

## 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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서 론

가 .  
 ,  
 collagen, thrombin, epinephrine, aden-  
 osine diphosphate(ADP)  
 , thromboxane malondiald-  
 ehyde 가 ,<sup>1-8)</sup>  
<sup>9-14)</sup> ,  
 가 ,<sup>15)16)</sup>  
 가 가  
<sup>17)</sup>  
 가 , ADP  
 , collagen thrombin  
 가 ,<sup>18)</sup>  
 가 aggr-  
 egometer ,  
 platelet factor 4, - thro-  
 mboglobulin, serotonin, malondialdehyde, thrombo-  
 xane B<sub>2</sub> ,  
<sup>19)</sup>  
 가  
 collagen throm-  
 boxane 가 ,<sup>6)7)20)21)</sup>  
 ,  
 / 가 가 ,  
 / 가 가  
<sup>22)</sup>  
 phospholipase 가 arac-  
 hidonic acid thromboxane  
 가 ,<sup>23)24)</sup>

phosphatidyl - inositol arachidonic  
 thromboxane  
 thromboxane 가  
<sup>8)</sup>  
<sup>13)</sup>  
 platelet factor 4, - thromboglobulin  
 collagen serotonin 가  
 가 ,<sup>4)8)</sup> - granule  
 dense granule 가  
 가 ,<sup>25)</sup>  
 가  
 phosphatidylinositol turnover 가,<sup>26)</sup>  
 Ca<sup>2+</sup> mobilizing system ,<sup>27)</sup>  
 Na<sup>+</sup>/H<sup>+</sup> antiport  
<sup>28)</sup>  
 phospholipase 가  
 Integrin glycoprotein(Gp) IIb/IIIa  
 40000~80000 가 ,  
 (fibrinogen)  
<sup>29-31)</sup> 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 ,<sup>32)</sup>  
 PAC1  
 Gp IIb/IIIa  
 iodine<sup>125</sup> PAC1  
 fluorescein isothiocyanate(FITC) phycoc-  
 erythrin(PE) PAC1 (flow  
 cytometry) Gp IIb/IIIa  
<sup>33-36)</sup>  
 Gp IIb/IIIa

Gp IIb/IIIa FITC - conjugated PAC1 8~9 30 21 G sc - alp 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 x g 20  
 - 70  
 Sodium citrate

### 대상 및 방법

대상

가 240 mg/dL  
 가 20 ( 7 ; 55.4 ± 8.7 )  
 가 21 ( 8 ; 51.1 ± 13.7 )

방법

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 IgG<sub>1</sub>(Code No. x 0927, DAKO Co., Denmark), ADP(Prod. No. A2754), thrombin(Prod. No. T4648), prostaglandin(PG) I<sub>2</sub>(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)

5 가 12 x 75 - mm polystyrene tube 5 μL  
 540 x g 5  
 cholesterol oxidase  
 glycerol triphosphate oxidase  
 phosphotungstic acid - MgCl<sub>2</sub>  
 Friedwald formula

FITC - conjugated anti - CD41  
 5 μL FITC - conjugated anti - CD41 10 μL( 20 μg/mL), PGI<sub>2</sub> 5 μL( 1 μM), isotonic HEPES buffer(137 mM NaCl, 2.7 mM KCl, 1 mM MgCl<sub>2</sub>, 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 IgG<sub>1</sub>

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, 0.05 μM, 0.1 μM, 0.5 μM, 1 μM, 20 μM, Thrombin 10 U/mL, 20 mU/mL, 50 mU/mL, 100 mU/mL, FITC-conjugated PAC1, ADP, thrombin, PGI<sub>2</sub> 5 μL (1 μM), 15, isotonic HEPES buffer 500 μL, 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 μm CaliBRI - TE beads (Cat. No. 34952, Becton Dickinson Co., San Jose, CA, USA), FITC-conjugated IgG<sub>1</sub>, 4000-5000, 70 μm nozzle, laser beam, Gain logarithmic 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 intensity) (Fig. 2), ADP thrombin (percentage of antibody-positive platelets), 1 μM PGI<sub>2</sub>, 99-99.2% (Fig. 3), (BI; binding index)

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

통계 분석

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

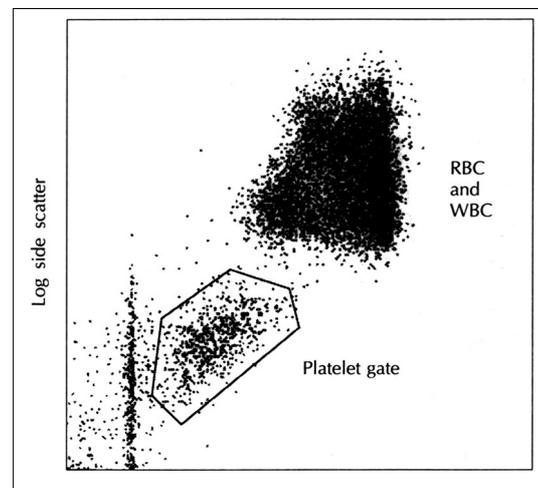


Fig. 1. Scatter profile of whole blood. The platelets could be separated from RBCs and WBCs on the basis of their forward- and side-light scatter, and a narrow gate was placed around the platelets for analysis. For each sample the cytometer analyzed 10000 cells within the platelet gate.

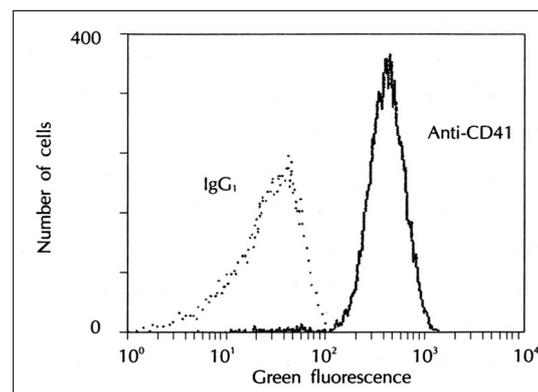
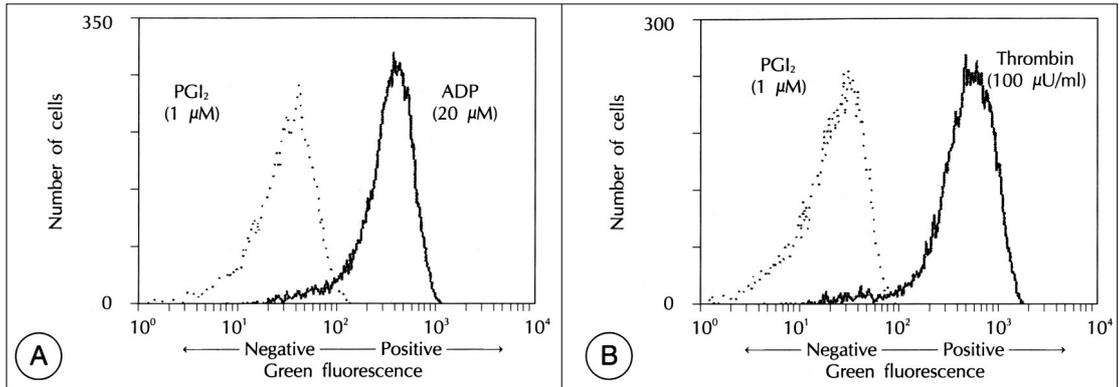


Fig. 2. Flow cytometric analysis of resting platelets. Whole blood was incubated with either FITC-conjugated anti-CD41 or control FITC-conjugated IgG<sub>1</sub>. A histogram of FITC ("green") fluorescence of these cells are shown. Mean fluorescence intensity was measured.



**Fig. 3.** Flow cytometric analysis of activated platelets. Whole blood was incubated with FITC-conjugated PAC1 and either with PGI<sub>2</sub> or ADP (a), or PGI<sub>2</sub> 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 μM of PGI<sub>2</sub>.

**Table 1.** Baseline characteristics of study groups

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

Values are mean and SD.

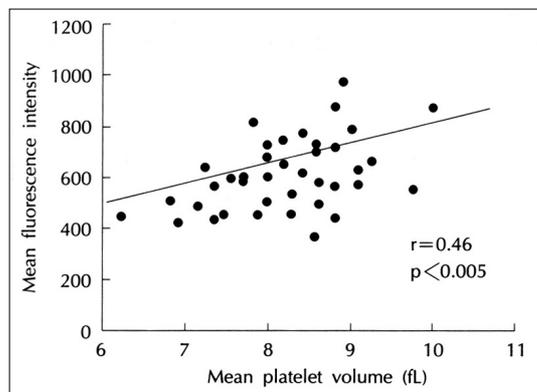
BMI=body mass index ; HDL=high density lipoprotein ; LDL=low density lipoprotein ; NS=not significant

가 , anti - CD41 가 .  
PAC1  
가 (step - 275.7 ± 31.4 mg/dL 205.3 ± 33.7 mg/dL  
wise) dL , 134.6 ± 21.4 mg/dL 73.9 ± 22.8  
windows SPSS . mg/dL 가 (p<0.001),  
결과 , 가 .  
휴지기 혈소판의 유세포 분석  
일반적 특성의 비교 FITC - conjugated IgG<sub>1</sub>  
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 <sub>1</sub>	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 IgG<sub>1</sub>, PAC1 or anti-CD41  
NS=not significant



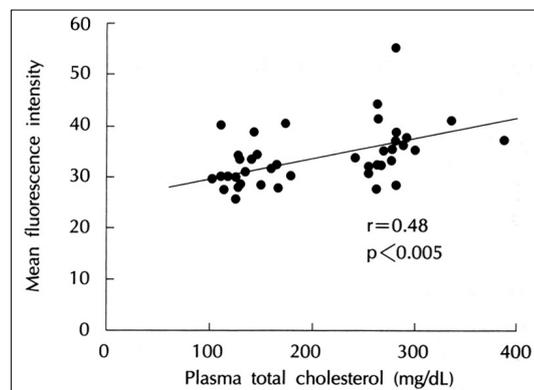
**Fig. 4.** Correlation between mean platelet volume and binding of anti-CD41 to unstimulated platelets. Anti-CD41 bound to platelets was estimated by mean fluorescence intensity (arbitrary unit) after incubating with FITC-conjugated anti-CD41.

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.

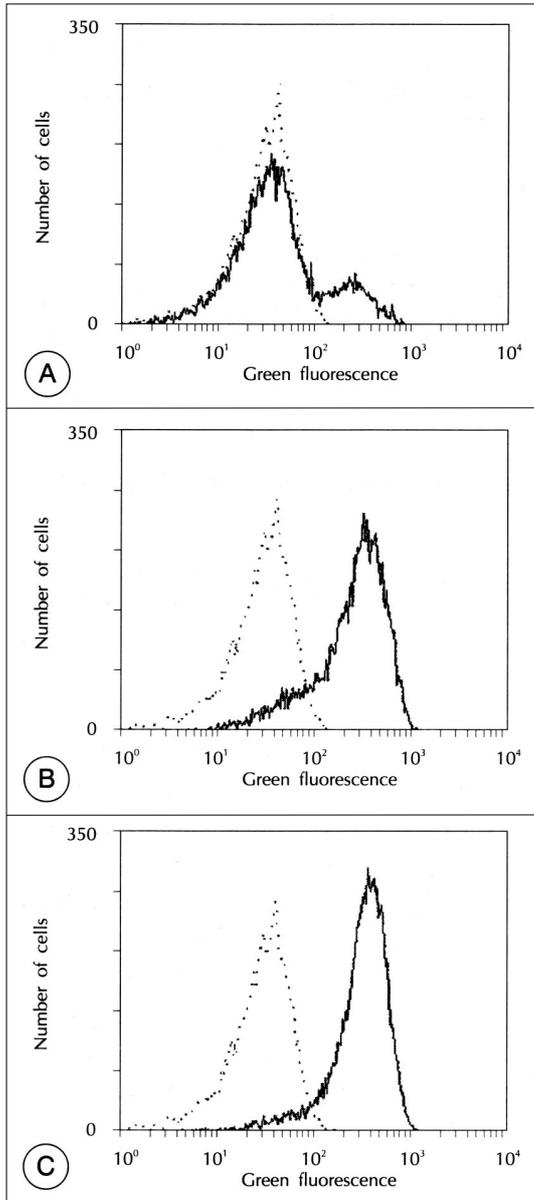
**Table 4.** Multivariate linear relations of PAC1 bound to unstimulated platelets with plasma lipids and binding of anti-CD41

Independent variables	Beta coefficient	Partial R <sup>2</sup> 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 (R<sup>2</sup>=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

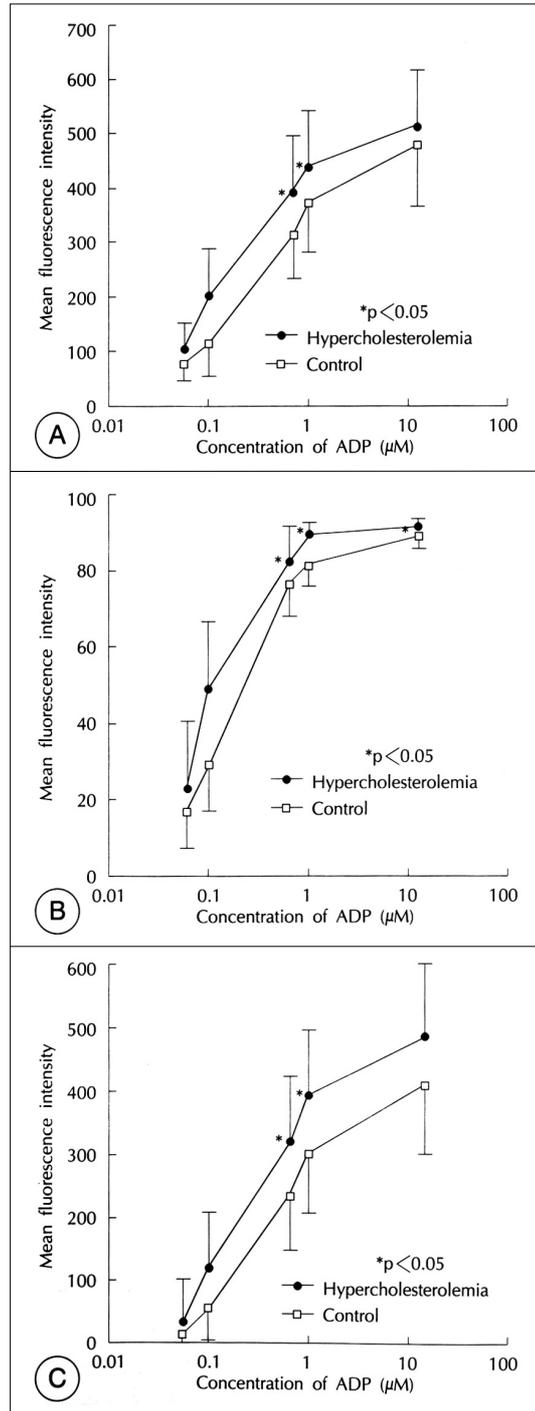


**Fig. 6.** Flow cytometric analysis of ADP-stimulated platelets. Whole blood was incubated with FITC-conjugated PAC1 and either with 1  $\mu\text{M}$  of  $\text{PGI}_2$  (—) or with ADP (· · ·). The more the concentrations of ADP increased ((a) 0.05  $\mu\text{M}$  (b) 0.05  $\mu\text{M}$  and (c) 20  $\mu\text{M}$ ), the more platelets glycoprotein IIb/IIIa were activated.

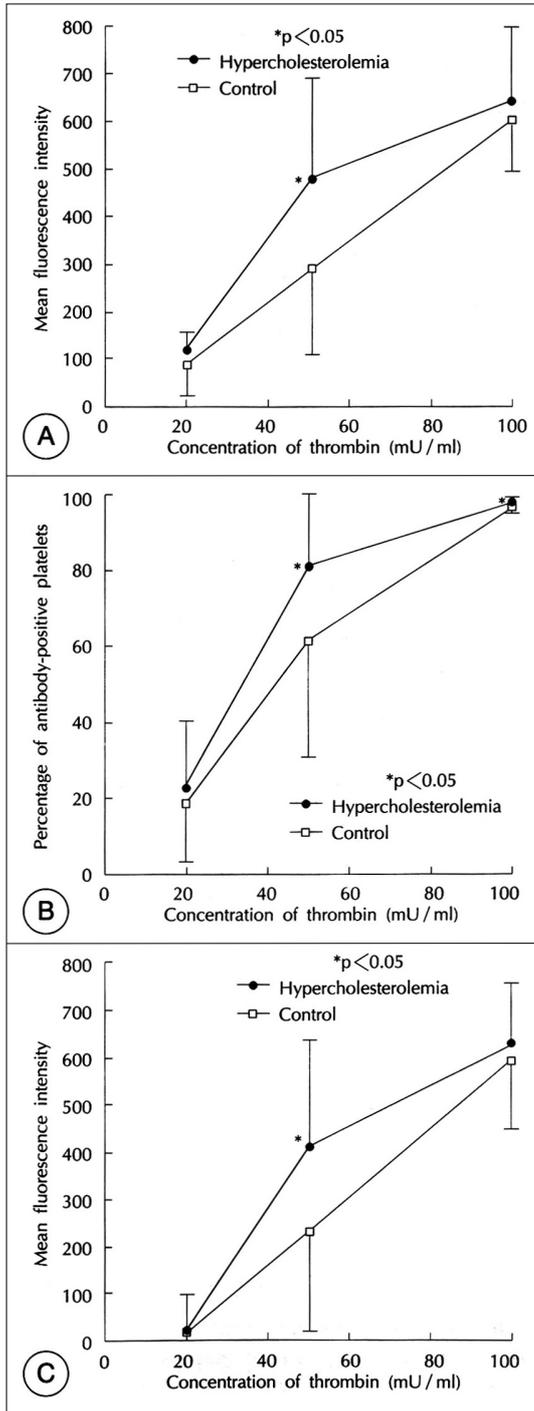
(Table 4).

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  $\mu$ M 0.1  $\mu$ M ADP  
MFI  
가  
0.5  $\mu$ M 1  $\mu$ M ADP  
MFI, , BI  
가 ( $p < 0.05$ ).  
20  $\mu$ 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  
( $p < 0.05$ ).

### 고찰

Gp IIb/IIIa  
가 , Gp  
IIb/IIIa ADP thrombin  
Gp IIb/IIIa 가  
Gp IIb/IIIa  
가 Gp IIb/IIIa  
가 , 7E3<sup>40)</sup> PAC1  
 , 7E3 ADP  
Gp  
IIb/IIIa 가 PAC1  
Gp IIb/IIIa  
,<sup>32)</sup> PAC1  
 . Anti - CD41 Gp IIb/IIIa  
Gp IIb/IIIa  
Gp IIb/IIIa  
.<sup>41)</sup> PAC1  
Gp Iib/ IIIa

iodine<sup>125</sup> PAC1 , Gp IIb/IIIa , anti - CD41 , 가 , 가 , 가 Gp IIb/IIIa PAC1 , 가 ADP thrombin Gp IIb/IIIa IIIa , 가 가 Gp IIb/IIIa , 가 , PAC1 anti - CD41 Gp IIb/IIIa , 가 가 , Gp IIb/IIIa PAC1 Gp IIb/IIIa , Gp IIb/IIIa ADP thrombin Gp IIb/IIIa , , double syringe technique . ADP (20 μM) , 5 PAC1 PAC1 가 , 1 μM PAC1 Thrombin 50 mU/ mL 100 mU/mL , PAC1 Gp IIb/IIIa ,<sup>32)</sup> 50 mU/mL , 4 PAC1 paraf - DiMinno<sup>19)</sup> 가 , Gp IIb/IIIa ormaldehyde 8 B79.7 , thr - omboxane A<sub>2</sub> mimetic , 275.7 ± 31.4 mg/dL 205.3 ± 33.7 B79.7 Gp IIb/IIIa , 가 , 가 , B79.7 Gp IIb/IIIa , 44)가 , thromboxane A<sub>2</sub> mi - metic ,<sup>14)</sup> , Gp IIb/IIIa PAC1 anti - CD41 가 ,

Gp IIb/IIIa 연구배경 : Integrin glycoprotein(Gp) IIb/IIIa

Gp IIb/IIIa 가 가 Gp IIb/IIIa 가 가

1)3)6 - 8)11)13)14) 가 가

2)22) (membrane fluidity) 가 가

가 46) phospholipase A<sub>2</sub> 가 47)

Gp IIb/IIIa 가가 thromboxane (flow cytometry) FITC - conjugated PAC1

48) Gp IIb/IIIa가 가 가 jugated PAC1 adenosine diphosphate(ADP) thrombin Gp IIb/IIIa

49)50) ADP thrombin 결과 : PAC1 (p<0.005), ADP thrombin Gp IIb/IIIa

aggregometer 51) - thrombin bin Gp IIb/IIIa

bulin 52) anti - CD41 PAC1 (r = 0.48, p = 0.002)가

Gp IIb/IIIa 가 41) , PAC1 (r = 0.47, p = 0.002)가

Gp IIb/IIIa 가가 PAC1 가 (r = 0.40, p = 0.01).

anti - CD41 Gp IIb/IIIa Gp IIIa 가 PAC1

P1<sup>A2</sup> (polymorphism) . ADP thrombin Gp IIb/IIIa

53) P1<sup>A2</sup> Gp IIIa PAC1

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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