 mol% cholesterol and measuring ~0·22 μm (Schreier et al 1993). However, we recently observed (Benameur et al 1993) that the incorporation of dexamethasone, into cholesterol-containing liposomes resulted in very low entrapment (< 2%); on the other hand, esterified dexamethasone, and in particular dexamethasone-21-palmitate (DMP) was successfully encapsulated (70%). Other corticosteroid esters have also been incorporated with high efficiency (Shaw et al 1976; Cleland et al 1982; Bonanomi et al 1987; Fidler et al 1989). In our previous study we demonstrated by theoretical conformational analysis and experimental measurements that dexamethasone inserts into the bilayer of SUVs with a high efficiency. However, before proceeding to in-vivo trials, more detailed

Correspondence: H. Benameur, Laboratory of Pharmacy and Biopharmacy, Campus de La Plaine, Free University of Brussels C.P. 207, Boulevard du Triomphe, 1050 Brussels, Belgium.

information on the pharmacology of DMP-containing SUVs (DMP-SUVs) is required.

The first step in this direction is to probe the biological effects of DMP-SUVs using a standard in-vitro test. The lymphocyte transformation test was selected on account of its broad range of applications and its reliability for assessing the immunoregulatory actions of glucocorticoids (Heilmann et al 1973; Fauci & Dale 1975). Furthermore, stimulated lymphocytes secrete cytokines such as interleukins and interferons, the production of which is inhibited by glucocorticoids (Cupps & Fauci 1982). Therefore, the effects of dexamethasone and DMP-SUVs on stimulated γ-interferon production were also measured.

Materials and Methods

Materials

Egg yolk phosphatidylcholine (EPC) was obtained from Lipoid, Germany (EPC Type I). Cholesterol and dexamethasone (9α-fluoro-16α-methylprednisolone) were purchased from Sigma (St Louis, MO, USA). All these materials were stored at −20°C. Dexamethasone-21-palmitate was kindly provided by Roussel Uclaf (Romainville, France). [6-^{3}H]-Thymidine (27 Ci mmol^{-1}) was from Amersham International (Amersham, UK) and concanavalin A was from Boehringer Mannheim, Belgium.

Preparation of liposomes

Liposomes were prepared as described previously (Benameur et al 1993). Briefly, 14·40 mg EPC and 5·60 mg cholesterol were mixed with 1 mg DMP in 1 mL chloroform. The thin-film method was used. Solvent was evaporated under N_2 and the lipid film was hydrated with 1 mL phosphate-buffered saline (300 mOsm (kg H_2O)$^{-1}$) at pH 7·4. The multilamellar vesicles (MLVs) obtained were sonicated with a sonicator probe in an ice-bath under N_2 (60 W, 20 min, Soniprep 150) to form SUVs, which were purified by minicolumn centrifugation (Sephadex G50 medium). The DMP-SUVs at 4:3:0·3 molar ratio (EPC : cholesterol : DMP) were then diluted to reach a working concentration of $\sim 10^{-5}$ M of the drug. The actual concentration varied from 0·8 to 1·2 × 10^{-5} M due to small variations in SUV recovery from the Sephadex column. The size distribution of vesicles was determined by dynamic laser-light scattering using a Malvern Zetasizer 3. The average size of SUVs and DMP-SUVs ranged between 50 and 80 nm. All liposomes (DMP-SUVs and SUVs) and free dexamethasone were sterilized by filtering through a 0·22 μm filter (Millex GH Millipore). As shown previously (Benameur et al 1993), no adsorption of radiolabelled liposomes or [^{3}H]DMP on the filter was observed. When incubated at 37°C in plasma or PBS, DMP-SUVs were stable for at least 7 days and no hydrolysis of DMP occurred, based on the absence of radiolabelled DMP in the supernatant.

Turbidity measurements

The turbidity of liposome dispersion is affected by vesicle aggregation (Ohsawa et al 1985). To verify that concanavalin A did not induce liposome precipitation or agglutination, we measured the turbidity of sonicated dispersions of SUVs and DMP-SUVs at a phospholipid concentration of 15 μM (EPC:cholesterol : DMP; 4 : 3 : 0·3), according to the method of Defrise-Quertain et al (1984). At the end of sonication, the samples were brought to 37°C and their absorbance was monitored at 550 nm against a buffer blank, using a Shimadzu spectrophotometer and 1-cm cells, before and after addition of concanavalin A or protamine.

Cell populations

Peripheral blood mononuclear cells (PBMCs) were isolated from heparinized blood of healthy volunteers. Different volunteers were used for preliminary experiments (n = 1), dose-response relationship (n = 2), main experiments with lymphocyte transformation testing (n = 6), and γ-interferon production (n = 3). The blood was diluted 1 : 1 with Hank's balanced salt solution, layered on Ficoll-Paque (sp. gr. 1·077; Pharmacia Fine Chemicals, Uppsala, Sweden) and centrifuged at 400 *g* for 30 min. The cell-containing interface was collected, washed three times in Hank's buffer and resuspended in RPMI 1640 medium (Gibco Laboratories) supplemented with 2 mM L-glutamine, 100 int. units mL^{-1} penicillin, 100 μg mL^{-1} streptomycine sulphate, 5 × 10^{-5} M β-mercaptoethanol and 10% heat-inactivated foetal-calf serum. Cells were counted and their viability was assessed by counting the percentage of cells which excluded trypan blue.

Lymphocyte transformation test

PBMCs were cultured in flat-bottomed 96-well plates (Nunc) at 37°C in humidified 5% CO_2. Each well received 200 μL cell suspension (final volume) including 20 μL test solution (either DMP-SUVs, empty SUVs, free dexamethasone, or blank control). The number of replicates was at least 5 for each condition. In some experiments, the test solutions were added 24 h after PBMCs (final volume was still 200 μL). The biological activity of DMP-SUVs could not be compared with that of DMP itself, since the characteristics of DMP (octanol : water coefficient measured at pH 7·4, log P = 3·6) prevented its dissolution in RPMI or phosphate-buffered saline. Unstable suspensions were obtained, with DMP forming a precipitate within a few minutes in spite of sonication. Therefore, a solution of free dexamethasone was used as a positive control. The maximal final concentration of free dexamethasone, which was used in most experiments, was 10^{-6} M, and the concentration of DMP-SUVs from stock solutions was adjusted to reach an equivalent molarity of 10^{-6} M. This corresponds to 2 μg liposome mL^{-1} (15 μM EPC). The same concentration was used for empty SUVs.

Twenty-four hours after starting cell culture, concanavalin A was added to each well at a final concentration of 10 μg mL^{-1}. In some experiments, as mentioned above, SUVs were also added at this instant. Lymphocyte proliferation was assessed later by [^{3}H]thymidine incorporation. The conditions for maximal [^{3}H]thymidine incorporation were determined in preliminary experiments. Both the number of cells mL^{-1} (from 10^4 to 10^6) and the time and duration of [^{3}H]thymidine addition were varied; the total period of PBMCs incubation ranged from 1 to 4 days. The best results were obtained with 5 × 10^5 cells mL^{-1}, a total cell incubation time of 90 h, and [^{3}H]thymidine addition 48 h after concanavalin A stimulation (i.e. 72 h after starting cell

culture). [^{3}H]Thymidine was thus present for 18 h. These conditions were used throughout all experiments reported in this paper.

At the end of the 90-h incubation period, the cells were collected onto a glass fibre filter paper disk and washed three times with deionized water with a semiautomated Microcell harvester (Nunc). After drying overnight, filter disks were counted for β-emission in a liquid scintillation counter (Pharmacia LKB) with 10 mL Packard liquid scintillation fluid. The proliferative response of PBMCs was calculated following the method of Burford-Mason & Gyte (1979), which proposes using the molar uptake of the selected base [^{3}H]thymidine by the lymphocytes as an appropriate method of expressing lymphocyte transformation rather than either crude counts min^{-1} or a stimulated index (stimulated value/unstimulated value).

Quantitative determination of γ-interferon

PBMCs were cultured in tubes in supplemented RPMI medium at a concentration of 10^6 cells mL^{-1}. Quintuplet tubes of the following four conditions were incubated for a total period of 48 h: cells without concanavalin A; cells with 10 μg mL^{-1} concanavalin A (control); cells with 10^{-6} M free dexamethasone and 10 μg mL^{-1} concanavalin A; cells with 10^{-6} M DMP-SUVs (calculated as the equivalent molarity of DMP) and 10 μg mL^{-1} concanavalin A. The mitogen was present during the last 24 h. At the end of the incubation period the tubes were spun at 400 *g* for 10 min. The supernatants were collected and stored at −20°C until use. The levels of γ-interferon in the supernatants were assayed by an ELISA kit (Eurogenetics, Belgium). The amount of γ-interferon (units mL^{-1}) for each condition was determined from the average absorbance of all replicates and interpolated from a standard curve. A total of three experiments was performed.

Statistical analysis

Data are expressed as mean ± s.e.m. Statistical differences between means were assessed by Student's *t*-test; the level of significance was set at $P = 0.05$.

Results

Preliminary experiments

The aim of the present study was to probe the biological activity of SUV-incorporated DMP, based on inhibition of lymphocyte proliferation. After determining the optimal conditions for [^{3}H]thymidine incorporation by concanavalin-stimulated PBMCs, we discovered that the time of liposome addition with respect to concanavalin A stimulation was critical. To demonstrate the antiproliferative effect of DMP-SUVs it was necessary to pre-incubate the PBMCs with drug-loaded liposomes for 24 h before adding the mitogen (Table 1). Because liposomes had to be added before concanavalin A to be effective, we reasoned that concanavalin A could induce a rapid aggregation of SUVs preventing them from interacting with PBMCs. However, this hypothesis was disproved by turbidity measurements using protamine, a recognized inducer of liposome aggregation (Fig. 1). Moreover, the size distribution of DMP-SUVs was not altered by concanavalin A addition (mean size: 49·4 ± 11·3 without vs 59·8 ± 11·6 with concanavalin A). In all subsequent experiments, DMP-SUVs or free dexamethasone was added to PBMCs 24 h before concanavalin A addition. The effects of varying this period of preincubation were not explored further.

Table 1. [^{3}H]Thymidine uptake of PBMCs after stimulation by concanavalin A either simultaneously with (t = 0) or 24 h after (t = 24) addition of either no additive (control), empty SUVs, 10^{-6} M DMP-SUVs, or 10^{-6} M free dexamethasone.

	[^{3}H]Thymidine uptake (pmol)	
	Stimulation t_0	Stimulation t_{24}
Control	181·6 ± 26·6	234·4 ± 20·0
SUVs	154·3 ± 31·3	204·0 ± 30·3
DMP-SUVs	137·9 ± 36·3	36·3 ± 4·7
Dexamethasone	34·7 ± 15·8	14·3 ± 1·9

Mean ± s.e.m. values are given of five independent measurements.

In all these experiments, DMP-SUVs were added at high concentration, corresponding to an equivalent molarity of 10^{-6} M dexamethasone (see below) and to a lipid concentration of 15 μM. Empty SUVs (15 μM total lipid concentration) had no effect on lymphocyte activation, even when preincubated for 24 h (Table 1). Under all conditions tested, cell viability remained > 90% even after 3-days incubation with SUVs or DMP-SUVs.

Dose–response relationship

Fig. 2 displays the relative inhibition of PBMC proliferation induced by various concentrations of either free dexamethasone or DMP-SUVs. At 10^{-7} and 10^{-6} M, as well as at 10^{-10} M, both formulations had identical effects, whereas DMP-SUVs were slightly less efficient than free dexamethasone at intermediate concentrations ($P = 0.03$ at 10^{-8} M; $P = 0.002$ at 10^{-9} M). To ensure maximal efficacy, DMP-SUVs were used at 10^{-6} M in the following experiments.

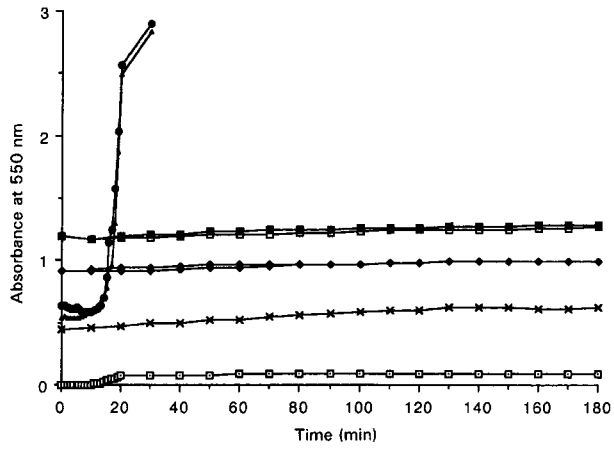

FIG. 1. Turbidity measurement of SUVs and DMP-SUVs incubated in phosphate-buffered saline at 37°C with or without addition of concanavalin A (10 μg mL^{-1}) or protamine (0·1 mg mL^{-1}) at time 0. A rise in absorbance corresponds to liposome aggregation or precipitation (Defriese-Quertain et al (1984)). Protamine, but not concanavalin A, increased absorbance at 550 nm (values remained above 3 for 180 min). × PBS + concanavalin A; ⊡ PBS + protamine; ◇ SUV; □ DMP-SUV; ◆ SUV + concanavalin A; ■ DMP-SUV + concanavalin A; ▲ SUV + protamine; ● DMP-SUV + protamine.

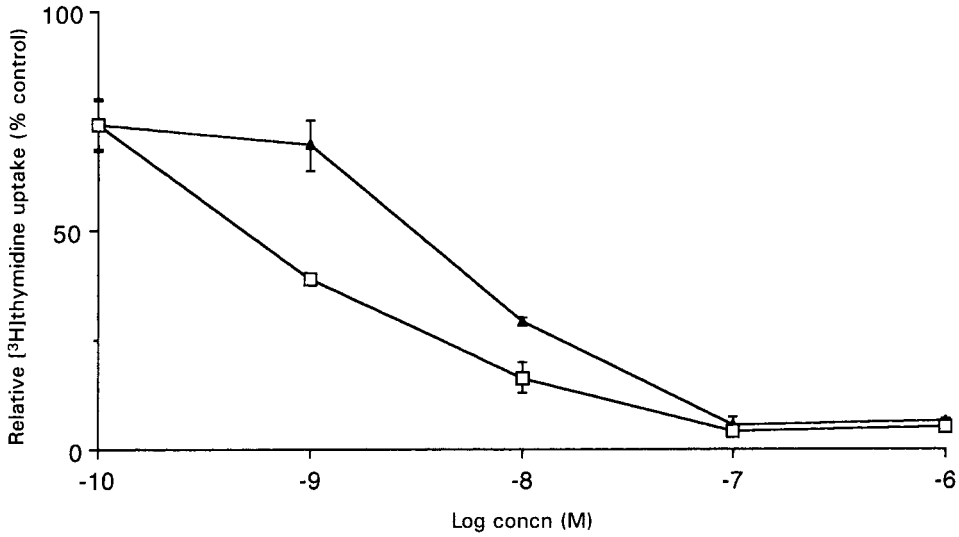

FIG. 2. Effects of dexamethasone and DMP-SUVs at different concentrations on concanavalin A-stimulated [^{3}H]thymidine uptake by PBMCs, expressed as a percentage of control values. Each point is a mean ± s.e.m. of five independent measurements. ▲ DMP-SUVs; □ dexamethasone.

Lymphocyte proliferation

The effects of dexamethasone and DMP-SUVs on the in-vitro proliferation of PBMCs obtained from six volunteers were tested under the conditions previously defined. The results are displayed in Tables 2, 3. Although the levels of lymphocyte proliferation varied considerably among donors, they were always greatly reduced in the presence of either dexamethasone or DMP-SUVs. There was no statistically significant difference between the antiproliferative effects of 10^{-6} M free dexamethasone and those of DMP-SUVs at the same drug concentration (percent inhibition; 94 vs 94: Table 3). Similar results were obtained with PBMCs stimulated by an antigen (*Candida albicans*; data not shown).

γ-Interferon production

Table 4 shows the effects of dexamethasone and DMP-SUVs on concanavalin A-stimulated γ-interferon production. The addition of either 10^{-6} M native dexamethasone or DMP-SUVs at the same dexamethasone concentration completely abrogated γ-interferon production. The percent inhibition was identical for native dexamethasone and liposomal DMP (Table 4).

Discussion

In the present study we have demonstrated that dexamethasone inserted as a palmitic ester into the bilayer of SUVs retained its biological activity as measured by two standard

Table 2. [^{3}H]Thymidine uptake of concanavalin A-stimulated PBMCs after addition of either no additive (control), 10^{-6} M DMP-SUVs or 10^{-6} M dexamethasone.

	[^{3}H]Thymidine uptake (pmol)					
	A	B	C	D	E	F
Control	363 ± 27	690 ± 68	457 ± 101	1033 ± 151	585 ± 53	674 ± 52
DMP-SUVs	59 ± 7	168 ± 37	22 ± 4	34 ± 6	48 ± 5	21 ± 3
Dexamethasone	12 ± 2	46 ± 4	49 ± 21	46 ± 2	42 ± 2	35 ± 3

Mean ± s.e.m. values are given of five independent measurements. Letters A to F represent individual healthy donors.

Table 3. Inhibition by dexamethasone and DMP-SUVs of concanavalin A-stimulated lymphocyte proliferation.

	Control	Dexamethasone	DMP-SUVs
[^{3}H]Thymidine uptake (pmol)	633·81 ± 94·2	38·29 ± 5·56	36·08 ± 6·68
Inhibition (%)		94	94

All values in the table are means ± s.e.m. of six experiments.

Table 4. Inhibition by dexamethasone and DMP-SUVs of concanavalin A-stimulated γ-interferon production.

	Control	Dexamethasone	DMP-SUVs	Unstimulated cells
Absolute values (units mL^{-1})	1·67 ± 0·53	0·07 ± 0·05	0·06 ± 0·02	0·03 ± 0·01
Inhibition (%)		96	96	

All values in the table are means ± s.e.m. of three experiments.

in-vitro tests. As both the lymphocyte transformation and γ-interferon assays are complex tests which explore multiple variables related to immunoproliferation, it should be stressed that our study was not intended to probe the cellular mechanisms of action of DMP-SUVs on lymphocytes, but simply to provide evidence that dexamethasone is presented to the cells in a biologically active form. This confirmation step is crucial before proceeding to in-vivo trials of this promising formulation of a powerful immunosuppressive and anti-inflammatory agent. However, several points encountered while examining this particular form of in-vitro liposome-cell interaction deserve comment.

First, we discovered that a 24-h pre-incubation of PBMCs with liposomes was required to demonstrate the antiproliferative effects of DMP-SUVs. Conversely, if DMP-SUVs and concanavalin A were added together to the PBMC suspension, the thymidine uptake was not significantly inhibited compared with no SUV or dexamethasone-free SUV additions. A deficient methodology can be excluded, since free dexamethasone added concomitantly with concanavalin A was a very efficient inhibitor of lymphocyte proliferation under the same conditions. It follows that the effects of DMP-SUVs on PBMCs are not mediated by free dexamethasone which might have been released from liposomes. Such a mechanism would be highly improbable anyway, because the dexamethasone molecule was incorporated in the lipid bilayer as a poorly water-soluble ester and remained therein for at least 7 days when DMP-SUVs were incubated in human plasma. Therefore, the lack of inhibition with simultaneous DMP-SUV and concanavalin A additions suggests two possible explanations. The first is that concanavalin A prevented liposomes from interacting with PBMCs by inducing rapid aggregation or precipitation of the lipid vesicles. However, turbidity and size measurements were not compatible with this hypothesis, although we cannot rule out the remote possibility that concanavalin A directly interfered with SUV-cell contact, e.g. by competing for the same binding sites. The second, and more likely, explanation is that concanavalin A-stimulated PBMCs quickly became unresponsive to the effect of DMP-SUVs and remained so for the next few days despite continuous presence of the liposomes. In other words, it is likely that some kind of direct physical interaction between SUVs and lymphocytes occurred and that this interaction was modified, once blastogenesis had been initiated. This suggestion is supported by earlier observations that while phosphatidylcholine liposomes containing various amounts of cholesterol are able to modify the cholesterol content of lymphocyte membranes and hence to inhibit or enhance lymphocyte proliferation, they can only do so efficiently when added before mitogenic stimulation (Alderson & Green 1975; Chen & Keenan 1977; Ip et al 1980). Presumably, the early steps of lymphocyte activation involve such extensive changes in membrane composition (Anel et al 1990) that liposome-lymphocyte interaction is greatly reduced or that DMP is no longer delivered to the host cell. In a similar regard, it should be noted that the SUVs used in the present study (molar ratio of EPC : cholesterol 4 : 3) should not alter the composition of the lymphocyte membrane significantly; indeed, a high concentration of dexamethasone-free SUVs had no effect whatsoever on lymphocyte activation. Further studies will be required to resolve how mitogenic stimulation modulates liposome–lymphocyte interaction.

Another relevant point is the range of concentrations over which DMP-SUVs were shown to be effective. Although an SUV concentration corresponding to 10^{-6} M dexamethasone, which is supramaximal with respect to lymphocyte inhibition, was selected to demonstrate the biological activity of liposomal DMP unequivocally, the dose–response relationship established in two different experiments suggests that DMP-SUVs inhibited lymphocyte proliferation by ~25% at a calculated dexamethasone concentration as low as 10^{-10} M and by 70% at 10^{-8} M (see Fig. 2). Yet, based on dexamethasone availability, the liposomal formulation should be much less efficient than a similar dose of free dexamethasone because dexamethasone was esterified and incorporated in liposomes which were presented to the cells as a dispersion, and previous studies of liposome–lymphocyte interaction have concurred with the finding that the maximum uptake of untargeted liposomes by either human PBMCs (Ozato et al 1978; Weinstein et al 1978) or murine lymphocytes (Huang et al 1978) was less than 0·1% of the total number of SUVs added, even after prolonged periods. We have preliminary evidence confirming such low levels of association under our present conditions (data not shown). Therefore, the effect of DMP-SUVs would be expected to correspond to that of free dexamethasone at a concentration one-hundredth less. Although our limited number of dose-response experiments does not allow us to compare the efficiency of DMP-SUVs and free dexamethasone with confidence over such a wide range of concentrations, we nevertheless favour the idea, based on the high sensitivity of PBMCs to liposomal DMP, that DMP-SUVs provide PBMCs with the active agent moiety (either dexamethasone or the ester) in a way that improves the efficiency of the glucocorticoid, at least as measured by standard lymphocyte proliferation assays.

How this could happen is a fascinating issue. If confirmed, this observation will require careful investigation of the cellular processing of DMP-SUVs. The main mechanism may involve fusion of DMP-SUVs with lymphocytes, or adsorption of SUVs followed by DMP transfer to the lymphocyte membrane. Once inside the cell, DMP may need to be subjected to esterase action, or the esterified moiety may directly activate glucocorticoid receptors. The

DMP-SUVs that have become cell-associated represent a kind of reservoir for slow release of active compound into the cells. Although this liposome–lymphocyte interaction may have some relevance to the expected in-vivo activities of DMP-SUVs, their suggested use as aerosols for the treatment of inflammatory lung diseases should shift the primary target of investigations towards the interaction between DMP-SUVs and alveolar macrophage.

To conclude, we have demonstrated, using in-vitro bioassays, that our liposomal DMP formulation is biologically active and thus may be considered a promising alternative to conventional glucocorticoid therapy in-vivo, especially if the DMP-SUV potential for reduced toxicity, enhanced activity and perhaps prolonged time-course can be confirmed. Furthermore, the formulation of this SUV preparation makes it well suited for aerosol delivery to the lungs (Schreier et al 1993).

Acknowledgement

We thank Dr K. Huygen, Institut Pasteur Brussels, for the demonstration of a technique for lymphocyte culture. We are obliged to Professor H. Schreier, University of Gainesville, Florida, and Dr M. Friede, SmithKline Beecham, Belgium, for their critical comments.

References

Alderson, J. C. E., Green, C. (1975) Enrichment of lymphocytes with cholesterol and its effect on lymphocyte activation. FEBS Lett. 52: 208–211

Anel, R., Naval, J., Gonzelez, B., Torres, J. M., Mishal, Z., Uriel, J., Pineiro, A. (1990) Fatty acid metabolism in human lymphocytes. I. Time-course of changes in fatty acid composition and membrane fluidity during blastic transformation of peripheral blood lymphocytes. Biochim. Biophys. Acta 1044: 323–331

Benameur, H., De Gand, G., Brasseur, R., Van Vooren, J. P., Legros, F. J. (1993) Liposome incorporated dexamethasone palmitate: chemical and physical properties. Int. J. Pharm. 89: 157–167

Bonanomi, M. H., Vevart, M., Stimpel, M., Roos, K. M., Fehr, K., Weder, H. G. (1987) Studies of pharmacokinetics and therapeutic effects of glucocorticoids entrapped in liposomes after intra-articular application in healthy rabbits and in rabbits with antigen-induced arthritis. Rheumatol. Int. 7: 203–212

Burford-Mason, A. P., Gyte, G. M. L. (1979) An alternative method of expressing results of lymphocyte transformation experiments. J. Immunol. Methods 28: 391–394

Chen, S. S.-H., Keenan, R. M. (1977) Effect of phosphatidylcholine liposomes on the mitogen-stimulated lymphocyte activation. Biochem. Biophys. Res. Commun. 79: 852–858

Cleland, L. G., Vermont Roberts, B., Garrett, R., Allen, T. M. (1982) Cortisol palmitate liposomes: enhanced anti-inflammatory effect in rats compared with free cortisol. Agents Action 12: 348–352

Cupps, T. R., Fauci, A. S. (1982) Corticosteroid-mediated immunoregulation in man. Immunol. Rev. 65: 133–155

Defrise-Quertain, F., Chatelain, P., Delruelle, M., Ruysschaert, J. M. (1984) Model studies for drugs entrapment and liposome stability. In: Gregoriadis, G. (ed.) Liposome Technology Volume III, CRC Press Inc., pp 1–16

De Silva, M., Hozleman, B. L., Thomas, D. P., Wraight, P. (1979) Liposomes in arthritis: a new approach. Lancet 8130: 1320–1322

Dingle, J. T., Gordon, J. L., Hozleman, B. L., Knight, C. G., Page Thomas, D. P., Phillips, N. C., Shaw, I. H., Fildes, F. J. T., Oliver, J. E., Jones, G., Turner, C. H., Lowe, J. S. (1978) Novel treatment for joint inflammation. Nature 271: 372–373

Fauci, A. S., Dale, D. C. (1975) The effect of hydrocortisone on the kinetics of normal human lymphocytes. Blood 46: 235–243

Fidler, I. J., Raz, A., Fogler, W. I., Kirsh, R., Bugelski, K., Poste, G. (1989) Design of liposomes to improve delivery of macrophage-augmenting agents to alveolar macrophages. Cancer Res. 49: 3976–3984

Fildes, F. J. T., Oliver, J. E. (1978) Interaction of cortisol-21-palmitate with liposomes examined by differential scanning calorimetry. J. Pharm. Pharmacol. 30: 337–342

Gregoriadis, G. (1976) The carrier potential of liposomes in biology and medicine. New Engl. J. Med. 295: 704–710

Heilmann, D. H., Gambrill, M., Leichner, J. P. (1973) The effect of hydrocortisone on the incorporation of tritiated thymidine by human blood lymphocytes cultured with phytohaemagglutinin and pokeweed mitogen. Clin. Exp. Immunol. 15: 203–212

Huang, L., Ozato, K., Pagano, R. E. (1978) Interactions of phospholipid vesicles with murine lymphocytes. I. Vesicle-cell interactions. J. Membr. Biochem. 1: 27–42

Ip, S. H. C., Abraham, J., Cooper, R. A. (1980) Enhancement of blastogenesis in cholesterol-enriched lymphocytes. J. Immunol. 124: 87–93

Mayer, L. D., Bally, M. B., Hope, K. J., Cullis, P. R. (1986) Techniques for encapsulation of bioactive agents into liposomes. Chem. Phys. Lipids 40: 333–345

Mizushima, Y., Hamano, T., Yokoyama, K. (1982) Tissue distribution and anti-inflammatory activity of corticosteroids incorporated in lipid emulsion. Ann. Rheum. Dis. 41: 263–267

Ohsawa, T., Miura, H., Harada, K. (1985) Improvement of encapsulation efficiency of water-soluble drugs in liposomes formed by freeze-thawing method. Chem. Pharm. Bull. 33: 3945–3952

Ozato, K., Kirk Ziegler, H., Henney, C. (1978) I. Transfer of antigenic determinants to lymphocyte membranes after interactions with hapten-bearing liposomes. J. Immunol. 121: 1376–1382

Phillips, N. C., Thomas, D. P. P., Knight, C. G., Dingle, J. T. (1979) Liposome-incorporated corticosteroids. II. Therapeutic activity in experimental arthritis. Ann. Rheum. Dis. 38: 553–557

Schreier, H., Gonzalez-Rothi, R. J., Stecenko, A. A. (1993) Pulmonary delivery of liposomes. J. Contr. Rel. 24: 209–223

Shaw, I. H., Knight, C. G., Dingle, J. T. (1976) Liposomal retention of a modified anti-inflammatory steroid. Biochem. J. 158: 473–476

Szoka, F., Papahadjopoulos, D. (1980) Comparative properties and methods of preparation of lipid vesicles (liposomes). Ann. Rev. Biophys. Bioeng. 9: 467–508

Tremblay, G. M., Therien, H. M., Rocheleau, H., Cormier, Y. (1993) Liposomal dexamethasone effectiveness in the treatment of hypersensitivity pneumonitis in mice. Eur. J. Clin. Invest. 23: 656–661

Weinstein, J. N., Blumenthal, R., Sharrow, S. O., Henkart, P. A. (1978) Antibody-mediated targeting of liposomes binding to lymphocytes does not ensure incorporation of vesicle contents into the cells. Biochim. Biophys. Acta 509: 272–288