Conjugation of Low-Molecular-Weight Heparin and Deoxycholic Acid for the Development of a New Oral Anticoagulant Agent

Yong-kyu Lee, MSc; Jong Hee Nam, MD; Ho-Chul Shin, DVM, PhD; Youngro Byun, PhD

**Background**—Heparin administration is usually limited to intravenous or subcutaneous injection. Oral delivery of heparin is an alternative to this and has been in great demand for treating patients who are at a high risk of deep vein thrombosis or pulmonary embolism. In this study, new heparin derivatives were synthesized to enhance the oral absorption of heparin in the gastrointestinal tract.

**Methods and Results**—By using heparin of 3000 Da [LMWH(3 kDa)], heparin of 6000 Da [LMWH(6 kDa)], and unfractionated heparin (UFH), we synthesized 3 kinds of conjugates of heparin and deoxycholic acid (DOCA): LMWH(3 kDa)-DOCA, LMWH(6 kDa)-DOCA, and UFH-DOCA. After oral administration of 100 mg/kg of heparin-DOCA, the maximum activated partial thromboplastin times of the LMWH(3 kDa)-DOCA, LMWH(6 kDa)-DOCA, and UFH-DOCA were 31.0 ± 6.0, 87.8 ± 11.1, and 51.0 ± 8.7 seconds, respectively. The peak plasma concentrations of LMWH(3 kDa)-DOCA, LMWH(6 kDa)-DOCA, and UFH-DOCA were 0.06 ± 0.02, 0.76 ± 0.15, and 0.41 ± 0.13 IU/mL, respectively. The bioavailability of LMWH(6 kDa)-DOCA at the 20-mg/kg dosage was calculated to be 7.8%.

**Conclusions**—LMWH(6 kDa)-DOCA was found to have a high anticoagulant effect when administered orally and could be used as a new oral anticoagulant agent. Furthermore, the present work proposed a new method for oral delivery of macromolecules and polysaccharide drugs. (Circulation. 2001;104:3116-3120.)

**Key Words:** heparin ■ deoxycholic acid ■ conjugates ■ drugs

Heparin is one of the most potent anticoagulants and is widely used for the treatment and prevention of deep vein thrombosis (DVT) and pulmonary embolism (PE). The main disadvantage of heparin treatment, however, is that it is available to patients only by parenteral administration. On discharge from the hospital, patients are usually switched from heparin to warfarin because warfarin can be administered orally. Warfarin, however, has a slow onset and is subject to a high possibility of drug-to-drug interactions, which is why there is a great need for oral heparin.

Heparin is not absorbed in the gastrointestinal (GI) tract because of its large molecular weight and its negatively charged structure. The hydrophilic property of heparin makes it difficult for heparin molecules to penetrate through the epithelial cells because of its low permeability and repulsion forces of the polar head group of the epithelial membrane. To facilitate heparin absorption in the GI tract, several forms of heparin dosage have been developed, such as liposomes, complexes of heparin with hydrophobic organic bases, enteric coating, and aerosol formulation. There also have been attempts to evaluate the enhancing effect of EDTA, acidic buffer, or sulfated surfactants on heparin absorption in the GI tract. Recently, sodium N-[8-(2-hydroxybenzoyl)amino] caprylate (SNAC) and sodium N-[10-(2-hydroxybenzoyl)amino] decanoate (SNAD) were developed as potent promoters of heparin absorption in the GI tract.

In our previous study, we synthesized new heparin derivatives by coupling unfractionated heparin (UFH) with several kinds of hydrophobic agents in order to increase the hydrophobic property of UFH. Among those UFH derivatives, the conjugate of UFH and deoxycholic acid (DOCA) (UFH-DOCA) showed the highest absorption in the GI tract. Two possibilities were proposed to explain the results: one was the increased hydrophobic property of UFH-DOCA, and the other was the interaction between the coupled DOCA and bile receptors in the ileum. In this study, we evaluated the anticoagulant effect of the conjugate of heparin and DOCA (heparin-DOCA) in the oral administration according to the molecular weight of heparin, with a particular focus on the oral absorption of the conjugate of low-molecular-weight heparin (LMWH; 6 kDa) and DOCA.
Methods

Synthesis of Heparin-DOCA

Three kinds of heparin were used in this study: UFH, LMWH(6 kDa), and LMWH(3 kDa). UFH (167 IU/mg), whose average molecular weight was ~12 000 Da, was obtained from Pharmacia Hepar Co. LMWH(6 kDa) and LMWH(3 kDa) were obtained from Kordia Co. These heparins were extracted from porcine intestinal tissues. Heparin-DOCA was synthesized as described in our previous study. In brief, DOCA (196 mg) was mixed with dicyclohexylcarbodiimide (165 mg) and hydroxy succinimide (92 mg) in 15 mL of dimethylformamide. The mixture reacted for 5 hours at room temperature under the nitrogen atmosphere, and the precipitated dicyclohexurea was filtered. The unreacted dicyclohexyl carbodiimide was precipitated by addition of 1 mL of distilled water dropwise and filtered. The filtered solution was poured into 15 mL of distilled water. The remaining hydroxysuccinimide was dissolved in water, and the activated DOCA was precipitated and filtered. The activated DOCA reacted with heparin in the cosolvent of dimethylformamide and water (1:1) for 4 hours at room temperature. The remaining activated DOCA was removed by precipitation in water. After the heparin-DOCA solution had been lyophilized, heparin-DOCA was obtained as a white powder.

The synthesized heparin-DOCA was characterized by Fourier transform infrared spectroscopy and Fourier transform NMR to confirm the conjugation of heparin and DOCA. The molecular weight and hydrophobicity of heparin-DOCA were measured by light-scattering and reversed-phase chromatography, respectively. In the analysis of reversed-phase chromatography, 5 mL of the heparin-DOCA solution (1 mg/mL) was loaded in a phenyl-sepharose CL-4B column (HR 16/30, Pharmacia Co). Heparin-DOCA was fractionated by the gradient elution of 1.7 mol/L ammonium sulfate at the rate of 1 mL/min, and the fractionated amount of heparin-DOCA was determined by azure A assay.

In Vivo Experiments

Male Sprague-Dawley rats were fasted for 12 hours before the administration of heparin-DOCA. Rats weighing 250 to 260 g were anesthetized with light diethyl ether and given heparin-DOCA through an oral gavage tube that was carefully passed down the esophagus into the stomach. The gavage tube was made of stainless steel with a blunt end to avoid causing lesions on the tissue surface. The heparin-DOCA solution was prepared in sodium bicarbonate buffer (pH 7.4). The total volume of the administered heparin-DOCA solution was 0.3 mL, with the dose amount varying from 20 to 100 mg/kg. Blood (450 μL) was collected serially from a capillary in the retro-orbital plexus at each time and directly mixed with 50 μL of sodium citrate (3.8% solution). The blood samples were immediately centrifuged at 2500g at 4°C for 15 minutes. At least 9 rats were used for each case.

The activities of heparin-DOCA conjugate in the prevention of the fibrin clot formation were measured by activated partial thromboplastin time (aPTT) assay. Each of the platelet-poor plasma samples containing heparin standards (0 to 2.8 IU/mL), 0.1 mL and plasma samples containing heparin derivatives (0.1 mL) was incubated with 0.1 mL of aPTT reagent for 2 minutes at 37°C. After the incubation, 0.1 mL of 0.02 mol/L calcium chloride was added, and the time was recorded from this point until the fibrin clot was formed. The clotting time was linearly proportional to the bioactivity of heparin when it was <200 seconds.

The concentrations of heparin-DOCA conjugate were also determined by factor Xa (FXa) chromogenic assay. Each of the heparin standards and plasma samples containing heparin derivatives (25 μL) was mixed with 200 μL of antithrombin III solution (0.1 IU/mL), where antithrombin III concentration was in excess to the heparin concentration. This solution was incubated at 37°C for 2 minutes, followed by the addition of 200 μL of FXa (4 nanokatals per milliliter). The resulting solution was incubated for an additional minute. FXa substrate (200 μL, 0.8 μmol/mL) was then added and incubated at 37°C for 5 minutes. The reaction was terminated by addition of 200 μL of acetic acid (50% solution). The absorbance of the final solution at 405 nm had a linear relationship with the concentration of heparin in the range of 0 to 0.8 IU/mL.

Pharmacokinetic Analysis

LMWH(6 kDa)-DOCA was administered to rats in two groups, 1 receiving the intravenous bolus injection and the other receiving the oral dose. LMWH(6 kDa)-DOCA (2 mg/kg) was injected via the tail vein of the intravenous bolus injection group, whereas LMWH(6 kDa)-DOCA (20 mg/kg) was administered orally to the oral dose group as described above. The blood samples (225 μL) were obtained from the tail vein by intravenous catheter at each time interval for 300 minutes. The blood samples were immediately mixed with 25 μL of sodium citrate (3.8% solution), followed by centrifugation at 2500g at 4°C for 15 minutes. The concentration of LMWH(6 kDa)-DOCA in the plasma was immediately measured by FXa assay. The elimination half-life (t1/2) was determined by linear regression analysis after the transformation of concentration into logarithmic values. The slope of the regression line representing the elimination phase was k. The area under the curve (AUC) in the concentration-time profile was calculated with the linear trapezoidal method, and the volume of distribution was calculated as dose/AUC/k. The clearance was calculated as the ratio of dose to AUC. The bioavailability of oral administration compared with intravenous injection was calculated by the ratio of the respective AUCs estimated after the administration of each different dosage.

Results

Characterizations of Heparin-DOCA

The coupling of DOCA to heparin was proved by the presence of amide bonds that were formed by coupling reactions between the carboxyl group of DOCA and the amine group of heparin. In the Fourier transform infrared spectrum, the peaks at 1720 and 1585 cm⁻¹ indicated the presence of amide bonds in heparin-DOCA. In the [¹H]NMR and the [¹3C]NMR spectra, the amide peak also occurred at 7.58 and 178 ppm, respectively. It was thus confirmed that heparin was successfully coupled with DOCA. The average molecular weights of LMWH(3 kDa), LMWH(6 kDa), and UFH, measured by light scattering, were 2910, 6150, and 12 386 Da, respectively. In our previous study, the maximum coupling ratio of DOCA to UFH was obtained when the feed mole ratio of UFH to DOCA was ~1:200. In this condition, the average molecular weights of LMWH(3 kDa)-DOCA, LMWH(6 kDa)-DOCA, and UFH-DOCA were 3410, 7576, and 16 320 Da, respectively. The molecular weights of LMWH(6 kDa)-DOCA and UFH-DOCA were in the range of 7200 to 8600 Da and from 6600 and 39 500 Da, respectively. The increased molecular weight of heparin-DOCA was due to the coupled DOCA. The coupling ratio of DOCA to heparin was calculated by subtracting the molecular weight of heparin from the molecular weight of heparin-DOCA, and then dividing by the molecular weight of DOCA. This value indicated the average number of DOCA molecules that were coupled with 1 heparin molecule. The calculated coupling ratios of DOCA to heparin were 1.3, 3.6, and 10.0 for LMWH(3 kDa)-DOCA, LMWH(6 kDa)-DOCA, and UFH-DOCA, respectively, as shown in Table 1. That is, in the case of LMWH(6 kDa)-DOCA, 3.6 molecules of DOCA were coupled with 1 molecule of LMWH(6 kDa). The bioactivity of heparin-DOCA decreased as the coupling ratio of DOCA to heparin increased. All kinds of heparin-DOCA, however, showed >70% relative bioactivity compared with the unmodified heparin, as shown in Table 1.
Figure 1 shows the elution curves of UFH and heparin-DOCA in reversed-phase chromatography. As the concentration of ammonium sulfate in the eluting solution increased, the hydrophobicity of the eluted heparin-DOCA was increased. UFH was eluted only by PBS, because UFH is highly hydrophilic. The elution curve of LMWH(3 kDa)-DOCA showed 3 peaks, and the concentration ranges of ammonium sulfate of each peak were 0 (buffer only), 0.06 to 0.34, and 0.45 to 1.47 mol/L, respectively. The total eluted amounts of LMWH(3 kDa)-DOCA in each peak were 11.2%, 68.9%, and 19.8%, respectively. In the case of LMWH(6 kDa)-DOCA, there were 2 major peaks in the elution curve in the range of 0 to 0.17 and 1.13 to 1.47 mol/L ammonium sulfate. The total eluted amounts of LMWH(6 kDa)-DOCA in each peak were 29.7% and 70.3%, respectively. Conversely, UFH-DOCA showed a broadly distributed elution curve in the range of 0.28 to 1.53 mol/L ammonium sulfate.

Oral Absorption of Heparin-DOCA

Figure 2 demonstrates the absorption of heparin-DOCA in the GI tract for the different molecular weights of heparin. After oral administration of 100 mg/kg of heparin-DOCA, the maximum clotting times of LMWH(3 kDa)-DOCA, LMWH(6 kDa)-DOCA, and UFH-DOCA were observed at 1 hour after dosing and were 31.0±6.0, 87.8±11.1, and 51.0±8.7 seconds (P<0.005), respectively. The baseline of the clotting time averaged 20 seconds. These results indicated that LMWH(6 kDa)-DOCA was significantly absorbed in the GI tract and that its maximum clotting time was 6 times higher than that of LMWH(3 kDa)-DOCA. The concentration profiles of 3 kinds of heparin-DOCA with time obtained from FXa assay were similar to the clotting time profiles from aPTT assay. The peak plasma concentrations of LMWH(3 kDa)-DOCA, LMWH(6 kDa)-DOCA, and UFH-DOCA were 0.06±0.02, 0.76±0.15, and 0.41±0.13 IU/mL, respectively.

When 20 mg/kg of LMWH(6 kDa) was orally administered to rats, the clotting time was around 20 seconds (Figure 3), which was the same value as the baseline. This result indicated that LMWH(6 kDa) was not absorbed in the GI tract. When the dosage of LMWH(6 kDa) was increased to 100 mg/kg, the clotting time increased slightly and fell to the baseline at 2 hours after dosing. Conversely, when 20, 50, and 100 mg/kg of LMWH(6 kDa)-DOCA were orally administered, the maximum clotting times were 52.5±4.7, 68.4±7.2, and 87.8±11.1 seconds (P<0.005), respectively. The clotting time reached the baseline.

### Table 1. Characteristics of Heparin-DOCA

<table>
<thead>
<tr>
<th>Average Molecular Weight, Da</th>
<th>Bioactivity by aPTT Assay, IU/mg</th>
<th>Bioactivity by FXa Assay, IU/mg</th>
<th>Coupling Ratio of DOCA to Heparin</th>
</tr>
</thead>
<tbody>
<tr>
<td>LMWH(3 kDa)</td>
<td>2910</td>
<td>50.8±4.9</td>
<td>124.7±0.8</td>
</tr>
<tr>
<td>LMWH(3 kDa)-DOCA</td>
<td>3410</td>
<td>40.0±7.7</td>
<td>121.5±1.6</td>
</tr>
<tr>
<td>LMWH(6 kDa)</td>
<td>6150</td>
<td>127.1±2.4</td>
<td>148.4±0.2</td>
</tr>
<tr>
<td>LMWH(6 kDa)-DOCA</td>
<td>7576</td>
<td>108.5±4.9</td>
<td>134.3±0.8</td>
</tr>
<tr>
<td>UFH-DOCA</td>
<td>12386</td>
<td>184*</td>
<td>167*</td>
</tr>
</tbody>
</table>

*Used as a standard.

![Figure 1](http://example.com/figure1.png)  
**Figure 1.** Elution profiles of heparin-DOCA in reversed-phase chromatography. ◦ indicates UFH; ●, LMWH(3 kDa)-DOCA; ○, LMWH(6 kDa)-DOCA and □, UFH-DOCA.

![Figure 2](http://example.com/figure2.png)  
**Figure 2.** Clotting time profiles (A) and concentration profiles (B) of heparin-DOCA after oral administration in rats. ◦ indicates LMWH(3 kDa)-DOCA; ●, LMWH(6 kDa)-DOCA; and ○, UFH-DOCA.
maximum at 1 hour after dosing and fell to the baseline at 4 hours after dosing.

The pharmacokinetic parameters of LMWH(6 kDa) and LMWH(6 kDa)-DOCA were calculated as shown in Table 2. The $t_{1/2}$ and the clearance of LMWH(6 kDa) were 5.7 hours and 1.7 mL · min$^{-1}$ · kg$^{-1}$, respectively. Conversely, the $t_{1/2}$ and the clearance of LMWH(6 kDa)-DOCA were 4.5 hours and 2.3 mL · min$^{-1}$ · kg$^{-1}$, respectively. The $t_{1/2}$ of LMWH(6 kDa) was slightly decreased by grafting with DOCA. Conversely, the volume of distribution of LMWH(6 kDa) and LMWH(6 kDa)-DOCA were almost the same. When LMWH(6 kDa)-DOCA was administered by the intravenous bolus injection (2 mg/kg) and the oral dose (20 mg/kg), the values of AUC were 860.1 and 667.8 μmol · min$^{-1}$ · mL$^{-1}$, respectively. The calculated bioavailability of LMWH(6 kDa)-DOCA for oral dose was 7.8%.

Discussion

The aPTT assay and FXa assay gave different results concerning the bioactivity of heparin with respect to its molecular weight. As the molecular weight decreased, the bioactivity measured by FXa assay slightly decreased, whereas the bioactivity measured by aPTT assay greatly decreased. The bioactivities of UFH and UFH-DOCA measured by FXa assay were similar to those measured by aPTT assay. The bioactivities of LMWH(3 kDa)-DOCA and LMWH(3 kDa) measured by FXa assay, however, were much lower than those measured by aPTT assay. The reason for this is that high-molecular-weight heparin, such as UFH, has an inhibition activity against thrombin as well as FXa, whereas low-molecular-weight heparin (<5400 Da) has mainly anti-FXa activity. In this study, the efficiency of absorbed heparin to prevent blood clotting was evaluated by aPTT assay, and the concentration of heparin-DOCA in plasma was evaluated by FXa assay.

Heparin-DOCA was synthesized by coupling the carboxylic group of DOCA with the amine groups of heparin. Heparin-DOCA showed as much high bioactivity as the unmodified heparin, because there are no amine groups in the active site of heparin. Also, the steric hindrance of the coupled DOCA might be low, because DOCA is a small molecule. The coupling ratio of DOCA to heparin decreased with the decrease of molecular weight of heparin because the number of amine groups of heparin had decreased. Therefore, the hydrophobicity of heparin-DOCA decreased with the decreasing molecular weight of heparin and the coupling ratio of DOCA to heparin.

Several factors affected the anticoagulant effect of heparin-DOCA in oral administration: the hydrophobicity, which is decided by the coupling ratio of DOCA to heparin; the molecular weight of heparin-DOCA; and the bioactivity, which is an inherent property of heparin. The hydrophobicity of heparin-DOCA is important to increase its permeability through the mucous cell layer in the GI tract. Also, the coupling ratio of DOCA to heparin may be important to enhance the interaction between the coupled DOCA and the bile receptor in the ileum. Among the 3 kinds of heparin-DOCA, LMWH(6 kDa)-DOCA showed the highest anticoagulant effect in oral administration. LMWH(6 kDa)-DOCA had higher bioactivity, a higher coupling ratio of DOCA to heparin, and greater hydrophobic property than LMWH(3 kDa)-DOCA. Conversely, LMWH(6 kDa)-DOCA had similar bioactivity and a lower molecular weight than UFH-DOCA.

In the pharmacokinetic results of LMWH(6 kDa)-DOCA, the coupled DOCA decreased the $t_{1/2}$ of LMWH(6 kDa)-DOCA and increased its clearance, because LMWH(6 kDa)-DOCA might have slightly higher binding affinity with proteins than LMWH(6 kDa) in the plasma. On the basis of these results, we proposed that heparin-DOCA might exist as a conjugate form in the blood circulation without dissociation of the coupled DOCA from heparin, because they were covalently coupled with heparin by a stable amide bond. For LMWH(6 kDa)-DOCA, the clotting time at 20 mg/kg was in the range of the therapeutic window, which was 1.5 to 2.5 times the baseline, and its bioavailability at this dosage in oral administration was calculated to be as high as 7.8%.

**TABLE 2. Pharmacokinetic Parameters of LMWH (6 kDa) and LMWH(6 kDa)-DOCA After Oral and Intravenous Administration**

<table>
<thead>
<tr>
<th>Heparin</th>
<th>Dose, mg/kg</th>
<th>$C_{\text{max}}, \mu\text{g/mL}$</th>
<th>AUC, $\mu\text{g} \cdot \text{mL}^{-1} \cdot \text{min}^{-1}$</th>
<th>$V_{\text{d}}, \text{L/kg}$</th>
<th>$CL, \text{mL} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$</th>
<th>$t_{1/2}, \text{h}$</th>
<th>$F, %$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intravenous</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LMWH(6 kDa)</td>
<td>2</td>
<td>9.4±2.6</td>
<td>1176±212</td>
<td>0.85±0.08</td>
<td>1.7±0.4</td>
<td>5.7</td>
<td>...</td>
</tr>
<tr>
<td>LMWH(6 kDa)-DOCA</td>
<td>2</td>
<td>9.7±2.4</td>
<td>860±150</td>
<td>0.90±0.18</td>
<td>2.3±0.5</td>
<td>4.5</td>
<td>...</td>
</tr>
<tr>
<td>Oral</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LMWH(6 kDa)</td>
<td>100</td>
<td>1.3±0.4</td>
<td>120±15</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>0.0</td>
</tr>
<tr>
<td>LMWH(6 kDa)-DOCA</td>
<td>20</td>
<td>5.3±0.5</td>
<td>668±250</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>7.8</td>
</tr>
</tbody>
</table>

$C_{\text{max}}$ indicates maximum concentration or LMWH; $V_{\text{d}}$, volume of distribution; $CL$, clearance; and $F$, bioavailability.
Recently, SNAC and SNAD have been developed as potent promoters of heparin absorption in the GI tract. Because SNAC and SNAD act as enhancers for the permeation of heparin through the mucous layer without any chemical reaction or conjugation with heparin, they caused no change to the heparin properties. Conversely, the heparin-DOCA developed in our study was chemically modified and could be orally administered without any enhancers or additives. It was reported that when 300 mg/kg of SNAC was administered orally in combination with 100 mg/kg heparin in rats, the peak clotting time in aPTT assay was ≈101 seconds. Salatar et al also reported that oral administration of LMWH with its carrier SNAD caused significant elevation in FXa levels. After oral administration of 15 mg/kg LMWH mixed with 300 mg/kg SNAD, the peak level of FXa in rats was ≈1 IU/mL at 30 minutes. The bioavailability of an oral administration of 150 mg/kg delivery agent and 100 mg/kg heparin in rat was 6%. In our study, when 100 mg/kg of LMWH(6 kDa)-DOCA was administered orally in rats without any enhancers, the mean peak aPTT responses showed ≈90 seconds. Also, we found that the FXa level of LMWH(6 kDa)-DOCA was 0.76±0.15 IU/mL after 100 mg/kg of LMWH-DOCA was administered to rats without any delivery agents, and the bioavailability was 7.8% at the 20 mg/kg dose. The efficacy of LMWH-DOCA in the total dosage was higher than that of LMWH combined with SNAC or SNAD.

Although significant adverse events in patients in whom SNAC was orally administered have not been reported, nausea was noted in some patients, and emesis occurred occasionally. Some reported adverse events included headache, diarrhea, abdominal pain, dyspepsia, and pharyngitis, although a causal relationship between these events and orally administered SNAC was not determined. Conversely, although heparin-DOCA was administered orally at as much as 200 mg/kg, heparin-DOCA did not show any damage on the surface of the GI tract, and side effects, such as bleeding and heparin-induced thrombocytopenia, were not detected (data not shown). Further work will be performed to evaluate clinical toxicities of heparin-DOCA.

It has been reported that heparin can induce the release of tissue factor pathway inhibitor (TFPI). When heparin was administered intravenously or subcutaneously, the bound intravascular TFPI on the endothelium was released from the vascular endothelium. The released amount of bound TFPI decreased with the decrease in the molecular weight of heparin. Other sulfated polysaccharides, such as pentosan polysulfate, fucoidan, and β-1,3-glucan sulfates, also caused the release of bound TFPI from the vascular endothelium. These results demonstrated that the release of bound TFPI from vascular endothelium depended on sulfate groups in the heparin structure. Therefore, it was expected that the effect of heparin-DOCA conjugate on the release of TFPI might be similar to that of heparin, because DOCA molecules were coupled with only amine groups of heparin when heparin-DOCA conjugate was synthesized.

In conclusion, LMWH(6 kDa)-DOCA was found to have a high anticoagulant effect in oral administration and could be used as a new oral anticoagulant agent. Furthermore, the present work proposed a new method of oral delivery of macromolecules and polysaccharide drugs.

Acknowledgments

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References

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