Development of liposome containing mycophenolic acid

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Abstract

Mycophenolic acid (MPA) is an immunosuppressive drug, originally developed as a potential antibiotic, antineoplastic and antipsoriatic drug. It is a potent and specific inhibitor of de novo purine synthesis and blocks proliferation of both T and B lymphocytes. MPA has been approved for maintenance immunosuppressive therapy of allogeneic graft rejection following solid organ transplantation. The aim of this study was to develop the liposome containing MPA to enhance skin permeation after topical administration. It was found that liposome of MPA could be prepared by modified ethanol injection method using phopholipid from soybean (SPC), tween 80 and PEG as a wall material. The optimum ratio was 84: 16: 1 (weight ratio) resulted in entrapment efficiency of 61.2% and the size ranging between 300 to 400 nm.

Keywords: Mycophenolic acid, Liposome

1. Introduction

Topical application has been an attractive option to pharmaceuticals, particularly for diseases located in the skin and related tissue. However, skin penetration of the drug is often the limiting factor in dermal delivery. The permeability barrier of the skin is mainly due to intercellular lipids of the stratum corneum (Biruss and Valenta, 2006). In the past decades, lipid vesicles or liposomes became the center of interest as drug carriers for topical treatments as well as active ingredients in skin care products. The application of liposomes on the skin surface has been reported by numerous studies to improve permeability for various entrapped drugs through the major barrier the stratum corneum. The penetration enhancing effect of liposomes may due to the interactions between the intracellular lipids in the skin and the liposome bilayer. In addition, they are able to enhance skin permeation as well as moisturize skin.

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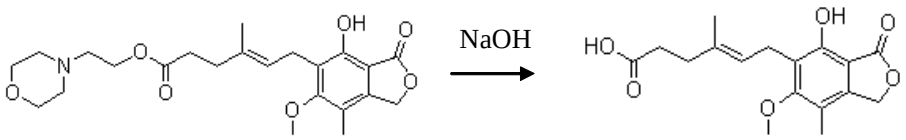

Mycophenolate mofetil Mycophenolic acid

Figure 1. Structure of Mycophenolate mofetil (MMF) and Mycophenolic acid (MPA)

Figure 1 shows the structure of mycophenolic acid (MPA) and its produg, mycophenolate mofetil (MMF). With increased bioavailability, MMF has been primarily used systemically as an immunosuppressive agent in transplantation medicine since 1995. MMF can be metabolized into the active form, MPA, by hydrolytically active enzymes. MPA is a specific inhibitor of de novo purine synthesis and blocks proliferation of both T and B lymphocytes. In addition, it possesses antibiotic, antineoplastic and anti-HIV activities. For dermatology, success has been reported in using MMF (or MPA) in the therapy of skin disorders such as allergic contact dermatitis and psoriasis (Shoji *et al.*,1994). However, it has been reported the low efficiency of MPA in a topical preparation due to the insufficient of its skin penetration (Geilen and and Mrowietz, 2000). Since liposomes can enhance the skin absorption, the liposome formulations for MPA from various lipid compositions and other additives have been developed in this study. The liposomal MPA is expected to be advantageous over MPA for skin prenetration enhancement.

2. Experimental

2.1. Preparation of liposomal MPA

Liposomes were prepared by modified ethanol injection method. In brief, required amount of lipids and additives (Table 1) were dissolved in 3 ml of ethanol containing MPA (1%). Ethanolic solution was rapidly added into 3 ml of phosphate buffer pH 8.0. under sonication. Then, the ethanol was evaporated under rotary evaporator to give about 3 ml of the colloidal dispersion of the liposome.

2.2. High performance liquid chromatography (HPLC)

Assay of MPA was carried out using HPLC (Shimudzu, Japan) equipped with a UV detector. Detection was carried out at 254 nm. Column used was C-18 (0.5μm, Hypersil , 250 mm x4.6 mm, Thermo) attached with guard column (ODS, Hypersil , 20mm x 4 mm, Thermo). MPA was eluted using Acetonitrile : 0.05% Phosphoric acid (40 : 60 v/v) as mobile phase at a flow rate of 1.0 ml/min.

2.3. Characterization of liposomes

2.3.1Size distribution

Mean vesicle size and size distribution of drugloaded liposomes was determined using Mastersizer E (Malvern Instruments, Malvern, UK) based on photon correlation spectroscopy.

Analysis (n = 3) was carried out at room temperature.

2.3.2 Encapsulation efficiency (EE)

EE was determined after separation of the non-entrapped drug using dialysis technique. The amount of total MPAin liposome and non-entrapped MPA was then detected by HPLC. Percent encapsulation was calculated using Eq. (1).

$$\% \text{ EE} = \left[1-\left(\frac{RE_F}{RE_T}\right)\times 100\right]$$

Where RE_F is the amount of free MPA and RE_T is the total amount of MPA present in liposome

3. Results and discussion

Figure 2 shows a typical HPLC chromatogram of MPA. The retention time of MPA was about 4 minutes followed by the peak of internal standard, diclofenac sodiumthe at about 7 minutes. It is clearly observed the well separation between these two peaks. The inter- and intra-day reproducibility of the method show that the relative standard deviation varies between 0.7 to 3.0 % and the bias is less than 2.5%.

Liposomes containing Mycophenolic acid (1% w/v) was formulated with various ratios of lipid composition and the total lipid contents as shown in Table 1. All formulations were determined for their physicochemical properties in terms of particle size and entrapment efficiency. It was found that at the total lipid of 200 μmol/ml, liposomes formulated using SPC: CHOL and SPC:Tween 80 gave good colloidal appearances as shown in Figure 3. In our study, the best liposome formulations were found in SPC: Tween 80: PEG with the ratio of 84: 16: 1 (by weight) and SPC: CHOL: DP with the ratio of 7:3:1 (by molar) showing the entrapment efficiency of about 61 % and 80%, respectively, and, their average sizes were in range of 300-600 nm.

Due to the biphasic character, liposomes can act as carriers for both lipophilic and hydrophilic drugs. The different entrapment efficiency of MPA in liposome formulations may depend upon its solubility and partitioning characteristic. A major problem of the low entrapment efficiency of liposomal MPA may due to its Log P_{oct} (1.6), therefore it can partition easily between the lipid and aqueous phases and very easily lost from the liposomes.

Table 1 Particle size and % entrapment properties of liposome containing various ratios of lipid compositions

Compositions	Ratio	Particle size (nm) Mean SD, (n=3)	% entrapment(n=3)
SPC:CHOL	7:2[a]	444.7 75.80	58.04 1.05
SPC:CHOL: PEG	7:2:1[a]	543.8 23.85	53.91 0.02
SPC:CHOL: DP	7:3:1[a]	591.8 57.84	79.75 0.05
SPC: Tween 80	84:16[b]	400.0 45.78	62.21 0.01
SPC: Tween 80: PEG	84:16:1[b]	350.50 42.28	61.25 0.05

Total lipid 200 μmol/ml; MPA 1% w/v

[a]molar ratio, [b] weigh ratio

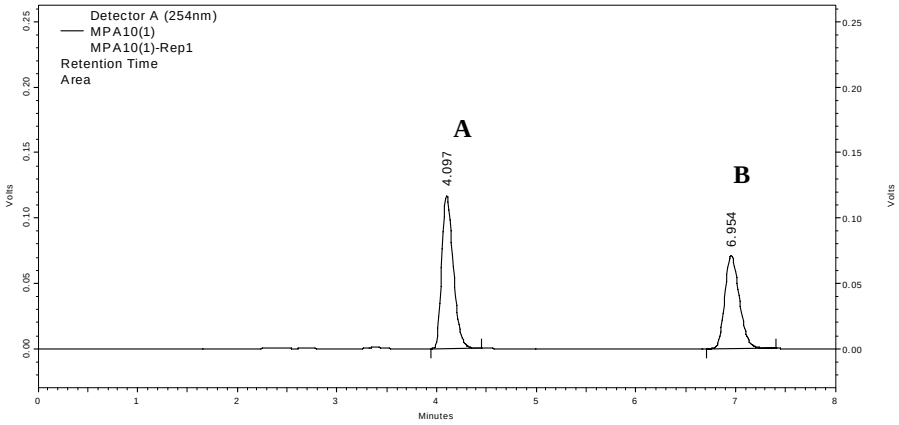

Figure 2. Chromatogram of MPA (A) and Diclofeanac sodium (B)

A B

Figure 3. Liposome of MPA in the formulation of SPC:Tween 80; 84:16 weight ratio (A) and SPC:CHOL; 7: 2 (molar ratio) (B)

4. Conclusion

From this study, MPA liposome could be developed by modified ethanol injection method. The best formulation found at this time was SPC: Tween 80: PEG with the ratio of 84: 16: 1 (by weight) and SPC: CHOL: DP with the ratio of 7:3:1 (by molar) and MPA content of 1%. However, the formulation developments as well as the evaluation in terms of stability and skin permeation efficiency are required for further study.

Acknowledgement

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