

백서의 실험적 급성 심근경색후 폐와 심근에서의 안지오텐신 전환효소 유전자 발현의 변화 양상 및 이에 대한 레닌-안지오텐신계 차단제의 효과 분석

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Sequential Changes of Angiotensin-Converting Enzyme Gene Expression in Lung and Myocardium after Myocardial Infarction in Rat : Different Patterns of Expression and Regulation between Circulating and Local System

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ABSTRACT

Background : We evaluated 1) the sequential changes of angiotensin-converting enzyme (ACE) mRNA expression in lung (main site for circulating ACE synthesis) and left ventricle, and 2) whether such expression is modified by ACE inhibitor or angiotensin II receptor blocker treatment after acute myocardial infarction (MI) in rats. **Methods :** Placebo, captopril (2 g/liter drinking water) or TCV-116 (10 mg/kg/day gavage) was administered 3 days before left coronary occlusion and continued for 6 weeks. At 1, 3 and 6 weeks after operation, hemodynamic measurement was performed and pulmonary and myocardial ACE mRNA expression was analyzed by quantitative RT-PCR using rcrRNA as an internal standard. **Results :** Mean BP and LVEDP increased in untreated rats compared with captopril- and TCV-116-treated rats (post-MI 6week, $p<0.05$). Pulmonary ACE mRNA increased in acute phase (post-MI 1 week, max. 1.4 fold, $p<0.05$ vs. pre-MI) and returned to pre-MI value thereafter. In contrast, cardiac ACE mRNA expression showed slightly decreasing tendency in acute phase, and was increased up to 1.6 fold in chronic phase after MI (post-MI 3 and 6weeks, $p<0.05$ vs. pre-MI). No changes in pulmonary ACE gene expression were found with RAS blockade. However, in acute phase after MI, cardiac ACE mRNA increased with both captopril and TCV-116 treatment (post-MI 24hour and 1week, max. 2 fold, $p<0.05$ vs. untreated group). **Conclusion :** 1) In contrast to the pulmonary ACE mRNA that is activated in acute phase, the cardiac ACE mRNA is activated in chronic phase after MI. 2) RAS blockade does not affect the change of pulmonary

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KEY WORDS : Acute myocardial infarction · Angiotensin converting enzyme · Gene expression · Captopril · TCV-116.

가
, ACE
mRNA
(
)
(ACE)

[illegible]

(5' - TAATACGACTCACTATAGG - GCAGTATT
 - TCATGCAGTACAA - AGGCCATGGTTTGCAG
 GAA - 3')
 3' - primer ;
 5' - (poly dT) - (ACE 3' primer sequence) - (GS -
 TM 3' primer sequence) - 3'
 (5' - TTTTTTTTTTTTTTTTTT - GGAAGTGG -
 GAACTGGATGATGA - GTTGGGCTCAAATA
 TA CG GTGG - 3')

12 Target RNA ACE primer 437 bp
 , spacer gene
 ACE primer 222 bp 가
 PE 50 , 200 bp 가 , electrophoresis
 , gel . gen -
 polygraphs model 7(Grass
 Instruments) , PCR 94 1
 가 denaturation , 60 1 30
 extention 35 . glass milk(BIO
 101kit, Boehringer Mannheim) , T7
 조직의 채취 RNA polymerase in vitro transcript -
 (post op : 24 , 1 , 2 , 3 , 6) ion(Riboprobe in vitro transcription system, Pro -
 mega, USA) , rcRNA ,
 - 70 .

정량적 역전사 PCR(Quantitative reverse transcri-
 ption polymerase chain reaction : QRT-PCR)
 Huh ¹⁵⁾ .

QRT - PCR RNA primer
 Internal standard ACE primer
 rcRNA(recombinant RNA)
 180 bp GSTM(glutathion transferase)
 , ACE specific primer seq -
 uence T7 promoter, poly(dT) tail spacer
 gene . recombinant PCR
 primer .
 5' - primer ;
 5' - (T7 promoter sequence) - (ACE 5' primer
 sequence) - (GSTM 5' primer sequence) - 3'

total RNA
 - 70 RNAzol - B(CIN -
 NA/BIOTECX Lab, Inc, : Guanidine thiocyan -
 ate, 2 - Mercaptoethanol, Phenol)
 RNA . 100 mg
 RNAzol 2 ml (homogenizer)
 30 3 .
 sample 1/10 chloroform 15
 15 15000
 rpm 15 4 ,
 isopropanol 가
 - 20 45 15000 rpm
 15 RNA
 75% ethanol 12000 rpm 8
 RNA . RNA
 0.5% SDS(sodium dodecylsulfate, pH 7.2)

suspension . - 70 - 20 가 85 5
 RNA 260nm spect - 2.5 unit Taq DNA (Boehringer Mannheim,
 rophotometer , 가 denaturation , PCR 95 1
 0.8% agarose gel(ethidium bromide amplification exponential phase
 stained) UV transilluminator(UVP) DNA .
 RNA (degradation) cDNA RNA PCR
 18S 28S band .
 QRT - PCR (reverse transcription) PCR PCR
 1 ug Total RNA rcRNA PCR 0.02 uCi/ul 32P - dCTP 가
 , MMLV reverse transcript - PCR 2% agarose gel
 ase(Promega, USA) , 37 60 . PCR gel X - ray
 single standard cDNA , film densitometer band
 99 5 , 5 .
 cDNA 2
 , 10 mM Tris(pH 8.3), 50 mM KCl, 1.5 mM 통계학적 분석
 MgCl₂, 200 uM dNTP 25 pmol sense pri - ± ,
 mer antisense primer가 PCR non parametric test(Mann -
 . Hot start 95 5 Whitney U, SPSS for window) .

Table 1. Hemodynamic profiles

	SBP	DBP	MAP	LVEDP	HR
Post MI (1 wk)					
Sham (n = 6)	137.5 ± 5.0	102.1 ± 5.4	116.7 ± 5.4	7.3 ± 0.8	384.7 ± 12.8
MI-vehicle (n = 7)	127.1 ± 5.1	96.8 ± 6.0	108.9 ± 5.5	21.7 ± 4.2*	387.9 ± 24.0
MI-captopril (n = 7)	101.4 ± 8.7* [†]	82.1 ± 10.1	91.8 ± 9.0* [†]	14.8 ± 2.1*	320.7 ± 17.8
MI-TCV-116 (n = 7)	93.9 ± 4.5* [†]	67.1 ± 6.0* [†]	81.8 ± 5.6* [†]	10 (n = 1) [‡]	348.7 ± 22.1
Post MI (3 wk)					
Sham (n = 5)	142.0 ± 6.6	106.5 ± 2.7	116.0 ± 3.7	6.6 ± 1.0	351.6 ± 34.3
MI-vehicle (n = 5)	135.0 ± 5.1	106.0 ± 5.1	118.0 ± 5.4	22.0 ± 3.4*	351.6 ± 15.3
MI-captopril (n = 8)	101.3 ± 4.6* [†]	75.0 ± 6.7* [†]	88.4 ± 5.4* [†]	11.0 ± 1.7 [†]	352.8 ± 12.8
MI-TCV-116 (n = 7)	92.9 ± 7.2* [†]	73.6 ± 7.6* [†]	83.2 ± 7.3* [†]	13.3 ± 2.5* [†]	326.0 ± 14.7
Post MI (6 wk)					
Sham (n = 5)	140.0 ± 4.5	112.1 ± 8.9	122.0 ± 4.9	5.6 ± 0.6	366.4 ± 11.3
MI-vehicle (n = 11)	132.7 ± 4.3	109.5 ± 3.5	118.6 ± 3.7	30.7 ± 4.5*	351.9 ± 13.1
MI-captopril (n = 9)	110.0 ± 2.0* [†]	82.2 ± 2.4* [†]	97.6 ± 2.3* [†]	14.0 ± 4.9 [†]	329.3 ± 22.6
MI-TCV-116 (n = 8)	102.8 ± 6.7* [†]	87.5 ± 5.7* [†]	94.1 ± 6.0* [†]	13.1 ± 4.0 [†]	336.3 ± 14.8

Sham, sham operated rats ; MI-vehicle, untreated infarcted rats ; MI-captopril, infarcted rats treated with captopril ; MI-TCV-116, infarcted rats treated with TCV-116 ; SBP, systolic arterial pressure (mmHg) ; DBP, diastolic arterial pressure (mmHg) ; MAP, mean arterial pressure (mmHg) ; LVEDP, left ventricular end diastolic pressure (mmHg) ; HR, heart rate (beats per minute). All values are shown as mean ± SEM. * : p<0.05 vs. Sham, [†] : p<0.05 vs. MI-vehicle, [‡] (n = 1) : case number = 1



Fig. 1. Typical autoradiogram of RT-PCR for sequential changes of ACE mRNA expression in lung at 24 hours, 1, 2, 3 and 6 weeks after experimental MI in rats.

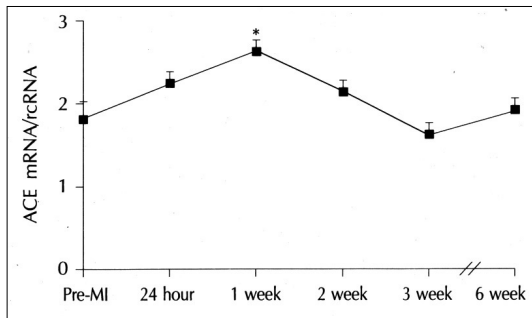


Fig. 2. Sequential changes of ACE mRNA expression in lung after experimental MI in rats. Each mRNA value is corrected for rcRNA value. All data are shown as mean \pm SEM. n = 4 for each time point. * : p < 0.05 vs. pre-MI.

결 과

혈역학적 변화(Table 1)

sham captopril TCV -
116

심근경색후 폐 및 심근에서의 ACE mRNA 발현

24 1
1.4
가 (p < 0.05 vs. pre - MI)
(Figs. 1 and 2). ACE
mRNA
가 가
가 3 6
1.6 가 (p < 0.05
vs. pre - MI) (Figs. 3 and 4).

Captopril 혹은 TCV-116 투여에 의한 ACE mRNA 발
현 변화

ACE mRNA
(Figs. 5 and 6). Captopril
TCV - 116 ACE mRNA

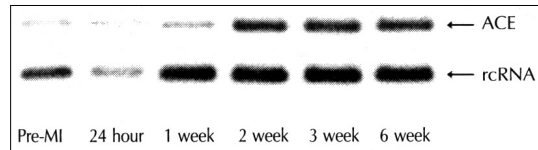


Fig. 3. Typical autoradiogram of RT-PCR for sequential changes of ACE mRNA expression in uninfarcted myocardium at 24 hours, 1, 2, 3 and 6 weeks after experimental MI in rats.

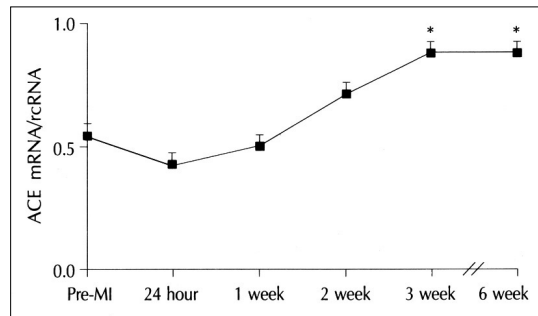


Fig. 4. Sequential changes of ACE mRNA expression in uninfarcted myocardium after experimental MI in rats. Each mRNA value is corrected for rcRNA value. All data are shown as mean \pm SEM. n = 4 for each time point. * : p < 0.05 vs. pre-MI.

가 (max. 2 fold, p < 0.05 vs. MI -
vehicle),
(Figs. 7 and 8).

고 안

(24 1),
2) (3, 6)
ACE mRNA ACE
mRNA 가
ACE mRNA 3
가
ACE mRNA ACE
가 II가

심근경색후 폐 및 심근의 ACE mRNA 발현

ACE mRNA, Hirsch 가, 30, ACE mRNA, 13) ACE 가 Sham 가, 16) 가 Sham, Huang 11), ACE mRNA, ACE, Kelly 12), 12, 4, ACE, 13) Chiba, 17), 18), ACE, 19), ACE

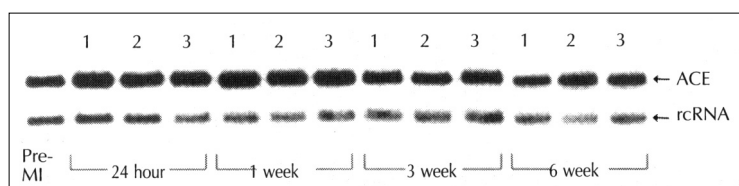


Fig. 5. Effects of captopril or TCV-116 on the expression of ACE mRNA expression in lung at 24 hours, 1, 3 and 6 weeks after experimental MI in rats. 1, untreated infarcted rats ; 2, infarcted rats treated with captopril ; 3, infarcted rats treated with TCV-116.

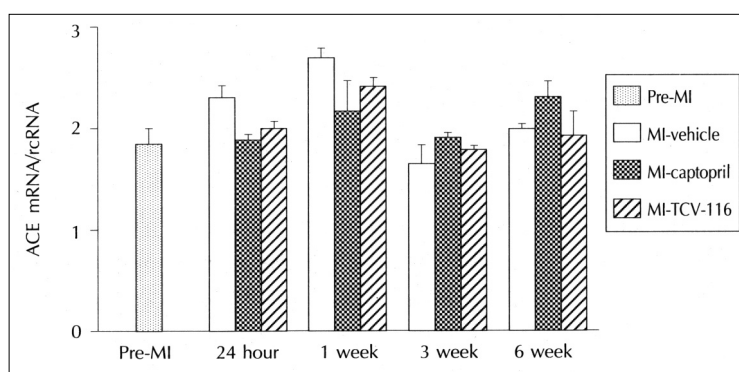


Fig. 6. Effects of captopril or TCV-116 on the expression of ACE mRNA expression in lung after experimental MI in rats. Each mRNA value is corrected for rcRNA value. All data are shown as mean \pm SEM. Pre-MI, normal control rats ; MI-vehicle, untreated infarcted rats ; MI-captopril, infarcted rats treated with captopril ; MI-TCV-116, infarcted rats treated with TCV-116 (n = 4 for each group and time point).

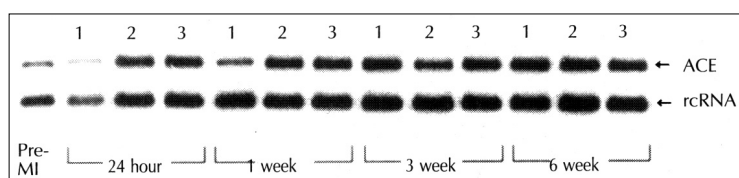


Fig. 7. Effects of captopril or TCV-116 on the expression of ACE mRNA expression in uninjured myocardium at 24 hours, 1, 3 and 6 weeks after experimental MI in rats. 1, untreated infarcted rats ; 2, infarcted rats treated with captopril ; 3, infarcted rats treated with TCV-116.

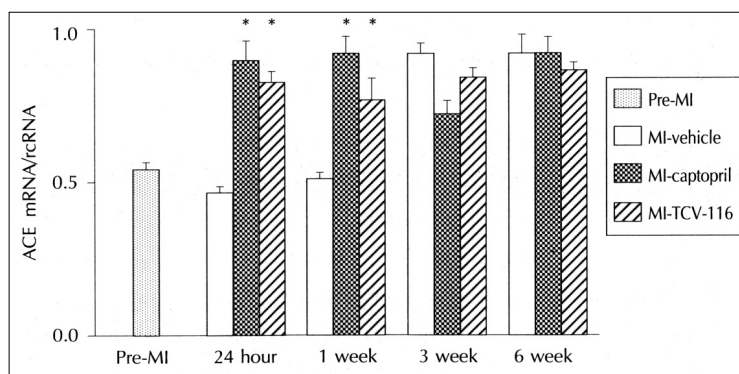


Fig. 8. Effects of captopril or TCV-116 on the expression of ACE mRNA expression in uninjured myocardium after experimental MI in rats. Each mRNA value is corrected for rcRNA value. All data are shown as mean \pm SEM. Pre-MI, normal control rats ; MI-vehicle, untreated infarcted rats ; MI-captopril, infarcted rats treated with captopril ; MI-TCV-116, infarcted rats treated with TCV-116 (n = 4 for each group and time point). * : p < 0.05 vs. MI-vehicle.

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ACE mRNA

방 법 :

(, n =

5 - 11 for each time point),

(captopril 2 g/liter drinking water) (ca -

ptopril , n=7 9 for each time point)

(TCV - 116 10 mg/kg/day)

(TCV - 116 , n=7 8 for each time po -

int)

3 1, 3,

6 RNA

. ACE mRNA RT -

PCR

결 과 :

1)

captopril TCV - 116

(6 , 118.6

± 3.7 , 97.6 ± 2.3 , 94.1 ± 6.0 , 30.7

± 4.5 , 14.0 ± 4.9 , 13.1 ± 4.0 ; , captopril

, TCV - 116 , $p < 0.05$ vs. MI - vehicle).

2) ACE mRNA 24

1 가 (p<0.05 vs. pre - MI),

ACE m -

RNA 가

3

6 1.6 가

(p<0.05 vs. pre - MI).

3) ACE mRNA -

captopril TCV - 116

ACE mRNA ,

가 (max. 2

fold, p<0.05 vs. MI - vehicle),

결 론 :

1) ACE mR -

NA , ACE mRNA

2) -

ACE mRNA

ACE mRNA 가 .

중심 단어 :

. Captopril . TCV - 116.

감사문

RT - PCR

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