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Controlled Release of Cyclosporin A from Liposomes-in-Microspheres as an Oral Delivery System

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Abstract The aim of this study was to prepare cyclosporin A-loaded liposome (CyA-Lip) as an oral delivery carrier, with their encapsulation into microspheres based on alginate or extracellular polysaccharide (EPS) p-m10356. The main advantage of liposomes in the microspheres (LIMs) is to improve the restricted drug release property from liposomes and their stability in the stomach environment. Alginate microspheres containing CyA-Lip were prepared with a spray nozzle; CyA-Lip-loaded EPS microspheres were also prepared using a w/o emulsion method. The shape of the LIMs was spherical and uniform, and the particle size of the alginate-LIMs ranged from 5 to 10 μm, and that of the EPS-LIMs was about 100 μm. In a release test, release rate of CyA in simulated intestinal fluid (SIF) from the LIMs was significantly enhanced compared to that in simulated gastric fluid (SGF). In addition, the CyA release rates were slower from formulations containing the liposomes compared to the microspheres without the liposome. Therefore, alginate- and EPS-LIMs have the potential for the controlled release of CyA and as an oral delivery system.

Keywords: cyclosporin, liposome, polysaccharide microsphere, intestinal lymphatics

INTRODUCTION

Cyclosporin A (CyA), with a molecular weight of 1,202 Da, consists of 11 amino acids, and is a neutral, lipophilic and non-polar cyclic undecapeptide. CyA is a powerful immunosuppressive agent used for the prevention of allograft rejection in organ and tissue transplantation [1]. However, CyA can not always be selected for clinical treatment due to its very low aqueous solubility (0.04 mg/mL at 25°C) and bioavailability [2]. The oral administration of CyA over a long period may also result in harmful side effects such as, hypochromic anemia, marrow hypoplasia, and lymphopenia [3]. Fortunately, there have been many efforts to improve the therapeutic efficacy and decrease the side effects of CyA. Among the various approaches for improving the bioavailability of poorly water-soluble drugs, particulate formulations provide better pharmacokinetic profiles and increased oral bioavailability of the drugs [4-7].

The particulate formulations proposed by many researchers can be briefly summarized into two techniques; namely, the polymer- and lipid-based formulation techniques [8]. The lipid-based formulations can reduce the incomplete dissolution of lipophilic drugs and increase the amount of drug transported via the intestinal lymphatic system; thereby, increasing their absorption from the gastrointestinal tract. However, despite the great interest as a drug delivery carrier, the lipid based formulation often suffers from the problem of instability. On the other hand, the polymer based systems are normally more stable than the lipid-based system, but the latter are more biocompatible than the former [9-11]. Thus, we designed that an appropriate combination of the two systems could improve their ability and avoid the disadvantages as a drug delivery carrier. The lipid based formulation could improve the drug loading, encapsulation efficiency and control drug release properties. The polymer based formulation encapsulating the lipid based formulation could protect the drug loaded into the lipid based formulation within the stomach environment.

In the present study, the liposome-in-microsphere (LIM) system has been demonstrated as an intestinal delivery of CyA. Alginate, natural polysaccharides obtained from marine algae, and extracellular polysaccharide (EPS) produced by *Hahella chejuensis* 96CJ10356,

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which is non-pigmented mutant, were used to improve the instability of liposome within the stomach environment. These polysaccharides have various advantages, such as biocompatibility, they are non-toxic and potential bioactivity. The LIM system designed for intestinal delivery of CyA may reduce the side effects and improve their low bioavailability [12]. CyA-loaded liposome (CyA-Lip) was encapsulated into alginate or EPS p-m10356 with the delivery systems, and the systems were characterized according to their size distribution, zeta potential, morphology, and stability under storage condition. An *in vitro* CyA release test from the delivery system was also investigated in stimulated gastric and intestinal environments.

MATERIALS AND METHODS

Materials

The L-α-dipalmitoylphosphatidylcholine (DPPC), phosphatidylserine (PS), cholesterol (Chol), and sodium alginate of low viscosity (250 cps for a 2% (w/v) solution at 25°C) were purchased from Sigma (St. Louis, MO, USA). The CyA was a gift from Chong Kun Dang Pharm. Co. (Seoul, Korea). The EPS p-m10356 was obtained from the Korea Ocean Research & Development Institute. The solvents used for chromatographic analysis were of HPLC grade.

Preparation and Characterization of CyA-Lip

Liposome consisting of the lipid composition of DPPC: PS:Chol (in molar ratio 7:3:2) was prepared by the thin film hydration method, as described previously [13]. A lipid mixture and 2 mg of CyA were dissolved in 3 mL of chloroform-methanol (2:1, v/v) solution. The organic solvent was evaporated, using a rotary evaporator under vacuum at 50°C, and the residue was then hydrated with PBS buffer (0.01 M, pH 7.4). The prepared liposomal dispersion was sonicated for 5 min in an ice water bath using a tip-type sonicator (Sonics & Materials Inc., USA). The size distribution and zeta potential of CyA-Lip were measured using an ELS-8000 (Otusuka Electronics, Osaka, Japan) over a 10-day period, and incubating at 37°C. The morphology of CyA-Lip was observed using a JEM-200 FXII model transmission electron microscope (TEM, JEOL Ltd., Toyko, Japan). To observe its morphology, CyA-Lip was negatively stained with 0.2% (w/v) phosphotungstic acid buffer, adjusted to pH 6.8 with 1 N of KOH solution.

Preparation and Characterization of LIMs

CyA-Lip was encapsulated with alginate or EPS p-m10356 using the following methods. Firstly, to encapsulate CyA-Lip with alginate, a CyA-Lip dispersion was mixed with 4% (w/v) alginate solution, with this solution added dropped into a 10% (w/v) $CaCl_2$ solution using a micro-nozzle (pore size: 0.41 mm) of a spray dryer (SD-1000, Eyela, Japan). After hardening in cross-linking

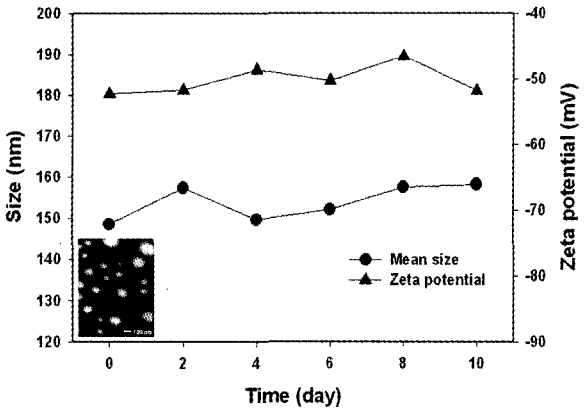

Fig. 1. Changes in the mean size and zeta potential values of CyA-liposomes during storage at 37°C. The image in the graph is a TEM photograph of CyA-loaded liposomes and the bar indicates 100 nm.

solution for 2 h, the alginate-LIMs were washed thrice with PBS buffer (pH 7.4). Secondly, EPS-LIMs were prepared using an emulsion method, as demonstrated previously [14]. A 3% (w/v) EPS p-m10356 and CyA-Lip solution was added to soybean oil under stirring, and the EPS p-m10356 was then cross-linked by the addition of acetic acid to the emulsion. After sufficient time for cross-linking, the EPS-LIMs were collected by centrifugation for 10 min at 12,000 rpm, and then washed three times with ethyl ether. To analyze the morphology, the alginate- and EPS-LIMs were observed under a scanning electron microscope (SEM, Jeol, JSM 5400, Japan) and optical microscope (Olympus, CH-2, Japan). The CyA-Lip in the LIMs were allowed to harden in epoxy and then observed by TEM.

In vitro Release Studies

The release properties of CyA from the alginate- and EPS-LIMs were investigated in simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) at 37°C for 2 days. At predetermined times, the fluid containing the released CyA was removed, with same volume of fresh fluid added. The concentration of CyA released into the fluid was determined by HPLC.

RESULTS AND DISCUSSION

The image of CyA-Lip obtained by TEM is shown in Fig. 1. The shape of the CyA-Lip was found to be spherical and non-aggregated, with a mean size of about 150 nm. ELS analysis confirmed that the mean size of the CyA-Lip was 148.5 nm. The stability of CyA-Lip was investigated by monitoring the changes in the mean size and zeta potential values. There were no the changes in the mean size or zeta potential for 10 days in PBS buffer at 37°C (Fig. 1). Despite the usefulness of liposomes in many field of research, the instability inconveniently limits their applications as a drug delivery system. In particular, con-

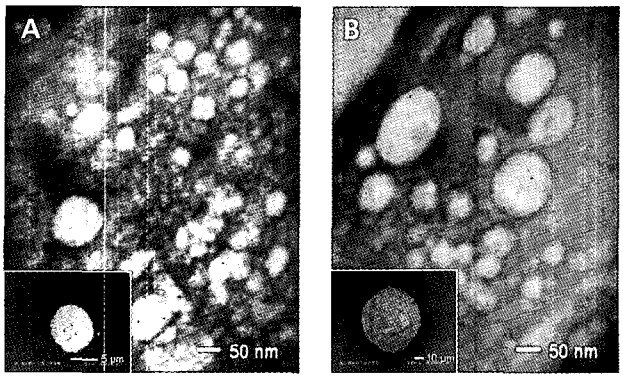

Fig. 2. TEM photographs of the cross sectioned (A) alginate microspheres containing CyA-liposomes and (B) EPS p-m10356 microsphere containing CyA-liposomes. The insets of the TEM photographs are SEM images of (A) alginate microspheres containing CyA-liposomes and (B) EPS p-m10356 microspheres containing CyA-liposomes.

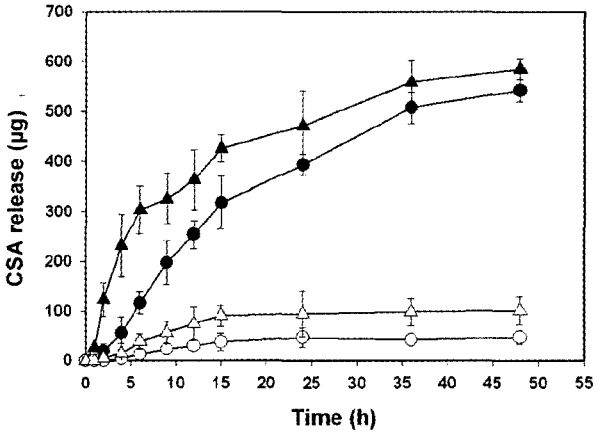

Fig. 3. Release profiles of CyA from alginate microspheres containing CyA-liposomes (circle symbol) and without the liposomes (triangle symbol) in SGF (open symbol) and SIF (closed symbol) at 37°C.

ventional liposomes composed of PC are unstable, but negatively charged liposomes composed of PS are relatively more stable due to the repulsion between the vehicles [15]. However, the liposomes are unstable in the gastrointestinal tract, where they are susceptible to the action of acids, bile and enzymes following oral administration [16,17]. Thus, the CyA-Lip was encapsulated with alginate or EPS p-m10356 to increase the stability of the vehicle in the stomach and control the drug release properties. Fig. 2 shows the TEM results of the section of the LIMs where the CyA-Lip was encapsulated into the alginate or EPS microspheres. The loading efficiencies of CyA in the LIMs were 58 ± 1.2 and 57 ± 0.87% for alginate- and EPS-LIMs, respectively.

In the release studies, the stability of the LIMs containing CyA in the stomach environment, and the control of the release kinetics of CyA from the LIMs were investigated. Figs. 3 and 4 show the *in vitro* release profiles of

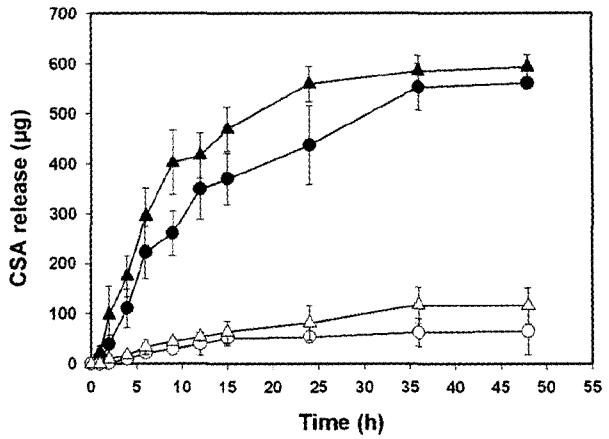

Fig. 4. Release profiles of CyA from EPS microspheres containing CyA-liposomes (circle symbol) and without the liposomes (triangle symbol) in SGF (open symbol) and SIF (closed symbol) at 37°C.

CyA from the alginate- and EPS-LIMs in both SGF and SIF. As seen in these figures, the release rates of CyA in the SGF were limited with all groups tested, while those in SIF increased significantly. Furthermore, the CyA release rates were slower for formulations containing the PS-Lip compared to those containing the alginate and EPS microspheres without the liposome. These results indicate that alginate and EPS p-m10356 can protect the CyA-Lip from the gastric fluid, and the release rates of CyA can be controlled using the LIMs system. In an oral delivery system, the fact that the system composed of only liposomes was not effective, possibly due to their instability and susceptibility, as confirmed through previous research [18,19]. These problems associated with the liposome as an oral carrier can be improved by encapsulation with alginate and EPS p-m10356, indicating the CyA can be effectively delivered to the intestinal lymphatic site when loaded to the liposomes. Under acidic conditions, alginate or EPS microspheres do not swell or erode, as they are precipitated in acidic fluid, while under neutral conditions, the swelling properties of the microspheres are further enhanced, which indicates the influence of the environmental conditions on the drug release properties [20,21]. In a future study, the *in vivo* release rates and animal tests will be performed to investigate the potential of using the LIMs for the intestinal delivery of CyA.

CONCLUSION

CyA-Lip were prepared, characterized and encapsulated into alginate and EPS microspheres for intestinal drug delivery through an oral route. The results of an *in vitro* release test showed the microspheres containing CyA-Lip were a useful system for intestinal drug delivery via oral administration. In addition, their potential for the controlled release over an extended period could be useful as an oral drug carrier. Therefore, these results indi-

cate that alginate- and EPS-LIMs are potential systems for the oral delivery of CyA.

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