

흰쥐의 심근 허혈/재관류 손상에 의한 세포고사에 미치는 에스트로겐의 영향

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Effects of Physiologic Concentration of Estrogen on Ischemia/Reperfusion-induced Apoptosis in Rat Myocardium

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ABSTRACT

Background : Ischemia/reperfusion injury is very important issue in the era of thrombolysis and primary coronary angioplasty in acute myocardial infarction. However, the mechanism of ischemia-reperfusion injury is not fully clarified. Estrogen is well known to have protective actions against ischemic heart disease. We tested hypothesis that estrogen may protect myocardium by reducing ischemia/reperfusion-induced apoptosis.

Methods : Sprague-Dawley rats that underwent ovariectomy, female controls, and male rats were subjected to 45minutes of left coronary artery occlusion followed by 4 or 24hours of reperfusion. Coronary artery occlusion was performed 1 week after ovariectomy or sham operation. And sham operation was also performed in each group to confirm the effects of ischemia/reperfusion. **Results** : Ischemia/reperfusion induced apoptosis in myocardium, especially at border zone, whereas sham operation did not induce apoptosis. After 4 hours of reperfusion the percentages of apoptotic myocytes in border and center zone of reperfused area were $35.7 \pm 3.7\%$, $29.0 \pm 4.2\%$ in ovariectomized rats ($n = 3$), $40.8 \pm 3.7\%$, $29.5 \pm 2.5\%$ in female control rats ($n = 3$), and $39.0 \pm 1.6\%$ ($p = 0.10$), $32.4 \pm 1.6\%$ ($p = 0.43$) in male rats ($n = 3$). After 24hours of reperfusion the percentages in border and center zone of reperfused area were $20.6 \pm 3.1\%$, $12.9 \pm 4.8\%$ in ovariectomized rats ($n = 3$), $19.6 \pm 4.1\%$, $14.0 \pm 2.0\%$ in female control rat ($n = 3$), and $21.4 \pm 6.6\%$ ($p = 0.93$), $15.1 \pm 2.4\%$ ($p = 0.85$) in male rats ($n =$

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3). There is difference of apoptosis neither between male and female rats nor between control and ovariectomized female rats. **Conclusion** : Estrogen did not affect the ischemia/reperfusion-induced apoptosis in rat myocardium. (**Korean Circulation J 1999;29(2):956-964**)

KEY WORDS : Apoptosis · Ischemia-reperfusion · Estrogen · Rat.

Na-H exchange inhibitor,⁴⁾ 5)

서 론

가 , 6)

가 () . 가 , 7) 8)

9-11)

12)

가 -

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가

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가

연구방법

가 (necrosis) 연구 디자인 (Fig. 1)

(apoptosis) , 8 10

(Sprague - Dawley) 1)

Gottlieb 1) 가 - (14),²⁾ (14),³⁾ (8

, Fliss 2)

45

4 , 24

2 DNA gel electrophoresis

TUNEL

1

sham

3)

실험 방법

(ketamine hydrochloride()50 mg/kg body weight + xylazine()10 mg/kg body weight)

4 - 0 silk Estr - adiol 1

Estradiol

Solid phase ¹²⁵I radioimmunoassay (Coat - A - count, diagnostic products corporation, Los Angeles, CA, USA) Estradiol

(mo - del 683, Harvard Apparatus) 1.2 ml, 75 /min 4

(left atrial appendage)

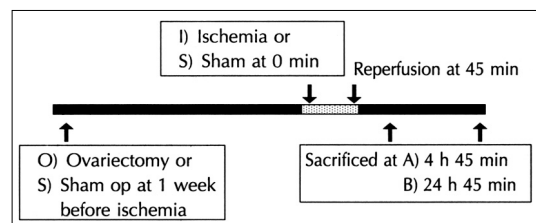


Fig. 1. 연구 디자인(Study design).

허혈/재관류 손상에서 세포고사가 개재하는지, 그리고 이에 대한 에스트로겐의 효과는 어떠한지를 보기 위하여, 수컷과 암컷 그리고 난소를 제거한 암컷 등, 세 군을 대상으로 실험을 하였다. 즉 45분 관동맥 결찰 후 재관류를 시켜서 4시간째와 24시간째에 심근을 얻어서 세포고사의 질적, 양적 분석을 하였으며, 여기에서 허혈/재관류 사술에 대한 대조군으로서 sham 수술군을 두었다. 한편 에스트로겐의 효과를 평가하기 위하여 수컷과 암컷을 비교했을 뿐만 아니라, 미리 1주일전에 난소를 제거하여 혈중 에스트로겐이 대폭 감소한 암컷과 난소 제거 수술에 상응하는 sham 수술을 받은 암컷 사이도 비교하였다(In order to evaluate the effect of estrogen on the ischemia/reperfusion-induced apoptosis of cardiomyocytes, three groups of rats ; male, female, and ovariectomized female, were studied. Qualitative and quantitative analyses were done at 4 and 24 hours reperfusion after 45 minutes' ligation of left coronary artery.)

7 - 0 silk 45

24

5% Evans blue (Sigma - aldri - ch.USA) (Fig. 2)

, 4 24

- 70

In Situ Nick End Labelling(TUNEL)

DNA DNA 3 - OH deo - xynucleotide 가 terminal deoxynu - cleotidyl transferase(TdT) TdT - medi - ated digoxigenin antidigoxigenin - peroxidase

(mo - rehydration 20 ug/mL proteinase K 15 incubation

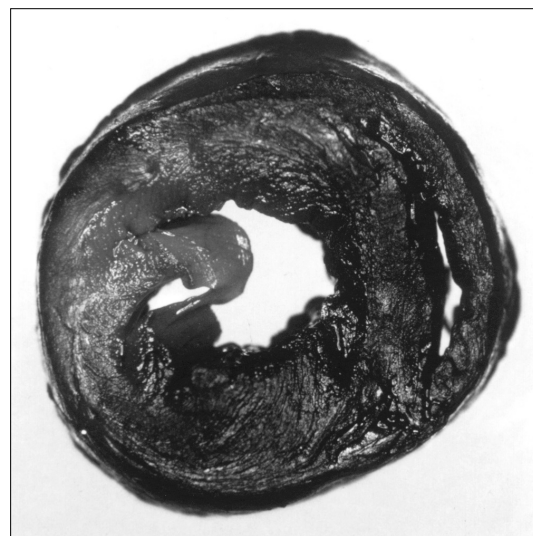


Fig. 2. 허혈/재관류 부위를 나타내는 흰쥐 심장의 단면도(Transverse section of rat heart which underwent ischemia/reperfusion). 살아 있는 흰 쥐에서, 좌관동맥을 결찰한 후 에반스-블루 염료를 대동맥에 관류시킨 후 희생시켜 얻은 심장의 단면도로서, 결찰되었던 좌관동맥 부위는 붉은 색을 띠고 있으며 이 부위가 연구대상이 되었던 허혈/재관류 부위이다. 한편 허혈/재관류 손상을 받지 않는 그 외의 부분은 푸른색으로 염색되어 있다(After ligation of left coronary artery, evans-blue dye was perfused into coronary artery from aorta in vivo. Ischemia/reperfusion area is shown red, whereas the other area is blue).

5 2 slide , endo -
genous peroxidase 3% hydrogen
peroxide 5 slide ,
5 3 . ApopTag(Oncor) kit
TdT(Oncor)L 37 1
humidified chamber incubation .
Negative control TdT phosph -
ate - buffered saline(PBS) . ApopTag kit
stop/wash buffer slide 30
incubation 1 x PBS 5 3 .
ApopTag kit antidigoxigenin - peroxidase 2
30 humidified chamber in -
cubation . 1 x PBS 5 3 3,3 - dia -
minobenzidine(DAB, Zymed) 3 .
Mayer hematoxylin counte -
r stain .
400 2
TUNEL .

Analysis of DNA ladder

- 1) 200 mg
homogenization buffer(100 mmol/L NaCl, 10 mmol/L
Tris - HCl [pH8.0], 25 mmol/L EDTA) ho -
mogenzier 30 .
- 2) 100 ug 15 1.25 mL
lysis buffer(100 mmol/L NaCl, 10 mmol/L Tris -
HCl [pH8.0], 25 mmol/L EDTA, 1.0% SDS)
13,000 g 15
- 3) 37 30 DNase - free RNase(100 ug/
mL, Boehringer Mannheim) , proteinase
K(100 ug/mL,Boehringer Mannheim) 50 30
incubate , ethanol precipitation DNA
- 4) Pellet TE buffer(10 mmol/l Tris - HCL [pH
8.0], 1 mmol/l EDTA) spectro -
photometer .
- 5) DNA 20 ug 5 ug/ml ethidium bro -
mide가 2% NuSieve(FMC BioProducts) aga -
rose gel(NuSieve : agarose = 3 : 1)
DNA ladder .

통계 처리

Estradiol , Wilcoxon - rank sum test
Kru -
skal - Wallis test . ±
(PC - SAS for windows version 6.12)

결 과

에스트로젠 농도

1 estr -
adiol 23.0 ± 19.5 pg/ml ,
127.6 ± 49.0 pg/ml (p<0.05),

Table 1. 암컷과 난소를 절제한 암컷에서 측정된 에스트라다이올의 혈중치(Estradiol level in female and ovariectomized female rats)

Group	Plasma concentration of estradiol
Ovariectomized female rat (N = 12)	23.0 ± 19.5 pg/ml p<0.05
Normal female rat (N = 12)	127.6 ± 49.0 pg/ml

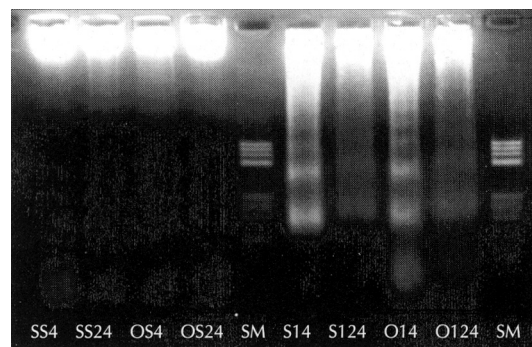


Fig. 3. 심근의 허혈/재관류 손상 후 관찰되는 세포고사의 증거인 DNA ladder 현상(DNA ladder from rat heart which underwent ischemia/reperfusion). 난소제거수술 혹은 대조 sham 수술을 받은 암컷 흰 쥐를, 허혈/재관류 수술을 시행한 군과 이에 상응하는 sham 수술을 한 군에서 각각 4시간, 24 시간째 심장을 얻었다. 심장의 허혈/재관류 부위에서 디옥시 리보 핵산을 추출하여 2% 아가로스 젤에 전기 영동한 사진이다. 허혈/재관류 수술을 한 심장에서만 세포고사가 발견되는데 이는 난소 제거 수술 여부에 관계가 없음을 알 수 있다(DNA ladder was observed only in myocardium that underwent ischemia/reperfusion injury regardless of the presence of ovary).

SM : size marker, SS4&SS24 : female rats with sham-ovary operation sacrificed at 4&24 hours after sham-heart operation, OS4&OS24 : female ovariectomized rats sacrificed at 4&24 hours after sham-heart operation, S14&S124 : female rats with sham-ovary operation that underwent ischemia and 4&24 hours of reperfusion, O14&O124 : female ovariectomized rats that underwent ischemia and 4&24hours of reperfusion.

.(Table 1)

DNA ladder

TUNEL 염색 (Table 2, Figs. 4 and 5)

DNA ladder (Fig. 3)

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TU -

-

DNA NEL

ladder가 , -

Table 2. 수컷, 암컷, 난소를 절제한 암컷등 세군의 흰쥐에서, 허혈/재관류 후 4시간 24시간에 관찰한 심근세포의 세포고사 지수(The apoptotic indices in center and border zone of reperfused myocardium at 4 and 24 hours after ischemia/reperfusion in male and female and ovariectomized female rats).

		Center zone of ischemic area	Border zone of ischemic area
Ovariectomized female rat (n = 3)	4 hours-reperfusion	29.0 ± 4.2%	35.7 ± 3.7%
	4 hours-sham op	0.0 ± 0.0%	0.0 ± 0.0%
	24 hours-reperfusion	12.9 ± 4.8%	20.6 ± 3.1%
	24 hours-sham op	0.0 ± 0.0%	0.0 ± 0.0%
Normal female rat (n = 3)	4 hours-reperfusion	29.5 ± 2.5%	40.8 ± 3.7%
	4 hours-sham op	0.0 ± 0.0%	0.0 ± 0.0%
	24 hours-reperfusion	14.0 ± 2.0%	19.6 ± 4.1%
	24 hours-sham op	0.0 ± 0.0%	0.0 ± 0.0%
Male rat (n = 3)	4 hours-reperfusion	32.4 ± 1.6%	39.0 ± 1.6%
	24 hours-reperfusion	15.1 ± 2.4%	21.4 ± 6.6%

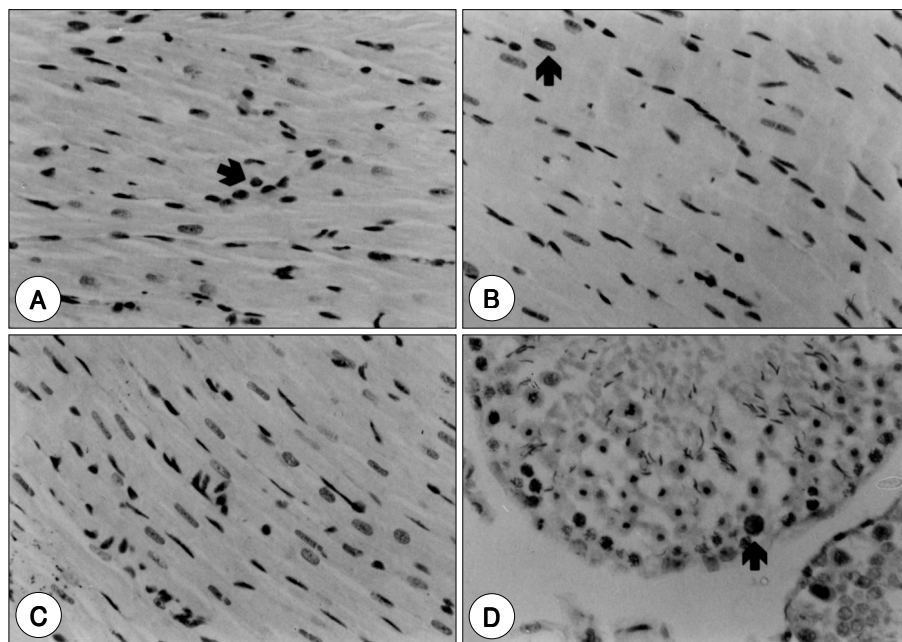


Fig. 4. 난소를 절제한 암컷 흰쥐에서 심근 허혈/재관류 손상에 의한 세포고사의 발생(Apoptosis of cardiomyocytes in reperfused myocardium in ovariectomized female rats).TUNEL 염색 결과, 난소를 절제한 암컷 흰 쥐에서 허혈/재관류 손상 후 4시간(A)과 24시간(B)째에 허혈 - 경계 부위에서 세포고사에 빠진 세포들이 다 수 관찰된다. 24시간째 보다 4시간째에 보다 많은 세포고사 양상을 볼 수 있다. 한편, (C)에서 보는 바와 같이, 난소 절제한 암컷 흰 쥐에서 sham-heart 수술을 받은 경우에는 심장에서 세포고사 양상을 관찰 할 수 없다. (D)는 TUNEL염색의 양성 대조 조직으로 이용한 흰쥐의 정소를 염색한 소견이다. 전형적인 TUNEL-양성 세포를 볼 수 있다(TUNEL-positive cells are observed in the border zone of reperfused myocardium at 4 and 24 hours after reperfusion, whereas they are not observed in myocardium after sham operation). 현미경 배율 = 400배 확대, 화살표는 세포고사를 가리킴.

4 24 가 drito ²¹⁾ 17Beta - oestradiol -
가 , Kolodgie ¹⁶⁾
-
.
.
Node ²²⁾ 17 beta - estradiol
-
가
Lang ²³⁾
가
Wellman ²⁴⁾²⁵⁾
가
허혈 - 재관류 손상에 대한 에스트로겐의 영향
- 가
-
-
가 4
24
Kolodgie ¹⁶⁾ 2
가
-
가
Hale ¹⁷⁾
-
가
가
1
가
Kim ¹⁸⁾¹⁹⁾ 2 17 - Beta estr -
adiol glutathione 가
McHugh ²⁰⁾ conjugated es -
trogen
hydroxyl radical
phenolic
anti - peroxidative
ring enzyme
1 가
가
Squa -

가 1 estradiol
 , 23 ± 20 pg/ml, 128 ± 49 pg/ml (p=0.02). TU -
 NEL
 4 (n=3) :
 35.7 ± 3.7%, 29.0 ± 4.2%, (n=3) :
 40.8 ± 3.7%, 29.5 ± 2.5%, (n=3) : 39.0 ± 1.6%,
 가 가 32.4 ± 1.6% (p=0.10,
 p=0.43) , 24 (n=3) : 20.6
 ± 3.1%, 12.9 ± 4.8%, (n=3) : 19.6
 4 가 ± 4.1%, 14.0 ± 2.0%, (n=3) : 21.4 ± 6.6%, 15.
 1 ± 2.4% 가 (
 , p=0.93, p=0.85).

결 론 :

24

요 약

서 론 :

중심 단어 :

감사문

(04 - 97 - 009)

/ 가

REFERENCES

대상 및 방법 :

8 10

1

45

4

24

TUNEL

DNA -

ladder

결 과 :

/ 4

가

. 24

sham

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