

치료받지 않은 고혈압환자에서 혈중 단핵구 β ARK1의 농도와 좌심실질량의 비교연구

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Association between β ARK1 Level of Circulating Mononuclear Leukocytes and Left Ventricular Mass in Non-treated Hypertensive Patients

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ABSTRACT

Background : Beta-adrenergic receptor Kinase 1 (β ARK1) is a serine/threonine kinase attached, which inhibits the coupling of β -adrenergic receptor with G-protein. Myocardial β ARK1 level is usually elevated in heart failure and hypertrophy, but it is not known whether the circulating β ARK1 level is related with the degree of cardiac hypertrophy. This study was performed to evaluate the association of the β ARK1 level in circulating mononuclear leukocytes (MNL) in untreated hypertension with left ventricular mass in hypertensive patients.

Method : Nineteen non-treated hypertensive patients were included for this study. High blood pressure was confirmed when systolic BP is over 150 mmHg or diastolic BP is over 95 mmHg. Echocardiography was performed to evaluate the degree of hypertrophy by measuring the left ventricular mass index (LVMI) and relative wall thickness (RWT), and test the LV function by measuring the ejection fraction (EF) according to ASE guideline. At the same time, blood was collected from each patient and MNL were isolated by gradient centrifuge with Ficoll-400. Total RNA was purified from MNL and semi-quantitative RT-PCR was performed. After reverse transcription, PCR was done with primers for human β ARK1 and GAPDH as external control.

β ARK1 levels were expressed by ratio to GAPDH level and estimated the relations with clinical and Echocardiographic parameters. **Result** : We studied confirmed 19 hypertensive patients (10 men and 9 women, mean age of 50.6 years). Echocardiographically measured indices (mean \pm SD) were as follows ; LVMI (137.3 \pm 30.6 g/m²), PWT (0.53 \pm 0.09) and EF (54.6 \pm 8.5%). Ratio of β ARK1 levels to GAPDH was from 0.10 to 0.96 (0.62 \pm 0.25). β ARK1 levels were correlated with LVMI (correlation coefficient : r = 0.502, p = 0.029) and RWT (r = 0.627, p = 0.004). But Systolic BP (r = -0.009, p = 0.93), diastolic BP (r = 0.07, p = 0.85) or EF (r = 0.045, p = 0.84) were not related to level of β ARK1. **Conclusions** : The β ARK1 level of circulating MNL was correlated well with the degree of the cardiac hypertrophy estimated by LVMI and RWT. This data

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suggests that activation of sympatho-adrenal system would exert a major role in developing cardiac hypertrophy and we can expect the decreased responsiveness to catecholamine in the heart of hypertensive patients.

ARK1 in circulating MNL might be used as a predictor or marker for LV hypertrophy in hypertensive patients. **(Korean Circulation J 2000;30(12):1530-1539)**

KEY WORDS : ARK1 (beta-adrenergic receptor Kinase 1) · Hypertrophy · LVMI (Left Ventricular Mass Index).

17)

서 론 가 (blunting) ,

가 (homologous desen-

sitization) 가

G - protein G - protein -

coupled receptor kinase(GRK) 18)

GRK serine/threonine kinase family ,

12% 30% 6 GRK

가 5-7)

GRK2(ARK1),

GRK3(ARK2) GRK5가

17)19)20) ARK1

G - protein - su -

bunit

C - terminal

G - protein uncoupling

renin - angio -

tensin neurohumoral system 가 Takashi 21)

8 - 15)

down regulation

(inotropic), norepine -

(lucitropic) (chronotropic) phrine 가

16)

7 transmembrane 가 Choi

G - protein adenyI cyclase ARK1 가

cyclic AMP 가 22)

$LVM(g) = 0.8[1.04(IVSTd + LVIDd + PWTd)^3 - LVIDd^3] + 0.6$
 $BSA(m^2) = 0.007184[height(cm)]^{0.725}[weight(kg)]^{0.425}$
 $Ejection\ Fraction(\%) = \frac{[(LVIDd^2 - LVIDs^2) - LVIDd^2] \times 100}{\dots}$
 $Relative\ Posterior\ Wall\ Thickness = 2PWTd/LVIDd$
 $Left\ Ventricular\ Mass\ Index(g/m^2) = LVM/BSA$
 (LVM : Left Ventricular Mass, LVIDd :
 , LVIDs : , IVSTd :
 , PWTd : , BSA :
)

total RNA 20

cc
 ACD(acid citrate Dextrose solution)
 Byum²⁷⁾
 PBS 1 : 2 Ficoll - 400
 x 400 g 30 Buffi
 coat
 Trireagent(BM[®]) total RNA
 total RNA
 RNA

Reverse transcription
 2 µg total RNA reverse transcription
 Buffer(50 mM Tris - HCl, pH 8.3, 75 mM KCl, 3 mM MgCl₂, 5 mM DTT, 0.5 mM dNTP) random hexamer(Gibco BRL[®]), reverse transcriptase(Perkin Elmer[®])
 5 25 10 annealing 37
 60 extension 95 5 denature
 DNA

PCR protocol
 Reverse transcription DNA tem -
 plate polymerase chain reaction(PCR)
 . Template DNA PCR Buffer(20 mM Tris -
 HCl, pH 8.4, 50 mM KCl, 1.5 mM MgCl₂, 0.1%

Triton X - 100, 0.01% Gelatin) 1 mM dNTP, 1 pmol primers, 3 unit Taq polymerase
 94 1 denature , 55 1 an -
 nealing, 72 1.5 extension cycle

primer sequence
 ARK1 : 1,050 bp(1,549 - 1,532)
 sense primer :
 5' - GCAACATGTACGCCATGAAGTGC
 antisense primer :
 5' - TCATCGAAGGAGCCAATGTGC
 GAPDH : 580 bp(603 - 581)
 sense primer :
 5' - GCTTTTAACTCTGGTAAAGTGG
 antisense primer :
 5' - TACCGCCACAGTTCCCGGAGG

PCR cycle
 ARK1 GAPDH 27 cycle 35
 cycle 3cycle , PCR product
 gel running PCR
 cycle cycle glyceraldehyde -
 3 - phosphate dehydrogenase(GAPDH) ARK1
 DNA cycle
 가 가 plateau cycle

ARK1
 cycle total RNA
 GAPDH ARK1 PCR . PCR pro -
 duct gel loading gel running
 UV illumination , SigmaGel[®]
 . ARK1 reverse transcripta - se -
 polymerase chain reaction(RT - PCR) DNA
 GAPDH ARK1
 가

통계적 방법
 ±
 , M - mode
 ARK1 SPSS

p 0.05

심초음파상의 지표

결 과

임상양상

19
 10 , 9 . 24 70 (:
 10.4 ± 12.3), 150 cm 178.1 cm(
 : 160.3 ± 10.5 cm, 43 kg 93.7 kg
 (: 62.4 ± 14.5 kg), 1.45 m²
 2.14 m²(: 1.66 ± 0.23 m²),
 168.68 ± 17.94
 mmHg, 103.68 ± 6.42 mmHg
 70.3 ± 8.3 (Table 1).

31.4 ± 3.5 mm, 46.8 ±
 2.8 mm, 12.4 ± 2.0 mm
 12.4 ± 1.8 mm
 0.35 0.70 0.53 ± 0.09
 (Ejection Fraction) 42.24% 68.85%
 54.6 ± 8.5% (LVM) 140.12
 g 348.72 g 243.3 ± 61.7 g
 (LVMI) 91.87 g/m² 206.26 g/m²
 137.3 ± 30.6 g/m² (Table 2).

PCR cycle의 결정

PCR cycle GAPDH
 ARK1 cycle PCR (Fig. 2)

Table 1. Clinical characteristics (n = 19)

Gender	Men 10, Female 9
Age (yr)	50.4 ± 12.3
Height (cm)	160.3 ± 10.5
Body weight (kg)	62.4 ± 14.5
Body surface area (m ²)	1.66 ± 0.23
SBP (mmHg)	
Systolic	168.7 ± 17.9
Diastolic	103.6 ± 6.4
Heart rate (/min)	70.3 ± 8.3

Values are mean ± SD.

Table 2. Echocardiographic data (n = 19)

LVD (S) (mm)	31.4 ± 3.5
LVD (D) (mm)	46.8 ± 2.8
ST (D) (mm)	12.4 ± 2.0
PWT (D) (mm)	12.4 ± 1.8
RWT	0.53 ± 0.1
LVEF (%)	54.6 ± 8.5
LVM (g)	243.3 ± 61.7
LVMI (g/m ²)	137.3 ± 30.6

Values are mean ± SD.

LVD (D) : Left ventricular internal dimension at end-diastole

LVD (S) : Left ventricular internal dimension at end-systole

ST (D) : Ventricular septal thickness at end-diastole

PWT (D) : Left ventricular posterobasal free wall thickness at end-diastole

RWT : Relative posterior wall thickness

LVEF : Left ventricular ejection fraction

LVM : Left ventricular mass

LVMI : Left Ventricular mass index

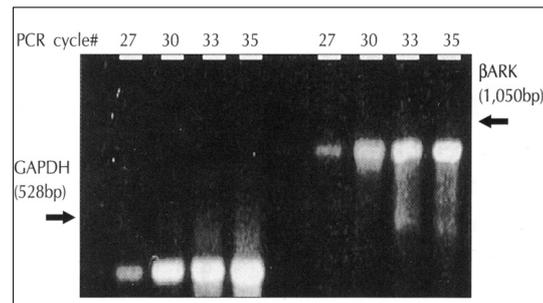


Fig. 2. Semiquantitative RT-PCR. Semiquantitative reverse transcriptase-polymerase chain reaction (RT-PCR) was performed to determine the adequate cycles for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and beta-adrenergic receptor kinase 1 (ARK1).

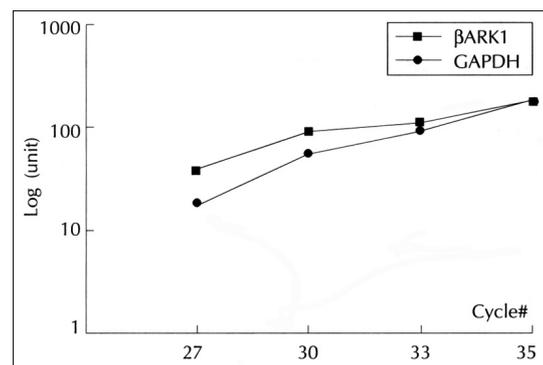


Fig. 3. Quantitation of PCR product. Proper polymerase chain reaction (PCR) cycle is determined as glyceraldehyde-3-phosphate dehydrogenase (GAPDH) 30 cycle and beta-adrenergic receptor kinase 1 (ARK1) 33 cycle.

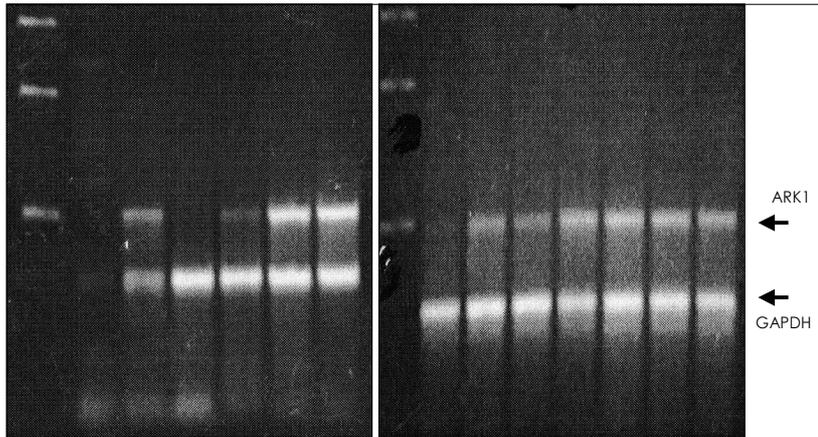


Fig. 4. RT-PCR sample of patients. After completion of PCR, each sample was run on the agarose gel and quantitated relative density of ARK1 compared with glyceraldehy-de-3-phosphate dehydrogenase (GAPDH) by SigmaGel[®] with UV exposed photography. Ratio of ARK1 levels to GAPDH levels was from 0.10 to 0.96 (0.62 ± 0.25).

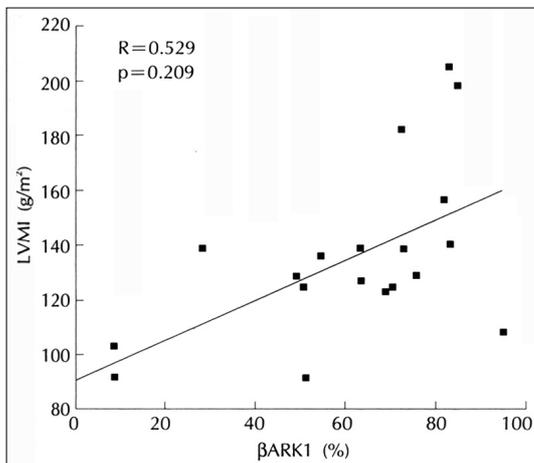


Fig. 5. Correlation between ARK1 and LVMI. ARK1 : beta-adrenergic receptor kinase 1. LVMI : left ventricular mass index.

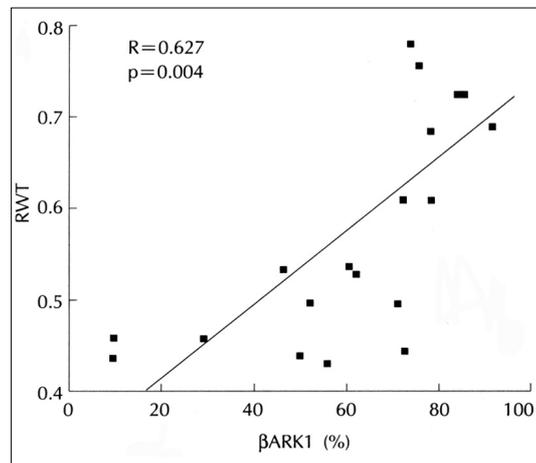


Fig. 6. Correlation between ARK1 and RWT. ARK1 : beta-adrenergic receptor kinase 1. RWT : relative posterior wall thickness.

(Fig. 3). GAPDH ARK1
 27 cycle 35 cycle
 cycle GAPDH 30 cycle,
 ARK1 33 cycle PCR

ARK1 (r = 0.502, p = 0.029)
 ARK1 (r = 0.627, p = 0.004)
 (Figs. 5 - 8).

고 찰

β ARK1 발현의 반정량분석

GAPDH ARK1 (Fig. 4) 0.10
 0.96(0.62 ± 0.25) 가

ARK1 가 (sarcomere)
 가 가
 (r = -0.009, p = 0.97),
 (r = 0.07, p = 0.85) (r = 0.045, p = 0.84)

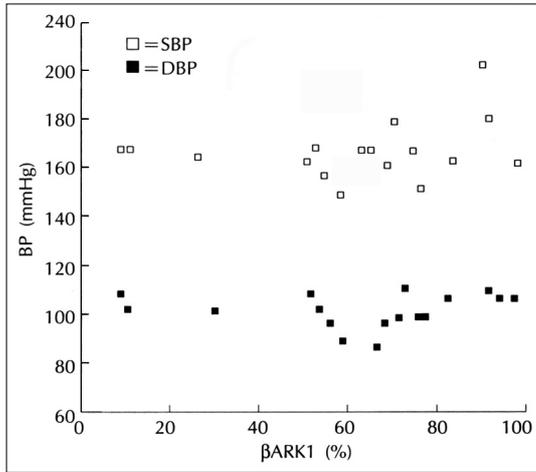


Fig. 7. Correlation between β ARK1 and BP. β ARK1 : beta-adrenergic receptor kinase 1. SBP : systolic pressure. DBP : diastolic pressure.

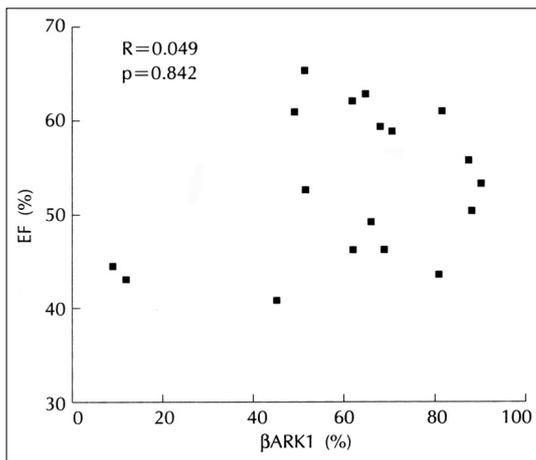


Fig. 8. Correlation between β ARK1 and EF. β ARK1 : beta-adrenergic receptor kinase 1. EF : ejection fraction.

uncoupling . GRK in vitro in vivo - G - protein 31)

adenylyl cyclase 가 , ARK1(GRK - 2) 가 cytosolic A - kinase GRK - 5/6 32)

ARK1 ARK1 가 ARK1

adenylyl cyclase GRK 가 가 ARK1 가 29)33)

. Choi GRK 가 가 22)

가 ARK1 ARK1

가 GRK 가 34)35)

가 가 36)

adenylyl cyclase 가 37)

forskolin adenylyl cyclase A - kinase ARK1

- G - protein

adenylyl cy -

clase

G - protein 28 - 30)

가

G - protein

. GRK가

receptor G-protein

ARK1
가
ARK1

ARK1

ARK1

중심 단어 : ARK1(beta-adrenergic receptor Ki-nase 1)

대상 및 방법 :

3
가 150 mmHg
95 mmHg
19
(LVMl), (RWT), (EF)
ARK1
RNA
GAPDH
RT-PCR
ARK1

결 과 :

19
가 10 , 가 9
50.42 ± 12.36
168.68 ± 17.94 mmHg,
103.68 ± 6.42 mmHg LVMl
137.3 ± 30.6 g/m², RWT 0.53 ± 0.09
EF 54.6 ± 8.5% . GAPDH ARK1
0.10 0.96(0.62 ± 0.25) . ARK1
LVMl
(correlation coefficient : r=0.502, p=0.029)
, RWT (r=0.627,
p=0.004) , ARK1
(r= -0.009, p=0.93), (r=0.07, p=
0.85) EF(r=0.045, p=0.84)

결 론 :

ARK1

ARK1

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