Oral Delivery of New Heparin Derivatives in Rats

Yong-kyu Lee,[1] Sun Hee Kim,[2] and Youngro Byun[1,3]

Received March 15, 2000; accepted July 7, 2000

Purpose. In this study, conjugates of heparin and deoxycholic acid were synthesized in order to enhance the heparin absorption in the GI tract. Oral delivery of heparin is a preferred therapy in the treatment of patients who are at high risk of deep vein thrombosis and pulmonary embolism.

Methods. Several different kinds of heparin derivatives were synthesized, and their absorption in the GI tract was determined by activated partial thromboplastin time (aPTT) and factor Xa (FXa) assay. Any histological changes caused by heparin derivatives were examined by hematoxylin and eosin (H&E) stain and transmission electron microscopy (TEM).

Results. After administering heparin-DOCA orally, the clotting time in aPTT assay was increased with the increase of the coupled DOCA amount. The maximum clotting time of heparin-DOCA was 136±33 sec at 200 mg/kg of oral dose. This value was 7 times higher than the baseline. The absorption of heparin-cholesterol, heparin-palmitic acid, and heparin-lauric acid conjugates in the GI tract was lower than that of heparin-DOCA. Histological examination of the GI tract indicated that heparin derivatives did not cause any damage to the microvilli and the cell layer.

Conclusions. DOCA coupled with heparin greatly enhanced absorption of heparin in the GI tract, and this enhancing effect was induced without changing the tissue structure of the GI wall.

KEY WORDS: oral delivery; heparin derivatives; deoxycholic acid; chemical conjugate.

INTRODUCTION

Heparin is a potent anticoagulant agent that interacts strongly with antithrombin III to prevent the formation of a fibrin clot (1). Heparin is used in the treatment of patients who have a high risk of deep vein thrombosis and pulmonary embolism (2,3). However, heparin is currently available to patients only by parenteral administration since it is both hydrophilic and highly negatively charged, and has a molecular weight that is as high as 12,000 dalton (4,5). Compared to parenteral administration, oral administration of heparin would be extremely convenient for patients needing daily treatments. This is why the development of oral heparin is highly desired.

Many research groups have tried to develop new formulations or enhancers for oral heparin delivery. There have been several different dosage forms, such as liposomes, hydrophobic organic bases, spermine and lysine salts, or monoolein complex, in order to facilitate heparin absorption into the gastrointestinal (GI) mucosa (6–8). Formulations using enteric coating materials, an intrapulmonary aerosol of sodium heparin, or heparin complex were also developed for oral delivery of heparin (9). Other researchers attempted to evaluate the effects of ethylenediaminetetraacetic acid (EDTA), acidic buffer, or sulfated surfactants on the heparin absorption in the GI tract (10). Recently, n-[8-(2-hydrocybenzoyl)amino] caprylate (SNAC) was developed as a potent promoter of heparin absorption from the GI tract (11,12).

In this study, we have synthesized new heparin derivatives by coupling heparin with deoxycholic acids (DOCA), since DOCA is a naturally occurring substance and can increase the hydrophobic property of heparin. We evaluated the effects of the coupled DOCA on the heparin absorption in the GI tract in rats. Histological evaluations of the GI tract were also performed by H&E stain and TEM after administering the heparin derivatives orally.

MATERIALS AND METHODS

Materials

Heparin sodium (140 IU/mg), whose average molecular weight is 12,000 dalton, was obtained from Pharmacia Hepar Co. (Franklin, OH). Deoxycholic acid (DOCA) and cholesterol were obtained from Sigma Chemical Co. (St. Louis, MO). Palmitic acid and lauric acid were purchased from Aldrich (Milwaukee, WI). Dicyclohexylcarbodiimide (DCC) and hydroxysuccinimide (HOSu) were purchased from Sigma Chemical Co. (St. Louis, MO). Dimethylformamide (DMF) was obtained from Merck (Darmstadt, Germany) and used without further purification. APTT(activated partial thromboplastin time) reagent, Factor Xa (FXa), antithrombin III (ATIII), FXa substrate, and Azure A were purchased from Sigma Chemical Co. (St. Louis, MO).

Synthesis of Heparin Derivatives

The conjugate of heparin and DOCA (heparin-DOCA) was synthesized, as reported in our previous study (13). 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 reacted for 5 h at room temperature in a vacuum and the precipitated dicyclohexylurea was filtered. The unreacted DCC was precipitated by adding 1 ml of distilled water dropwise, 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 reacted with heparin in the co-solvent of DMF and water (1:1) for 4 h at room temperature. The remaining activated DOCA was removed by precipitating it in water. After lyophilizing

[1] Department of Materials Science and Engineering, Kwangju Institute of Science and Technology, Kwangju 500-712, Korea.

[2] Department of Clinical Pathology, Samsung Medical Center, Seoul 135-710, Korea.

[3] To whom correspondence should be addressed at Department of Materials Science and Engineering, Kwangju Institute of Science and Technology, 1 Oryong-dong, Puk-gu, Kwangju 500-712, Korea. (e-mail: yrbyun@kjist.ac.kr)

ABBREVIATIONS: GI tract, gastrointestinal tract; aPTT, activated partial thromboplastin time; Fxa, factor Xa; ATIII, antithrombin III; H&E, hematoxylin and eosin; TEM, transmission electron microscopy; Heparin-DOCA, the conjugate of heparin and deoxycholic acid; DOCA, deoxycholic acid; DCC, dicyclohexylcarbodiimide; HOSu, hydroxysuccinimide; DMF, dimethylformamide.

the heparin-DOCA solution, heparin-DOCA was obtained as white powder.

For the heparin–cholesterol conjugate, most of the experimental procedures were the same with those of heparin-DOCA, except for the alkylation step. Since cholesterol has a hydroxy group and no carboxyl groups, a carboxyl group had to be developed in order to couple the cholesterol with the amine group of heparin. The cholesterol (200 mg) was reacted with 40 mg of choloroacetic acid at 40°C for 5 h, thereby developing a carboxyl group by an alkylation process of a hydroxy group of cholesterol. Palmitic acid and lauric acid were also coupled with heparin, respectively. The coupling method was the same as that of heparin-DOCA, that is, the carboxyl group of alkanoic acid was coupled with the amine groups of heparin.

The synthesized heparin-DOCA was further purified by reverse phase chromatography. A phenyl-sepharose CL-4B column (HR 16/30 I.D.) was washed with 100ml of distilled water, 40 ml of 50mM phosphate buffer (pH 7.0), 40 ml of 50 mM phosphate buffer (pH 7.0) containing 1.7 M ammonium sulfate, and 40 ml of 50 mM phosphate buffer. Five milliliters of the heparin-DOCA solution (1 mg/ml) was loaded in the column, and the heparin-DOCA was fractionated by step elution of an ammonium sulfate solution. The phosphate buffer was eluted for 20 min, followed by the ammonium sulfate solution (50 mM phosphate buffer (pH 7.0) + 1.7 M ammonium sulfate) with the flow rate of 1 ml/min. Heparin-DOCA was obtained in the elution process by using the ammonium sulfate solution, as described in our previous study. The heparin-DOCA solution was dialyzed in distilled water and lyophilized.

The synthesized heparin derivatives were characterized by FT-IR and ^{13}C-NMR to prove the coupling between heparin and hydrophobic agents as described in our previous study (13). The amount of coupled hydrophobic agents in the heparin derivatives were determined by measuring their molecular weights by Light Scattering (series 4700, Malvern Instruments Ltd., Worcestershire, UK).

Measurement of Bioactivity of Heparin Derivatives

Anticoagulant activities of heparin derivatives were determined by aPTT assay and FXa chromogenic assay. The activities of heparin derivatives in the prevention of fibrin clot formation were measured by aPTT assay. Each of the platelet-poor, plasma-containing heparin standards (0.1 to 0.7 U/ml, 0.1 ml) and plasma samples containing heparin derivatives (0.1 ml) were incubated with 0.1ml of aPTT reagent for 2 min at 37°C. After the incubation, 0.1 ml of 0.02 M calcium chloride was added, and the time was recorded from this point until the fibrin clot was formed. The bioactivity of the heparin derivative was calculated by comparing the clotting time with the heparin standard curve. The clotting time was linearly proportional to the activity of heparin in the plasma.

The activity and the concentration of heparin derivatives were also determined by FXa chromogenic assay. Each of the heparin standards and plasma samples containing heparin derivatives (25 μl) were mixed with 200 μl of AT III solution (0.1 IU/ml), where the ATIII concentration was in excess of the heparin concentration. This solution was incubated at 37°C for 2 min, and 200 μl of FXa (4 nkcat/ml) was added. The resulting solution was then incubated for an additional 1 min. The concentration of FXa was also in excess of the heparin concentration. FXa substrate (200 μl, 0.8 μmol/ml) was then added and incubated at 37°C for 5 min. The reaction was terminated by adding 200 μl of acetic acid (50% solution). The bioactivity and the concentration of heparin in the plasma sample were calculated from the absorbance at 405 nm.

In vivo Experiments

Sprague–Dawley rats (male, 250–260g) were fasted for 12 h before dosing. The rats were anesthetized with diethyl ether and administered with a single oral dose of heparin derivatives through an oral gavage that was carefully passed down the esophagus into the stomach. The gavage is made of stainless steel with a blunt end to avoid causing lesions on the tissue surface. The heparin derivative solution was prepared in a sodium bicarbonate buffer (pH 7.4). The total administered volume of the heparin derivative solution was 0.3 ml. The dose amount was varied at 50, 80, 100, and 200 mg/kg. There were 9 rats in each group. Blood (450 μl) was collected serially by capillary from 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 2500×g and 4 °C for 5 min. The clotting time and the concentration of heparin derivative in the plasma were measured by aPTT assay and FXa assay, respectively.

Histological Evaluation of the Gastrointestinal Tract

Heparin-DOCA was administered to rats by oral gavage as described above. The mole ratio of coupled DOCA to heparin in heparin-DOCA was 10; that is, ten molecules of DOCA were coupled with one molecule of heparin, and the dose amount was 200 mg/kg. At 1, 2, and 3 hours after dosing, rats were anesthetized with diethyl ether, and were sacrificed by cutting the diaphragm. Gastric, duodenal, jejunal, and ileal tissues were removed from the rats and fixed in neutral buffered formalin for processing. The GI tissues before administering heparin-DOCA were prepared as control samples. 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 at –20°C, and picked up on a glass slide. The tissue sections were then washed with xylene and absolute alcohol, in order to remove paraffin. The prepared 5 μm sections were stained with the use of hematoxylin and eosin (H&E). At least 4 rats were used for each case. For the transmission electron microscopy) evaluation, the gastric, duodenal, jejunal, and ileal tissues were fixed with 1% osmium tetroxide in phosphate-buffered saline (PBS) (0.1 M, pH 7.4), and then hydrated by changing the alcohol concentration gradually from 50 to 100 %. The hydrated tissues were infiltrated with propylene oxide and embedded with an epon mixture. The embedded tissues were sectioned as 50–60 nm thickness slides. These slides were stained very lightly with uranyl acetate and lead citrate for 1 min, and were observed with TEM (Hitachi 7100, Tokyo, Japan).

RESULTS AND DISCUSSION

In the synthesis of heparin-DOCA, the carboxyl group of DOCA was coupled with amine groups of heparin. If other

functional groups of heparin, such as sulfonyl, carboxyl, or hydroxyl groups, had been used in the coupling reaction in order to increase the binding mole ratio of DOCA to heparin, the activity of heparin derivatives would have completely disappeared. This is because there are sulfonyl, carboxyl, and hydroxyl groups in the active site of heparin, and the coupled DOCA can block the active sites of heparin. However, since there are no amine groups in the active site of heparin, only the amine group of heparin is available for the conjugation.

The coupling of DOCA to heparin was proved by the presence of an amide bond that was formed by a coupling reaction between the carboxyl group of a hydrophobic agent and the amine group of heparin. In the FT-IR spectrum, the peak at 1,720 cm^{-1} indicated the presence of amide bonds in all kinds of heparin derivatives. In the ^{13}C-NMR spectrum, the amide peak at 178 ppm also occurred in all cases of heparin derivatives. It was thus confirmed that heparin was successfully coupled with hydrophobic agents. The amount of coupled hydrophobic agents in a heparin derivative was calculated by subtracting the molecular weight of heparin from the molecular weight of the heparin derivative, as shown in Table 1. The molecular weight of heparin, measured by light scattering, was 12,386 dalton. For heparin-DOCA, the maximum coupling ratio of DOCA to heparin was ten.

The absorption of heparin-DOCA in the GI tract was determined according to the dose amount in the range of 50–200 mg/kg. In this experiment, the mole ratio of coupled DOCA to heparin in heparin-DOCA was 10. When raw heparin was administered orally to rats, the clotting time, as measured by aPTT assay, was about 18 sec, and it did not change over time. The average value of the baseline was 18 sec, indicating that the raw heparin was never absorbed in the GI tract. When the physical mixture of heparin and DOCA was administered orally, the aPTT value was about 20 sec, and this value did not change with time. On the other hand, when heparin-DOCA was orally administered, the clotting time increased as shown in Fig. 1. Since the blood sampling was carried out at one-hour intervals and the maximum clotting time was shown at the first hour, the real maximum clotting time could not be determined. However, the clotting time at one hour was linearly increased with the increase of dosage. When heparin-DOCA was given at 50, 80, 100, and 200 mg/kg, the clotting times at one hour were 25.7±2.6, 43.1±4.0, 51.2±9.3, and 136±33 sec, respectively. When heparin-DOCA was administered at 200 mg/kg, the clotting time at one hour highly increased, to more than 7 times the baseline. Since the therapeutic window of heparin is 1.5–2.5 times the baseline, the therapeutic effect can be seen at a dose of 80–100 mg/kg. Therefore, the coupled DOCA to heparin greatly enhanced the absorption of heparin in the GI tract, whereas DOCA, which was physically mixed with heparin, could not enhance the heparin absorption.

Table 1. Characteristics of Heparin and Heparin Derivatives

Compound	Mole ratio of coupled hydrophobic agent	Bioactivity (IU/mg)	Molecular weight
Heparin	—	140	12,386
Heparin-DOCA	2.5	130 ± 1.0	13,357
Heparin-DOCA	5.0	113 ± 2.8	14,403
Heparin-DOCA	10.0	100 ± 4.3	16,320
Heparin-cholesterol	4.5	122 ± 6.7	13,791
Heparin-lauric acid	5.0	118 ± 5.0	13,400
Heparin-palmitic acid	4.4	123 ± 2.7	13,500

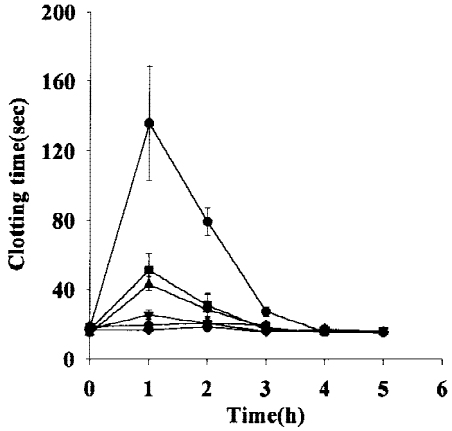

Fig. 1. Clotting time profiles of heparin-DOCA after administering orally in rats. The clotting time was measured by aPTT assay; raw heparin: (♦) 100 mg/kg; (●) the physical mixture of heparin (200 mg/kg) and DOCA (200 mg/kg); heparin-DOCA: (▼) 50 mg/kg, (▲) 80 mg/kg, (■) 100 mg/kg, (●) 200 mg/kg. The data are plotted as mean ± SD, n = 9.

The concentration of heparin-DOCA in the plasma was determined by FXa assay as shown in Fig. 2. The concentration profiles of heparin-DOCA over time were similar to the results of aPTT assay (Fig. 1). The concentration of absorbed heparin-DOCA increased with the increase of the dosage. The therapeutic target range was 0.1 to 0.2 IU/ml. For a 200 mg/kg dose of heparin-DOCA, the mean concentration peak at one hour was about 9–10 times the baseline, and the concentration at that time was around 1.0 IU/ml. The plasma concentration of heparin-DOCA returned to the baseline after 3 h. Therefore, the absorption of heparin-DOCA in the GI tract was confirmed again.

In order to determine the heparin-DOCA absorption in the GI tract according to the coupled ratio of DOCA to hep-

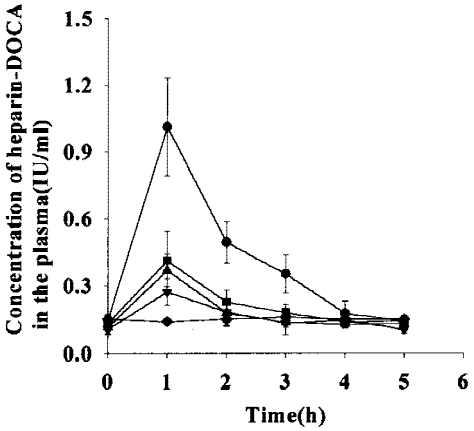

Fig. 2. Change of the concentration profile of heparin-DOCA according to the dose amount after administering orally in rats. The concentration was measured by FXa chromogenic assay; raw heparin: (♦) 100 mg/kg; heparin-DOCA: (▼) 50 mg/kg, (▲) 80 mg/kg, (■) 100 mg/kg, (●) 200 mg/kg. The data are plotted as mean ± SD, n = 9.

arin, three kinds of different heparin-DOCA were synthesized with such varied mole ratios of coupled DOCA to heparin as 2.5, 5.0, and 10.0. As shown in Table 1, the bioactivity of heparin-DOCA slightly decreased as the mole ratio of DOCA to heparin was increased. When the mole ratios of coupled DOCA to heparin were 2.5, 5.0, and 10.0, the bioactivity, based on weight, was 130±1.0, 113±2.8, and 100±4.3 IU/mg, respectively. However, since the molecular weight of heparin-DOCA increased as the mole ratio of DOCA to heparin increased, the bioactivity of heparin-DOCA, based on mole concentration, decreased only about 5%; that is, the bioactivities of heparin and heparin-DOCA (coupled DOCA: heparin = 10:1) were 1,734 and 1,632±7 IU/mol, respectively.

Figure 3 shows the change in the clotting time according to the coupled mole ratio of DOCA to heparin. In this experiment, the dosage of heparin-DOCA was 100 mg/kg. When the mole ratio of the coupled DOCA to heparin was increased, the bioactivity of heparin-DOCA decreased slightly as shown in Table 1, whereas the maximum clotting time increased. This result indicates that the coupled DOCA to heparin facilitated the absorption of heparin in the GI tract.

In order to show the effect of a hydrophobic agent coupled with heparin on GI absorption, four kinds of heparin derivatives were synthesized: heparin-DOCA, heparin-cholesterol, heparin-palmitic acid, and heparin-lauric acid. In each case, the mole ratio of coupled hydrophobic agent to heparin was controlled in the range of 4 to 4.5 as shown in Table 1. The bioactivity of each heparin derivative was similar to each other and was in the range of 113 to 123 IU/mg. This may be explained by the fact that the amine groups of heparin were used for the coupling reaction, and that they were not in the active site of heparin. The absorptions of different kinds of heparin derivatives such as heparin-DOCA, heparin-cholesterol, heparin-palmitic acid, and heparin-lauric acid in the GI tract are shown in Fig. 4.

In this experiment, the dosage of heparin derivatives was 100 mg/kg, and the clotting time of the plasma was measured by aPTT assay. After heparin-cholesterol, heparin-palmitic acid, and heparin-lauric acid had been administered, the maximum clotting times at one hour were 32±6.1, 29±8.3, and

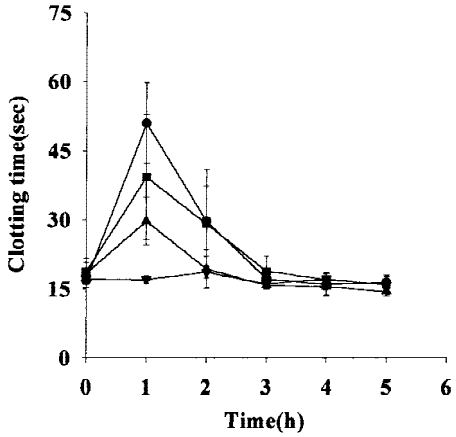

Fig. 3. Clotting time profiles of heparin-DOCA according to coupling ratio of DOCA to heparin; (▼) raw heparin, (▲) 2.5 mole ratio, (■) 5.0 mole ratio, (●) 10.0 mole ratio. The data are plotted as mean ± SD, n = 9.

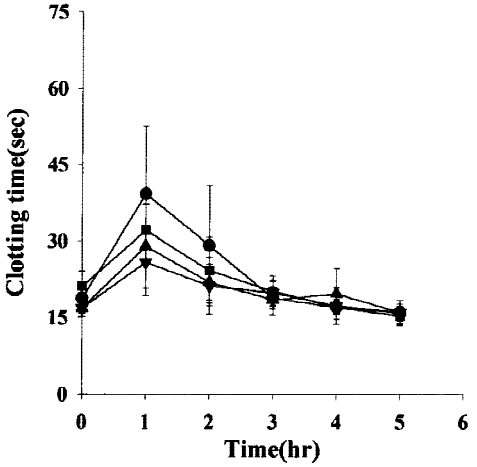

Fig. 4. Clotting time profiles of heparin derivatives according to coupled hydrophobic agents to heparin; (▼) heparin-lauric acid conjugate, (▲) heparin-palmitic acid conjugate, (■) heparin-cholesterol conjugate, (●) heparin-DOCA. The data are plotted as mean ± SD, n = 9.

25.9±6.6, respectively. The carbon numbers of cholesterol, palmitic acid, and lauric acid were 24, 16, and 12, respectively. The hydrophobicity of the coupled agent is proportional to the number of carbons. Thus the maximum clotting time increased with the increase of hydrophobicity of coupled agent. This result indicated that the hydrophobicity of the heparin derivative was an important property for increasing the absorption of heparin derivative in the GI tract. But even if cholesterol is more hydrophobic than DOCA, it is important to note that the heparin-DOCA showed a higher clotting time than the heparin-cholesterol. Therefore, DOCA may have other properties besides hydrophobicity that may have enhanced the absorption of the heparin derivative in the GI tract.

One possible property may be related to the interaction between the coupled DOCA and the bile acid receptors at the ileum. So far, conjugated bile acids bound with small molecular weight peptides have long been known to involve a sodium-dependent transporter mainly in the apical membrane of the ileal enterocyte (9). However, it is difficult for heparin-DOCA to diffuse into the GI wall by active transport since the heparin-DOCA has a high molecular weight.

We propose two possibilities. One is the amphiphilic property of heparin-DOCA. Hydrophilic heparin can attain a slightly hydrophobic property by binding with hydrophobic DOCA, thereby acquiring an amphiphilic property. This property might have improved the permeability of the heparin derivative into the GI wall. The other possibility is the interaction between the coupled DOCA of heparin-DOCA and the DOCA receptors in the GI wall, especially in the ileum site. This interaction might have increased the adhesion of heparin-DOCA on the GI wall, thereby increasing the possibility of heparin-DOCA absorption.

After a single oral administration of heparin-DOCA, the histological change in the GI wall was evaluated by an H&E stain and by TEM. For the heparin-DOCA used in these experiments, the mole ratio of coupled DOCA to heparin was 10, and the dose amount was 200 mg/kg. After heparin-DOCA was administered orally, the stomach and the small

intestine were removed at 1, 2, and 3 h, since the clotting time had returned to the baseline after 3 h. As shown in Fig. 5, evidence of damage to the GI wall, such as occasional epithelial cell shedding, villi fusion, and congestion of mucosal capillary with blood and focal trauma, were not found in parts of the stomach, duodenum, jejunum, and ileum. These results confirm that increased absorption of heparin derivatives was not caused by the disruption of the gastrointestinal epithelium.

Figure 6 shows the morphology of microvilli after they have been exposed to heparin derivatives by TEM. The control samples showed healthy tight junctions, microvilli, and mitochondria. After 1, 2, and 3 h, the cell appearance in all sections showed no signs of damage such as microvilli fusion, dissolution, disoriented cell layer with porosity, or the cytotoxic effect. Microvilli exposed to heparin derivatives were also found to be as healthy as the control. The absence of tissue damage indicates that the enhancing effect of the coupled DOCA on heparin absorption in the GI tract was not caused by changing the tissue structure. Further research will be performed to prove its enhancing mechanism.

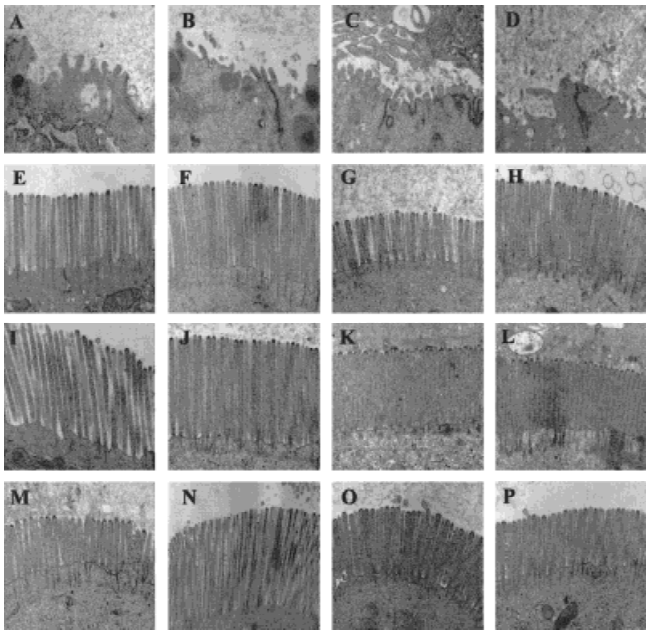

Fig. 6. TEM pictures of membrane or microvilli in the gastrointestinal tissue which were isolated from rats after oral administration of 100 mg/kg. Panels A, B, C, and D show a cross section of the stomach after 0, 1, 2, and 3 h, respectively. Panels E, F, G, and H show a cross section of the duodenum after 0, 1, 2 and 3 h, respectively. Panels I, J, K, and L show a cross section of the jejunum after 0, 1, 2 and 3 h, respectively. Panels M, N, O, and P show a cross section of the ileum after 0, 1, 2, and 3 h, respectively. The original magnification is 25,000× in all panels.

CONCLUSIONS

This study proposes a new heparin derivative that could be administered orally. Sufficient amounts of heparin-DOCA can be absorbed in the GI tract in order to maintain the plasma concentration in the therapeutic window without changing the tissue structure of the GI wall. This study proposes two possibilities for the enhanced absorption of heparin-DOCA by coupled DOCA. One is the added hydrophobic property, and the other is the interaction between the coupled DOCA and bile acid receptors in the ileum. Furthermore, the results of the present study suggest that the effect of the DOCA interaction might be more important to oral heparin delivery than the added hydrophobicity. However, further study is needed in order to prove the interaction of the coupled DOCA and its receptors in the GI wall.

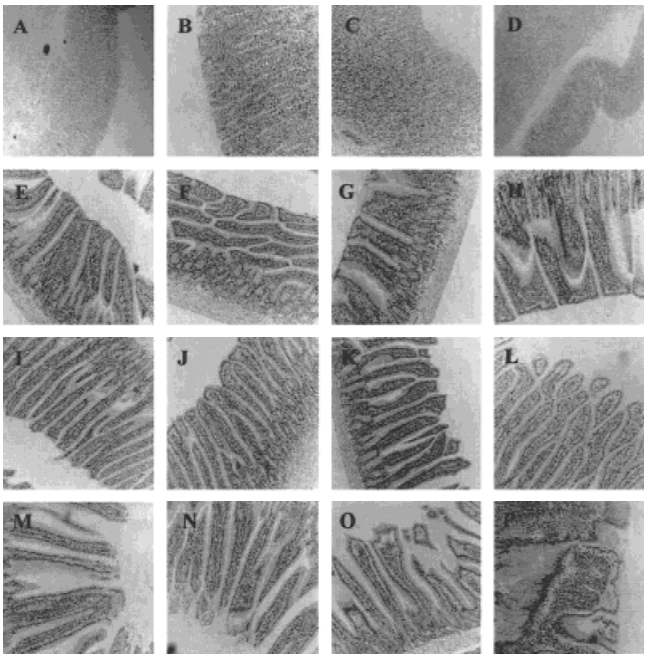

Fig. 5. Micrographs of hematoxylin- and eosin-stained gastrointestinal tissues which were isolated from rats after oral administration of 100 mg/kg. Panels A, B, C, and D show a cross section of the stomach after 0, 1, 2, and 3 h, respectively. Panels E, F, G, and H show a cross section of the duodenum after 0, 1, 2, and 3 h, respectively. Panels I, J, K, and L show a cross section of the jejunum after 0, 1, 2, and 3 h, respectively. Panel M, N, and O, P show a cross section of the ileum after 0, 1, 2, and 3 h, respectively. The original magnification is 100× in all panels.

ACKNOWLEDGMENT

This study was supported by an "Advanced Research Program Grant" from Mediplex Co. (Korea).

REFERENCES

1. P. S. Damus, M. Hicks, and R. B. Rosenberg. Anticoagulant action of heparin. *Nature* **246**:355 (1977).
2. J. K. Thompson-Ford. Low molecular weight heparin for the treatment of deep vein thrombosis. *Pharmacotherapy* **18**:749–758 (1998).
3. R. D. Rosenberg. Biochemistry and pharmacology of low molecular weight heparin. *Semin. Hematol.* **34**:2–8 (1997).
4. G. F. Pineo and R. D. Hull. Unfractionated and low molecular-weight heparin. *Curr. Concepts. Thromb.* **82**:587–599 (1998).
5. M. D. Gonze, J. D. Manord, A. Leone-Bay, R. A. Baughman, L. Garrard, W. C. Sternbergh III, and S. R. Money. Orally administered heparin for preventing deep venous thrombosis. *Am. J. Surg.* **176**:176–178 (1998).
6. A. DalPozzo, M. Acquasaliente, and M. R. Geron. New heparin complexes active by intestinal absorption. I. Multiple ion pairs with basic organic compounds. *Thromb. Res.* **56**:119–124 (1989).
7. I. D. Caramazza, G. Atri, M. L. Bossi, F. D. Deponti, L. Angelo, and A. Crema. Intraduodenal absorption of the new UF-heparin

salt ITF 1057 in the conscious dog. *Thromb. Res.* **62**:785–789 (1991).
8. A. K. Morton, H. E. Edwards, J. C. Allen, and G. O. Phillips. An evaluation of the oral administration of commercial and fractionated heparin samples in rats. *Int. J. pharm.* **9**:321–355 (1982).
9. T. K. Sue, L. B. Jaque, and E.Yuen. Effects of acidity, cations and alcoholic fractionation on absorption of heparin from gastrointestinal tract. *Can. J. Physiol. Pharm.* **54**:613–617 (1976).
10. R. H. Engel and S.J. Riggi. Intestinal absorption of heparin facilitated by sulphated or sulfonated surfactants. *Proc. Soc. Exp. Biol. Med.* **130**:706–710 (1969).
11. T. M. Rivera, A. Leone-Bay, D. R. Paton, H. R. Leipold, and R. A. Baughman. Oral delivery of heparin in combination with sodium N-[8-(2-Hydrocybenzoyl)amino] caprylate: Pharmacological considerations. *Pharm. Res.* **14**:1830–1834 (1997).
12. A. Leone-Bay, D.R. Paton, and J. J. Weidner. The development of delivery agents that facilitate the oral absorption of macromolecular drugs. *Med. Res. Rev.* **20**:169–186 (2000)
13. Y. Lee, H. T. Moon, and Y. Byun. Preparation of slightly hydrophobic heparin derivatives which can be used for solvent casting in polymeric formulation. *Thromb. Res.* **92**:149–156 (1998).