## Formulation of topical liposomes encapsulated with triamcinolone and comparison of their anti-inflammatory effects with available conventional topical ointment in mice

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## Abstract

#### Objective

Triamcinolone is a steroidal anti-inflammatory drug widely used in the treatment of skin diseases. Liposomes are microscopic vesicles that composed of lipid bilayers enclosing aqueous compartments. In this study, different liposomal formulations encapsulated with triamcinolone were prepared and their anti-inflammatory effects were compared with a conventional topical ointment.

#### Materials and Methods

In this study liposomes containing 0.1% triamcinolone were prepared by the fusion method using lecithin, cholesterol and penetration enhancers. Encapsulation efficiency was determined by UV spectrophotometry. Liposome size was examined by optical microscopy. The anti-inflammatory effect of liposomal formulations was evaluated by "xylene-induced ear edema" method in mice and then results were compared with a conventional topical ointment.

#### Results

Among different formulations only two formulations were stable and suitable regards to encapsulation efficiency, size, and the lack of triamcinolone precipitation. The first formulation did not have penetration enhancer and the second one contained a penetration enhancer. Liposome size was varied from 2 to 5 micron, and encapsulation efficiency in the first and second formulation was  $80.33 \pm 3.51\%$  and  $90.50 \pm 2.78\%$ , respectively. In vivo study showed that both conventional ointment and liposomal triamcinolone decrease ear edema compared to the control liposome (p<0.01). The percent of edema inhibition (Mean  $\pm$  SEM) in comparison with control was  $44\% \pm 6.0$ ,  $71\% \pm 6.4$  and  $78\% \pm 5.4$  for conventional ointment, first and second liposomal formulations respectively. The anti-inflammatory of liposomal formulations were significantly more than conventional triamcinolone ointment (p<0.05).

#### Conclusion

Results show that liposomal triamcinolone have more anti-inflammatory effect than conventional triamcinolone ointment. Thus, to provide the same effect as conventional triamcinolone ointment, the lower concentration of triamcinolone in liposome formulation is needed, this in turn will cause less side effects.

Keywords: Anti-inflammatory effect, Liposome, Ointment, Triamcinolone, Topical delivery, Skin.

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# Introduction

Liposomes are microscopic vesicles composed of phospholipid bilayers that enclose aqueous inner compartments. They encapsulate both hydrophilic and lipophilic drugs to increase drug effectiveness and specificity. Hydrophilic drugs be can incorporated into lipid bilayers, and hydrophilic drugs are usually entrapped in the aqueous compartment (1-4).

Liposome applications vary from topical delivery to intercellular delivery and three main usages of liposomes in the medical field are: drug delivery, increasing immune responses and gene transfer. Liposomes have been a carrier for different drugs due to their suitable characteristics; such as, protection against metabolic degradation, increasing half life, sustain release effects, targeting ability, biocompatibility and biodegradability (5-7).

Liposomes are applied topically on the skin for the following three main goals (8-11):

- 1) to increase transdermal absorption
- 2) to localize drug effect in skin
- 3) to sustain release effects.

In 1976 at first topical liposomes were prepared and patented. The main advantages of topical liposomes in comparison with conventional topical dosage forms are: adhesion to skin keratin, raising water uptake by epidermis, enhancement of drug effects and other general advantages of liposomes. Results of several studies in animal models have shown that most liposome formulations increase drug concentration in the skin after topical application and in comparison with conventional topical dosage forms such as, cream, ointment and gel, they are more effective. Examples of drugs that have been formulated liposomes for as topical application are progesterone, minoxidil, retinoids and topical anesthetics (12). In 1983 minoxidil formulation liposomal was developed and results showed that delivery of drug to site of action (hair follicles) was increased (13). Various liposomal products containing tetracaine and lidocaine were prepared and experiments on these formulations confirmed the superiority of liposomal 0.5% tetracain products compared to a product in conventional dosage forms (1%) (14). Liposomal tobramycin and silversulfadiazine formulations, prepared by Price *et al.*, had better antibacterial effects than classical forms (15).

Triamcinolone is one of steroidal antiinflammatory drug used in different diseases, such as, atopic dermatitis, contact dermatitis, seborreheic dermatitis and psoriasis. This drug is the acetonide derivative of the fluorinated steroids and belongs to the glococorticoids with long effect (16, 17).

The objective of this study was to develop topical liposomal formulation containing triamcinolone with simple and applied method to reduce side effects and increase anti-inflammatory effects of triamcinolone.

# **Material and Methods**

*Material:* Lecithin, cholesterol, oleic acid and propylenglycol were purchased from Merck (Germany) and all solvents were of analytical grade.

Preparation of liposomal triamcinolone by solvent evaporation method: In this method for liposome preparation, egg lecithin and cholesterol were dissolved in organic solvent (methanol:chloroform, 2:1 V/V). The solvent was removed by rotatory evaporation (Heidolph, Germany) until a thin film of the lipid mixture formed in the inner wall of round bottom flask. To remove trace of solvent, lipid film was frozen at -70°C and connected to freeze drier for 4 hours. For hydration process, phosphate buffer saline (PBS) was added to the dried lipid film at temperature above the Tm and vortexed for 30 min. To complete annealing, the liposome was remained in this temperature for five

hours. In some formulations, Oleic acid and methyl salicylate were added to the lipid phase (2, 18). Different formulations were numbered according to the Table 1.

**Preparation of liposomal triamcinolone by fusion method:** First, components of lipid phase and propylene glycol were weighted and kept at 60°C water bath for dissolution of lecithin and cholesterol in propylene glycol. Next, triamcinolone was dissolved in suitable amount of acetone and was added to lipid phase. To evaporate acetone, the mixture was kept at 50°C. Then the aqueous phase was warmed up to 50°C and added to lipid phase and continued by vortex to form liposome (19).

Determination of encapsulation efficiency:

First, liposomes were centrifuged in 1500 rpm for 5-10 min to precipitate triamcinolone crystals (unencapsulated). Then, certain amount of MLV liposomes was dissolved in specific amount of ethanol and UV absorbance in  $\lambda$ max (240 nm) was measured and by using standard curve amount of triamcinolone was determinated. Optical microscope was used for evaluation of triamcinolone crystals.

*Evaluation of liposomes under optical microscope:* For morphological characterization of liposomes and evaluation of triamcinolone crystals, sample of liposomes was observed under an optical microscope (Olympus, Germany). Also, the

Table 1. Ingredients of different formulations.

size of liposomes was determined by using of micrometer.

Evaluation of anti-inflammatory effect by "xylene-induced ear edema" method: For in vivo study, "xylene-induced ear edema" method was used. In this method, male albino mice 25-30 g were selected. Mice were divided into groups of five and xylene was adminstrated as irritant. First, different formulations were applied to the anterior and posterior surfaces of the right and left ear. Regarding to the low viscosity of liposomes for in vivo study, they were mixed with plastybase by 1:1 ratio (this base is used in triamcinolone conventional dosage forms). This process results to a better viscosity for liposomal preparation and with same concentration as conventional dosage forms.

After one hour, formulations were cleaned from surface of ears by alcohol and cotton and  $50\mu$ l xylene was applied to the anterior and posterior surfaces of the right ear and the left ear was considered as a control. Two hours after xylene application, mice were killed and both ears were removed. Circular sections were taken using a cork borer with a diameter of 3mm, and weighted. The increase in weight caused by the irritant was measured by subtracting the weight of the untreated left ear section from that of the treated right ear section (20, 21).

Formula number	Ingredients	Percent	Formula number	Ingredients	Percent
	Lecithin	15		Lecithin	15
Formula 1	Cholesterol	2		Cholesterol	2
	Propylene glycol	7	Formula 2	Propylene glycol	7
				Oleic acid	1
	Triamcinolone	0.2		Triamcinolone	0.2
	Aqueous phase	Up to 100		Aqueous phase	Up to 100
	Lecithin	15		Lecithin	15
	Cholesterol	2		Cholesterol	2
Formula 3	Propylene glycol	7		Propylene glycol	7
				Oleic acid	1
	Methyl salicylate	2	Formula 4	Methyl salicylate	2
	Triamcinolone	0.2		Triamcinolone	0.2
	Aqueous phase	Up to 100		Aqueous phase	Up to 100

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6) *Statistical Analysis:* One-way ANOVA statistical test was used to assess the significance of the differences among various groups. In case of significant F value multiple comparison Tukey test was used to compare the means of different treatment groups. Results with p<0.05 were considered to be statistically significant.

## Results

## Characterization of liposomes

After liposome preparation, evaluation of different formulation showed that formulations containing methyl salicylate are instable and phase separation occurs in them. Therefore, formulations containing methyl salicylate were omitted.

In the other hand, comparison between liposomes prepared by fusion method and solvent evaporation method, showed that liposome prepared by fusion were more hemogene and they had better entrapment efficiency. Thus, fusion method was used for liposome preparation.

After liposome preparation, for characterization of liposomes, liposome size was measured by optical microscope. The results showed that size varies between 1 to 20 µm and 1 to 5 µm for liposomes prepared by solvent evaporation and fusion method, respectively. Encapsulation efficiency for liposomes prepared by solvent evaporation method for formula 1 and 2 was  $65.94\% \pm$ 5.04,  $72.38\% \pm 4.91$  and by fusion method was  $80.33\% \pm 3.51$ ,  $90.50\% \pm 2.78$ , respectively.

In evaluation by optical microscope no triamcinolone crystals were observed. Since liposomes prepared by fusion method had higher entrapment efficiency, more homogeneity, lack of usage of organic solvent in the process of liposome preparation and simplicity, for the rest of the study this method was used for the preparation of liposomes containing triamcinolone.

# Evaluation of anti-inflammatory effect of triamcinolone liposomes

Table 2 shows the anti-inflammatory effects of liposome preparation containing triamcinolone by "xylene-induced ear edema" method.

Table 2. Evaluation of anti-inflammatory effect of liposomal triamcinolone by "xylene-induced ear edema" method after one hour.

Sample	Weigh difference ± SEM (mg)	Inhibition percent
Control Tria. oint, Formula 1 Formula 2	$\begin{array}{c} 2.66 \pm 0.08 \\ 1.56 \pm 0.11 \\ 2.26 \pm 0.16 \\ 2.20 \pm 0.11 \end{array}$	$41\% \pm 4.1***$ $15\% \pm 6.1$ $17\% \pm 4.1$

(\*\*\*p<0.001, n=5, Tukey-Kramer)

Samples used in this study were liposomal (formula 1). triamcinolone number 1 liposomal triamcinolone number 2 (formula 2), and triamcinolone ointment. Liposome without any triamcinolone was used as a Statistical analysis was done by control. Tukey-Kramer test between different groups. Results in Table 2 and statistical analysis show that triamcinolone ointment effects after one hour were better than liposomal formulations and this in case antiinflammatory effects of ointment were more considerable. Also, difference between two liposomal triamcinolone forms was not significant (p > 0.05).

According to the for results. more investigation on four groups, time between application of formulations and xylene was increased to two hours and different results were achieved (Table 3). In this case, triamcinolone ointment effects did not change but the effects of liposomal forms were increased significantly. The anti-inflammatory effects of both liposomal formulations was significantly (p<0.05) more than triamcinolone ointment. The antiinflammatory effect of liposomal formula 2

Table 3. Evaluation of anti-inflammatory effect of liposomal triamcinolone by "xylene-induced ear edema" method after two hours.

Sample	Weigh difference ± SEM (mg)	Inhibition percent
Control Tria. oint. Formula 1 Formula 2	$\begin{array}{c} 1.60 \pm 0.15 \\ 0.90 \pm 0.10 \\ 0.46 \pm 0.10 \\ 0.34 \pm 0.09 \end{array}$	44% ± 6.2*** 71% ± 6.2*** 78% ± 5.6***

(\*\*\*p<0.001, n=5, Tukey-Kramer)

was more than formula 1 but this difference was not significant. Figure 1 shows the antiinflammatory effects of three samples after one or two hours application.



Figure 1. Percent of edema inhibition of liposomal triamcinolone by "xylene-induced ear edema" method after one and two hours.

## Discussion

As mentioned before, topical liposomes have several advantages; such as, enhancement of local effects, reducing side effects, dose adjusting and achievement of prolong effects. The goal of our study was to evaluate the anti-inflammatory effects of liposomal triamcinolone in animal model.

According to the lipophilic nature of triamcinolone, expected site of drug in liposome is phospholipid bilayers. Also due to restricted loading capacity in bilayers and fairly high encapsulation efficiency in our samples, it seems lipid phase ratio and drug amount were chosen properly that minimum un-encapsulated drug was seen in microscope evaluation of liposomes. These results are similar to the results of Kulkami and *et al.* In that study encapsulation behavior of four selected steroids in MLV liposomes were studied and encapsulation efficiency in liposomes composed of lecithin and cholesterol was around 80% (8).

Since liposomes are more like a thick suspension and it is hard to apply them directly to the skin for in vivo study, the plastybase was added to the liposomal preparation (1:1) in order to get a cream-like preparation. Microscopical examination of this mixture showed that plastybase has no influence on the structure, stability and size of liposomes.

In this study, fusion and solvent evaporation methods were used for the preparation liposomes of containing triamcinolone. Since fusion resulted in better homogeneity and encapsulation efficiency, liposomes were prepared by this method for in vivo study. This method is simple, contains few steps, does not need to be in contact with organic solvents like ethanol, chloroform or ether which are usually used in the other methods of liposome preparation and is one of the best method for the preparation of topical and cosmetics liposomes.

Previous studies have shown that hydrophilic or hydrophobic character of drug in choosing liposome type is critical. Hydrophilic substances should preferably be encapsulated in large unilamellar vesicle that contains a large aqueous region; for hydrophobic substances entrapment, MLV liposomes are suggested because these liposomes are composed of more bilayers. Since fusion method results to MLV liposomes; therefore, this method is suitable for the encapsulation of lipophilic drugs like triamcinolone (4, 8). Likewise in Hsiu-Ying Yu study, SUV liposomes with negative charge for transdermal studies and MLV liposomes with positive charge for intradermal studies were suggested (22).

In this study in vivo results showed that the effects of triamcinolone ointment reach to its maximum during one hour and then it is constant but liposomes need more time to reach to their anti-inflammatory effects. In the ointment the triamcinolone is free and immediately after application can penetrate and induce its effect; however, in the liposomal form the drug is not free. During and after penetration of liposomes to the different layers of skin the drug gradually releases and then inserts its effect. In general the effect of liposomal triamcinolone was more than triamcinolone ointment. As mentioned before the drug is free in the ointment and can penetrate easily from the skin and not only the drug would be in the different skin layers but also it can reaches to the systemic circulation. However, in the liposome form, due to the similarity of liposomes structure to the building blocks of epidermis, liposomes are concentrated in the upper part of the epidermis; by this way in turn the encapsulated drug also concentrates more in the epidermis (4, 23, 24). Therefore, the effect of drug is increased topically and its systemic side effects are decreased. In similar study by Mezei and et al., liposomal formulation was applied to rabbit skin twice daily for 5 days and was compared to conventional product. Results showed liposomal forms delivered 4-times more drugs as compared to lotion and gel form; whereas drug concentration in deep layers was less that decreases side effects of triamcinolone (4, 12). In the study was done

by Fresta and *et al.*, triamcinolone skin lipid liposomes were prepared and results showed 6 and 1.3 times higher blanching effect than that obtained with triamcinolone ointment and synthetic phospholipids–base liposome formulation, respectively (25). Similar studies have been carried out for other steroids that show ideal effect of liposomal forms. For example can be refer to Lasch study about hydrocortisone and Korting study about betametasone (26, 27).

Better anti-inflammatory effect of formula 2 compared to formula 1 can be consequent of penetration enhancing effect of oleic acid. Oleic acid increases penetration by reducing the order of intercellular lipid domains in skin (28).

## Conclusion

This study showed that liposomal formulations have better triamcinolone topical anti-inflammatory effects compared to conventional ointment. Consequently, less concentration of triamcinolone is needed to prepare liposomal formulation to achieve the same anti-inflammatory effect as conventional dosage forms, this in turn will reduce the adverse effects after topical application. On the other hand, according to the simplicity of fusion method, it is possible to use this method in the industrial scale.

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