

가토 경동맥 외번모델에서 히루딘(Hirudin)의 항 혈소판 응집효과

조홍근¹ · 강석민³ · 이상학³ · 백금련² · 박시훈¹ · 신길자¹ · 장양수³ · 정광희²

Antiplatelet Effect of Hirudin in a Rabbit Carotid Artery Eversion Model

Hong Keun Cho, MD¹, Seokmin Kang, MD³, Sang Hak Lee, MD³, Keum Ryun Pack, MD²,
Si Hoon Park, MD¹, Gil Ja Shin, MD¹, Yangsoo Jang, MD³ and Kwang Hoe Chung, MD²

¹Division of Cardiology, College of Medicine, Ewha Women University, Seoul

²Cardiovascular Institute, ³Division of Cardiology, Yonsei University College of Medicine, Seoul, Korea

ABSTRACT

Background and Objectives : Thrombin and its interaction with platelets play a pivotal role in arterial thrombus formation. Hirudin, an anticoagulant agent derived from medicinal leeches (*Hirudo medicinalis*), is a unique and specific thrombin inhibitor with no effect on other serine protease. We investigated the inhibitory effect of hirudin on platelet deposition in a rabbit carotid artery eversion model of acute arterial thrombosis. **Materials and Methods** : The everted arterial segments were perfused with 111Indium-labeled human platelets only (control, n = 8), and a mixed solution of 111Indium-labeled human platelets and hirudin (30, 45, 60, 90 μ g/ml, n = 3, respectively). Platelet deposition was calculated by a gamma-counter and confirmed by scanning electron microscopy. **Results** : 1) Indium-111 labeling efficiency of platelets was $87.0 \pm 6.6\%$, and the aggregation of platelets was not changed after labeling. The number of platelets perfused through each arterial segment was $4.3 \pm 0.2 \times 10^8$ platelets/ml. 2) The control group showed a platelet deposition rate of $23.9 \pm 7.0\%$ and a number of platelet deposition of $9.8 \pm 2.5 \times 10^8$ platelets/cm². 3) Platelet deposition of arteries perfused with hirudin (60 μ g/ml) was significantly decreased compared with that of the control group (2.9 ± 0.6 vs $9.8 \pm 2.5 \times 10^8$ /cm², p<0.05). 4) The number of deposited platelets in hirudin-perfused arteries was dose dependently decreased (30 μ g/ml : $6.7 \pm 1.4 \times 10^8$ /cm², 45 μ g/ml : $4.8 \pm 1.7 \times 10^8$ /cm², 60 μ g/ml : $2.9 \pm 1.8 \times 10^8$ /cm², 90 μ g/ml : $2.9 \pm 1.4 \times 10^8$ /cm² ; p<0.05 vs. control, respectively). 5) Scanning electron microscopic examination revealed significantly reduced platelet deposition in hirudin-perfused groups compared with control group. **Conclusion** : Hirudin inhibits effectively platelet deposition and arterial thrombus formation in a rabbit carotid artery eversion model. The antiplatelet effect of hirudin in this model suggests that hirudin may be an useful antithrombotic agent therapeutically useful in the prevention of acute arterial thrombus formation. (Korean Circulation J 1999;29(10):1121-1128)

KEY WORDS : Antiplatelet effect · Hirudin · Carotid artery eversion model.

: 1999 5 7
: 1999 9 1
: , 120 - 752 134
: (02) 361 - 7354 · : (02) 365 - 1878
E - mail : khchung@yumc.yonsei.ac.kr

서론

20

1)

2)

가 (prothrombin) (thrombin) (phospholipid) 가 collagen 가 (plug)가 가 (Hirudo medicinalis) 65 polypeptide (Fig. 1). thrombin (Hirudo medicinalis)

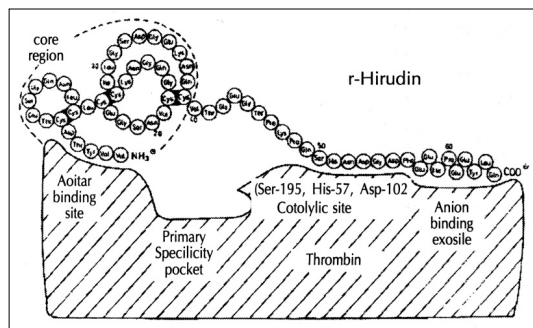


Fig. 1. The structure of amino acid sequence of hirudin.

1122

stoichiometric

가 (eversion) 8-9)

재료 및 방법

재 료

(1 ml 가 1.7×10^9) 24 48 ABO (Chrono-log Co., Havertown, PA, USA)가 , ADP 10 μ M/ml Indium - 111(In - 111) 10-11) 5 ml 37 Tyrode 12) (pH = 6.5) 5 ml 10 ml 1 2 640 g 10 2 ml Tyrode 5 Tyrode 2 ml , 37 10 가 . In - 111 - oxine (Ame-rsharm, Buckinghamshire, UK) 5 μ l(3.9 μ Ci) 가 37 1 8 ml 가 1 2 640 g 10 ml (Packard, Warenbillerd Downers, , USA)

(labeling efficiency)

9 ml 가 가 4.3×10^8 /ml

Korean Circulation J 1999;29(10):1121-1128

가 (Fig. 2). 37 HE -
PES - Tyrode ¹²⁾(pH = 7.33) ,
6F , 14G

Labeling efficiency(%) = [ppt(cpm)/{ppt(cpm) +
sup(cpm)}] × 100
ppt : platelet pellet
sup : supernatant solution
cpm : counts per minute
In - 111

가 (Jeil Co., Seoul,
Korea) 37 가
HEPES - Tyrode
(Terumo Co., Tokyo, Japan)
가 shear rate 400
500/sec shear rate range
1.1 1.2 mm
5 ml/min ¹³⁾ Sh -
ear rate
rate = $4Q/r^3$ Q (ml/min) r
(cm)

3 4 kg New Zealand white rabbit 7
Ketamine(30
mg/ kg) (ear vein)

HEPES - Tyrode 1
In - 111
2
(30 µg/ml, 45 µg/ml, 60 µg/ml, 90
µg/ml, 120 µg/ml) In - 111
2 HE -
PES - Tyrode 2 ,

(perfusion system)
Riemann ¹¹⁾

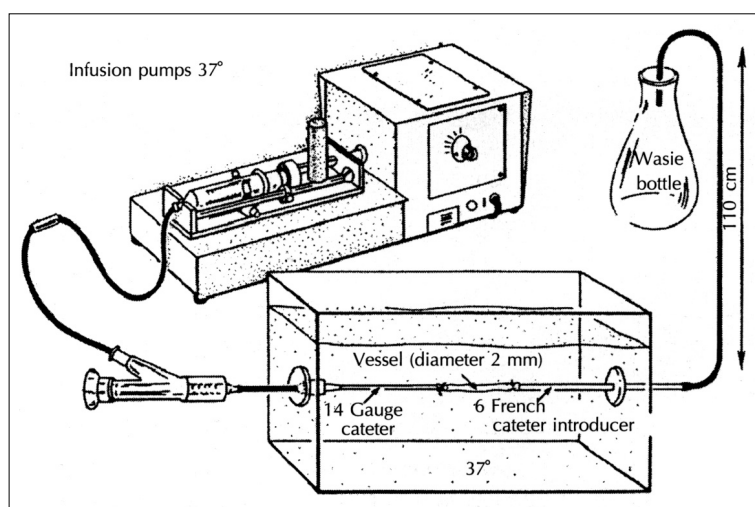


Fig. 2. Perfusion system used for rabbit carotid artery eversion model.

(%)
 (%) = { In - 111
 (cpm) ÷ In - 111
 (cpm) } × 100
 (deposited platelets)

±
 Windows for SPSS release 7.5
 Mann - Whitney U - Wilcoxon
 Rank Sum W
 value 0.05 p -

결 과

Deposited platelets(platelets/cm²) = RA/SA × 2 rL

RA : radioactivity of vessel segment(cpm)

SA : specific radioactivity per platelet(cpm)

r : vessel lumen radius(cm)

L : length of vessel counted(cm)

(scanning electron microscopy)

SEM S - 800(Hitachi Co.,
 Tokyo, Japan)
 2% glutaraldehyde PBS 1 2
 , 1% OsO₄ 2
 . 50%, 60%, 70%, 80%, 90%, 95%, 100%
 10 , Hitachi HCP - 2
 CO₂ 20 20 , 37.5
 5 (critical point dry)
 Eiko IB - 3 ion coater
 300 coating

혈소판 응집능, 혈소판의 In-111 표지율, 관류된 혈소판 수

In - 111

(Fig. 3),

In - 111

87.8 ± 6.6%

4.3 ±

0.2 × 10⁸ platelets/ml

대조군의 침착 혈소판 수 및 혈소판 침착율

(8)

9.8 ± 2.5 × 10⁸

platelets/cm²

23.9 ± 7.0 %

히루딘 투여군에서 혈소판 침착 억제효과

60 µg/ml (3)

(2.9 ± 0.6 vs. 9.8 ±

2.5 × 10⁸ platelets/cm², p<0.05)(Fig. 4).

(3)

(30 µg/ml : 6.7 ± 1.4 ×

10⁸/cm², 45 µg/ml : 4.8 ± 1.7 × 10⁸/cm², 60 µg/ml :

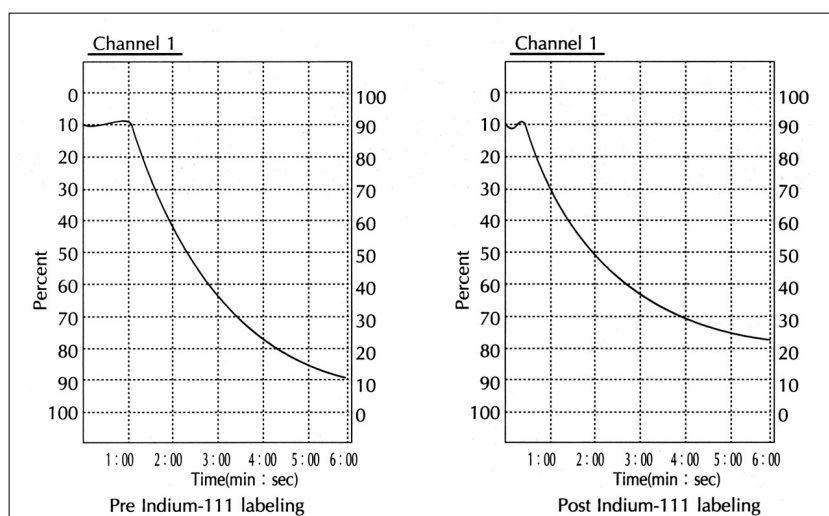


Fig. 3. The effect of Indium-111 labeling on ADP-induced platelet aggregation.

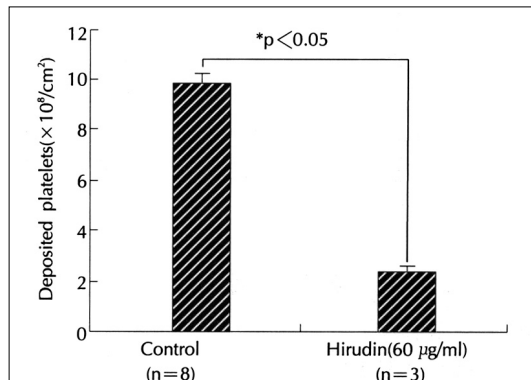


Fig. 4. Comparison of the deposited platelets between control and hirudin-perfused group in rabbit carotid artery eversion model.

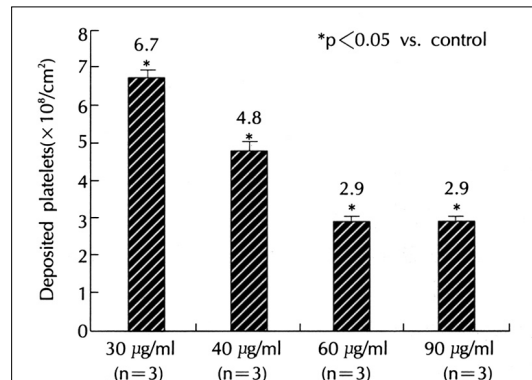


Fig. 5. Comparison of the deposited platelets at different concentrations of hirudin in rabbit carotid artery eversion model.

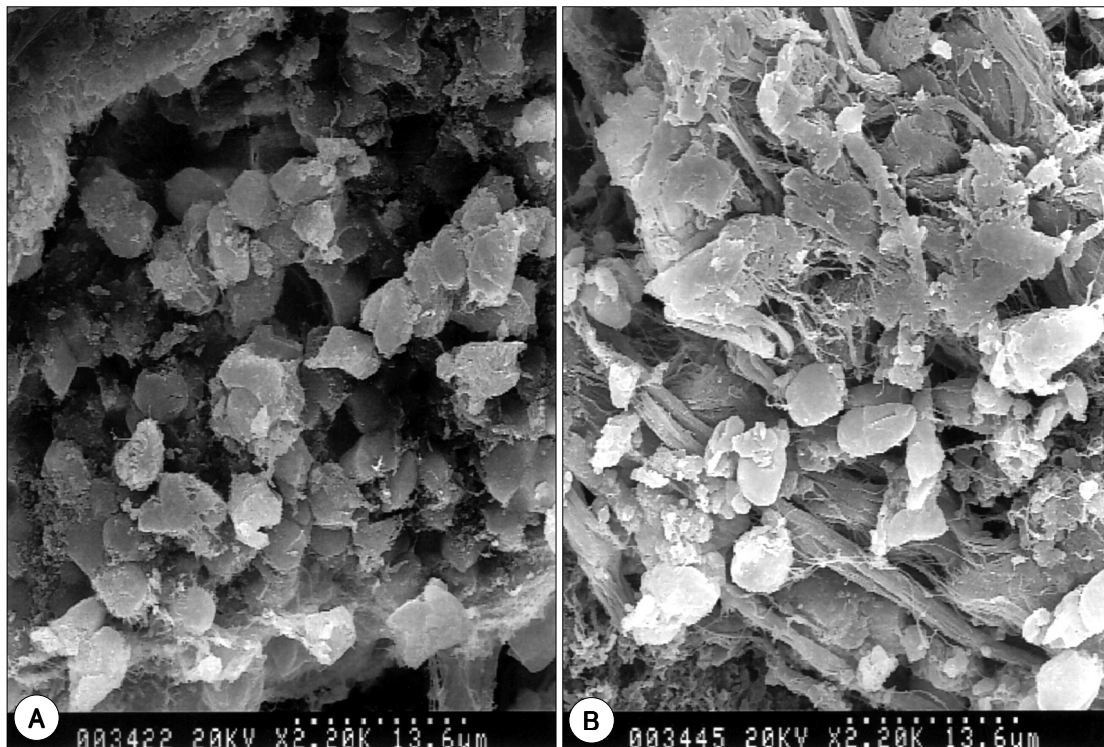


Fig. 6. Scanning electron microscopy of the everted rabbit carotid artery. The platelet deposition in hirudin-perfused group (B) was decreased compared with the control group (A).

$2.9 \pm 1.8 \times 10^8/\text{cm}^2$, 90 $\mu\text{g/ml}$: $2.9 \pm 1.4 \times 10^8/\text{cm}^2$;
 $p < 0.05$ vs.) (Fig. 5).

고 찰

주사 전자현미경 소견

가

60 $\mu\text{g/ml}$

(Fig. 6).

m
 가 (fibrin) (end
 Xa to end anastomosis)
 가
 가 (adventitia) tissue factor
 가
 가
 가 (turbule -
 (low molecular weight) nce) shear force가
 pentasaccharide
 Xa
 Xa
 가
 가 (14)16)
 가 (6)
 antithrombin Xa
 , RGD(Arg -
 factor Xa protease Gly - Asp) analogue
 inhibitor (7) (17)18)
 가 Xa tick anticoagulant peptide(TAP)
 가 (19)
 가
 가 (20)
 50 100 µg/kg/min 60 가
 Dacron (21)
 (19)22)23) (19) 100 µg/kg/min
 2.35 ± 0.17 µmol/l
 (24)25) 30 µg/ml
 (26)
 가 (8-10) 가 5 m

가 , 가
가 .

중심 단어 :

감사문

1997

요약

연구배경 :

가

재료 및 방법 :

가

In - 111

111

In -

결 과 :

1) ln - 111

In - 111

 $87.8 \pm 6.6\%$ $4.3 \pm$

0.2 x 10⁸ platelets/ml . 2) (8)

 $9.8 \pm 2.5 \times 10^8 \text{ platelets/cm}^2$ $23.9 \pm 7.0\%$

3) 60 µg/ml (3)

(2.9 ± 1.8 vs.

$9.8 \pm 2.5 \times 10^8$ platelets/cm², $p < 0.05$). 4)

(30 $\mu\text{g/ml}$: $6.7 \pm 1.4 \times 10^8/\text{cm}^2$, 45

$$\mu\text{g/ml} : 4.8 \pm 1.7 \times 10^8/\text{cm}^2, 60 \mu\text{g/ml} : 2.9 \pm 1.8 \times$$
 $10^8/\text{cm}^2$, 90 $\mu\text{g}/\text{ml}$: $2.9 \pm 1.4 \times 10^8/\text{cm}^2$). 5)60 $\mu\text{g/ml}$

REFERENCES

- 1) Verstraete M. *New developments in antiplatelet and anti-thrombotic therapy.* Eur Heart J 1995;16 (Suppl 1):16-23.
- 2) Ware JA, Heistad DD. *Platelet-endothelium interactions.* N Engl J Med 1993;328:628-35.
- 3) Markwaradt F. *Inventory of coagulation inhibitors from animals feeding on blood.* Thromb Haemost 1994;72: 477-80.
- 4) Chung KH. *Novel antithrombotic drugs in development.* Kor J Hemost Thromb 1996;3:1-12.
- 5) Harenberg J. *Pharmacology of low molecular weight heparins.* Semin Thromb Hemost 1990;16 (Suppl 1):2-8.
- 6) Weitz JI, Hudoba M, Massel D, Maraganore J, Hirsh J. *Clotbound thrombin is protected from inhibition by heparin-antithrombin III but is susceptible to inactivation by antithrombin III-independent inhibitors.* J Clin Invest 1990;86:385-91.
- 7) Markwart F. *Hirudin and derivatives as anticoagulant agents.* Thromb Haemost 1991;66:141-52.
- 8) Jang IK, Gold HK, Ziskind AA, Fallon JT, Holt RE, Leinbach RC, et al. *Differential sensitivity of erythrocyte-rich and platelet-rich arterial thrombi to lysis with recombinant tissue-type plasminogen activator.* Circulation 1989;79: 920-8.
- 9) Jang IK, Gold HK, Ziskind AA, Leinbach RC, Fallon JT, Collen D. *Prevention of platelet-rich arterial thrombolysis by selective thrombin inhibition.* Circulation 1990; 81:219-25.
- 10) Lee SH, Chung KH, Paek KR, Kang SM, Song KS, Jang YS, Kim SS. *Effect of tick anticoagulant peptide on platelet deposition in a rabbit carotid artery eversion model.* Kor J Hemost Thromb 1998;5:129-37.
- 11) Riemann CD, Massey CV, McCarron DL. *Ionic contrast agent-mediated endothelial injury causes increased platelet deposition to vascular surfaces.* Am Heart J 1993; 125:71-8.
- 12) Watson SP, Authi KS. *Platelets.* New York, Oxford University Press;1996. p.52, p.132.
- 13) Gross DR. *Animal models in cardiovascular research,* 2nd ed. Dor-drecht, Kluwer Academic Publishers;1994. p.375-6.
- 14) Heras M, Chesebro JH, Penny WJ, Bailey KR, Badimon L, Fuster V. *Effects of thrombin inhibition on the develop-*

- ment of acute platelet-thrombus deposition during angioplasty in pigs. *Circulation* 1989;79:657-65.
- 15) Agnelli G, Pascucci C, Cosmi B, Nenci GG. The comparative effects of recombinant hirudin (CGP39393) and standard heparin on thrombus growth in rabbits. *Thromb Haemost* 1990;63:204-7.
 - 16) Meyer BJ, Fernandez-Ortiz A, Mailhac A, Falk E. Local delivery of r-hirudin by a double-balloon perfusion catheter prevents mural thrombosis and minimize platelet deposition after angioplasty. *Circulation* 1994;90:2474-80.
 - 17) Haskell EJ, Prager NA, Sobel BE, Abenschein DR. Relative efficacy of antithrombin compared with antiplatelet agents in accelerating coronary thrombolysis and preventing early reocclusion. *Circulation* 1991;83:1048-56.
 - 18) Klement P, Borm A, Hirsh J, Maraganore JM, Wilson G, Weiz JI. The effect of thrombin inhibitors on tissue plasminogen activator induced thrombolysis in a rat model. *Thromb Haemost* 1992;68:64-8.
 - 19) Sitko GR, Ramjit DR, Stabilito II, Lehman D, Lynch JJ, Vlasuk GP. Conjunctive enhancement of enzymatic thrombolysis and prevention of thrombolytic reocclusion with the selective factor Xa inhibitor, tick anticoagulant peptide. *Circulation* 1992;85:805-15.
 - 20) Serruys PW, Herman JP, Simon R. A comparison of hirudin with heparin in the prevention of restenosis after coronary angioplasty. *Helvetica Investigators. N Engl J Med* 1995;333:757-63.
 - 21) Schaffer LW, Davidson JT, Vlasuk GP, Siegl PKS. Anti-thrombotic efficacy of recombinant tick anticoagulant peptide. *Circulation* 1991;84:1741-8.
 - 22) Lefkovits J, Malycky JL, Rao J, Hart CE, Plow E, Topol EJ, Nicolini FA. Selective inhibition of factor Xa is more efficient than factor VIIa-tissue factor complex blockade at facilitating coronary thrombolysis in the canine model. *J Am Coll Cardiol* 1996;28:1858-65.
 - 23) Nicolini FA, Lee P, Malycky JL, Lefkovits J, Kottke-Marchant K, Plow EF, Topol EJ. Selective inhibition of factor Xa during thrombolytic therapy markedly improves coronary artery patency in a canine model of coronary thrombosis. *Blood Coagul Fibrinolysis* 1996;7:39-48.
 - 24) Ragosta M, Gimble LW, Gertz D, Dunwiddie CT, Vlasuk GP, Haber HL, et al. Specific factor Xa inhibition reduces restenosis after balloon angioplasty of atherosclerotic femoral arteries in rabbits. *Circulation* 1994;89:1262-71.
 - 25) Jang Y, Guzman LA, Lincoff AM, Gottsauner-Wolf M, Forudi F, Hart CE, et al. Influence of blockade at specific levels of the coagulation cascade on restenosis in a rabbit atherosclerotic femoral artery injury model. *Circulation* 1995;92:3041-50.
 - 26) Abendschein DR, Recchia D, Meng YY, Oltrona L, Wiczkline SA, Eisenberg PR. Inhibition of thrombin attenuates stenosis after arterial injury in minipigs. *J Am Coll Cardiol* 1996;28:1849-55.