

고콜레스테롤혈증 환자에서 활성화된 혈소판 Glycoprotein IIb/IIIa의 증가

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Increased Activation of Platelet Glycoprotein IIb/IIIa in Hypercholesterolemic Patients

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ABSTRACT

Background : Platelet function is directly influenced by lipoproteins, and platelets from hypercholesterolemic patients display increased reactivity which is related to initiation, progression, and development of thromboembolic complications in atherosclerosis. But the exact mechanism of this effect is unclear. **Methods** : In this study, total and activated numbers of platelet glycoprotein (Gp) IIb/IIIa were evaluated in twenty patients (7 men ; age, 55.4 ± 8.7 years) with hypercholesterolemia (plasma total cholesterol level over 240 mg/dL and normal triglyceride level) and twenty one subjects (8 men ; 51.1 ± 13.7 years) with normal plasma cholesterol and triglyceride levels. Flow cytometry was used to detect the binding of fluorescein isothiocyanate (FITC)-conjugated anti-CD41 or PAC1 to platelet Gp IIb / IIIa in whole blood. When whole blood was incubated with PAC1, platelets were also activated with adenosine diphosphate (ADP) or thrombin. **Results** : PAC1 was more bound to unstimulated platelets from patients with hypercholesterolemia ($p < 0.005$), and binding of PAC1 correlated significantly with plasma total cholesterol ($r = 0.48$, $p = 0.002$) and LDL-cholesterol ($r = 0.47$, $p = 0.002$) levels. Binding of PAC1 to unstimulated platelets increased as binding of anti-CD41 increased ($r = 0.40$, $p = 0.01$). On multivariate linear regression analysis, plasma total cholesterol level and binding of anti-CD41 were independent variables that determined binding of PAC1. After ADP- or thrombin-stimulation, binding of PAC1 to platelets and percentage of antibody positive cells were also greater in patients with hypercholesterolemia ($p < 0.05$). There was a significant positive correlation between mean platelet volume and binding of anti-CD41 to unstimulated platelets ($r = 0.46$, $p < 0.005$), but the latter was not different between hypercholesterolemia and control groups. **Conclusion** : Unstimulated platelets from patients with hypercholesterolemia had similar total number of Gp IIb/IIIa to those from control subjects, but had more activated Gp IIb/IIIa. After ADP- or thrombin-stimulation, platelet Gp IIb/IIIa was also more activated under hypercholesterolemia. (**Korean Circulation J 1998;28(12):2030-2041**)

KEY WORDS : Platelet glycoprotein IIb/IIIa · Hypercholesterolemia · Flow cytometry.

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서론

phosphatidyl - inositol arachidonic
acid thromboxane
thromboxane 가
8)
13)
platelet factor 4, - thromboglobulin
collagen serotonin 가
4)8) - granule
dense granule 가
25)
가
phosphatidylinositol turnover 가, 26)
Ca²⁺ mobilizing system , 27)
Na⁺/H⁺ antiport
28)
phospholipase 가
17)
가 , ADP
collagen thrombin
가 18)
가 agg -
regometer ,
platelet factor 4, - thro -
mboglobulin, serotonin, malondialdehyde, thrombo -
xane B₂ ,
19)
가
arachidonic acid, thrombin,
collagen throm -
boxane 가 6)7)20)21)
가
/ 가 가 ,
/ 가 가
22)
phospholipase 가 arac -
hidonic acid thromboxane
가 23)24)
Integrin glycoprotein(Gp) IIb/IIIa
40000~80000 가 ,
(fibrinogen) 29 - 31) Gp
IIb/IIIa , ADP, epinep -
hrine, thrombin
Gp IIb/IIIa 가
가
Gp IIb/IIIa
murine monoclonal antiplatelet antibody PAC1
Gp IIb/IIIa ep -
itope 32)
PAC1
Gp IIb/IIIa
iodine¹²⁵ PAC1
fluorescein isothiocyanate(FITC) phycoe -
rythrine(PE) PAC1 (flow
cytometry) Gp IIb/IIIa
33 - 36)
Gp IIb/IIIa

Gp IIb/IIIa FITC - conjugated PAC1 8~9 30 21 G scalp needle

Gp IIb/IIIa ADP thrombin 10 mL 2 mL EDTA bottle

Gp IIb/IIIa Coulter counter

Gp IIb/IIIa 8 mL heparin

IIIa 9 mL 3.8% sodium citrate 1 mL(1/10 v/v)가

FITC - conjugated anti - CD41 Heparin

Gp IIb/IIIa 4 2500 × g 20

- 70

Sodium citrate

대상 및 방법

대 상

가 240 mg/dL

가 20 (7 ; 55.4 ± 8.7)

cholesterol oxidase

glycerol triphosphate oxidase

phosphotungstic acid - MgCl₂

Friedwald formula

가 21 (8 ; 51.1 ± 13.7)

방 법

FITC - conjugated PAC1 (Cat. No. 340507, Becton Dickinson Co., San Jose, CA, USA), FITC - conjugated anti - CD41 (Code No. F7088, DAKO Co., Denmark), FITC - conjugated murine IgG₁ (Code No. x 0927, DAKO Co., Denmark)

ADP (Prod. No. A2754), thrombin (Prod. No. T4648), prostaglandin (PG) I₂ (Prod. No. P6188), 4 - (2 - hydroxyethyl) - 1 - piperazineethane - sulfonic acid (HEPES ; Prod. No. H7523), bovine serum albumin (BSA ; Prod. No. A2153), glycyl - L - prolyl - L - arginyl - L - proline (GPRP ; Prod. No. G1895) Sigma (St. Louis, MO, USA)

FITC - conjugated anti - CD41

5 μL FITC - conjugated anti - CD41 10 μL (20 μg/mL), PGI₂ 5 μL (1 μM), isotonic HEPES buffer (137 mM NaCl, 2.7 mM KCl, 1 mM MgCl₂, 5.6 mM glucose, 1 mg/mL BSA, 20 mM HEPES pH 7.4) 30 μL가 polystyrene tube 15

, isotonic HEPES buffer 500 μL

1% paraformaldehyde

4

FITC - conjugated anti - CD41

FITC - conjugated IgG₁

FITC - conjugated PAC1

5 μL FITC - conjugated PAC1 15 μL (30 μg/mL), ADP thrombin 5 μL, isotonic HEPES buffer 25 μL가 polystyrene tube

ADP 20 mM ,
 가 0.05 μ M, 0.1 μ M,
 0.5 μ M, 1 μ M, 20 μ M . Thro -
 mbin 10 U/mL ,
 20 mU/mL, 50 mU/mL, 100
 mU/mL가 . FITC -
 conjugated PAC1 ADP
 thrombin PGI₂ 5 μ L(1 μ M)
 15 ,
 isotonic HEPES buffer 500 μ L
 , 1% paraformaldehyde
 , 4
 . Thrombin fibrin polymerization
 thrombin GPRP
 (2.5 mM) 가 .³⁷⁻³⁹⁾

 Becton Dickinson FACStar
 . Argon laser 488 nm , 200
 mW . FACStar 2 μ m CaliBRI - TE be -
 ads(Cat. No. 34952, Becton Dickinson Co., San Jose,
 CA, USA), , FITC - conjugated IgG₁

 4000~5000 70 μ m nozzle
 laser beam . Gain logarit -
 hmic scale , (light scatter)
 , Hewlett - Packard Consort 30
 H - P 217 computer(Palo Alto, CA, USA)
 . forward scatter(FSC) side
 scatter(SCC) profile , ,
 (debris), machine noise' (Fig. 1).
 gating ,
 PAC1 anti - CD41 , 10000
 FITC
 (MFI ; mean fluorescence inten -
 sity) (Fig. 2), ADP thrombin
 (percentage
 of antibody - positive platelets)
 ,
 1 μ M PGI₂
 99~99.2%
 (Fig. 3). (BI ; binding index)

{BI = MFI \times (percentage of antibody - positive
 platelets)/100}.

통계 분석

Student's t - test , chi -
 square test . FITC - conjugated anti - CD41
 MFI

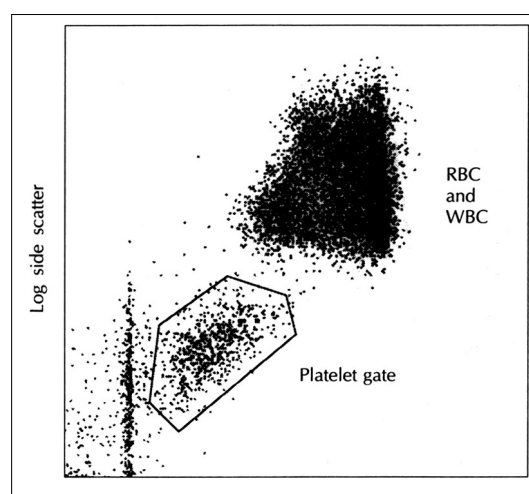


Fig. 1. Scatter profile of whole blood. The platelets could be separated from RBCs and WBCs on the basis of their forward- and side-light scatter, and a narrow gate was placed around the platelets for analysis. For each sample the cytometer analyzed 10000 cells within the platelet gate.

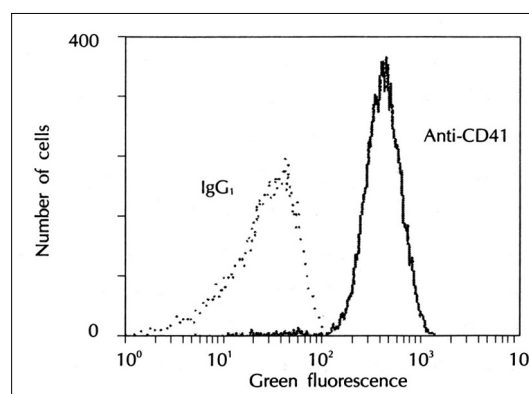


Fig. 2. Flow cytometric analysis of resting platelets. Whole blood was incubated with either FITC-conjugated anti-CD41 or control FITC-conjugated IgG₁. A histogram of FITC ("green") fluorescence of these cells are shown. Mean fluorescence intensity was measured.

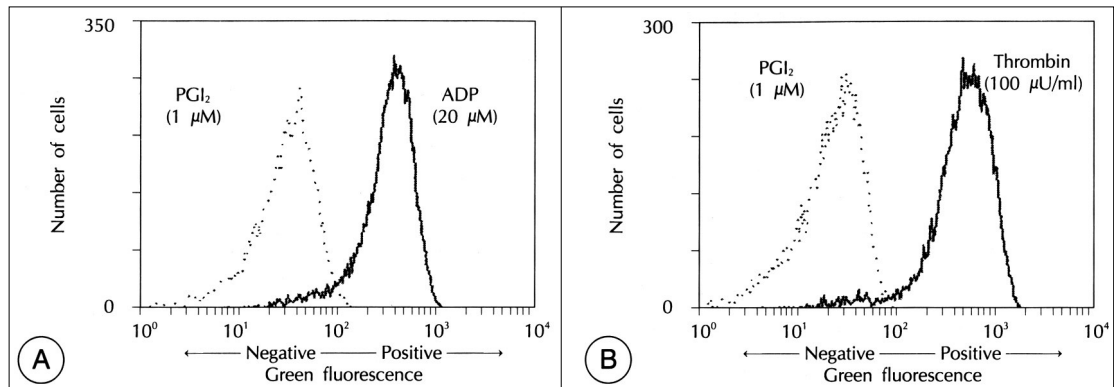


Fig. 3. Flow cytometric analysis of activated platelets. Whole blood was incubated with FITC-conjugated PAC1 and either with PGI₂ or ADP (a), or PGI₂ or thrombin (b). Histograms of FITC ("green") fluorescence of these cells are shown. Mean fluorescence intensity and the percentage of platelets positive for antibody were measured. Antibody-positive cells were defined as those platelets with a fluorescence intensity 99.0% to 99.2% of unstimulated platelets that had been prepared in the presence of 1 μ M of PGI₂.

Table 1. Baseline characteristics of study groups

	Control group (n=21)	Hypercholesterolemia group (n=20)	p value
Age (yr)	51.1 \pm 13.7	55.4 \pm 8.7	NS
Male	8 (38%)	7 (35%)	NS
BMI (kg/m ²)	21.63 \pm 3.10	23.29 \pm 2.72	0.078
Smoker	7 (33%)	6 (30%)	NS
Platelet count ($\times 10^3/\text{mm}^3$)	248.7 \pm 74.9	252.1 \pm 49.9	NS
Mean platelet volume (fL)	8.18 \pm 0.98	8.26 \pm 0.61	NS
Plasma lipid profile			
Total cholesterol (mg/dL)	134.6 \pm 21.4	275.7 \pm 31.4	<0.001
Triglyceride (mg/dL)	95.6 \pm 46.6	121.3 \pm 46.5	NS
HDL-cholesterol (mg/dL)	41.6 \pm 7.2	46.1 \pm 13.8	NS
LDL-cholesterol (mg/dL)	73.9 \pm 22.8	205.3 \pm 33.7	<0.001

Values are mean and SD.

BMI=body mass index ; HDL=high density lipoprotein ; LDL=low density lipoprotein ; NS=not significant

가 , anti - CD41 가 .

PAC1

가 (step - 275.7 \pm 31.4 mg/dL 205.3 \pm 33.7 mg/dL

wise) dL , 134.6 \pm 21.4 mg/dL 73.9 \pm 22.8

windows SPSS mg/dL 가 (p<0.001),

결과

휴지기 혈소판의 유세포 분석

일반적 특성의 비교 FITC - conjugated IgG₁

가 , MFI

MFI 가 (Table 2). FITC - con -

(Table 1). jugated anti - CD41

Table 2. Flow cytometric analysis of unstimulated platelets

	Control group (n=21)	Hypercho- lesterolemia group (n=20)	p value
Unloaded platelets	15.4 ± 1.2	15.4 ± 0.8	NS
Control IgG ₁	34.0 ± 3.4	34.3 ± 3.8	NS
Anti-CD41 bound	582.9 ± 134.1	609.7 ± 143.2	NS
PAC1 bound	31.4 ± 4.2	36.3 ± 6.0	<0.005

Values are mean fluorescence intensity (arbitrary unit ; mean and SD) after incubating with fluorescein isothiocyanate (FITC)-conjugated IgG₁, PAC1 or anti-CD41
NS=not significant

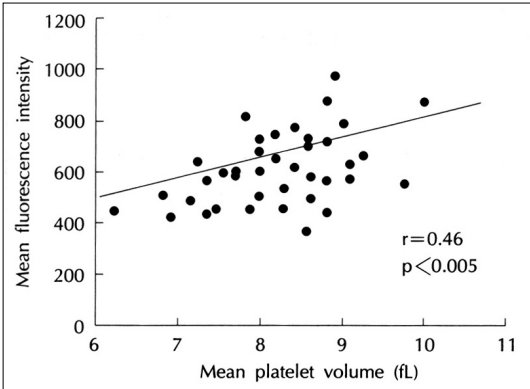


Fig. 4. Correlation between mean platelet volume and binding of anti-CD41 to unstimulated platelets. Anti-CD41 bound to platelets was estimated by mean fluorescence intensity (arbitrary unit) after incubating with FITC-conjugated anti-CD41.

MFI
($r = 0.46, p < 0.005$)가
Gp IIb/IIIa
(Fig. 4). , anti - CD41
, anti - CD41
FITC - conjugated PAC1
MFI (p<
0.005), ADP thrombin
PAC1
PAC1
PAC1 ($r = 0.48, p =$
0.002)가 가 가

Table 3. Univariate relations of PAC1 bound to unstimulated platelets with plasma lipids and binding of anti-CD41

	Correlation coefficients	p value
Total cholesterol	0.48	0.002
LDL-cholesterol	0.47	0.002
Triglyceride	0.17	0.28
HDL-cholesterol	0.07	0.66
Anti-CD41 bound	0.40	0.01

Binding of anti-CD41 or PAC1 to platelets was estimated by flow cytometry. Mean fluorescence intensity was measured after incubating with fluorescein isothiocyanate (FITC)-conjugated anti-CD41 or PAC1 respectively.
HDL=high density lipoprotein ; LDL=low density lipoprotein

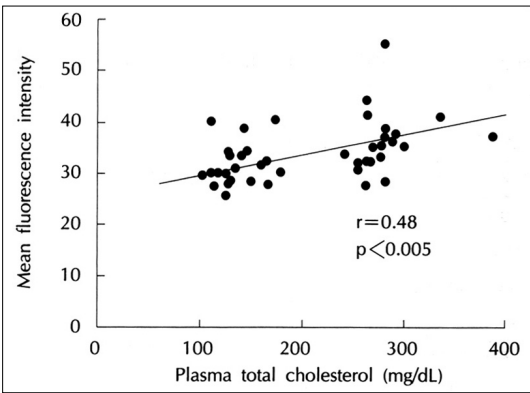


Fig. 5. Correlation between plasma total cholesterol level and binding of PAC1 to unstimulated platelets. PAC1 bound to platelets was estimated by mean fluorescence intensity (arbitrary unit) after incubating with FITC-conjugated PAC1.

Table 4. Multivariate linear relations of PAC1 bound to unstimulated platelets with plasma lipids and binding of anti-CD41

Independent variables	Beta coefficient	Partial R ² value	p value
Total cholesterol	0.033	0.23	0.0016
Anti-CD41 bound	0.015	0.16	0.0098
Intercept	18.241		<0.001

Overall R=0.595 (R²=0.35), p=0.0002
Binding of anti-CD41 or PAC1 to platelets was estimated by flow cytometry. Mean fluorescence intensity was measured after incubating with fluorescein isothiocyanate (FITC)-conjugated anti-CD41 or PAC1 respectively.

Gp IIb/IIIa (Fig. 5).
anti - CD41 PAC1
가 (Table 3).
가 PAC1 anti - CD41

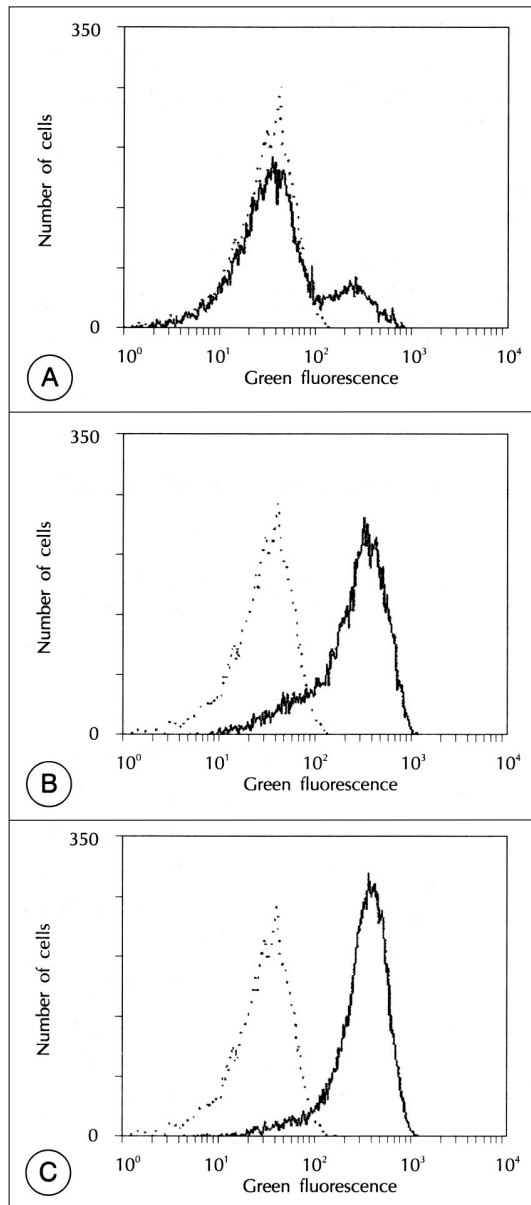


Fig. 6. Flow cytometric analysis of ADP-stimulated platelets. Whole blood was incubated with FITC-conjugated PAC1 and either with 1 μ M of PG₂ (—) or with ADP (---). The more the concentrations of ADP increased ((a) 0.05 μ M (b) 0.05 μ M and (c) 20 μ M), the more platelets glycoprotein IIb/IIIa were activated.

(Table 4).

ADP로 자극한 혈소판의 유세포 분석

ADP 가 가 FITC- conjugated PAC1
MFI

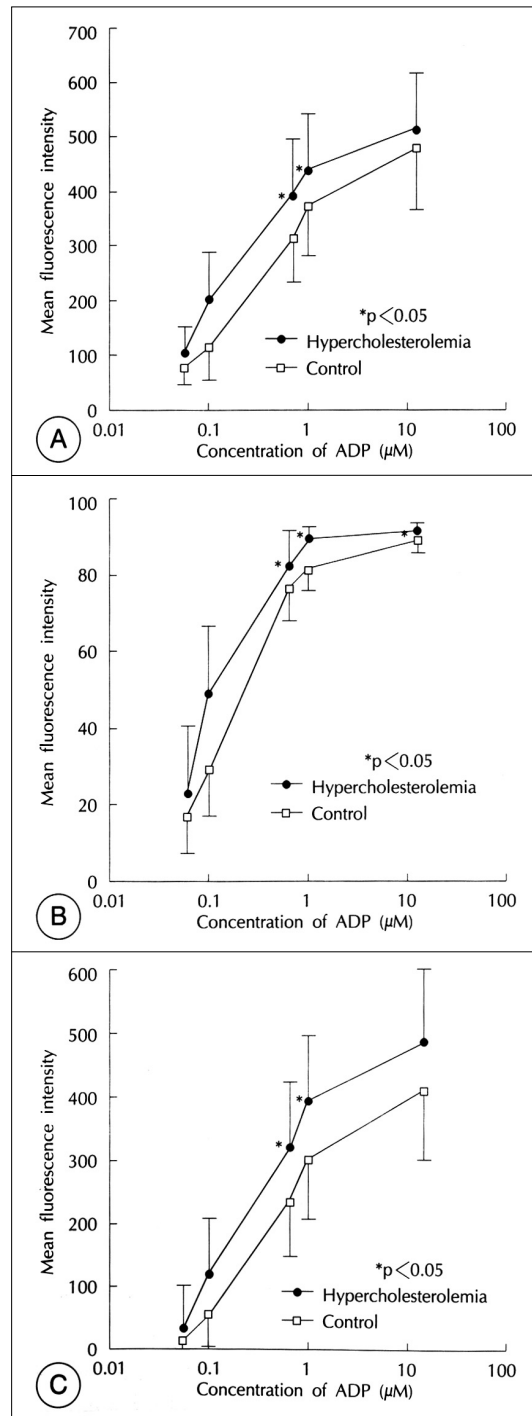


Fig. 7. Dose-response curve of PAC1 binding to glycoprotein IIb/IIIa in response to stimulation with ADP. Mean fluorescence intensity (arbitrary unit) (a), percentage of antibody-positive platelets (b), and binding index (arbitrary unit) (c) were depicted (mean and SD). Each value was higher in hypercholesterolemia group (* $p < 0.05$).

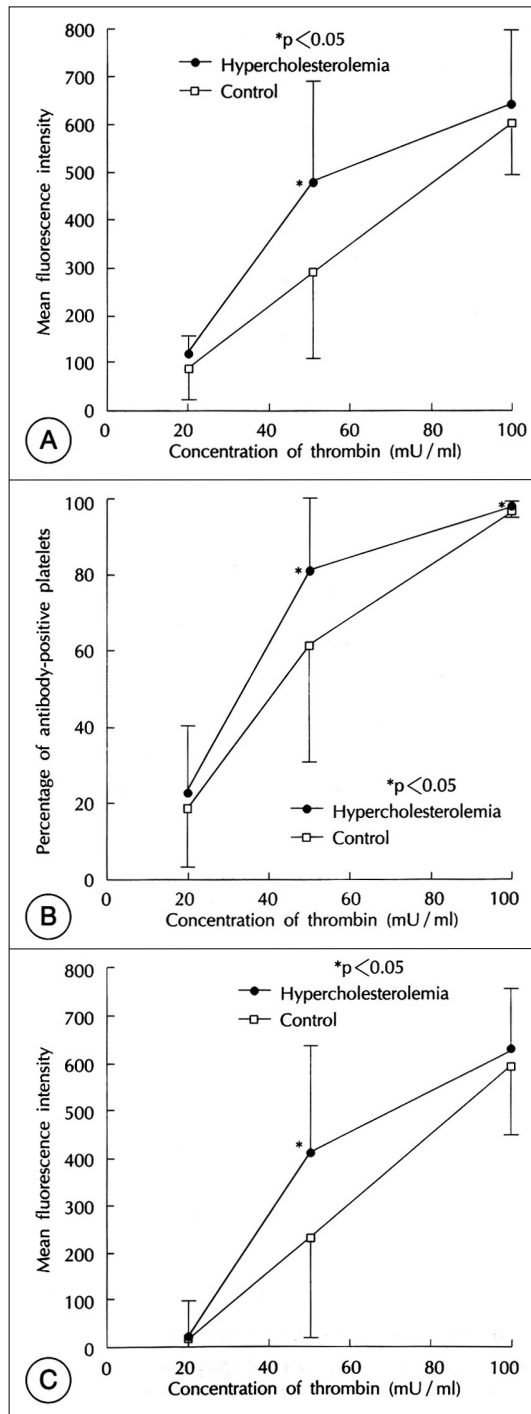


Fig. 8. Dose-response curve of PAC1 binding to glycoprotein IIb/IIIa in response to stimulation with thrombin. Mean fluorescence intensity (arbitrary unit) (a), percentage of antibody-positive platelets (b), and binding index (arbitrary unit) (c) were depicted (mean and SD). Each value was higher in hypercholesterolemia group (* $p < 0.05$).

가 (Fig. 6). 0.05 μ M 0.1 μ M ADP
MFI
가
0.5 μ M 1 μ M ADP
MFI, , BI
가 ($p < 0.05$).
0.05). , 20 μ M ADP
가 (Fig. 7).

Thrombin으로 자극한 혈소판의 유세포 분석
Thrombin 가 가 FITC - conjugated
PAC1 MFI
가 . 20 mU/mL thrombin
FITC - conjugated PAC1
가 , 50 mU/mL
MFI, , BI
가
($p < 0.05$)(Fig. 8). 100 mU/mL thrombin
가
($p < 0.05$).

고 찰

Gp IIb/IIIa
가 , Gp
IIb/IIIa ADP thrombin
Gp IIb/IIIa 가
Gp IIb/IIIa
가 Gp IIb/IIIa
가 , 7E3⁴⁰⁾ PAC1
7E3 ADP
Gp
IIb/IIIa 가 PAC1
Gp IIb/IIIa
³²⁾ PAC1
Anti - CD41 Gp IIb/IIIa
Gp IIb/IIIa
Gp IIb/IIIa
⁴¹⁾ PAC1
Gp Iib/ IIIa

iodine¹²⁵ PAC1 Gp IIb/IIIa
가 , anti - CD41
가 ,
가 Gp IIb/IIIa
PAC1
가 ADP thrombin
Gp IIb/
IIIa
가 가 Gp IIb/IIIa 가
, PAC1 anti - CD41
가 가 Gp IIb/IIIa
Gp IIb/IIIa
. PAC1
Gp IIb/IIIa
Gp IIb/IIIa
ADP thrombin Gp IIb/IIIa
. ADP
(20 μM)
, 5 PAC1 PAC1 가 , 1 μM
가 PAC1
. Thrombin
1 , 50 mU/
PAC1 mL 100 mU/mL , PAC1
Gp IIb/IIIa ,³²⁾ 50 mU/mL
4 PAC1 paraf - DiMinno¹⁹⁾ 가
ormaldehyde , Gp IIb/IIIa
8 B79.7 , thr -
omboxane A₂ mimetic
275.7 ± 31.4 mg/dL 205.3 ± 33.7 B79.7
mg/dL 가 Gp IIb/IIIa
가 , B79.7
가 Gp IIb/IIIa ,
⁴⁴⁾가 ,
thromboxane A₂ mi -
metic 가
. ¹⁴⁾ ,
anti - CD41 Gp IIb/IIIa PAC1
가 ,

Gp IIb/IIIa

Gp IIb/IIIa 가

가

가

Gp IIb/IIIa

연구배경 :

가 가

Integrin

glycoprotein(Gp) IIb/IIIa

Gp IIb/IIIa

방 법 :

가 240 mg/dL

가 20 (7 ; 55.4 ± 8.7)

가 21 (8 ; 51.1 ± 13.7)

Gp IIb/IIIa

fluorescein isothiocyanate(FITC) - conjugated anti - CD41

Gp IIb/IIIa FITC - conjugated PAC1

(flow cytometry)

FITC - con -

aden -

jugated PAC1

osine diphosphate(ADP) thrombin

Gp IIb/IIIa

결 과 :

PAC1

(p<0.005), ADP throm -

Gp lib/

bin

IIIa

anti - CD41

PAC1

(r = 0.48, p=0.002)가

PAC1

(r = 0.47, p=0.002)가

anti - CD41

PAC1 가 (r=0.40, p=0.01).

anti - CD41

Gp IIb/IIIa

Gp IIIa

가 PAC1

ADP thrombin

Gp IIb/

PAC1

(polymorphism)

⁵³ P1^{A2}

Gp

IIIa

가

(p<0.05). Anti - CD41

(r = 0.46, p<0.005)가

,

anti - CD41

결 론 :

Gp IIb/IIIa

가

Gp IIb/IIIa

ADP thrombin

가 가 , Gp IIb/IIIa

가

중심 단어 : glycoprotein IIb/IIIa

.

감사문

1995

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