Journal of Controlled Release 194 (2014) 323–331

Contents lists available at ScienceDirect

Journal of Controlled Release

journal homepage: www.elsevier.com/locate/jconrel

Novel self assembling nanoparticles for the oral administration of fondaparinux: Synthesis, characterization and *in vivo* evaluation

Bettina Ralay-Ranaivo [a], Didier Desmaële [a], Elsa P. Bianchini [b], Elise Lepeltier [a], Claudie Bourgaux [a], Delphine Borgel [b], Thierry Pouget [c], Jean François Tranchant [c], Patrick Couvreur [a], Ruxandra Gref [a,*]

[a] UMR CNRS 8612, Institut Galien Paris-Sud, 5 Rue J.B. Clément, 92296 Châtenay-Malabry Cedex, France
[b] EA 4531, Faculté de pharmacie de Châtenay-Malabry, 5 Rue J.B. Clément, 92296 Châtenay-Malabry Cedex, France
[c] LVMH Recherche Parfums et Cosmétique, 185 Av. de Verdun, 45804 Saint Jean de Braye, France

ARTICLE INFO

Article history:
Received 31 March 2014
Accepted 31 July 2014
Available online 13 August 2014

Keywords:
Cationic squalenyl derivative
Fondaparinux
Nanoparticle
Self-assembly

ABSTRACT

Fondaparinux (Fpx) is the anticoagulant of choice in the treatment of short- and medium-term thromboembolic disease. To overcome the low oral bioavailability of Fpx, a new nanoparticulate carrier has been developed. The nanoparticles (NPs) contain squalenyl derivatives, known for their excellent oral bioavailability. They spontaneously self-assemble upon both electrostatic and hydrophobic interactions between the polyanionic Fpx and cationic squalenyl (CSq) derivatives. The preparation conditions were optimized to obtain monodisperse, stable NPs with a mean diameter in the range of 150–200 nm. The encapsulation efficiencies were around 80%. Fpx loadings reached 39 wt.%. According to structural and morphological analysis, Fpx and CSq organized in spherical multilamellar ("onion-type") nanoparticles. Furthermore, *in vivo* studies in rats suggested that Fpx was well absorbed from the orally administered NPs, which totally dissociated when reaching the blood stream, leading to the release of free Fpx. The Fpx:CSq NPs improved the plasmatic concentration of Fpx in a dose-dependent manner. However, the oral bioavailability of these new NPs remained low (around 0.3%) but of note, the C_{max} obtained after oral administration of 50 mg/kg NPs was close to the prophylactic plasma concentration needed to treat venous thromboembolism. Moreover, the oral bioavailability of Fpx could be dramatically increased up to 9% by including the nanoparticles into gastroresistant capsules. This study opens up new perspectives for the oral administration of Fpx and paves the way towards elaborating squalene-based NPs which self assemble without the need of covalently grafting the drug to Sq.

1. Introduction

Since its introduction in the market in 2002, fondaparinux (Fpx, 1, Fig. 1) became the anticoagulant of choice in the treatment of short- and medium-term thromboembolic disease with the unfractionated heparin (UFH) and low molecular weight heparin (LMWH) [1]. Fpx is a synthetic analog of the antithrombin-binding pentasaccharide found in heparin [2]. Although its molecular weight (1728 Da) is much lower than the one of LMWH, Fpx remains a hydrophilic polyanionic macromolecule showing a very low bioavailability (>1%) by oral route [3]. This low oral absorption is the result of: i) poor transport through the intestinal epithelial barrier; ii) pronounced instability in acidic pH conditions in the stomach and iii) fast enzymatic degradation [4]. Due to these drawbacks, Fpx is only administrated via subcutaneous route [5].

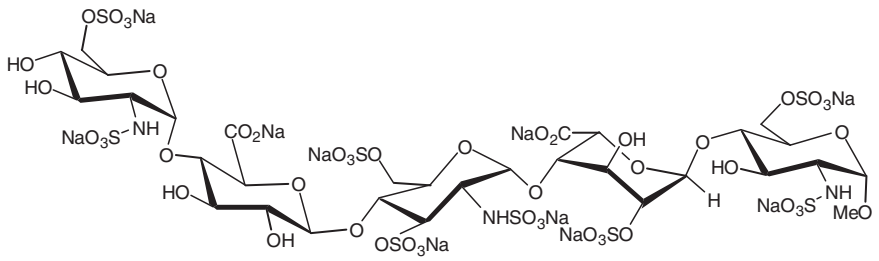
It is not questionable that oral delivery is the preferred route of administration. Few attempts have recently been made to develop emulsions as oral delivery forms of Fpx [6–8]. However, the preparation methods of the Fpx-based emulsions are complicated, need heating and up to five different excipients [6–8]. Moreover, the Fpx loadings were low, less than 1 wt.% [6,7] or 5 wt.% [8]. In this context, the aim of the present study was to develop an innovative oral form of Fpx, able to associate large amounts of the drug by using a simple method with only one excipient.

Thus, to improve the poor oral bioavailability of Fpx, we have envisioned associating it to squalene (SQ), a natural lipid well-known for its excellent oral absorption of more than 60% [9,10]. Moreover, SQ derivatives have the property to self-assemble as stable nanoparticles (NPs) in water [9,11]. It could therefore be expected that Fpx would be protected from degradation by encapsulation into SQ-based NPs. To form NPs, a first approach was to synthesize amphiphilic Fpx-SQ conjugates (data not shown). However, chemical conjugation of Fpx and SQ

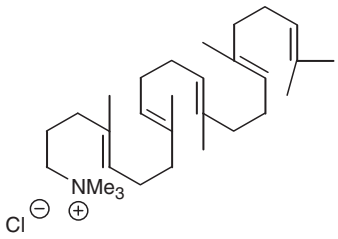
Abbreviations: Fpx, fondaparinux; NPs, nanoparticles; CSq, cationic squalenyl.

* Corresponding author. Present address: UMR CNRS 8214, Institut des Sciences Moléculaires d'Orsay, Université Paris-Sud, 91405 Orsay Cedex, France. Tel.: +33 (0)1 69 15 82 47.
E-mail address: ruxandra.gref@u-psud.fr (R. Gref).

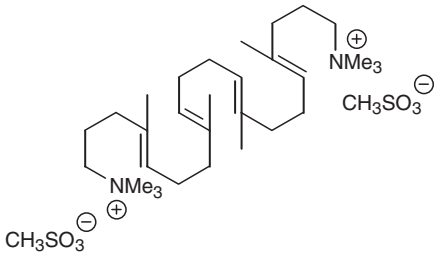
http://dx.doi.org/10.1016/j.jconrel.2014.07.060



1: Fondaparinux Sodium



2: Trimethyl (trisnor-squalenyl)ammonium chloride salt



3: 1,20-bis-trimethylammonium(hexanorsqualenyl) dimethanesulfonate salt

Fig. 1. Chemical structures of fondaparinux sodium (Fpx) and the cationic squalenyl derivatives salts (CSq).

was a tremendous synthetic challenge and had led to the loss of the anticoagulant properties of Fpx (data not shown). Alternative strategies based on ion-pairing to associate Fpx with SQ derivatives appeared to be much more appealing. Therefore, two lipophilic cationic squalenyl derivatives (CSq) (2–3, Fig. 1) were synthesized here to enable ion-pair formation with polyanionic Fpx.

Ion-pairing has previously been shown to be a potential approach for improving the oral bioavailability of heparin [12–16]. The advantage of this method is that cationic molecules such as polycationic-lipophilic-core dendrons [13], chitosan derivatives [14,15] or deoxycholylethylamine (DCEA) [16] efficiently interacted with heparin without changing its chemical structure, thus avoiding the risk of reducing heparin's anticoagulant activity [16,17]. As an illustration of this strategy, Lee et al. synthesized a cationic bile acid derivative to associate with LMWH through the formation of ion pairs [16]. However, the oral bioavailability of the complex was low (3%) with a high administered dose of 50 mg/kg [16]. Chitosan derivatives self-assembled with LMWH in nanocomplexes that were able to protect heparin from enzymatic degradation in the gastrointestinal tract (GIT) [14,15]. Despite these interesting results and to the best of our knowledge, no study has as yet considered the oral administration of Fpx using the ion pairing approach.

We report here on the synthesis and characterization of new CSq, on the formation and characterization of self-assemblies in aqueous media with high Fpx loadings and on the physicochemical stability of the resulting NPs. Finally, preliminary *in vivo* studies have investigated the anticoagulant activity of this nanoparticulate system after intravenous and oral administrations in rats.

2. Materials and methods

2.1. Drugs and chemicals

Fondaparinux (Fpx, Arixtra®, 10 mg/0.8 mL) was purchased from GlaxoSmithKline (UK). Squalene (SQ) was purchased from Sigma-Aldrich Chemical Co. (France), lithium chloride and trimethylamine hydrochloride from Alfa Aesar (France). Acetone, absolute ethanol, diethyl ether, dimethylformamide and dichloromethane were obtained from Carlo Erba (Italy). Filtered MilliQ water (Millipore®, France) was used. Glucose, glycerol, trehalose, sodium phosphate dibasic, sodium phosphate monobasic, Nile red and citrate concentrated solution were purchased from Sigma-Aldrich Chemical Co. (France). Hard gelatin capsules (size 9el) and capsule feeding needle were purchased from Harvard Apparatus (France). Eudragit L100® was obtained as a gift sample from IMCD (France).

2.2. General

IR spectra were obtained as solid or neat liquid on a Fourier Transform Bruker Vector 22 spectrometer. Only significant absorptions are listed. The ^{1}H and ^{13}C NMR spectra were recorded with Bruker Avance 300 (300 and 75 MHz, for ^{1}H and ^{13}C, respectively) or Bruker Avance 400 (400 and 100 MHz for ^{1}H and ^{13}C, respectively) spectrometers. Mass spectra were recorded with a Bruker Esquire-LC instrument. Elemental analyses were performed by the Microanalysis Service in ICSN–CNRS, Gif-Sur-Yvette — France. Analytical thin-layer chromatography was performed with Merck silica gel 60 F_{254} glass precoated plates (0.25 mm layer) and Merck aluminum oxide 60F254 neutral sheets. Column chromatography was performed with Merck silica gel 60 (230–400 mesh ASTM) and Fluka aluminum oxide type 507C neutral. All reactions involving air- or water-sensitive compounds were routinely conducted in oven- or flame-dried glassware under a positive pressure of nitrogen. Except as otherwise indicated, all reactions were carried out in distilled solvents. Triethylamine was distilled over calcium hydride. Chemicals obtained from commercial suppliers were used without further purification.

2.3. Synthesis and characterization of $Sq^{+}2$

2.3.1. (4E,8E,12E,16E)-4,8,13,17,21-pentamethyldocosa-4,8,12,16,20-pentaen-1-yl methanesulfonate (5)

To a stirred solution of trisnorsqualene alcohol 4 (582 mg, 1.5 mmol) in anhydrous CH_2Cl_2 (7 mL) at 0 °C, was added DMAP (10 mg) and dropwise, triethylamine (224 mg, 2.2 mmol) followed by methanesulfonyl chloride (205 mg, 1.8 mmol). The mixture was then slowly raised to room temperature and stirred for 2 h. The reaction was then quenched with brine and the mixture was extracted with