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Sulfated polysaccharide heparin used as carrier to load hydrophobic lappaconitine

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ABSTRACT

One-step self-assembly was used to prepare pH-sensitive lappaconitine-loaded low-molecular-weight heparin (LMWH-LA) and to demonstrate that the sulfur group promotes dissolution and has synergistic effect on the analgesic property of lappaconitine (LA). The LMWH-LA was characterized in terms of releasing behavior, pH-sensitivity, analgesic activity and anticoagulation property. The drug loading level of LA in low-molecular-weight heparin (LMWH) reached 24.3% (w/w). The LA, self-assembled in LMWH, released faster in an acidic environment than that in neutral or alkaline environments. Analgesic experiments showed that the LMWH-LA had earlier onset time and longer duration than the LA. Compared with LMWH, the LMWH-LA can reduce clotting time more effectively. These results suggest that the LMWH is a good template and has great potential to achieve synergistic effect of LA. In addition, similar macromolecular structure can be used as a new natural polymeric carrier for loading hydrophobic alkaloids.

1. Introduction

Lappaconitine (LA) is an alkaloid with good analgesic activity found in the root of *Aconitum sinomantanum Nakai*. However, due to its hydrophobicity, slow onset and short duration, its use is limited [1,2]. To improve solubility of LA, different salts form of LA have been prepared, and evaluated by hot plate test [3]. The sulfate form of LA in six organic and inorganic acids has good solubility and advantageous analgesic activity. Therefore, sulfur group might promote dissolution and synergistic effect on the analgesic property of LA.

In recent years, naturally occurring polysaccharides have captured an increasing amount of attention in the field of drug/gene delivery systems, due to outstanding biocompatibility, biodegradability, non-immunogenicity, extremely low toxicity, etc [4–7]. Sulfated polysaccharide is a type of macromolecule, which has sulfate groups, such as heparin, chondroitin sulfate, and carrageenan [8–10]. It has been reported that the sulfated polysaccharides exerted better biological activities, such as anti-coagulant, antiviral, immunostimulant, hypoglycemic, antioxidant and antitumour activities, in comparison with non-sulfated polysaccharides [11–13]. Heparin, a member of the glycosaminoglycan family, is one of the oldest drugs in the field of thrombosis, venous thrombosis and pulmonary embolism [14,15]. Like unfractionated heparin, low-molecular-weight heparins (LMWH) are glycosaminoglycans, consisting of chains of alternating residues of D-glucosamine and uronic acid, or glucuronic acid and iduronic acid [16]. It has been reported anticoagulant properties of LMWH due to the presence of 3-*O*-sulfation forms part of the antithrombin III binding site [16–20].

In this study, the LWMH was chosen as a template as it is a macromolecule with many sulfonic acid groups that, after ion exchange, show a strong acid and easily self-assemble to an alkaloid. LWMH was investigated not only as a drug-loaded polymer but also as an ionic polymer to improve the poor solubility of hydrophobic lappaconitine. First, lappaconitine loaded low-molecular-weight heparin (LMWH-LA) was prepared. Then, the release behavior, pH-sensitivity, analgesic activity and anticoagulation properties of LMWH-LA were investigated.

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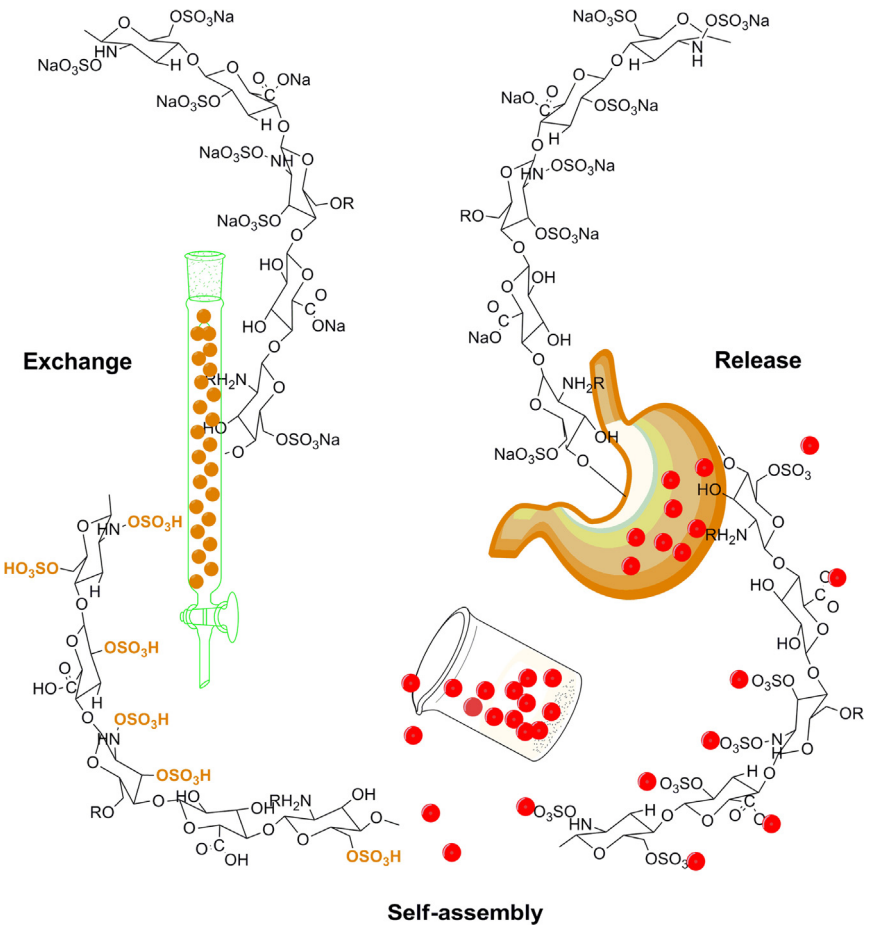

Scheme 1. General view of the reaction route.

2. Materials and methods

2.1. Materials

Low-molecular-weight heparin (Dalteparin, 70 IU/mg) was purchased from Wujiang Xiehe Biochemical Product Co., Ltd. (Jiangsu, China). Lappaconitine was extracted from *A. sinomantanum Nakai* in the laboratory with 98% ethanol. A 732 medical ion exchange resin with strong acid form was obtained from Shanghai Zhenxing Co. (Shanghai, China). Other reagents, such as ethanol was purchased from Sigma–Aldrich Canada Co. (Oakville, ON, Canada) and directly used without further purification.

2.2. Characterization

UV spectra were recorded on a UV-2501PC/2550 (Shimadzu Corporation, Japan). The FTIR spectra were recorded using a Bruker Vector-22 FT-IR spectrometer from 4000 to 500 cm^{-1}. Elemental analysis (C, H, N and S) was performed using a PE-2400 analyzer. X-ray photoelectron spectra were obtained on an X-ray photoelectron spectrometer (ESCALab MKII), using Mg KR radiation (1253.6 eV) as the exciting source. The morphology was observed using environmental scanning electron microscopy (Philips XL-30 ESEM) operated at an accelerating voltage of 20 kV. Molecular weight determination HPSEC-LLS measurements were carried out on size-exclusion chromatograph combined with multi-angle laser photometer (MALLS, $\lambda = 690$ nm; DAWN EOS, Wyatt Technology Co., USA). Ultrahydrogel™ column (7.8mm × 300 mm, Waters, USA) was used as SEC instrument. An optilab refractometer (DAWN, Wyatt Technology Co., USA) was simultaneously connected. The samples with desired concentrations were prepared and optical clarification of the samples was achieved by filtration into a scattering cell. The injection volume was 50 μL and the flow rate was 0.5 mL/min. The analgesic and anticoagulant properties assays were carried out using protocols approved by the Health Sciences Animal Welfare Committee at the Inner Mongolia Agricultural University.

2.3. Sample preparation

LMWH (1 g) was added into 100 mL of deionized water, and stirred well until completely dissolved at 15 °C. Stirring continued at the speed of 250 rpm for 20 min after the addition of 5 g of cation exchange resin. Then, with the removal of cation exchange resin, the exchange liquid was added 1 g LA. After 30 min stirring at 15 °C, extra lappaconitine was removed by the use of chloroform. LMWH-LA was obtained after freeze-drying. The reaction route, including preparation, self-assembly and control release, is shown in Scheme 1.

2.4. LA content

The LMWH-LA was diluted into six different concentrations by 0.1 mol/L HBr, and then filtered by micro-filtration membrane (0.45 μm). The absorbance of the solution was measured at 298 nm using a UV-spectrometer. The content of LA in LMWH-LA was calculated using lappaconitine hydrobromide standard curves. The loading capacity was 24.3% (w/w).