A newly developed oral heparin derivative for deep vein thrombosis:
Non-human primate study

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Received 23 May 2007; accepted 8 August 2007

Abstract

The development of orally active heparin will have tremendous clinical importance since it can be used to effectively prevent deep vein thrombosis (DVT) in a long-term chronic treatment. We developed in this study a new orally active heparin derivative (Db-LHD), which has heparin chemically conjugated with deoxycholic acid and DMSO molecules by secondary interactions. Db-LHD was prepared in the powder form in soft capsules. When we administered Db-LHD capsules to monkeys, its oral physiological availability was increased up to 16.6%. The maximum anti-FXa activity at 5 mg/kg of Db-LHD was more than twice the minimum effective anti-FXa activity (MEC, 0.1 IU/mL) for preventing DVT, and the anti-FXa activity in plasma was maintained for 10 h above the MEC in monkeys. Also, we evaluated anti-thrombogenic effect of Db-LHD in a rat thrombosis model. A subcutaneous administration of enoxaparin (100 IU/kg), which was the highest recommended dose for the prevention of venous thromboembolism, reduced thrombus formation by 38.9±14.2%. On the other hand, 5 mg/kg (425 IU/kg) of orally administered Db-LHD reduced thrombus formation by 51.0±2.0. We propose a new orally active heparin, Db-LHD, in a solid dosage form to effectively prevent DVT and PE.

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Keywords: Heparin; Deep vein thrombosis; Oral delivery; Bioavailability; Conjugate

1. Introduction

Venous thromboembolism is recognized in approximately 250,000 hospitalized patients annually, and about 100,000 patients die each year due to pulmonary embolism (PE) in the US [1,2]. Without therapy, these patients have a 50% chance of succumbing to recurrent thromboembolism [3]. Heparin is regarded as the most potent anticoagulant that can be used in preventing deep vein thrombosis (DVT) and pulmonary embolism (PE) [4,5]; however, heparin cannot be absorbed in the intestine owing to its large molecular size and hydrophilicity, and can only be given parenterally. Currently, the so-called ‘gold-standard’ treatment consisting of 5-day injection of parenteral heparin followed by 3 months of oral warfarin therapy can successfully prevent 95% of PE patients with proximal DVT [6]. Warfarin, however, has a slow onset, and is subjected to a low therapeutic efficacy and high possibility of drug–drug interactions [7]. Therefore, the development of orally active heparin would have tremendous clinical importance as it would become a choice drug to effectively prevent DVT and PE in a long-term chronic treatment. In addition, orally active heparin could also be widely used to prevent several kinds of thrombotic events in continuous long-term treatment.

To overcome the poor oral bioavailability of heparin, several research groups have attempted various methods for oral heparin delivery, such as liposomes, oil–water emulsions, complexes of heparin with hydrophobic organic bases, enteric coating, nanoparticle and aerosol formulations [8–12]. There
also have been attempts to evaluate the enhancing effects of EDTA, acidic buffer, sodium N-(8-(2-hydroxybenzoyl) amino) caprylate (SNAC) and sodium N-(10-(2-hydroxybenzoyl) amino) decanoate (SNAD) or sulfated surfactants on heparin absorption in the GI tract [13,14].

In the previous studies, we synthesized a chemical conjugate (LHD) of low molecular weight heparin (LMWH) and deoxycholic acid (DOCA), a kind of bile acids. LHD was successfully absorbed in rats when it was orally administered (7.8% physiological availability) [15–17]. These results offer two possible explanations for the mechanism of enhanced absorption of LMWH in the intestine: one can be attributed to the added hydrophobic property owing to the conjugated DOCA, and the other to the interaction between the conjugated DOCA and intestinal membrane. However, the enhancing effect of the conjugated DOCA on the absorption of LHD in the intestine was limited because LHD would form self-assembled nanoparticles in the intestine due to the hydrophobic aggregation of the conjugated DOCA molecules [18].

To maximize the effect of the conjugated DOCA on enhancing the absorption of LHD in the intestine, we have developed new strategies for making the soluble form of oral heparin [19–21]. In this study, we propose a new water soluble Db-LHD, that is, LHD bound with dimethylsulfoxide (DMSO) molecules by secondary interactions. We evaluated the absorption efficacy and prevention effect on thrombosis of Db-LHD in a small animal model. Finally, Db-LHD was prepared in the solid dosage form using a soft gelatin capsule, and we evaluated its absorption and toxicity after oral administration in non-human primate.

2. Materials and methods

2.1. Materials

Low molecular weight heparin (LMWH; Fraxiparin®, 4500 Da) was obtained from GlaxoSmithKline (Brentford, Middlesex, UK). Deoxycholic acid (DOCA), dicyclohexylcarbodiimide (DCC), hydroxysuccinimide (HOSu), 1-ethyl-3-(3-dimethylamino propyl) carbodiimide hydrochloride (EDAC), ethylenediamine, and dimethyl sulfoxide (DMSO) were purchased from Sigma Chemical Co. (St. Louis, MO). Dimethylformamide (DMF) was obtained from Merck (Darmstadt, Germany). Coatest anti-Factor Xa assay kits were purchased from Chromogenix (Milano, Italy).

2.2. Preparation of DMSO-bound LHD (Db-LHD)

The chemical conjugate of LMWH and DOCA was synthesized by conjugating the carboxylic group of DOCA with carboxylic group of LMWH as described in the previous study [22]. Briefly, DOCA (196 mg) was mixed with DCC (165 mg) and HOSu (92 mg) in 15 mL of DMF. The feed mole ratio of DOCA, DCC and HOSu was 1:1.6:1.6. The concentrations of DCC and HOSu were slightly higher than that of DOCA in order to activate DOCA completely. The mixture was reacted for 5 h at room temperature in vacuum and the precipitated dicyclohexylurea was filtered. The unreacted DCC was precipitated by adding 1 mL of distilled water and filtered. The filtrated solution was poured in 15 mL of distilled water. The remaining HOSu was dissolved in water and the activated DOCA was precipitated and filtered. The activated DOCA was mixed with ethylenediamine in DMF and reacted for 5 h at room temperature, thereby forming deoxycholyethylamine. The feed mole ratio of activated DOCA to ethylenediamine was 3:1. LMWH (100 mg) was dissolved in 2 mL of formamide and EDAC solution (11.5 mg) was added in order to activate carboxylic groups of LMWH. Deoxycholyethylamine was coupled with activated carboxylic groups of LMWH. The reaction mixture was incubated at 25 °C for 12 h. The feed mole ratio of deoxycholyethylamine to LMWH was 3:1. After the product was precipitated in acetone followed by lyophilization, LHD was obtained as white powder. The synthesized product was analyzed using FT-IR (1725X, Perkin-Elmer, Wellesley, MA), 1H-NMR (JNM-LA 300 WB FT-NMR, JEOL, Tokyo, Japan) and 13C-NMR (JEOL), and its anticoagulant activity was determined using anti-FXa chromogenic assay (Chromogenix kit, Chromogenic®, Milano, Italy), where S-2222 (Bz-Ile-Glu(γ-OR)-Gly-Arg-pNA-HCl) was used as the substrate [23].

In order to prepare Db-LHD, LHD was dissolved in 10% aqueous DMSO solution and freeze dried at −80 °C to obtain white powder. The amount of DMSO bound to LHD was obtained by calculating the weight change of the sample before and after being freeze dried. The prepared Db-LHD was analyzed using thermal gravimetry analysis (TGA, Mettler TG 50, Greifensee, Switzerland), differential scanning calorimetry (DSC, DSC 821e, Mettler Toledo, Columbus, OH) and FT-IR.

2.3. Absorption of Db-LHD after its oral administration

ICR mice (Korean Animal Center, Seoul, Korea) were fasted for 12 h before orally administering Db-LHD. Mice, anesthetized with light diethyl ether and Db-LHD that was dissolved in water, were orally administered through an oral gavage that was carefully passed down the esophagus into the stomach. The administered dosage ranged from 0 to 10 mg/kg, with each of the dose volume of Db-LHD solution being 0.2 mL.

Male cynomolgus monkeys (3.5–4.0 kg, Korea Research Institute of Chemical Technology (KRICT), Daejeon, Korea) were fasted for 12 h before drug administration. Db-LHD in dosages of 5 (425 IU/kg) and 10 mg/kg (850 IU/kg) were prepared in solution and in a soft gelatin capsule type, respectively. Also, Db-LHD (1 mg/kg) in PBS solution (pH 7.4) was intravenously administered in order to calculate the physiological availability for its oral administration.

All of the animal experiments were carried out in accordance with the procedures outlined in the Guide for the Care and Use of Laboratory Animals and approved by KRICT.

Blood (450 μL) was collected from a capillary in the retro-orbital plexus (mice) or the vein (monkeys) and directly mixed with 50 μL of sodium citrate (3.8% solution), followed by immediate centrifugation at 2500 ×g at 4 °C for 15 min. The anti-FXa activity that was induced by Db-LHD in plasma was measured by using anti-FXa chromogenic assay.
2.4. Venous thrombosis model

The animal model for DVT was prepared as described in the literature [20], Sprague–Dawley rats (SD male rat, 250–280 g), obtained from the animal care facility at the Korean Animal Center (Seoul, Korea), were kept under a 12:12 h light–dark cycle and were used after fasting them for 12 h. Briefly, LMWH (enoxaparin) of 100 IU/kg was administered by subcutaneous injection, whereas 3 (255 IU/kg), 5 (425 IU/kg) and 10 mg/kg (850 IU/kg) Db-LHD were orally administered to SD rats, respectively. After administration of enoxaparin and Db-LHD via subcutaneous and oral route, respectively, animals were anesthetized with ketamine (45 mg/kg) and xylazine (5 mg/kg) by means of intramuscular injection. After rats were anesthetized, both sides of the vena cava of rats were exposed and separated from the surrounding tissue. Each end (2 cm) of vena cava was loosely tied and the branched blood vessels were completely tied with 2–0 silk thread. At 60 min after enoxaparin or Db-LHD administration, 1 mL/kg human pooled plasma warmed to 37 °C was injected through the tail vein. Fifteen seconds later, the vena cava was ligated with 2–0 silk thread in situ to produce stasis. At 120 min after finishing the surgical operation, the veins were segregated and opened in a Petri dish filled with 3.8% sodium citrate. Thrombus formation was evaluated by measuring the dry weight of the thrombus.

2.5. Toxicity of Db-LHD in monkeys

Monkeys were divided into four groups (the number of monkeys in each group was three): the first group was a control group that orally received PBS only, and other three groups were orally administered 10 (850 IU/kg), 70 (5950 IU/kg), 500 mg/kg (42,500 IU/kg) of Db-LHD, respectively. After Db-LHD was orally administered once a day for 2 weeks, histology of the GI tract, and hematological and serological parameters were evaluated.

For the histological analysis, stomach, small intestine and colon tissues were removed from the mice and fixed in neutral buffered formalin for processing. The tissue specimens were washed with alcohol to remove any tissue water. Specimens were perfused with colored silicone and embedded in paraffin. The embedded specimens were cut into 5 μm sections by a microtome, and picked up on a glass slide. The tissue sections were then washed with xylene and absolute alcohol, respectively, in order to remove paraffin. The 5 μm sections prepared were stained with the use of hematoxylin and eosin (H&E).

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At 2 weeks, the blood samples collected from the retro-orbital puncture were analyzed immediately for hematological and serological parameters. The second blood sample tubes were centrifuged at 12,000 rpm at 4 °C for 10 min, which was stored at −20 °C until analysis for serological parameters. The following hematological parameters were measured: erythrocyte count (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet count, and total leukocyte count (WBC). Serological parameters measured included the following: alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), creatine, glucose, total protein, albumin, sodium and potassium.

2.6. Statistical analysis

The cumulative data from animal experiments were expressed as mean±SD, and a paired t-test was used for comparison between groups. A value of P ≤ 0.05 was considered as statistically significant.

3. Results

3.1. Characterization of Db-LHD

As described in our previous study [16], the conjugation of LMWH and DOCA was confirmed by the presence of amide bonds formed by coupling between carboxylic groups of heparin and amine groups of deoxychorylethylamine using FT-IR, 1H-NMR and 13C-NMR. The peaks at 1720 and 1585 cm−1 in the Fourier Transform infrared spectrum indicated the presence of amide bonds in LHD. In the 1H-NMR and the 13C-NMR spectrums, the amide peak also occurred at 7.58 and 178 ppm, respectively. The conjugation ratio of DOCA to LWMH was 2.4:1, and the anticoagulant activity of LMWH and LHD measured by anti-FXa chromogenic assay was 97 and 86 IU/mg, respectively.

LHD formed self-assembled nanoparticles in water (~350 nm) because the conjugated hydrophobic DOCA molecules were gathered inside against the hydrophilic aqueous environment [16]; however, Db-LHD was highly soluble in water without forming any nanoparticles as confirmed by dynamic light scattering (DSL, Spectra Physics Laser model 127-35, Mountain View, CA). The anticoagulant activity of Db-LHD measured by anti-FXa chromogenic assay was 85 IU/mg. The amount of bound DMSO on Db-LHD was about 20 wt.% of LHD, and the presence of bound DMSO of Db-LHD was confirmed using FT-IR and thermal analysis. As shown in Fig. 1(a), there were several peaks in the IR spectrum due to the presence of bound DMSO, such as the O–S stretch band at 1012 cm−1, a weak C–S–C deformation band at 702 cm−1, the rocking, asymmetric deformation and symmetric deformation bands of methyl group at 950, 1420 and 1320 cm−1, respectively. The thermogram of Db-LHD analyzed by DSC and TGA showed that DMSO molecules were bound to LHD by secondary interactions. The thermal degradation of LHD in the TGA thermogram started approximately at 183 °C (Tg), and progressed gradually within a broad temperature range (183–320 °C), whereas that of Db-LHD progressed abruptly in a narrow temperature range (230–240 °C) (Fig. 1(b)). The DSC thermogram of LHD showed a major exothermic peak due to thermal degradation at the peak temperatures of 240 °C, and the exothermic degradation peak of Db-LHD shifted to a slightly lower temperature range than that of LHD due to the bound DMSO molecules (Fig. 1(c)).

3.2. Absorption of orally administered Db-LHD in mice

After orally administering 100 mg/kg of LMWH or 10 mg/kg of Db-LMW, LHD and Db-LHD in mice, the maximum
anti-FXa activities in the plasma of LHD and Db-LHD were 0.38±0.13 and 0.67±0.16 IU/mL at 20 min, respectively (Fig. 2(a)). AUC and oral physiological availability were 1.03±0.28 IU min/mL and 2.9±0.8% for LHD and 3.22±0.46 IU min/mL and 9.2±1.3% for Db-LHD. On the other hand, the maximum anti-FXa activity in plasma of LMWH was below 0.1 IU/mL even at 100 mg/kg and that of Db-LMWH was not almost detected. Therefore, LMWH was not nearly absorbed in the GI tract even though DMSO molecules were bound. On the other hand, the conjugated DOCA molecules could make it possible for Db-LHD to be effectively absorbed in the GI tract, and this effect was significantly enhanced by the bound DMSO molecules. When 3, 5 or 10 mg/kg of Db-LHD was orally administered, the maximum anti-FXa activity in plasma was 0.20±0.02, 0.40±0.14 and 0.67±0.16 IU/mL, respectively (Fig. 2(b)). When the administered dose was 5 mg/kg, the maximum anti-FXa activity in plasma was 4 times higher than the minimum effective anti-FXa activity (0.1 IU/mL) for the prevention of DVT.

3.3. Inhibition of venous thrombosis by orally administered Db-LHD

When the phosphate buffer only was treated as a control, thrombus was formed as much as 28.5±3.0 mg. A subcutaneous administration of enoxaparin (100 IU/kg) reduced thrombus...
formation by 38.9±14.2%. The dose of enoxaparin recommended for the prevention of venous thromboembolism is in the range of 50–100 IU/kg, and the highest dose in this range was used as the control. On the other hand, 3 mg/kg (255 IU/kg), 5 mg/kg (425 IU/kg) and 10 mg/kg (850 IU/kg) of Db-LHLD that was orally administered reduced thrombus formation by 42.4±2.1, 51.0±2.0 and 65.9±0.8%, respectively (Fig. 3).

3.4. Non-human primate studies

The maximum anti-FXa activities in plasma of Db-LMWH and LHD were around 0.1 IU/mL at 10 mg/kg dose. On the other hand, the maximum anti-FXa activities in plasma of Db-LHLD formulated at 5 mg/kg dose in solution and capsule were 0.32±0.0 and 0.25±0.02 IU/mL, respectively (Fig. 4-a). The AUC of Db-LHLD after intravenous injection of 1 mg/kg was 13.2±5.5 IU min/mL and we calculated relative bioavailabilities by comparing AUC after oral administration of Db-LHLD (Fig. 4-b). For the same dosage of Db-LHLD, AUC and oral physiological availability were 10.9±1.7 IU min/mL and 16.6±2.6% for Db-LHLD in solution formulation and 10.8±1.9 IU min/mL and 16.4±2.8% for Db-LHLD in capsule. At 10 mg/kg dose, the maximum anti-FXa activities in plasma, AUC, and oral physiological availability were 0.35±0.08 IU/mL, 13.0±1.6 IU min/mL and 9.8±1.2% for Db-LHLD in solution formulation and 0.42±0.02 IU/mL, 17.8±1.3 IU min/mL and 13.5±1.0% for Db-LHLD in capsule. The conjugated DOCA molecules increased the absorption of LMWH in the intestine; on the other hand, when DMSO molecules were bound to LHD, its absorption was further increased above 2.5–3 times. When the Db-LHLD dose was 5 mg/kg, the maximum anti-FXa activity in plasma was greater than twice the minimum effective anti-FXa activity (MEC, 0.1 IU/mL) for the prevention of DVT, and the anti-FXa activity in plasma was maintained for 10 h above MEC. Particularly noteworthy is that Db-LHLD could be prepared in a solid dosage form such as in a soft gelatin capsule. When Db-LHLD was orally administered in capsules to

Fig. 3. Inhibition effect of thrombus formation by Db-LHLD and enoxaparin in rat venous thrombosis model. Seven animals were used in each group, mean±SD.

Fig. 4. Absorption profiles of orally administered Db-LHLD in monkeys. (a) The solution formulation of 5 mg/kg (○) and 10 mg/kg (△), the capsule formulation of 5 mg/kg (■) and 10 mg/kg (▲) were administered to monkeys. Db-LMWH (□) and LHD (●) of 10 mg/kg were orally administered as the control. (b) Anti-FXa activity profile after intravenous injection of Db-LHLD (1 mg/kg (◆)). The data were plotted as mean±SD, n=3.

Fig. 5. Two-week toxicodynamic profile of Db-LHLD. In order to evaluate toxicokinetic profiles of Db-LHLD in monkeys, anti-FXa activity after repeating oral administration of Db-LHLD was measured: ● PBS, ■ 10 mg/kg of Db-LHLD, ▲ 70 mg/kg of Db-LHLD, ▼ 500 mg/kg of Db-LHLD. The data were plotted as mean±SD, n=3.

Please cite this article as: S.K. Kim et al., A newly developed oral heparin derivative for deep vein thrombosis: Non-human primate study, J. Control. Release (2007), doi:10.1016/j.jconrel.2007.08.007
monkeys, the profile of anti-FXa activity in plasma was similar to that of its solution dosage form.

### 3.5. Subacute toxicity of Db-LHD

When 10, 70 and 500 mg/kg of Db-LHD were orally administered once a day for 2 weeks to monkeys, the maximum plasma FXa activities at day 1 were 0.3±0.0, 0.4±0.1 and 1.4±0.3 IU/mL, respectively, whereas their maximum plasma FXa activities at day 14 were changed to 0.6±0.1, 0.8±0.0 and 2.2±0.2 IU/mL, respectively (Fig. 5). These results showed that repeating administration of Db-LHD at such high doses as 500 mg/kg highly accumulated the plasma FXa activity. After oral administration of Db-LHD for 2 weeks, all animals

![Histology of tissue sections of intestinal membranes](image_url)
survived without any mortality. Also, there weren’t any abnormal behaviors displayed by monkeys that could be observed, and we did not observe any general symptoms such as diaphoresis, trembling, abdominal cramps, arthralgias, paralysis, vomiting, limping, weakness, etc. Even at 500 mg/kg dose of Db-LHD, none of the monkeys treated with Db-LHD showed erosions, hemorrhages and ulcerations of the small intestine. Also, any evidences of membrane damage such as mucosa hemorrhage or intestinal villi damage such as occasional epithelial cell shedding, villi fusion, congestion of mucosal capillary with blood and focal trauma were not detected by H&E stain, as shown in Fig. 6.

The hematological values of Db-LHD treated mice were similar to those of the control group (Table 1). The RBC count with hemoglobin level as an indicator of RBC balance was measured to investigate whether Db-LHD induced anemia by affecting RBC. Also, MCV, MCH and MCHC levels, which are related to the condition of RBC, were not changed in any of the groups. Moreover, the change of HCT value was not observed compared to the control. One of the important factors of heparin toxicity is the number platelets in plasma, indicating heparin-induced thrombocytopenia (HIT). However, the number of platelets in the condition of high concentration and repeated oral administration of Db-LHD was not decreased compared to the control, that is, Db-LHD would not induce severe thrombocytopenia.

Biochemical parameters, such as the AST and ALP values, were measured to evaluate the toxicity of Db-LHD on the liver and the kidney (Table 2). AST and ALP values were not changed after 14 days, whereas the ALT value was slightly increased. However, the increased ALT value was in normal values and this increase was a common phenomenon to patients who are in long-term heparin treatment. The AST/ALT ratio, which is the sensitive indicator of hepatocyte damage, was not changed in the Db-LHD treated group. Therefore, Db-LHD did not show any hepatotoxicity. Also, various biochemical levels, such as albumin, total protein, bilirubin and BUN, were not changed even at a high dose (500 mg/kg) of Db-LHD. In particular, the BUN/creatinin ratio was not changed, indicating that Db-LHD did not affect the function of the liver or kidney. The levels of inorganic ions in blood such as sodium and potassium was measured to check the alteration of biological balance induced by the oral administration of Db-LHD, and the levels of inorganic ions in blood were similar to that of the control.

## 4. Discussion

LHD was synthesized by conjugating heparin and deoxycholic acid to enable oral delivery of heparin since heparin cannot penetrate the intestinal cell membrane because of its low partition coefficient and diffusivity in tissues. LHD was not exhibit high absorption in the intestine since amphiphilic LHD makes self-assembled particles under aqueous condition due to hydrophobic interactions between the conjugated DOCA molecules [17].

The proposed technology in this study is to bind water miscible solubilizers, DMSO, to LHD by secondary interactions, such as hydrogen bonding and hydrophobic interaction. Moreover, it would produce DMSO/water molecules as the miscible solubilizers, DMSO, to LHD by secondary interactions. Such self-assembled LHDs in aqueous condition would not induce severe thrombocytopenia. More importantly, the LHD molecule favors the formation of hydrogen bonds with water due to hydrophobic interactions between the conjugated DOCA molecules. Therefore, LHD did not induce severe thrombocytopenia.
molecule. The DMSO/water molecule having high polarity as well as DMSO molecule can easily bind with hydroxyl groups of heparin.

When LHD in DMSO solution was freeze dried, some of DMSO molecules in the free state were lyophilized and others having secondary interactions with LHD remained on LHD. The binding of DMSO on LHD was confirmed by the shifted or stretched peaks in the FT-IR spectrum, which can occur only when DMSO molecules are bound to LHD by secondary interactions. The weight loss in TGA and the shift in the decomposition peak of DSC also confirmed that DMSO molecules were bound to LHD. Randomly intercalating DMSO and DMSO/water molecules in LHD could block the formation of molecular aggregation, thereby solubilizing LHD molecules in aqueous solution. Therefore, Db-LHD was highly soluble without forming any particles in aqueous solution. In particular, since the amount of bound DMSO was only about 20% based on the LHD weight, the final product would be obtained as a powder form, which could be formulated as a capsule.

The enhancement of heparin absorption by oral administration of LHD was evidenced by as much as 7.8% physiological availability in rats; however, the oral physiological availability in monkeys was only 1.7% (18). This study showed that the oral physiological availability of LHD was significantly enhanced up to about 16.6% due to the bound DMSO molecules. In particular, there was no difference in the oral physiological availability of LHD according to species, such as mice and monkeys. The minimum effective concentration required for the prevention of DVT and PE was 0.1 IU/mL. When 5 mg/kg Db-LHD was orally administered, its concentration in plasma was about twice higher than the minimum effective concentration for about 10 h. This indicates that a dosage of 5 mg/kg of Db-LHD would be enough to prevent DVT and PE in monkeys.

Especially, when we evaluated anti-thrombogenic effect of Db-LHD in rat thrombosis model, 3 or 5 mg/kg of Db-LHD would be enough to reduce thrombosis formation, compared to enoxaparin (100 IU, SC) at the recommended dose. Based on these results, it would be expected that the clinical dose would be about 200 mg once a day for a human adult since the oral bioavailability in monkeys is generally lower than that in human [24–26]; at this dose, the amount of bound DMSO is only 40 mg. DMSO in Class 3 is regarded as less toxic and a residual amounts of 50 mg DMSO per day or less is considered to be acceptable based on the FDA guideline [27–29]. In the previous study, the repeated administration of Db-LHD in 10% DMSO solution (the dose volume was 200 μL) for 30 days did not induce any symptoms related to toxicity, hematological or serological level changes [20].

When the high dose of Db-LHD (500 mg/kg) was orally administered in monkeys for 2 weeks, Db-LHD in plasma was accumulated and its peak plasma concentration was increased up to 2.2 IU/mL at 14 days. However, this dose did not show any toxicity. Since the target plasma level for the prevention of DVT and PE is 0.1 to 0.5 IU/mL, Db-LHD could be very safely used. In conclusion, we could propose Db-LHD as a non-toxic, orally active heparin which could be effectively applied to prevent DVT and PE. Furthermore, this technology might be applied to other macromolecule drugs for their oral delivery systems.

Acknowledgements

This study was supported by Mediplex Corp., Korea and was a part of the project entitled “Safety assessment of orally active cardiovascular drug using non-human primate,” supported by the Korea Food and Drug Administration (S-06-03-2-NVA-423-0-B).

References


