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ScienceDirect
International Journal of Pharmaceutics 325 (2006) 155–162
INTERNATIONAL JOURNAL OF PHARMACEUTICS
www.elsevier.com/locate/ijpharm

Pharmaceutical Nanotechnology

Novel *O*-palmitoylscleroglucan-coated liposomes as drug carriers: Development, characterization and interaction with leuprolide

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Received 6 March 2006; received in revised form 15 June 2006; accepted 17 June 2006
Available online 1 July 2006

Abstract

Polysaccharide-coated liposomes have been studied for their potential use for peptide drug delivery by the oral route because they are able to minimize the disruptive influences on peptide drugs of gastrointestinal fluids. The aim of this work was to synthesize and characterize a modified polysaccharide, *O*-palmitoylscleroglucan (PSCG), and to coate unilamellar liposomes for oral delivery of peptide drugs.

To better evaluate the coating efficiency of PSCG, also scleroglucan (SCG)-coated liposomes were prepared.

We studied the surface modification of liposomes and the SCG- and PSCG-coated liposomes were characterized in terms of size, shape, ζ potential, influence of polymer coating on bilayer fluidity, stability in serum, in simulated gastric and intestinal fluids and against sodium cholate and pancreatin.

Leuprolide, a synthetic superpotent agonist of luteinizing hormone releasing hormone (LHRH) receptor, was chosen as a model peptide drug.

After polymer coating the vesicle dimensions increased and the ζ potential shifted to less negative values. These results indicate that both SCG- and PSCG-coated liposomes surface and DSC results showed that PSCG was anchored on the liposomal surface.

The stability of coated-liposomes in SGF, sodium cholate solution and pancreatin solution was increased.

From this preliminary in vitro studies, it seems that PSCG-coated liposomes could be considered as a potential carrier for oral administration.

Keywords: Oral; Sleroglucan; *O*-Palmitoylscleroglucan; Coated-liposomes; Leuprolide

1. Introduction

Liposomes have been extensively studied for their potential use as drug carriers and development of stable liposomes is fundamental for this purpose. Many attempts have been made to enhance the stability of liposomes (New, 1990; Gregoriadis, 1991; Park et al., 1992; Sivakumar and Panduranga Rao, 2001). Among them, surface modification of liposomes is an attractive method to enhance vesicle in vitro and in vivo stability (Jones, 1995; Sagristá et al., 2000; Kato et al., 2004; Lukyanov et al., 2004; Han et al., 2006).

Liposomes have been studied for intraperitoneal and intravenous administration for the delivery of therapeutic or diagnostic agents to specific target tissues. However, there has been increased interest in their potential use for peptide drug delivery by the oral route because they are composed of physiological materials (Fukunaga et al., 1991).

The main problem associated with orally-administered liposomes is their poor stability in the gastrointestinal tract, due to pH, bile salts and pancreatic lipase presence in the GI tract (Kato et al., 1993).

To enhance liposome stability in order to decrease the leakage of entrapped solute and to improve the cellular uptake of liposomes, natural polysaccharides (i.e. mannan, pullulan, amylopectin, dextran, chitosan) were used to coat the outermost surface of liposomal vesicle (Vyas et al., 2005, 2004; Venkatesan and Vyas, 2000; Cansell et al., 1999; Guo et al., 2003).

Coating liposomes with polypeptides or ligands is also an important biomimetic strategy to realize molecular recognition

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doi:10.1016/j.ijpharm.2006.06.040

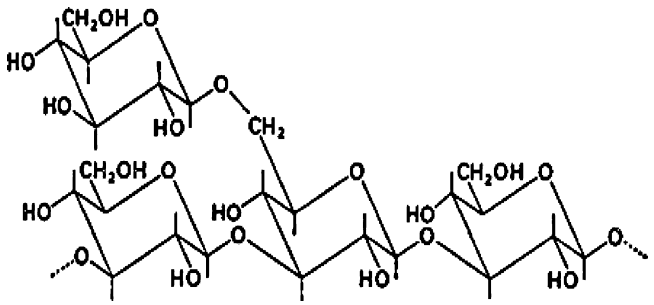

Fig. 1. Polymer structure.

on liposome surface and also to strengthen the mechanical properties of liposomes.

On the other hand, polysaccharide anchoring by adsorption was found to be thermodynamically unstable and pharmaceutically unacceptable (Sihorkar and Vyas, 2001). In order to eliminate these limitations, chemically modified polysaccharide were used to coat liposomes (Sunamoto and Iwamoto, 1986; Lee et al., 2005). In these partially hydrophobized polysaccharides acyl chains were allowed to react covalently with natural polysaccharides and subsequently integrate with the lipid constituents of liposome bilayer.

Scleroglucan is a biocompatible and biodegradable polymer of low toxicity, thus it has been extensively used in the pharmaceutical field (Maggi et al., 1996; Coviello et al., 1998, 2005).

Scleroglucan (SCG) (Fig. 1), a microbial polysaccharide, is a linear chain of 1,3-β linked D-glucopyranose units with single D-glucopyranose residues 1,6-β linked to every third unit of the chain.

The aim of this work was to synthesize and characterize a modified polysaccharide, *O*-palmitoylscleroglucan (PSCG), to coat unilamellar liposomes for oral delivery of peptide drugs.

To better evaluate the coating efficiency of PSCG, also scleroglucan (SCG)-coated liposomes were prepared.

The SCG- and PSCG-coated liposomes were characterized in terms of size, shape, ζ potential, influence of polymer coating on bilayer fluidity, stability in serum and in simulated gastric and intestinal fluids and against sodium cholate and pancreatin.

Leuprolide, a synthetic superpotent agonist of luteinizing hormone releasing hormone (LHRH) receptor, was chosen as a model peptide drug.

2. Materials and methods

2.1. Materials

Phospholipon 90 (Ph90) was a gift of Nattermann (Germany), cholesterol (CHOL) was purchased from Carlo Erba (Italy), calcein; HEPES salt {*N*-(2-idroxyethyl)-piperazine-*N*′-(2-ethanesulfonicacid)}; Sepharose 4B; Sephadex G-75; pancreatin (from porcine pancreas) and bovine serum were Sigma–Aldrich (Italy) products, sodium 5,6-carboxyfluorescein was purchased by Kodak, scleroglucan (SCG) was provided by Degussa (Germany), Leuprolide was a generous gift of Abbott (USA). All other products and reagents were of analytical grade.

Table 1
Sample composition, expressed as molar fractions

Samples	Phospholipon 90	CHOL
Ph90	1.00	0
P/C1	0.99	0.01
P/C5	0.95	0.05

2.2. Preparation of liposomes

Unilamellar vesicles were obtained by means of the "film" method as previously reported (Carafa et al., 2002), according to the composition reported in Table 1.

For this purpose, Ph90 and, when applicable, CHOL were dissolved in a $CHCl_3/CH_3OH$ (3:1) mixture in a round-bottomed flask.

When the peptide drug (1 mg/ml) was used, the same procedure was carried out adding leuprolide to the constituents before film preparation.

After evaporation of the solvents, the dried film was hydrated by addition of 5 ml of different aqueous phases:

1. HEPES buffer (0.01 M, pH 7.4) for vesicle characterization.
2. Sodium calcein 10^{-2} M in HEPES (0.01 M, pH 7.4) to determine the best sample composition, the entrapment efficiency and for stability studies.
3. Sodium 5,6-carboxyfluorescein 10^{-2} M in HEPES (0.01 M, pH 7.4) to make stability studies in calf serum.

The dispersion was then vortexed for about 5 min and then sonicated for 30 min at 25 °C using a tapered microtip operating at 20 kHz at an amplitude of 18% (Vibracell-VCX 400-Sonics, USA).

2.3. Vesicle purification

In order to separate formed vesicles from not structured materials, the vesicle dispersion was purified by gel-filtration on Sephadex G75 columns (50 cm × 1.2 cm), using HEPES buffer as eluent.

According to the quantitative evaluation of phospholipids proposed by Stewart (1980), carried out on the purified suspension of liposomes, the percentage of phospholipid actually structured in all samples to form the vesicles was determined.

2.4. Derivatization of scleroglucan

Palmitoylscleroglucan (PSCG) was prepared as described by Sunamoto and Iwamoto (1986) for the preparation of *O*-palmitoylpullulan. Briefly, 10 g of scleroglucan were dissolved in 100 ml of dry dimethylformamide at 60 °C. To the resultant solution 16 ml of dry pyridine and 6.1 g of palmitoyl chloride, dissolved in 20 ml of dimethylformamide were added. The mixture was stirred at 60 °C for 2 h followed by 1 h at room temperature. Then, it was slowly poured into 350 ml of absolute ethanol. The precipitate obtained was collected and washed with 200 ml of ethanol and 180 ml of dry diethyl ether. The solid