Antithrombotic Effect of Recombinant Batroxobin in $FeCl_3$-induced Carotid Artery Thrombosis Rat Model

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Antithrombotic Effect of Recombinant Batroxobin in $FeCl_3$-induced Carotid Artery Thrombosis Rat Model

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Yours sincerely,

EunHa, Park

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ABSTRACT

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Objective: The aim of this study is to investigate an antithrombotic effect of recombinant batroxobin (rBat) via $FeCl_3$-induced carotid artery thrombosis rat model.

Methods: Sprague-Dawley (SD) rats were injected intravenously with PBS (without KCl, pH 7.4), rBat 2 BU/kg, and rBat 10 BU/kg, respectively. An hour later, the thrombus was induced by 70% $FeCl_3$ application. Heparin was injected intravenously 5 minutes before the thrombosis induction and used as a positive control. The antithrombotic effect of the recombinant batroxobin was examined with criteria including the time to occlusion (TTO), thrombus weight (TW), thrombus area (TA), and blood flow (BF) in a $FeCl_3$-induced thrombosis rat. Histological analysis was performed to examine the effect of the recombinant batroxobin on thrombus area using hematoxylin & eosin. For determination of endothelialization, immunostaining of CD31 was performed on the sections of carotid arteries.

Results: Compared with PBS-treated group, heparin, rBat 2 BU/kg, and rBat 10 BU/kg-treated groups showed significant delay in TTO (PBS, 7.65 ± 2.79 min; Heparin, rBat 2 BU/kg, and rBat 10 BU/kg, 60.00 ± 0.00 min; $P < 0.001$ versus PBS-treated group) and exhibited inhibitory effect on TW (PBS, 2.58 ± 0.93 mg; Heparin, 1.45 ± 0.54 mg; rBat 2 BU/kg, 2.06 ± 0.92 mg and rBat 10 BU/kg, 1.53 ± 0.64 mg) and TA (PBS, 90.15 ± 4.84%; Heparin, 49.00 ± 19.39%; rBat 2 BU/kg, 69.84 ± 17.88 and rBat 10 BU/kg, 70.71 ± 18.71%). In addition, Immunohistochemical staining of CD31 revealed that PBS, heparin and

recombinant batroxobin-groups had no significant difference in injured vessels (PBS, 0.84 ± 1.00 score; Heparin, 1.34 ± 0.80 score; rBat 2 BU/kg, 1.03 ± 1.05 score and rBat 10 BU/kg, 1.00 ± 0.72 score).

Conclusions: These results suggest that recombinant batroxobin has outstanding antithrombotic activity in $FeCl_3$-induced thrombosis rat model and provide experimental evidences that recombinant batroxobin can be used to prevent vascular injury and thrombosis.

Key Words: Recombinant Batroxobin, Heparin, $FeCl_3$-induced carotid arterial thrombosis rat model, thrombosis

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I. INTRODUCTION

Thrombus formation plays a very important role in ischemic heart diseases and in peripheral arterial and cerebral vascular diseases [1-3]. Intravascular

thrombosis is a cause of myocardial infarction, stroke, deep vein thrombosis and restenosis [4, 5]. Abnormal platelet activation augmented blood clotting, vascular dysfunction and shear stress due to interrupted blood flow and atherosclerosis bring intravascular thrombosis [5-7]. Thrombin produced on the platelet surface initiates local activation of the coagulation cascade by activating the platelets, and this positive feedback results in the thrombotic process [8]. To treat thrombotic diseases, administration of an antithrombotic or thrombolytic drug is generally employed [9]. Antithrombotic drugs such as heparin, oral anticoagulants, aspirin, and anti-platelet drugs are available for the prevention of intravascular thrombosis. But narrow therapeutic window, bleeding risk, incidence of resistance, and unwanted drug interactions are the cause of major concerns [10]. Therefore, antithrombotic drugs with minimum side effects and better efficacies are required [10, 11].

Batroxobin is a thrombin-like enzyme derived from *Bothrops atrox,* moojeni venom [12, 13]. In contrast with thrombin, which converts fibrinogen into fibrin by cleavage of fibrinogen A and B chains, batroxobin splits off the fibrinopeptide-A but not fibrinopeptide-B from fibrinogen [14, 15]. In addition, thrombin activates various blood coagulation proteins such as factors V, VIII, and XIII, whereas batroxobin has no direct effect on these factors [16]. As a defibrinogenating drug, batroxobin is now being used to treat arteriosclerosis

obliterans [17, 18]. It reduces fibrinogen levels by causing dissociation of fibrinopeptide-A from the fibrinogen chain, and has been shown to enhance thrombolysis in working with tissue-type plasminogen activator (t-PA) [18].

But main drawback of the native batroxobin is the high price of purified proteins. To overcome this, the recombinant batroxobin was expressed in *Pichia Pastoris* by using gene recombination technology. Thrombin-like activity of the recombinant batroxobin was identified with *in vivo* bleeding time assay, and it was found that it exhibits similar biological activity with the native batroxobin [19].

In this study, we examined whether the activity of the recombinant batroxobin has similar effect with the antithrombotic effect of heparin. Consequently, it is suggested that the recombinant batroxobin could exhibit similar efficacy with heparin and be developed as a new antithrombotic drug without bleeding risk, incidence of resistance, and unwanted drug interactions.

II. MATERIALS AND METHODS

1. Reagents

The recombinant batroxobin was offered from the BioBud, Inc. (Korea). Ferric chloride ($FeCl_3$) was purchased from Sigma Aldrich (MO, USA). Hematoxylin & Eosin (H&E) was purchased from Merck (Germany). Masson`s Trichrome was purchased from BBC biochemical (WA, USA). Anti-CD31 (1:500) antibody was purchased from Santa Cruz Biotechnologies (CA,USA).

2. Animals

All experimental procedures were approved by the Institutional Animal Care and Use Committee of Yonsei University Health System (Approval No. 14-0173-1). Male Sprague-Dawley (SD) rats (220-250g, Orient Bio, Korea) were used in this study. A total of 32 SD rats were divided into 4 groups; each group was treated with intravenous injection of PBS (without KCl, pH 7.4), heparin, rBat 2 BU/kg, and rBat 10 BU/kg, respectively.

3. The $FeCl_3$-induced thrombosis rat model

Anesthesia was induced with 3% isoflurane in the induction chamber and maintained under 1.5~3% isoflurane in 2% oxygen and it was continued throughout the surgical procedure. An incision in the skin was created directly on top of the region of the left common carotid artery. A small piece of filter paper (1 x 1mm) soaked in $FeCl_3$ solution (70%, w/v) was then applied topically to the carotid artery of the SD rats for 5min. The rats were injected intravenously with PBS (without KCl, pH 7.4), rBat 2 BU/kg, or rBat 10 BU/kg. An hour later, thrombus was induced by 70% $FeCl_3$ solution application. Heparin was injected intravenously 5 minutes before the thrombosis induction [20]. To measure the occlusion time in the carotid artery, carotid blood flow was continuously monitored for 60 min after $FeCl_3$ application.

4. Measurement of blood flow and occlusion time

The time to occlusion (TTO) was determined by measuring the flow duration time after removal of $FeCl_3$-saturated filter paper until occlusion was observed. Occlusion was defined as blood flow is reduced to 0 mL/min for longer than 5 min. If occlusion did not occur within 60 min, the occlusion time was reported as

60 min, even though no occlusion was occurred during the observation period. Carotid blood flow (BF) and TTO were measured continuously using an ultrasonic perivascular 1PRB flowprobe (Transonic Systems, NY, USA) of the flowmeter (Transonic Systems, NY, USA). The data acquisition software was an in-house program written in Labchart 8.0 express (2015) (AD Instruments Inc., CO, USA).

5. Measurement of thrombus weight

All the experimental animals were sacrificed after the measurement of occlusion time. Injured carotid artery and opposite side of undamaged carotid artery were extracted and cleaned with saline, and the thrombus weights (TW) were measured. Thrombus weights were calculated by subtracting weight of the uninjured carotid artery from the weight of the injured carotid artery.

6. Histomorphologic analysis

The rat carotid arteries were dissected and fixed in 4% paraformaldehyde, transferred to 70% ethanol, dehydrated and embedded in paraffin. The paraffin blocks of blood vessels were then cut into 4 μm sections and stained with

hematoxylin and eosin (H&E) and masson's trichrome (MT). The stained sections were examined using light microscopy (SCN400, Leica, Germany). To measure the thrombus area (%) in H&E staining, the ratio of the thrombus to lumen area was calculated and the total lumen area was counted as 100%..

7. Immunohistochemistry

Paraffin embedded section slides in 4 μm thickness were heated and melted at 56℃ oven for 1hr to be deparaffinized and rehydrated with ethanol. The slides were blocked with 3% hydrogen peroxide in methanol for 10 min at RT and 5% horse serum in PBS for 10 min at RT, and incubated at 4℃ for overnight with anti-CD31 primary antibody, followed by biotinylated anti-mouse IgG anti-rabbit IgG (H+L) (Vector Laboratories, Inc. CA, USA) for 30 min at 37℃ and horseradish peroxidase (HRP)-conjugated streptavidin (Vector Laboratories, Inc. CA, USA) for 30 min at RT. Signal detection was performed with DAB (3,3'-diaminobenzidine) peroxidase substrate kit (Vector Laboratories, Inc. CA, USA).

8. Statistical analysis

The arterial thrombosis data were analyzed using one-way ANOVA followed by the Bonferroni post-hoc test. All results are presented as the mean ± SD. Values of $P < 0.05$ were considered to be significant. All analyses were performed using the SPSS (IBM, NY, USA).

III. RESULTS

1. Effect of recombinant batroxobin on time to occlusion

The effect of PBS, heparin, rBat 2 BU/kg, and rBat 10 BU/kg treatments on TTO in $FeCl_3$-induced thrombus formation was examined in carotid arterial thrombosis rat model using the flowmeter. Figure 1 shows that the TTO of the carotid artery was 7.65 ± 2.79 min in the PBS-treated group of the $FeCl_3$-induced carotid arterial thrombosis rat. The TTO of the carotid artery of heparin, rBat 2 BU/kg, and rBat 10 BU/kg-treated groups of the $FeCl_3$-induced carotid arterial thrombosis rats were all observed as 60.00 ± 0.00 min (Figure 1). The TTOs of the heparin, rBat 2 BU/kg, and rBat 10 BU/kg-treated groups were all delayed about 8 times compared with that of the PBS-treated group. There was statistically significant difference in TTO between heparin, rBat 2 BU/kg, and rBat 10 BU/kg-treated groups and PBS-treated group ($P < 0.001$). And there was no significant difference among heparin, rBat 2 BU/kg, and rBat 10 BU/kg-treated groups.

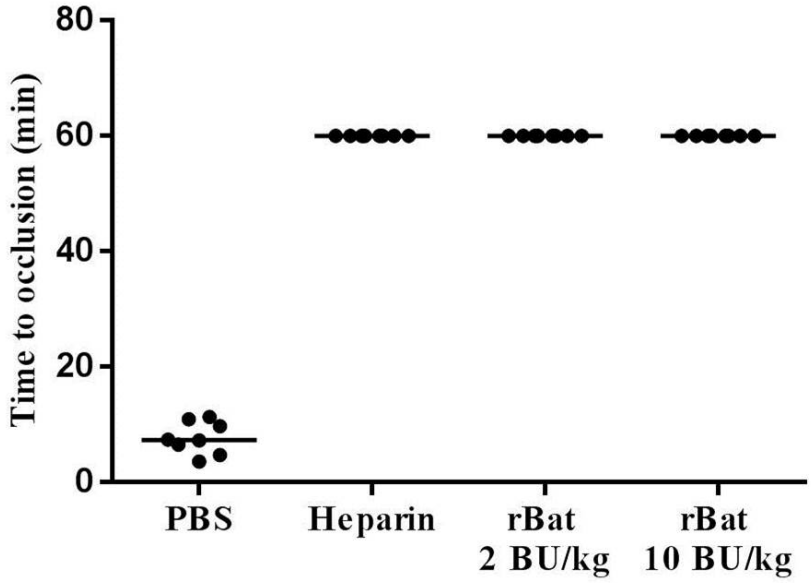


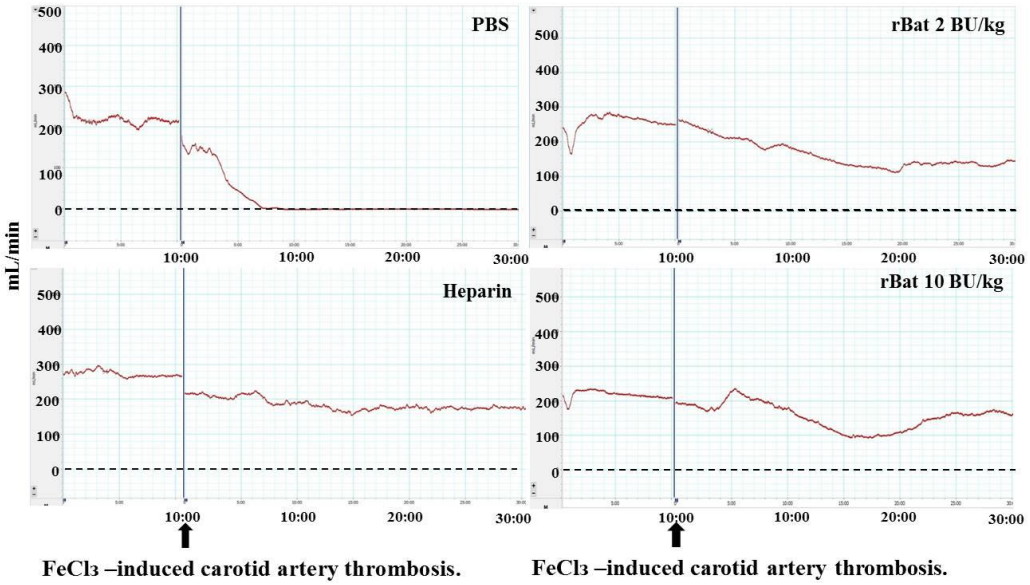
Figure 1. Effect of PBS, heparin, rBat 2 BU/kg, and rBat 10 BU/kg on TTO in $FeCl_3$-induced carotid artery thrombosis rat.

An hour prior to $FeCl_3$ application, the SD rats were injected with PBS, rBat 2 BU/kg, and rBat 10 BU/kg, respectively by intravenous injection. Heparin 200 U/kg was injected intravenously 5 minutes before the thrombosis induction. The left carotid artery was exposed, and a filter paper saturated with 70% $FeCl_3$ was placed on top of the exposed vessel for 5 min. The data are presented as the mean ± SD (n=8). The horizontal lines represent the mean of the displayed values for time delay to occlusion in each group (n=8). *$P < 0.001$ versus PBS-treated group.

2. Effect of recombinant batroxobin on the blood flow rate

Figure 2 shows that the effects of PBS, heparin, rBat 2 BU/kg, and rBat 10 BU/kg on the blood flow rate in $FeCl_3$-induced thrombus formation. Total occlusion was occurred and no blood flow was observed at 12 minute in PBS-treated group of $FeCl_3$-induced carotid artery thrombosis rat while no occlusion was observed for 60min in other groups. There were no significant difference in the changes of blood flow rate (%) among heparin (10 min, 90.70 ± 21.06 ; 20 min, 85.81 ± 22.28; 30 min, 86.08 ± 21.17; 40 min, 86.32 ± 20.47; 50 min, 84.83 ± 17.14; 60 min, 84.18 ± 15.08), rBat 2 BU/kg (10 min, 90.07 ± 18.40; 20 min, 59.78 ± 31.91; 30 min, 60.01 ± 39.41; 40 min, 73.87 ± 46.12; 50 min, 71.65 ± 33.60; 60 min, 69.05 ± 27.84), and rBat 10 BU/kg (10 min, 87.02 ± 20.99 ; 20 min, 56.39 ± 39.12; 30 min, 56.94 ± 42.55; 40 min, 59.87 ± 28.53; 50 min, 69.56 ± 24.72; 60 min, 71.63 ± 22.61)-treated groups.

A



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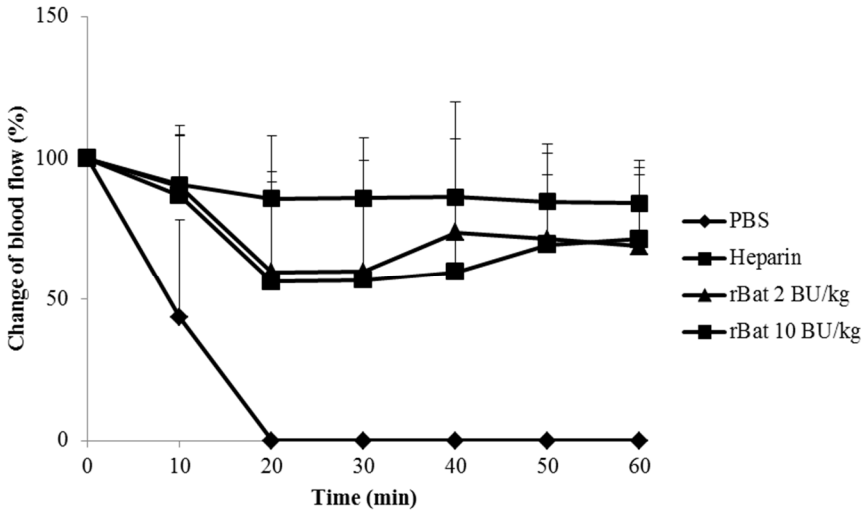

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Figure 2. Effect of PBS, heparin, rBat 2 BU/kg, and rBat 10 BU/kg on carotid artery blood flow in $FeCl_3$-induced carotid artery thrombosis rat.

An hour prior to $FeCl_3$ application, the SD rats were injected with PBS, rBat 2 BU/kg, and rBat 10 BU/kg (intravenous injection). Heparin 200 U/kg was injected intravenously 5 minutes before the thrombosis induction. The left carotid artery was exposed, and a filter paper saturated with 70% $FeCl_3$ was placed on top of the exposed vessel for 5 min. A: Blood flow profile was measured by flowmeter. Measurement was continued for 60 min after removal of $FeCl_3$-saturated filter paper. B: Average change of blood flow rate (%) in every 10 minutes was plotted. The data are presented as the mean ± SD of each group (n=8).

3. The reduction of the thrombus weight

Figure 3 exhibits the differences of thrombus weights of PBS, heparin, rBat 2 BU/kg, and rBat 10 BU/kg-treated $FeCl_3$-induced thrombosis rats. The thrombus weight of the carotid artery was 2.58 ± 0.93 mg in the PBS-treated group of the $FeCl_3$-induced carotid arterial thrombosis rats. The thrombus weight of the heparin-treated group (1.45 ± 0.54 mg) was reduced more than 1.8 times compared with that of the PBS-treated group. The thrombus weights of the rBat 2 BU/kg, and rBat 10 BU/kg-treated groups (2.06 ± 0.92 and 1.53 ± 0.64 mg, respectively) were reduced approximately 1.3 and 1.7 times, respectively, compared with that of the PBS-treated group. There was statistically significant reduction in thrombus weight in heparin-treated group compared to PBS-treated group ($P < 0.05$). Thrombus weights were not significantly reduced by recombinant batroxobin treatments, but exhibited slight decrease in dose-dependent manner. And there was no significant difference among heparin, rBat 2 BU/kg, and rBat 10 BU/kg-treated groups.

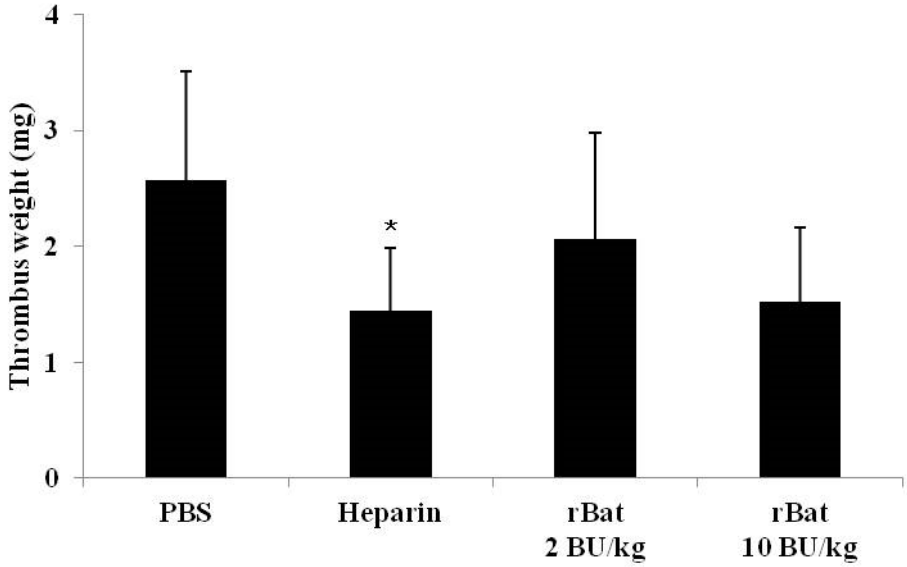


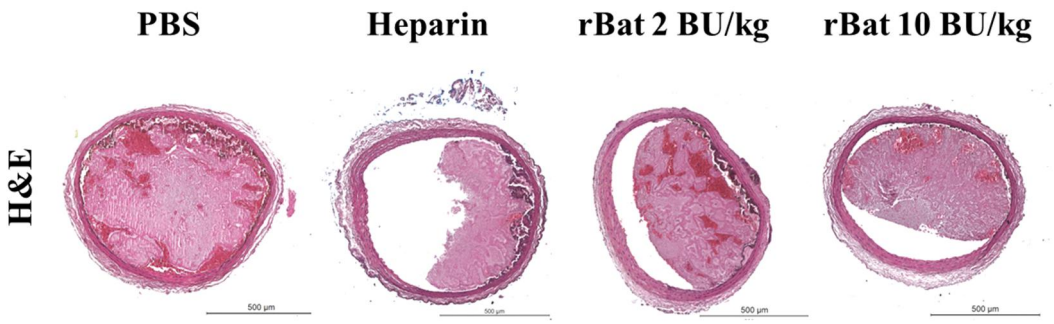
Figure 3. Thrombus weight of PBS, heparin, rBat 2 BU/kg, and rBat 10 BU/kg-treated groups in $FeCl_3$-induced carotid artery thrombosis rat.

Injured carotid artery and opposite side of uninjured carotid artery were extracted, cleaned with saline, and then the thrombus weights were measured. Thrombus weight was calculated by subtracting weight of the uninjured carotid artery from the weight of the injured carotid artery. The data are presented as the mean ± SD of each group (n=8). * $P < 0.05$ versus PBS-treated group.

4. The reduction of the thrombus area

All tissue sections were stained with hematoxylin and eosin. Representative histological images observed in the carotid arteries of SD rats after $FeCl_3$-induced thrombosis formation in each groups are displayed in Figure 4A. Figure 4B shows that the thrombus area of PBS, heparin, rBat 2 BU/kg, and rBat 10 BU/kg-treated groups in $FeCl_3$-induced thrombosis rat model. The thrombus area of the carotid artery was 90.15 ± 4.84% in the PBS-treated group of the $FeCl_3$-induced carotid arterial thrombosis rat. The thrombus area of the heparin-treated group (49.00 ± 19.39%) was reduced by 1.8-fold compared to that of the PBS-treated group. The thrombus area of the rBat 2 BU/kg and rBat 10 BU/kg-treated groups (69.84 ± 17.88 and 70.71 ± 18.71%, respectively) were both decreased about 1.3-fold compared with that of the PBS-treated group. Reduction of thrombus area was statistically significant in comparison between heparin and PBS-treated group ($P < 0.05$). Slight decrease in Thrombus area was also observed in recombinant batroxobin-treated groups but there were no statistical significance. And there were no significant difference in thrombus area among the heparin, rBat 2 BU/kg, and rBat 10 BU/kg-treated groups.

A



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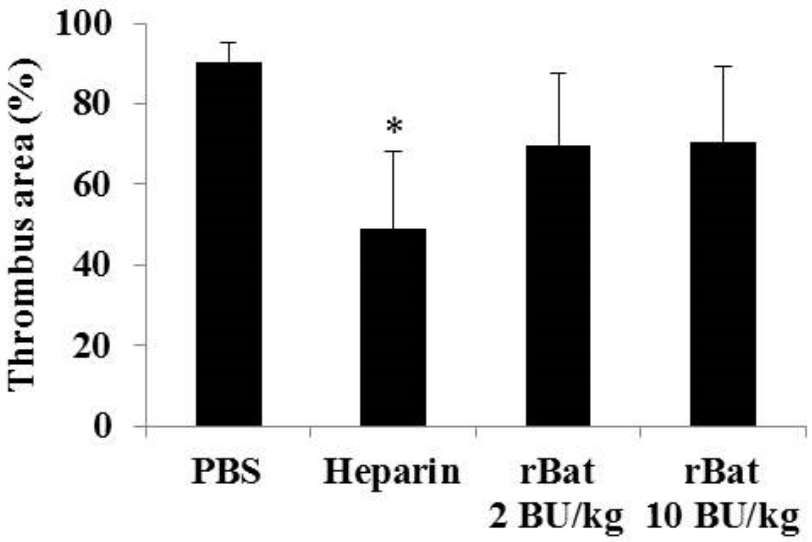

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Figure 4. Histological examination of $FeCl_3$-induced carotid artery thrombosis rat of the PBS, heparin, rBat 2 BU/kg, and rBat 10 BU/kg-treated groups.

Representative images of sections stained with hematoxylin and eosin (H&E, upper column, magnification x100) is displayed in Figure 4A. B: Analysis of thrombus area of PBS, heparin, rBat 2 BU/kg, and rBat 10 BU/kg-treated groups on $FeCl_3$-induced carotid artery thrombosis rat was depicted. The data are presented as the means ± SD of each group (n=8). *$P < 0.05$ versus PBS-treated group.

5. Immunohistochemical staining of endothelial cell

To examine endothelialization of the carotid artery, immunostaining for CD31, a commonly used marker for assessment of the endothelial cell was performed. Figure 5 exhibits the endothelialization score of PBS, heparin, rBat 2 BU/kg, and rBat 10 BU/kg-treated groups on $FeCl_3$-induced carotid artery thrombosis rat. Endothelialization score comparison between PBS-treated group (0.84 ± 1.00 score) and heparin-treated group (1.34 ± 0.80 score) was not statistically significant. However, The scores tended to slightly increase in heparin-treated group. The score were slightly increased in recombinant batroxobin groups (rBat 2 BU/kg, 1.03 ± 1.05 and rBat 10 BU/kg, 1.00 ± 0.72 score, respectively) when compared to PBS-treated group, but the effects were not statistically significant. And there was no significant difference among heparin, rBat 2 BU/kg, and rBat 10 BU/kg-treated groups.

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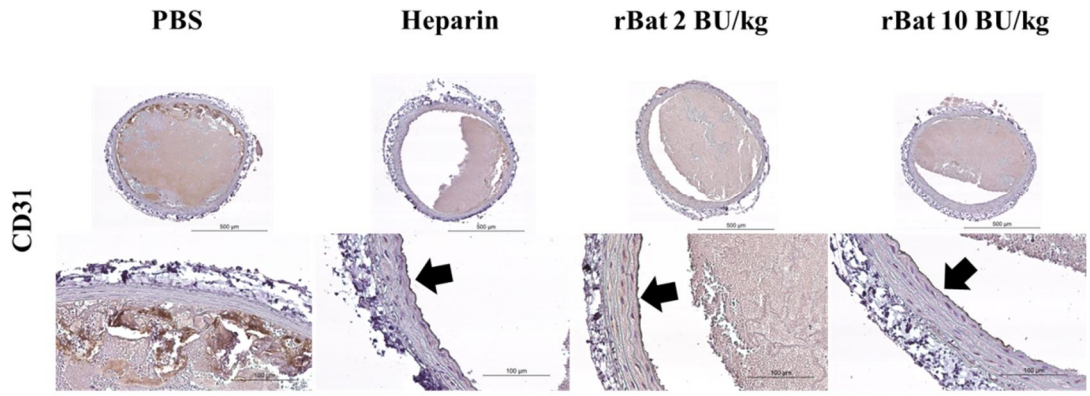

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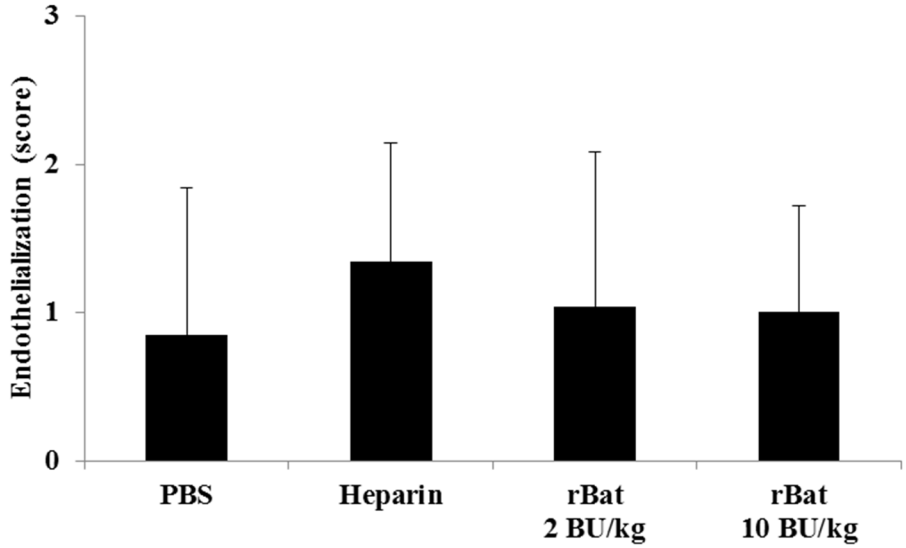

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Figure 5. Degree of intact endothelium in FeCl3-induced carotid artery model in PBS, heparin, rBat 2 BU/kg, and rBat 10 BU/kg-treated groups.

To determine the effect of each treatment on endothelialization, immunostaining of CD31 was performed on the sections of carotid arteries. A: Brown-stained line was considered positive. Arrow heads point to internal elastic lamina (IEL). B: The percentage of the luminal perimeter that stained for CD31 versus total perimeter was measured using LAS (Leica, Germamy). Endothelialization was then scored from 1 to 3 (0: 0%; 1: 25 to 50%; 2: 50 to 75%; 3:75 to 100%) and the scores were averaged with the data from 8 rats (2 sections per rat) in each treatment group. The data are presented as the mean ± SD of each group (n=8). *$P < 0.05$ versus PBS-treated group.

IV. DISCUSSIONS

In worldwide, the incidence of vascular disease has increased together with extended life expectancy [21]. Thrombin, the key component of blood coagulation, is produced on the platelet surface and initiates local activation of the coagulation cascade by activating the platelets, and this positive feedback results in the thrombotic process [8, 22]. To treat thrombotic diseases, administration of an antithrombotic or thrombolytic drug is generally employed [9]. The conventional management of thrombotic and cardiovascular disease is based on the use of heparin, oral anticoagulants, aspirin, and anti-platelet drugs. But narrow therapeutic window, bleeding risk, incidence of resistance, and unwanted drug interactions are the cause of serious concern, therefore antithrombotic drugs with minimum side effects and better efficacies are thus required [10, 11].

Batroxobin is a thrombin-like enzyme derived from *Bothrops atrox, moojeni* venom. In contrast with thrombin, which converts fibrinogen into fibrin by cleavage of fibrinogen A and B chains, batroxobin splits off the fibrinopeptide-A but not fibrinopeptide-B from fibrinogen [14, 15]. Snake venom thrombin-like enzymes such as batroxobin, crotalase and ancrod were known to have not only potent procoagulant activities but also defibrinogenating activities, which are associated with the reduction of fibrinogen level in plasma by clearing converted

fibrinogen and enhancing fibrinolytic activity [23-27]. Recombinant batroxobin and native batroxobin also decreased fibrinogen concentration in rat plasma [19, 28]. In addition, measured the coagulation parameters, including activated partical thromboplastin time (APTT), prothrombin time (PT), and thrombin time (TT) with batroxobin-treated rat plasma in order to investigate whether the recombinant batroxobin has an influence on other blood coagulation factors in vivo. And found out that the coagulation parameters were not significantly affected by the treatments of native batroxobin and recombinant batroxobin [16], but the coagulation parameters including APTT, PT, and TT are extended when the heparin is injected [31].

Native batroxobin faces challenges of high price and shortage in supply. Recombinant batroxobin rewarding these drawbacks was used for this research, which its cDNA was expressed in *Pichia pastoris*. Previously, the recombinant batroxobin was functionally characterized and mass production was also become available [19].

In this study, we examined whether the antithrombotic effect of recombinant batroxobin has similar effect as the antithrombotic effect of heparin. Recombinant batroxobin is the antithrombotic drug using as an injection, therefore chose heparin as antithrombotic drug for comparison, which is currently using as an injection. By using the $FeCl_3$-induced carotid artery thrombosis rat model, the

effect of recombinant batroxobin was evaluated. In the precedent study, rBat 2 BU/kg was injected 60 minutes before the thrombosis induction. And it most effectively reduced the fibrinogen level. Therefore rBat 2 BU/kg treated low concentration in this study, and treated rBat 10 BU/kg as a high concentration which is more than 5 times, then injected intravenously 60 minutes before the thrombosis induction. The TTO and carotid BF in the carotid artery were measured with continuous monitoring with flowmeter for 60 min after $FeCl_3$ application. The TTO of heparin, rBat 2 BU/kg, and rBat 10 BU/kg-treated groups was delayed more than 7.8 times compared with that of the PBS-treated group in $FeCl_3$-induced carotid artery thrombosis rat. For determination of endothelialization, immunostaining of CD31 was performed on the sections of carotid arteries. There was no significant difference among PBS, heparin, rBat 2 BU/kg, and rBat 10 BU/kg-treated groups. The present study established an *in vivo* animal model system of thrombosis to assess the efficacy of recombinant batroxobin and showed that heparin and recombinant batroxobin may have an antithrombotic effect through delaying TTO, reducing TW and reducing TA induced by the $FeCl_3$-induced carotid arterial thrombosis rat. In addition, further studies are required to identify whether heparin and recombinant batroxobin could exhibit synergistic antithrombotic effect.

We were not able to verify how long the effect of recombinant batroxobin

lasts in vivo based on our experiment design. Also, antithrombotic activity of recombinant batroxobin did not exhibited dose-dependency in our assay system. Therefore additional study is required to determine an appropriate dosage in clinical application. In addition, further studies are required to identify whether heparin and recombinant batroxobin could exhibit synergistic antithrombotic effect.

In summary, we identified that the activity of the recombinant batroxobin has similar effect with the antithrombotic effect of heparin. Consequently, it is suggested that the recombinant batroxobin could exhibit similar efficacy with heparin and could be developed as a new antithrombotic drug without bleeding risk, incidence of resistance, and unwanted drug interactions.

V. CONCLUSION

These results indicate that recombinant batroxobin possesses the antithrombotic activity comparable to heparin in $FeCl_3$-induced carotid arterial thrombosis rat model. Our study suggests that recombinant batroxobin has outstanding antithrombotic activity in $FeCl_3$-induced thrombosis rat and provides experimental evidences that recombinant batroxobin can be used to prevent vascular injury and thrombosis.

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ABSTRACT (in Korean)

$FeCl_3$에 의해 유도된 경동맥 혈전증 모델에서 재조합 배트록소빈의 항혈전 효과

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박은하

최근 조사된 현대인의 사망 원인 분석자료에 의하면 혈관 내 장애에 의한 사망률이 1위를 차지하고 있으며, 우리 나라의 경우 또한 국민소득의 향상과

식생활의 서구화에 따라 혈관 장애로 인한 사망률이 1위를 차지하고 있다. 이와 같이 혈관 내 장애와 관련된 질환의 증가는 생체가 노화되면서 대사과정에서 발생되는 활성산소에 의한 산화적 스트레스로 인하여 혈관의 손상과 혈전(thrombus)이 과다하게 형성되기 때문이다. 혈관에 혈전이 형성되면, 혈행 장애를 유발하여 뇌졸증, 심근경색과 같은 심각한 질환으로 이어질 수 있다. 트롬빈은 혈액 응고에 중추적 역할을 수행하는데, 과다한 트롬빈의 활성화는 혈전증을 유발하게 되며, 트롬빈 활성 저해물질은 다양한 혈전성 질환에 매우 유용한 예방 및 치료제로 사용될 수 있다. 현재까지 혈전성 질환의 예방과 치료에 헤파린, 쿠마린, 아스피린, 유로키네이즈 등의 다양한 항응고제, 항혈소판제, 혈전용해제 등이 사용되고 있으나, 출혈성 부작용과 위장장해 및 과민반응 등으로 그 사용이 한정되고 있는 실정이다. 따라서 인체에 부작용이 최소화된 안전성이 우수한 혈전 예방 물질을 발굴해야 할 필요성이 있다.

배트록소빈은 *bothrops atrox moojeni* 라는 뱀독에서 분리 정제된 트롬빈 유사효소로 최근 defibrinogenating agent로서 배트록소빈의 항혈전 효과가 새로이 임상적으로 관심을 받고 있다. 또한 혈액 내의 다른 응고인자에는 영향을 주지 않고 혈소판의 활성화에도 영향을 미치지 않아 출혈과 같은 부작용이 없다는 장점이 있다. 하지만 천연형의 배트록소빈은 고가로 완제품을 수입해야 하는 점과 대량생산의 한계가 있는 단점이 있다. 재조합 배트록소빈은 배트록소빈을 유전자 재조합기술로 효모에서 대량으로 정제한 물질로 천연형 배트록소빈과 유사한 효능을 가지는 것으로 알려져 있다.

본 연구에서는 재조합 배트록소빈의 항혈전 효과가 현재 사용되고 있는

헤파린의 항혈전 효과와 비슷한 정도의 효능을 보이는지 알아보고자 하였다. 랫드를 네 그룹으로 나누어 대조군에는 PBS, 실험군에는 헤파린, 재조합 배트록소빈 2 BU/kg, 재조합 배트록소빈 10 BU/kg를 정맥투여하여 flowmeter를 이용하여 혈류량과 혈전생성 시간을 측정하였다. 희생 시킨 후에는 혈전의 무게를 측정하고 조직학적 변화를 관찰하기 위하여 H&E 염색, 면역염색(CD31)을 실시하여 혈전의 면적을 측정하고 내피세포화를 비교 관찰하였다. 그 결과 혈전생성 시간은 대조군에 비해 모든 실험군에서 의미 있게 지연되는 것을 확인할 수 있었고 혈전의 무게와 면적에서는 대조군에 비해 모든 실험군에서 적게 측정되어서 혈전이 덜 생기는 경향을 확인할 수 있었다. 내피세포화를 비교 관찰한 결과에서는 대조군과 모든 실험군간에 유의적인 차이가 없는 것을 확인하였다.

결론적으로 적합한 농도의 배트록소빈은 혈관의 손상과 혈전을 예방하는 데 효과적으로 작용할 수 있다는 것을 확인하였다.

핵심어: 재조합 배트록소빈, 헤파린, 혈전증, 경동맥 혈전증 모델