

고양이를 이용한 허혈성 전조건 모델에서 Heat Shock Protein의 변화에 의한 지연 심근보호효과

박종선 · 홍그루 · 신동구 · 김영조 · 심봉섭

Delayed Cardioprotective Effect of Ischemic Preconditioning is Mediated by Heat Shock Protein in Cat Heart

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ABSTRACT

Background : It is well known that ischemic preconditioning protects the heart against infarction or arrhythmias from a subsequent ischemic injury. Two phases of the effect of preconditioning has been explored, early protection and second window of protection at 24 hours. The late protection was seen in some animal model, but the precise mechanism is controversial. This study was designed to evaluate the late cardioprotective effect and role of HSP70 in ischemic preconditioning of cat heart. **Methods** : Two groups of cats were studied. Control animals were subjected to an episode of 40-min coronary artery occlusion followed by 30-min reperfusion. Experimental animals were subjected to ischemic preconditioning before the 40-min ischemia/reperfusion. The preconditioning protocol was comprised of three 5-min episodes of ischemia interspersed by 10-min episodes of reperfusion. After sustained ischemia and reperfusion, left ventricular risk area and infarct area were measured by injection of Evans blue dye and triphenyltetrazolium staining, and myocardial HSP70 mRNA was examined in risk (left ventricular anterior wall) and nonrisk (left ventricular posterior wall) area using northern blot hybridization. HSP70 mRNA expression was quantified as a percent of GAPDH. The late cardioprotective effects of ischemic preconditioning were determined by infarct size (% area at risk). **Results** : Infarct size was markedly limited by ischemic preconditioning when compared with the control group ($18.5 \pm 6.9\%$ vs $38.5 \pm 11.1\%$; $p < 0.001$). HSP70 mRNA expression in risk area was much higher in preconditioning group than control group ($78 \pm 12\%$ vs $41 \pm 11\%$; $p < 0.01$). But, there was no significant difference of HSP70 mRNA expression in the posterior wall between control and ischemic preconditioning group. **Conclusions** : These data suggest that ischemic preconditioning have delayed myocardial protective effect from ischemia. The increase in myocardial HSP70 mRNA may be one of the contributing factors to the delayed cardioprotective effects of ischemic preconditioning in cats. (**Korean Circulation J 2001;31(1):16-23**)

KEY WORDS : Ischemic preconditioning · Delayed myocardial protection · HSP70.

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서 론

1985 Murry ¹⁾

2-4)

5) 가

4

7-9)

adenosine ¹⁰⁾¹¹⁾

가

가

“second window of protection” ¹²⁾¹³⁾

mRNA

가 가 heat shock protein

(HSP70) 가 ¹⁴⁾

heat shock protein(HSP70)

(Harvard model 607 respirator, USA)

PCO₂, PO₂,

pH 7.40, 25 30 mmHg, 80 100 mmHg, 7.35

36 37

4

()

23 G silastic needle (dP/dt)

5 F (5 F pediatric polyvinyl catheter)

heparin kg 1000 U

heparinized saline (10 U/cc)

가

20 가

MP100WS, BIOPACK system (USA) Acqknowledge 881

관상동맥폐쇄 및 재관류

3 5 mm

3-0 1

cm, 3 mm

대상 및 방법

실험재료 및 준비

2.5 4.0 kg(3.1 kg)

19

10 , 9

kg 2 mg ketamine

kg 1 mg

5 mm

ST

5

3

40

2% lidocaine kg 2 mg

(Fig. 1).

사용된 약물

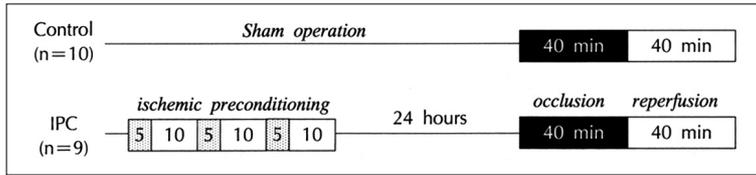


Fig. 1. Diagram of experimental time course, Ischemic preconditioning (IPC) was elicited by three 5 min episodes of ischemia and 10 min reperfusion.

triphenyl tetrazolium chloride(TTC), Evan's blue
(Sigma Chemical Co. St. Louis, USA)

(atrio - ventricular gr -
oove) 2 3 mm 4
5
sham operation
24 , pH 7.4, 37 , 0.1% TTC
15
24 Evans
blue TTC
ketamine 0.5 mg
HSP70
PCO₂, PO₂, pH 25 30 mmHg,
80 100 mmHg, 7.35 7.40, 36
37
HSP70 mRNA 측정
24 - 70
mRNA RNA
Chomczynski Sacchi¹⁵⁾ acid guani -
dium thiocyanatephenol chloroform
Rigbi¹⁶⁾ nick translation
20 μCi - ³²P - dCTP
Sephadex G50 gel filtration
hybridization
Northern hybridization RNA agarose
gel nitrocellulose capillary tra -
nsfer nitrocellulose 80
2 prehybridization 32P
5% 42 hybridiza -
Evans blue 30 ml tion Hybridization X - ray

실험방법
24
24
ketamine 0.5 mg
HSP70
PCO₂, PO₂, pH 25 30 mmHg,
80 100 mmHg, 7.35 7.40,
36
37
HSP70
(가) (가)
가) 1 mg
- 70
혈역학적 지수 측정
, , dP/dt ,
dP/dt , 30
, 30 5
심근괴사부위 및 위험지역 측정
5%
Evans blue 30 ml

Table 1. Effects of ischemic preconditioning on heart rate, left ventricular systolic pressure and rate-pressure product in each groups of cats

	Heart Rate (bpm)			LVSP (mmHg)			RPP (mmHg × bpm/10 ³)		
	B	O	R	B	O	R	B	O	R
Control (n = 10)	206 ± 20	129 ± 15	126 ± 17	206 ± 27	129 ± 22	117 ± 11	41.3 ± 3.54	16.6 ± 3.33	14.7 ± 3.19
IPC (n = 9)	202 ± 23	134 ± 30	125 ± 17	217 ± 30	144 ± 25	146 ± 25	42.3 ± 3.69	19.3 ± 3.75	18.3 ± 3.43

B : basal, O : occlusion, R : reperfusion, IPC : ischemic preconditioning, LVSP : left ventricular systolic pressure, RPP : rate-pressure product. Values are mean ± SD. There was no significant difference between groups

Table 2. Effects of ischemic preconditioning on maximal and minimal dP/dt in each groups of cats

	Max dP/dt (mmHg/sec)			Min dP/dt (mmHg/sec)		
	B	O	R	B	O	R
Control (n = 10)	1615 ± 272	1386 ± 277	1294 ± 234	1334 ± 107	1184 ± 85	1108 ± 68
IPC (n = 9)	1448 ± 266	1362 ± 283	1239 ± 158	1342 ± 195	1263 ± 166	1261 ± 298

B : basal, O : occlusion, R : reperfusion, IPC : ischemic preconditioning, B, LVSP : left ventricular systolic pressure, RPP : rate-pressure product. Values are mean ± SD. There was no significant difference between groups

autoradiography

HSP70 mRNA internal standard
glyceraldehyde 3 - phosphate dehydrogenase
(GAPDH) northern hybridization
scanning GAPDH
%

통계처리

SPSS/PC+

p<0.05

unpaired t - test

결 과

혈역동학적 측정

dP/dt

(Table 1

and 2).

위험지역 및 경색지역의 면적

18.5 ± 6.9%

38.5 ±

11.1%

(p<0.001)(Table 3, Fig. 2).

Table 3. Percent of risk area to total left ventricular weight and percent of infarct area to risk area

	Risk area/LV	Infarct area/risk area
Control (n = 10)	45.2 ± 5.6	38.5 ± 11.1*
IPC (n = 9)	41.5 ± 4.3	18.5 ± 6.9

IPC : ischemic preconditioning, LV : left ventricle
The ± denotes mean ± SD. * : p<0.001 vs control

HSP70 mRNA 분석

HSP70 mRNA

45 ± 15%, 39 ± 9%

HSP70 mRNA

78 ± 12%, 41 ± 11%

가

가

(p<0.01)(Table 4,

Figs. 3 and 4).

고 찰

Marber¹³⁾

Kuzuya¹²⁾

1 4

가

가

“second window of protection”

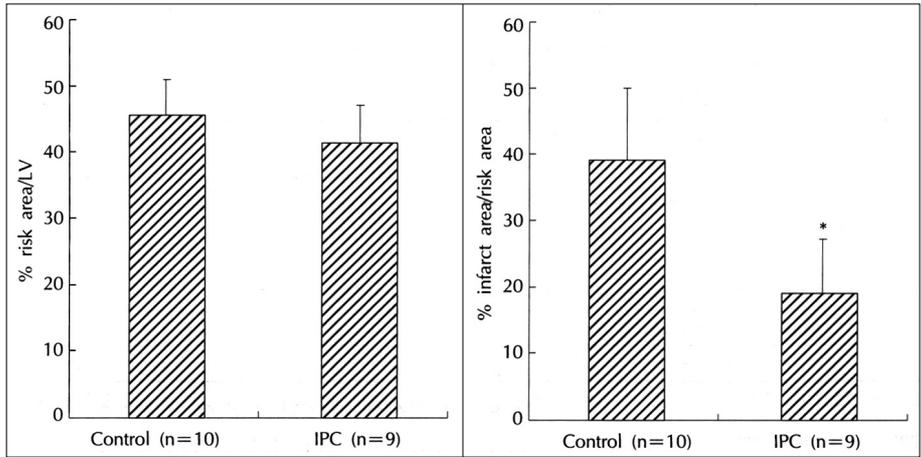


Fig. 2. The differences of risk area and infarct area in control (n = 10) and IPC group (n = 9). The infarct size was significantly decreased in ischemic preconditioning (IPC) group. * : p<0.001 vs. control.

Table 4. HSP70 mRNA expression of myocardial tissue in cats

	(HSP70 mRNA/GAPDH) × 100 (%)	
	Control	IPC pretreated
Risk area (anterior wall)	41 ± 11	78 ± 12*
Non-risk area (posterior wall)	39 ± 09	45 ± 15

IPC : ischemic preconditioning
The ± denotes mean ± SD. * : p<0.01 vs control

25)26)
Currie 27)
HSP 가
가
Yellon 37)
HSP70
Knowlton 23)
2
HSP
24
Tanaka 28)
24 , 48
가 , Ber -
nardo 29)
가 adenosine K_{ATP}
가
Donnelly 14)
8
HSP72가
24 HSP72
HSP 가
DNA
가
HSP
kinase
apoptosis

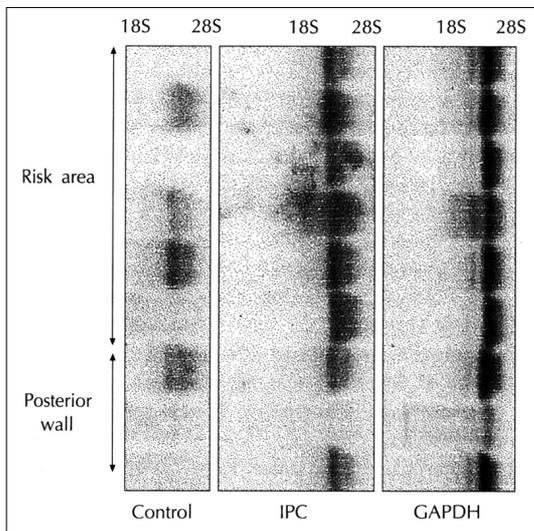
13)17)
19) 가
24
18.5 ± 6.9%
38.5 ± 11.1% 가
가
ATP -
adenosine 가
18)20)
adenosine
가
manganese - su -
peroxide dismutase(SOD)¹²⁾
가 superoxide anion
HSP²⁾
가
22) 23)24) 가 HSP

30)31) , Thornton 32)
 actino -
 mycin - D cyclohexide

Przyklek 33)
 가

HSP70 mRNA
 가 78 ± 12%
 HSP70 mRNA
 41 ± 11%

, Gho 34)



가
 가
 HSP70 mRNA
 가가
 가
 HSP70 mRNA
 가
 HSP

Fig. 3. Representative Northern blot for HSP70 comparing control and preconditioned group. The increase in HSP70 mRNA in preconditioned myocardial sample is noted. The right panel shows the same blot probed for glyceraldehyde-3-phosphatedehydrogenase (GAPDH) for comparison.

가
 가
 가
 HSP70

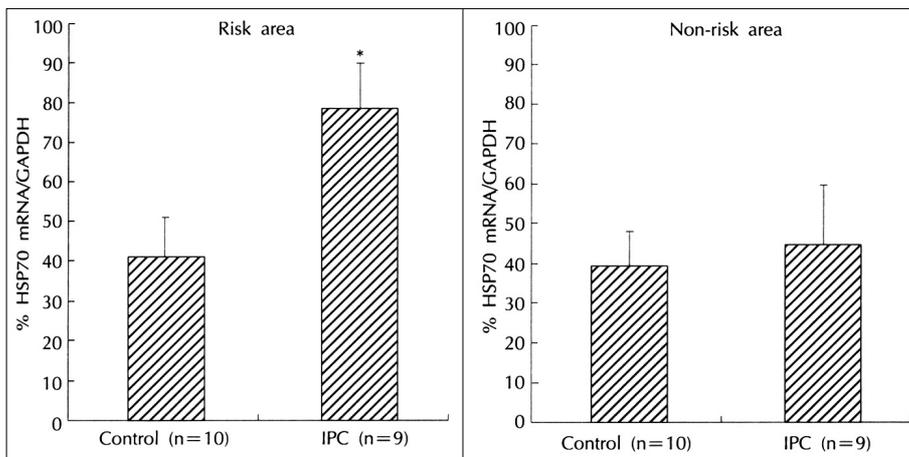


Fig. 4. The relative amounts of HSP70 mRNA in myocardial samples obtained from risk area and non-risk area of left ventricular wall. *: p<0.001 vs. control.

요 약

연구목적 :
 , 24
 가 가
 heat shock protein(HSP)
 방 법 :
 sham operation
 24
 40 30
 GADPH nothern blot
 HSP70 mRNA GAPDH
 HSP70 mRNA %
 5 10
 3
 40
 결 과 :
 (18.5 ± 6.9% vs 38.5 ±
 11.1% ; p<0.001). HSP70 mRNA
 가
 (39 ± 9% vs 45 ± 15% ; p=NS), HSP70
 mRNA
 (41 ± 11% vs 78 ± 12% ; p<0.01).
 결 론 :
 HSP70 가
 중심 단어 : Heat
 shock protein 70.

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