Enhancement of the Intestinal Absorption of Low Molecular Weight Heparin (LMWH) in Rats and Pigs Using Carbopol® 934P

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INTRODUCTION

Advances in anticoagulant therapy have led to low molecular mass heparins (low molecular weight heparin; LMWH) as improved anticoagulants compared to unfractionated heparins (UFHs) with respect to pharmacokinetic parameters and minimal bleeding after subcutaneous injection (1,2). There are a number of LMWH products on the market, with anti-Xa/anti-IIa ratios varying between 4:1 and 2:1 (3). These LMWHs have a molecular weight of approximately 4500 Da and, compared to UFHs (12,000 Da), show better distribution and less binding to non-anticoagulant-related plasma proteins and platelets (2). This particular molecular size may give improved intestinal permeation /absorption characteristics to LMWHs. LMWHs are of hydrophilic character and stable in the gastrointestinal tract. It is likely that LMWH will permeate across the intestinal epithelium via the paracellular routes. However, their relatively high molecular weight impedes this permeation and is due to the tight junctions that restrict the passage of large molecules across these routes. Recently, *N*-[8-(2-hydroxybenzoyl)amino]caprylate (SNAC) and *N*-[10-(2-hydroxybenzoyl)amino]decanoate (SNAD) have been referred to as complexing agents that improve the intestinal absorption of both unfractionated and low molecular weight heparins (4,5). Agents of this class can form complexes (by noncovalent interactions) with macromolecules such as heparins and LMWHs that may penetrate the intestinal barrier transcellularly. It is hypothesized that when the complex has passed the intestinal barrier, it dissociates to yield the original active three-dimensional conformation of the macromolecule (6).

The Food and Drug Administration-approved poly(acrylate) derivative Carbopol® 934P (C934P) has been reported to significantly increase the intestinal absorption of the peptide drug buserelin after intraduodenal administration in rats (7). Because of its Ca^{2+}-chelating ability, C934P interferes with the intercellular junctions and increases the paracellular permeability (7). In the present study, we investigate the potential enhancing effect of C934P on the intestinal absorption of LMWH in both rats and pigs *in vivo*.

MATERIALS AND METHODS

Materials

The low molecular weight heparin Opocrin® (83 anti-Xa Units/mg; average molecular weight 4500 Da) was obtained from S.P.A. (Modena, Italy). Carbopol® 934P (C934P) was a generous gift of BF Goodrich (Cleveland, OH). Hypnorm® (containing 0.315 mg/mL fentanyl citrate and 10 mg/mL fluanisone) was from Janssen Pharmaceuticals (Oxford, UK) and Dormicum® (5 mg/mL midazolam hydrochloride) from Hoffmann-La Roche (Mijdrecht, The Netherlands). Narketan® (ketamine) was purchased from Chassot (Vught, The Netherlands) and Stresnil ® (azaperon) from Janssen-Cilag (Tilburg, The Netherlands). Vials containing 3.8% sodium citrate for blood sample collection were obtained from Terumo Europe (Leuven, Belgium). Chromostrate® heparin anti-Xa assay kits were from Organon Teknika (Oss, The Netherlands). All other reagents were of analytical grade.

LMWH Absorption in Rats

The experimental procedure was slightly different from previously reported studies (7). In brief, male Wistar rats SPF (average body weight 250 g) were obtained from Harlan (Zeist, The Netherlands). The animals were fasted for 16 h before the experiment with free access to water. The animals were anaesthetized with Hypnorm® (1.5 mL/kg) and Dormicum® (500 μg midazolam/kg). Control (LMWH; Opocrin®) and polymer-containing [LMWH with 1% (w/v) C934P] solutions were prepared in physiological saline (0.9% NaCl). The pH of the formulations was readjusted with 0.1 M NaOH or 0.1 M HCl to values of 7.4. LMWH was administered intraduodenally at a concentration of 10,800 anti-Xa U/kg. The LMWH-C934P preparations were administered at 3600, 7200, and 10,800 anti-Xa/kg animal. To administer the LMWH formulations, a teflon tube connected to a syringe was inserted from a small incision into the body of the stomach and guided through the pylorus into the duodenum. Two milliliters of the prepared formulations were administered at a rate of 1 mL/min. Afterwards, the tube was removed, and the incision in the stomach was closed. Blood sampling was performed through a cannula previously inserted into the right carotid artery. To avoid blood clotting, infusion of physiologic saline at a constant rate (1 mL/h; 50 mL of Original Perfusor-Spr., Melsungen, Germany) maintained the patency of the cannula. Samples of 200 μL were withdrawn at 0, 30, 60, 90, 120, 180, 240, 300, 360, 420, and 480 min post-administration in vials containing sodium citrate. Blood samples were centrifuged (17000 g for 15 min), and serum was collected and stored at –20°C until analysis. The analysis of anti-Xa activity was performed by using the Chromostrate® assay.

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LMWH Absorption in Pigs

Four female pigs, weighing about 25 kg, were used. Before and during the experiments, the pigs were housed at the Central Laboratory Animal Institute of Utrecht University (Utrecht, The Netherlands). The animals followed a standarized diet to gain maximally 1 kg/week. One week before starting the LMWH absorption studies, a silicone fistula (T-shaped, 2 cm o.d. and 1.5 cm i.d.) was inserted into the duodenum, and a jugular vein was cannulated using the surgical procedure previously described (8). The patency of the jugular cannula was maintained by constant infusion of physiological saline.

The pigs received administrations according to a randomized cross-over setup. The LMWH formulations were administered every other day at 48-h intervals between administrations to ensure complete washout of the drug. Before administration, the animals were sedated by subcutaneous injection of an azaperon/ketamine mixture to facilitate intestinal administration. The duration of the sedation was approximately 15 min. During this period, three control blood samples of 3 mL were withdrawn. An elastic tube of 15 cm connected to a syringe of 50 mL containing the LMWH formulations was inserted into the fistula and guided into the duodenum for about 5 cm to ensure complete drug dosing. Twenty milliliters of LMWH formulations were administered at a rate of 10 mL/min. LMWH was administered without or with 1% (w/v) C934P at 5000 anti-Xa/kg in physiological saline (pH = 7.4). After drug dosing, the fistula was tightly closed and inspected for possible leaking. At the end of this procedure, the animals recovered to consciousness. Blood samples of 4 mL were withdrawn at 30, 60, 90, 120, 180, 240, 300, 360, and 420 min and collected in citrated vials. These samples were centrifuged for 15 min at 2000 g and 4°C. Plasma obtained was stored at –20°C until analysis with the Chromostrate® assay.

Analysis of Data

For both rats and pigs, the areas under the individual blood anti-Xa concentration vs. time curves (AUC) were calculated according to the linear trapezoidal rule. The intestinal absorption enhancement ratio (ER) was determined according to the following formula:

$$ER = \frac{AUC_{polymer}}{AUC_{control}}$$

The data were evaluated for statistically significant differences by one-way analysis of variance (ANOVA).

RESULTS

Figure 1 shows the serum anti-Xa levels after intraduodenal administration of LMWH (10800 anti-Xa U/kg) with or without 1% (w/v) C934P in rats. Polymer co-administration significantly increased the LMWH absorption and the corresponding anti-Xa concentrations. These levels were sustained at about 0.3 anti-Xa U/mL serum for about 6 h after administration. In contrast, administration of LMWH without poly(acrylate) resulted in poor absorption and low level of 0.03 anti-Xa U/mL. The $AUC_{0–360\ min}$ of the co-administration with C934P was significantly higher than that of LMWH alone ($P < 0.001$; Fig. 2), yielding an absorption ER of 10.5.

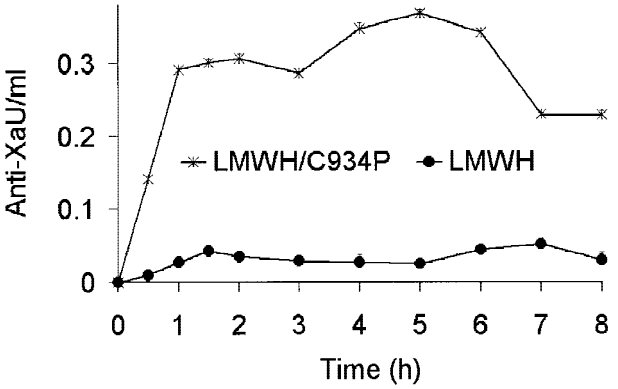

Fig. 1. Serum anti-Xa levels after intraduodenal administration of low molecular weight heparin (10800 anti-Xa U/kg) with or without 1% (w/v) C934P in rats (mean ± standard error of the mean; n = 6).

The effect of reducing the dose of LMWH (co-administered with 1% C934P) is presented in Figure 2. Co-administration of C934P with LMWH at 7200 and 3600 anti-Xa U/kg resulted also in increased $AUC_{0–360\ min}$. For these two LMWH doses, the observed serum levels were also sustained throughout the experiments (data not shown).

Co-administration of LMWH (5000 anti-Xa U/kg) with 1% (w/v) C934P in pigs resulted in enhanced plasma anti-Xa levels compared to intraduodenal administration of LMWH alone (Fig. 3). Plasma anti-Xa reached maximum levels of 0.25 U/mL at 2 h after administration and then gradually declined to 0.2 U/mL at 7 h. Dosing of LMWH without C934P resulted in almost constant plasma levels of 0.07 anti-Xa U/mL. $AUC_{0–420\ min}$ were 32.0 ± 8.6 and 88.4 ± 22.6 anti-Xa U/mL*min after administration of LMWH without and with 1% C934P, respectively, resulting in an absorption ER of 2.8. The AUC values were significantly different from each other ($P < 0.01$).

At the end of all experiments, the rats and pigs were sacrificed by an overdose of pentobarbital, and the gut was macroscopically inspected for possible lesions. All animals demonstrated normal intestinal tissue, and no bleeding or morphologic damage was observed.

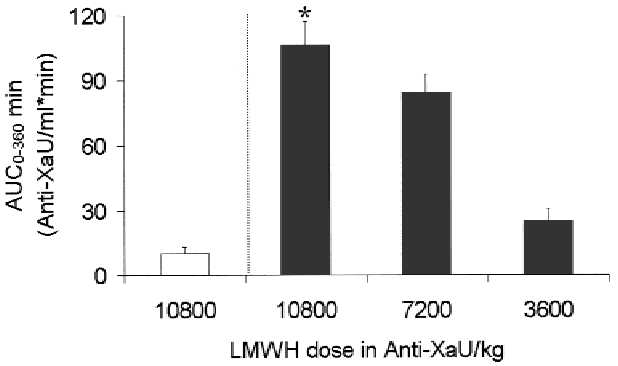

Fig. 2. Effect of the administered low molecular weight heparin dose on the $AUC_{0–360\ min}$. Open bar represents the AUC after intraduodenal administration of 10,800 anti-Xa U/kg rat. Closed bars represent the AUC after co-administration with 1% (w/v) C934P (mean ± standard error of the mean; n = 6). *Significantly different from low molecular weight heparin alone (10,800 anti-XaU/kg dose; $P < 0.001$).

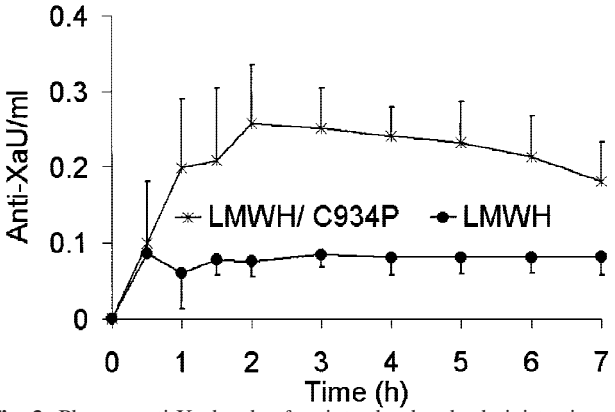

Fig. 3. Plasma anti-Xa levels after intraduodenal administration of low molecular weight heparin (5000 anti-Xa U/kg) with or without 1% (w/v) C934P in pigs (mean ± standard deviation; n = 4).

DISCUSSION

Intestinal absorption of hydrophilic and macromolecular drugs is a challenge to biopharmaceutical scientists. The hydrophilicity and large molecular size of these agents prevent the permeation across the transcellular routes of the intestinal epithelium and their systemic absorption. Recently, mucoadhesive polymers based on chitosan and poly(acrylate) have shown to increase the enteral absorption of peptide drugs *in vivo* (9). In the present study, the poly(acrylate) C934P was investigated for its ability to enhance the absorption of an anionic polysaccharide (molecular weight 4500 Da). C934P was chosen because of its physicochemical compatibility with LMWH polysaccharides. Both poly(acrylates) and heparin are of anionic character, and electrostatic interactions between these compounds in solution are prohibited. Moreover, LMWH –C934P mixtures result in clear hydrogels. Analysis of LMWH absorption levels was performed using an anti-Xa assay because this assay is more suitable to monitor LMWH levels than the activated partial thromboplastin time (aPTT) due to the fact that LMWHs scarcely affect the aPTT (10).

Results from the present intestinal absorption studies in rats and pigs showed that the mucoadhesive C934P managed to increase the absorption of LMWH to anti-Xa levels, which are considered to be sufficient for the prevention of thrombi formation (11). It has been reported that the plasma anti-Xa concentration required to induce a 50% antithrombotic effect is 0.12 U/mL, whereas concentrations exceeding 0.2 U /mL always result in an evident antithrombotic effect (11). In the present studies, administration of LMWH alone resulted in blood anti-Xa levels lower than the therapeutic concentrations; in both rats and pigs, these levels did not exceed 0.07 U/mL (Figs. 1 and 3). This indicates that intestinal LMWH absorption is restricted by the permeation barrier across the intestine. Co-administration of LMWH with C934P resulted in enhanced anti-Xa levels, which were sustained for 6 h after delivery. The mechanism of absorption enhancement is probably a combination of the mucoadhesive properties of C934P and its influence on the intercellular tight junctions to allow for paracellular drug permeation (7,9).

Deep vein thrombosis is a widespread disease that usually occurs as a complication in surgical procedures (e.g., hip replacement). It is caused by poor circulation from inactivity or prolonged bed rest due to conditions such as heart disease, and it has been also documented to occur after long-distance flights. For the treatment of deep vein thrombosis, LMWHs can be used subcutaneously (1). The prospect of an outpatient treatment for this disease could be advanced by the use of an orally administered LMWH dosage form.

Reported strategies to increase the intestinal absorption of LMWH also include the formation of a salt with a tertiary diamine (bis-*N,N*-dibutylamino-butylene carbonate; 11). This LMWH salt was shown to be better absorbed from rabbit duodenum than native LMWH, resulting in significantly increased anti-Xa levels. This improved absorption was speculated to be due to increased lipophilicity of the LMWH-counterion complex (12). Recently, the approach of complex formation led to an orally absorbable LMWH formulation using SNAD as complexing agent in high doses of 50 mg/kg (13). Enteral administration of LMWH/SNAD complexes resulted in significantly increased anti-Xa levels and diminished thrombi in rats (5) and in pigs with experimentally induced venous thrombosis (13). The anti-Xa levels measured in the present investigations appeared to be quite similar to those reported in the LMWH/SNAD studies. Compared with the aforementioned approach of complex formation, the use of poly(acrylate) polymers as absorption enhancers has the following advantages: 1) because of their polymeric nature (C934P has a molecular weight of $>10^6$ Da), there is almost no intestinal absorption *per se,* in contrast to SNAD, which is absorbed as a complex with LMWH and 2) poly(acrylates) allow for reversibly increased drug permeation only by the paracellular routes along the enterocytes (9). Additionally, C934P shows physicochemical (hydrogel formation) and mucoadhesive properties useful for the design of oral dosage forms. C934P is a Food and Drug Administration-approved material; however, solid dosage forms composed of this polymer and LMWH should also undergo toxicologic evaluation.

In conclusion, C934P has advantageous characteristics for developing suitable LMWH oral-delivery formulations to be used as outpatient treatment for the prevention of deep vein thrombosis and substituting for the present LMWH subcutaneous injections.

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