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Research paper

In situ mucoadhesive-thermosensitive liposomal gel as a novel vehicle for nasal extended delivery of opiorphin

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ABSTRACT

Previous studies proved the effectiveness of an intravenous PEGylated liposomal formulation of opiorphin (1 mg/mL) in protecting the drug from enzymatic degradation, and improving intensity and duration of its painkilling effect. Therefore, considering the advantages of nasal administration, the aim of this work was the development of a liposomal mucoadhesive thermo-sensitive in situ gel for the extended nasal delivery of opiorphin. With this purpose, the potential of a series of combinations of different polymers (i.e. chitosan, hydroxypropylmethylcellulose, Poloxamer, Carbopol) in forming solutions able to rapidly gel at the nasal cavity temperature (34 °C) has been investigated. The best formulations were further characterized for gel strength and mucoadhesion properties. The selected formulation, composed by Poloxamer 407 (26.5%) and Carbopol 934P (1%), showed short gelation time at 34 °C (10 s) and suitable mucoadhesion duration (5.5 h) and strength (27 g/cm^2). Due to the low volume administrable via the nasal route, a concentrated liposomal formulation of the peptide (16.5 mg/mL) was developed and loaded in the selected in situ gel formulation. *Ex-vivo* permeation studies, by excised nasal porcine mucosa, showed that the liposomal hydrogel formulation enabled a sustained and controlled delivery of opiorphin over more than 5 h, and highlighted the role of the liposomal carrier in enhancing up to 6 times permeability coefficient and permeation rate of the peptide through the lipophilic nasal mucosa compared to a free peptide-loaded gel.

1. Introduction

Opiorphin is a natural peptide recently isolated from human saliva [1], that shows a strong analgesic effect, even superior than that induced by morphine [2]. It appears as an interesting and promising therapeutic agent in the treatment of acute and chronic pain, also considering that many of the side effects given by morphine and morphine-like drugs should be absent. In fact opiorphin does not activate directly the opioid receptors, but it mainly acts by stopping the normal breakdown of enkephalins [1,3]. It has been shown that repeated treatments with opiorphin did not produce significant abuse liability and opioid dependence and failed to give rise to tolerance or morphine-cross-tolerance phenomena [4,5].

However, despite its strong pain-killing activity, the short duration of action after intravenous administration, probably caused by its rapid degradation by the peptidases in the bloodstream [2] may seriously hinder the successful use of opiorphin into clinical practice. In order to overcome this problem, we recently developed a PEGylated-liposomal formulation of opiorphin, which proved to be able to protect the drug and significantly enhance extent and duration of its analgesic effect after intravenous administration to rats, with respect not only to the simple peptide aqueous solution but also to the peptide loaded in conventional liposomes [6].

Nasal route has gained an increasing interest over recent decades as a potential alternative to the parenteral administration of a number of therapeutic agents, including peptides [7,8]. In fact, in addition to its non-invasiveness and painlessness, intranasal administration presents a series of important advantages such as the relatively large surface area available for drug absorption, the highly vascularized epithelium, the avoidance of hepatic first-pass metabolism of drugs and the possibility of delivery drugs to the brain [9]. In this latter respect, a review about the possible routes and mechanisms likely involved in intranasal delivery of drugs to the central nervous system (CNS) has been published [10]. Furthermore, the easy accessibility of the nasal cavity allows a quick self-administration, thus further improving patient compliance.

Therefore, intranasal delivery of opiorphin, as liposomal formulation, could represent a valid alternative to its intravenous administration. However, in spite of the high potential of nasal drug delivery, some important limitations on its adoption have to be considered, such as eventual presence of pathological conditions (such as colds, rhinitis,

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allergies), potential local tissue irritation by formulation components, possible enzymatic degradation. Moreover, the mucociliary clearance can reduce the in situ residence time of drugs and their absorption by transporting the drug to the nasopharynx and then to the gastrointestinal tract [11]. One of the most attracting and effective approaches to overcome this critic drawback is the addition in the formulation of suitable mucoadhesive polymers, owing to their ability to interact with the mucus layer, thus hampering the clearance of the delivery system and favoring the drug absorption [12,13]. In particular, it has been shown that mucoadhesive polymeric gels are able to establish an intimate contact with the nasal mucosa surface and thus prolong the drug in situ residence time [14,15]. However, due to their high viscosity, gels are difficult to apply and the dose of administered drug cannot be accurately determined. In situ forming hydrogels have been developed that can allow to overcome both these problems [16]. Among the possible mechanisms leading to in situ gel formation, the thermo-sensitive approach is considered particularly advantageous [17,18]. Mucoadhesive thermosensitive polymeric systems are fluid-like before nasal administration, and can be administered as drops, allowing high accuracy of drug dosage and ease of administration; however they undergo a fast sol-gel transition at the temperature of the deposition site, so that the increased viscosity of the resulting mucoadhesive gel system gives rise to an extended in situ residence time.

On the basis of these premises, the aim of this research work was the development of mucoadhesive, thermosensitive in situ gels for nasal delivery of opiorphin. With this purpose, the potential of a series of combinations of different mucoadhesive polymers (i.e. chitosan, hydroxypropylmethylcellulose, Poloxamer, Carbopol) in forming solutions able to rapidly gel at the temperature of the nasal cavity has been investigated. The best formulations were further characterized for gel strength and mucoadhesion properties.

Moreover, it was considered necessary to modify the previously developed opiorphin liposomal formulation [6], in order to increase the loaded drug amount and obtain a more concentrated formulation, suitable to the lower volume administrable via the nasal route. The new opiorphin liposomal formulation, characterized for vesicle size, homogeneity, Zeta potential and entrapment efficiency, was loaded into the selected in situ gel formulation and tested *ex-vivo* for drug permeation properties, using excised nasal porcine mucosa, compared to a free peptide-loaded gel.

2. Materials and methods

2.1. Materials

Cross-linked lipid polyethylene glycol 2000–distearoyl-phosphatidylethanolamine (PEG (2000)–DSPE) and cholesterol were from Avanti Polar Lipids Inc) (Alabaster, AL, USA). Egg L-α-phosphatidylcholine was from Fluka (Neu-Ulm, Germany), stearylamine from Merck GmBH (Germany), chitosan hydrochloride from Protasan (Germany), hydroxypropylmethylcellulose K100M (HPMC) from Colorcon GmBH (Germany), Poloxamer 407 (MW 40000) (P407) and Poloxamer 188 (MW 1800) (P188) from BASF (Ludwigshafen, Germany), beta-glycerophosphate disodium salt pentahydrate (βGP) from Sigma Aldrich (Milan, Italy), Carbopol 934P from Lubrizol (Cleveland, Ohio USA). The amino acid building blocks, resin and reagents used for opiorphin (OPI) synthesis were from Bachem AG (Switzerland). All other chemicals and solvents were of analytical grade.

2.2. Synthesis of opiorphin (OPI)

Opiorphin (OPI), a five-amino acid polypeptide (Glutamine-Arginine-Phenylalanine-Serine-Arginine), was synthesized by using N^{α}-Fmoc solid-phase methodology on preloaded H-Arg(Pbf)-2-chlorotrityl resin (200–400 mesh, loading 0.37 mmol/g, 1 eq, 1 g) according to the method previously developed [6]. The identity and purity of the synthesized peptide was determined by HPLC-DAD-ESI MS analysis, using an HP 1100 Liquid chromatograph (Agilent Technologies, CA, USA) endowed with a HP1040 DAD and connected to a mass spectrometer endowed with API-ES (Atmosphere-Pressure-Ionization-Electro-Spray) (Agilent Technologies, CA, USA). The analyses demonstrated that the synthetic procedure utilized enabled the obtainment of the peptide with a high purity, around 98%. The final yield was about 60%.

2.3. Preparation and characterization of opiorphin-loaded liposomes

PEGylated liposomes were prepared by the thin layer evaporation method, according to the previously developed procedure [6], by keeping constant the types and the respective molar ratios of the lipid phase components, but varying their total amounts. Briefly, the desired amounts of phosphatidylcholine, cholesterol, and stearylamine were dissolved in chloroform in a round bottom flask and added with PEG (2000)–DSPE. The solvent was then removed under reduced pressure in a rotary evaporator. The dry lipid film obtained was hydrated, under stirring, by adding 5 mL of NaCl 0.9% w/v solution containing the drug (16.5 mg/mL). The system was then subjected to 3 cycles of vortexing (1 min at 30 Hz) and heating (5 min at 58 °C) and finally sonicated (3 min at 30 W) in an ice bath. All samples were stored at 4 °C in sealed containers under light protection.

The liposomal formulations were characterized for mean vesicle size, polydispersity index (PDI) and Zeta potential by Photon Correlation Spectroscopy (Malvern ZetaSizer Nano ZS90, Malvern, UK) at 25 ± 0.1 °C. Before analysis, each liposomal dispersion was suitably diluted with NaCl 0.9% w/v, to avoid multiscattering phenomena. Six independent samples were taken from each batch and measured for both vesicle size and Zeta potential.

The drug entrapment efficiency (EE%) was determined by a direct method, after separation, by size exclusion chromatography, of loaded liposomes from free OPI. Purified liposomes were then destroyed by methanol addition; after 1 min sonication, the sample was centrifuged and the OPI concentration in the supernatant assayed by HPLC analysis, as described below. The entrapment efficiency (EE%) was calculated by the following equation:

$$EE\% = (W_L/W_T) \times 100.$$

where W_L is the amount of OPI actually loaded into the vesicle, and W_T the OPI amount initially added during the batch preparation.

Each result is the mean of three separate experiments.

2.4. HPLC assay of opiorphin (OPI)

OPI was assayed by HPLC (Merck Hitachi Elite LaChrom chromatograph, Darmstadt, Germany, equipped with UV–vis detector L-2400), according to a previously developed method [6]. The stationary phase was a Thermo-Scientific™ Hypersil BDS C18 column (100 × 4.6 mm, 2.4 μm). The mobile phase was: (A) water added with 0.05% v/v trifluoroacetic acid; (B) CH_3CN added with 0.05% v/v trifluoroacetic acid. The analysis was carried out under gradient: 0–9 min, A% (v/v) 95–85; B% (v/v) 5–15. The injection volume was 20 μL, the flow rate 1 mL/min. In these conditions The OPI retention time was 7.6 ± 0.2 min. The method was validated for linearity (r^2 = 0.999), LOQ (1.14 μg/mL) and LOD (0.34 μg/mL). Adequate controls have been performed to verify the absence of interference of the components of the liposomal carrier.

2.5. Storage stability studies of liposomal formulations

Stability of OPI-loaded PEGylated liposomal dispersions, as such or added with the components of the gel, was checked for 6 months. The colloidal dispersions were kept at 4 °C in sealed containers under light protection. At given time intervals, aliquots were withdrawn and examined for mean size, PDI, Zeta potential and EE%.