

혈관내피세포에 대한 Diamide의 독성과 Thioredoxin의 방어효과

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Cytotoxicity of Diamide and the Protective Effect of Thioredoxin on Diamide-Induced Vasculotoxicity in Vascular Endothelial Cells

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ABSTRACT

Background and Objectives : This study was designed to examine the effects of diamide and thioredoxin (TRX) on vascular endothelial cells in order to clarify the mechanism by which vascular damage is mediated by oxygen free radicals. **Materials and Methods :** The pulmonary artery endothelial cell (PAEC) line derived from bovine serum was cultured for 8 hours in media supplemented with various concentrations of diamide and TRX. The XTT assay, MTS assay, SRB assay, LDH activity and lipid peroxidation tests were performed. **Results :** In XTT and MTS assays, diamide significantly decreased the cell viability of cultured PAEC in a dose- and time-dependent manner. Diamide showed a decrease in the amount of total protein, although it showed an increase of lipid peroxidation and LDH activity in cultured PAEC. In regards to the protective effect of TRX on diamide-induced cytotoxicity, this showed an increase of total protein, however it showed a decrease of lipid peroxidation and LDH activity. **Conclusion :** Our results suggest that diamide has a vasculotoxic effect on cultured bovine PAEC and that TRX is very effective in the protection of diamide-induced cytotoxicity by due to the increase of total protein and the decrease of lipid peroxidation and LDH activity in these cultures. (Korean Circulation J 2001;31(11):1185-1193)

KEY WORDS : Stent · Coronary artery disease · Intravascular ultrasound.

서 론

가

(redox state)

가

: 2001 7 2

: 2001 8 24

: 2001 9 25

: 570 - 711

344 - 2

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가³⁾,
가²⁾,
transcrip -
tion factor nuclear factor kappa B(NF - kB)가
vascular cell adhesion molecule - 1(VC -

AM - 1) monocyte chemotactic 가
protein - 1(MCP - 1) chemokine ²⁾⁴⁾ (pulmonary ar -
, VCAM - 1 , tery endothelial cell, PAEC) diamide
⁵⁾ , thiorodoxin(TRX)
⁶⁾ thiobarbituric - reactive su - assay, lactate dehydrogenase(LDH) MTS
stance(TBARS) NF - kB , heme oxi - , SRB
genase mRNA 가 ⁷⁾ Lipid peroxidation .

재료 및 방법

가 ⁸⁾ 재 료
probucol vitamin E
⁹⁾ ,
가가 ¹⁰⁾ , NO
¹¹⁾ NO가 NF - kB VC - American Tissue Type
AM - 1, MCP - 1 Culture(ATTC) (pulm -
onary artery endothelial cell, PAEC) .

(amyotrophic la - Diamide, Thiorodoxin, XTT[2,
teral sclerosis, ALS) 3 - bis(2 - methoxy - 4 - nitro - 5 - sulfophenyl) - 5 -
¹²⁾¹³⁾ (phenylamino carbonyl) - 2H - tetrazolium hydrox -
¹⁴⁾¹⁵⁾ , ide] MTS[3 - 4(4,5 - dimethylthiazol - 2 - yl) - 5 -
(3 - carboxymethoxyphenyl) - 2 - (4 - sulfophenyl) -
2H - tetrazolium] Sigma .

^{16 - 18)} , 실험 방법
(excitotoxic amino ac -
ids, EAAs) ,
EAAs N - methyl - D - aspartate(NMDA) Diamide Thiorredo -
Ca²⁺ xin 100 mM, 10 mM, 1 mM
¹⁹⁾²⁰⁾ ,
가 ²¹⁾ 가
protein kinase C(PKC) cyclic AMP .

¹⁸⁾ PAEC Takahashi ⁴⁾
, PAEC phosphate buffered saline(PBS)
36 , 5% CO₂/95% air
가 , 10% fetal bov -
ine serum(FBS, Gibco) Eagle 's minimum
essential medium(EMEM, Gibco) . PA -

EC poly - L - lysine(Sigma) 96 - mu - phosphotungstic acid 2.0 mL 0.3 mL
 ltiwell 3 × 10⁶ cells/well 10 . TBA 1.0 mL
 , 3 가 90 1 가
 가 5 n - butanol . n - butanol
 553 nm

PAEC 가 LDH LDH
 PAEC 0.6% - D gl - Takahashi ²⁶⁾ . ,
 ucase가 MEM 3 1 100 LDH kit(Atron lab, Japan) 1.0 mL
 μM Diamide 2 37 10 . 10
 16 3.0 mL 570 nm

SRB
 SRB PAEC 0.4% sulforhodamine B
 XTT 200 μL 가 1
 XTT 1.0% acetic acid 3 . 10
 PBS 3 mM Tris base SRB - bound protein
 50 mg/mL XTT well ELISA reader 540 nm
 37 , 5% CO₂
 spectrophotometer 450 nm

MTS (× 125),
 MTS Goodwin ²⁵⁾ .
 Diamide
 phosphate buffered saline(PBS) 3
 5 mg/mL MTS well ANOVA
 37 , 5% CO₂ , p 0.05
 spectrophotometer 203 nm

결 과

Lipid peroxidation 산소자유기의 세포독성(세포 생존율 분석)
 Lipid peroxidation
 PAEC
 TBARS(thiobarbituric acid reactive su - XTT
 bstances) , 12N H₂SO₄ 10% Diamide가

1 μ M 100 μ M Diamide가 48.3%(p<0.05) 50 μ M MTS
 8 50 100 μ M Diamide 21.1%
 Diamide XTT assay (p<0.05) (Fig. 3).
 1 μ M Diamide
 (100%) 86.3%
 25 μ M 69.0% 50
 μ M 100 μ M Diamide
 51.2%(p<0.05) 37.5%(p<0.05)
 50 μ M XTT 50 (Fig. 1).
 Diamide가
 50 μ M Diamide가
 2
 16 XTT assay
 2
 (100%) 72.4%
 4 58.6%(p<0.05)
 8
 48.7%(p<0.05) , 16 21.
 1%(p<0.05) 8 XTT
 50 (Fig. 2).
 MTS
 Ca²⁺, Mg²⁺ -
 free Hank's balanced salt solution (HBSS, Gibco)
 3 Diamide가 1 μ M 100 μ M
 8
 1 μ M
 (100%) 74.1%
 25 μ M 50 μ M 58.5%(p<0.05)

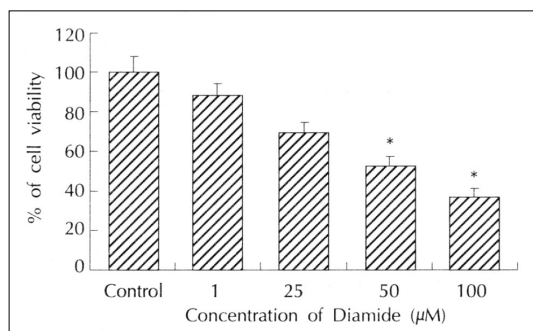


Fig. 1. Dose-response relationship of diamide in cultured bovine pulmonary vascular endothelial cell line (PVEC). Cytotoxicity was measured by XTT assay. The result indicates the mean \pm SEM (n=6). * : p<0.05.

항산화제의 효과

Lipid peroxidation

Diamide

Diamide

amide

lipid peroxidation

Diamide가 1 180 μ M

Di -

8
 TBARS(thiobarbituric acid reactive
 substances)

1 μ M Diamide

TBARS가

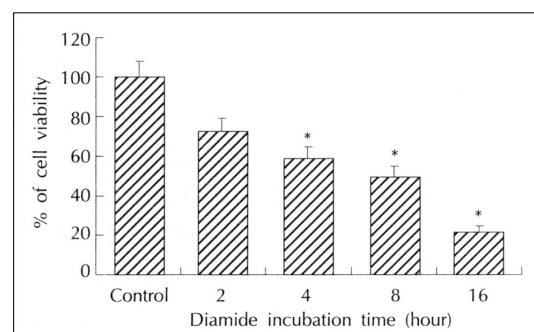


Fig. 2. Time-response relationship of diamide in cultured bovine pulmonary vascular endothelial cell line (PVEC). Cytotoxicity was measured by XTT assay after cells were incubated with 50 μ M diamide. The result indicates the mean \pm SEM (n=6). * : p<0.05.

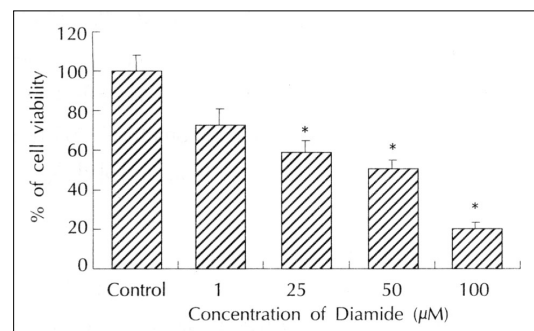


Fig. 3. Dose-response relationship of diamide in cultured bovine pulmonary vascular endothelial cell line (PVEC). Cytotoxicity was measured by MTS assay. The result indicates the mean \pm SEM (n=6). * : p<0.05.

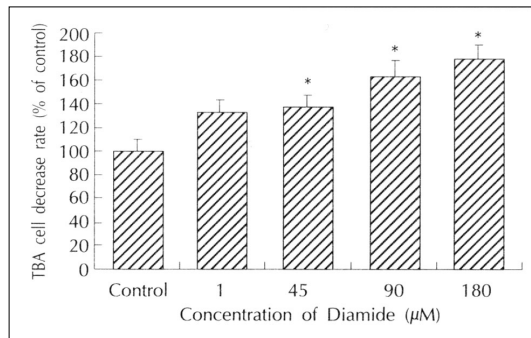


Fig. 4. Dose-response relationship of diamide in cultured bovine pulmonary vascular endothelial cell line (PVEC). Cytotoxicity was measured by lipidperoxidation assay. The result indicates the mean \pm SEM (n=6). * : p<0.05.

(100%)(45.6 \pm 6.2) 132.8%(68.5 \pm 7.2)
 . 45 μ M 90 μ M Diamide
 TBARS가 138.6%(71.5 \pm 7.9)(p<0.05) 162.2%(83.7 \pm 8.5)(p<0.05)
 90 μ M TBARS 50 .
 180 μ M Diamide TBARS가 176.4%(91.0 \pm 8.7)(p<0.05)
 가 (Fig. 4).

TRX(thioredoxin)

Lipid peroxidation Diamide
 TRX
 Diamide TBARS 50 90 μ M Dia-
 mide 8 2 1
 160 μ g/mL TRX가
 . 1 μ M TRX
 TBARS 78.3%(32.4 \pm 2.9) Diamide
 100%(41.4 \pm 3.6) .
 45 μ g/mL 80 μ g/mL TRX
 TBARS가 68.7%(28.4 \pm 2.5) 44.6%(18.5 \pm 1.6)(p<0.05)
 160 μ g/mL TRX
 TBARS가 36.4%(15.1 \pm 1.2)(p<0.05)
 (Fig. 5).

LDH

Diamide
 Diamide

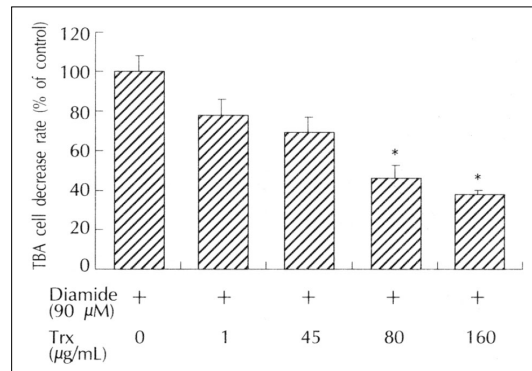


Fig. 5. Dose-response relationship of thioredoxin (Trx) for its protect effect on diamide by lipid peroxidation assay. Cultured bovine pulmonary vascular endothelial cell line (PVEC) were preincubated with Trx for 2 hours before exposure to diamide. The result indicates the mean \pm SEM (n=6). * : p<0.05.

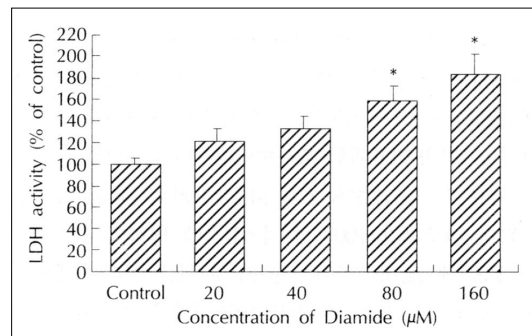


Fig. 6. Dose-response relationship of diamide in cultured bovine pulmonary vascular endothelial cell line (PVEC). Cytotoxicity was measured by LDH assay. The result indicates the mean \pm SEM (n=6). * : p<0.05.

LDH Diamide가 20
 160 μ M
 8
 LDH . 20 μ M
 Diamide 100%(15.3 \pm 1.3)
 121.6%(18.6 \pm 1.5) . 40 μ M 80
 μ M Diamide 134.0%(20.5 \pm 1.8) 158.2%(24.2 \pm 2.2)(p<0.05)
 160 μ M Diamide 187.6%(28.7 \pm 2.6)
 (p<0.05) Diamide
 가 . LDH MVV
 (midviability value) 80 μ M Diamide
 (Fig. 6).

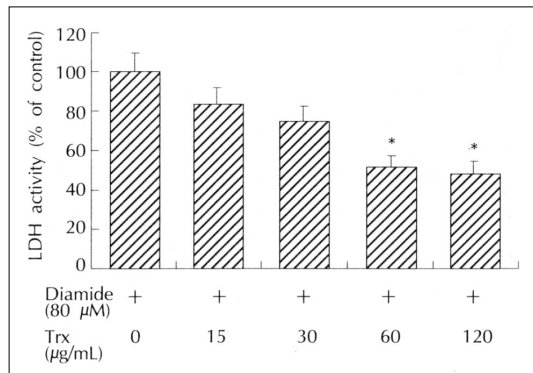


Fig. 7. Dose-response relationship of thioredoxin (Trx) for its protect effect on diamide by LDH assay. Cultured bovine pulmonary vascular endothelial cell line (PVEC) were preincubated with Trx for 2 hours before exposure to diamide. The result indicates the mean \pm SEM (n=6). *: p<0.05.

TRX
LDH
Diamide
TRX
Diamide midviability value(MVV) 80 μM Di -
amide 8 2 15
120 μg/mL TRX
15 μM TRX
TBARS 83.6%(14.5 \pm 1.2) Diamide
100%(17.4 \pm 1.3)
30 μg/mL 60 μg/mL TRX
TBARS 75.4%(13.1 \pm 1.5) 52.3%
(9.1 \pm 0.7)(p<0.05) 120 μg/mL TRX
LDH 48.7%(8.5 \pm 0.6)(p<0.05)
(Fig. 7).

SRB

Diamide
Diamide
10 μM 80 μM
Diamide 8
Diamide
10 μM Diamide
(100%) 85.4% 20
μM Diamide 76.7%
40 μM 80 μM Diamide

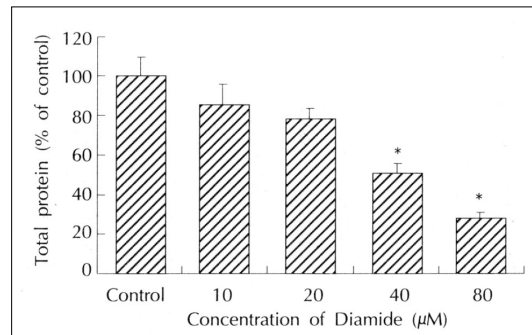


Fig. 8. Dose-response relationship of diamide in cultured bovine pulmonary vascular endothelial cell line (PVEC). Cytotoxicity was measured by SRB assay. The result indicates the mean \pm SEM (n=6). *: p<0.05.

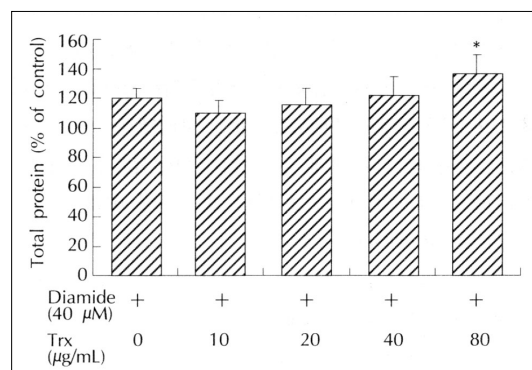


Fig. 9. Dose-response relationship of thioredoxin (Trx) for its protect effect on diamide by SRB assay. Cultured bovine pulmonary vascular endothelial cell line (PVEC) were preincubated with Trx for 2 hours before exposure to diamide. The result indicates the mean \pm SEM (n=6). *: p<0.05.

26.7% MVV 40 μM(p<0.05)
(Fig. 8).

TRX

Diamide
TRX
Diamide MVV 40 μM Diamide 8
2 10 80 μg/mL TRX
가
10 μg/mL TRX
80 μM Diamide
(100%) 109.2%
20 μg/mL TRX 116.

3% , 40 $\mu\text{g/mL}$ 80 $\mu\text{g/mL}$
121.4% 136.4% ($p < 0.05$)
(Fig. 9).

세포의 형태학적 변화

3
, 5 가
(vacuole)
(granulation), (fusion), (swelling)

고찰

PKC , phospho-
inositol (7)(10)
nuclear factor kappa
B(NF- κ B)가 vascular cell adhesion mo-
lecule-1(VCAM-1) MCP-1
가
(4)
Diamide
sulfhydryl group(-SH)
disulfide , gl-
utathione SOD(superoxide dismutase) 8
Diamide
XTT MTS . Diamide
XTT MTS
XTT MTS
Diamide

, Goodwin (25) XTT₅₀
MTS₅₀ 50 μM
(XTT₅₀, MTS₅₀ < 100 μM). T-cell (29)

Diamide가 (apoptosis)
Sato (28)
Diamide
thioredoxin(TRX) . TRX
(-SH)
(lipid peroxidation) TRX
, Diamide
TBARS(thiobarbituric acid reactive substan-
ces) 가 TRX 2
Diamide 8 Diamide
TBARS
TRX가
Diamide
TRX가 lactate dehydrogenase(LDH)
Diamide
LDH 가
TRX 2 Diam-
Diamide
LDH
TRX가 Diamide
가 TBARS assay
Diamide
SRB as-
say
TRX 2 Diamide
가
TRX가 Diamide
Diamide
, Diamide
TRX
, gl-

yceraldehyde 3 - phosphate

30)

N - me -
thyl - D - aspartate(NMDA) receptor Ca^{2+} - ch -
annel pathway (exitotoxic amino
acid, EAA) 가

요 약

배경 및 목적 :

Diamide
thioredoxin(TRX)

방 법 :

(pu -
lmonary artery endothelial cell, PAEC)
, XTT assay MTS assay, lactate dehydrogen -
ase(LDH) , SRB Lipid peroxidation

결 과 :

XTT MTS PAEC Dia -
mide

. PAEC Diamide
SRB
, LDH 가 lipid peroxid -
ation 가 . Diamide
TRX TRX Di -
amide 가
, LDH lipid peroxidation

결 론 :

Diamide PAEC
, TRX 가 Diamide
가 lipid pero -
xidation LDH Di -

2000

중심 단어 : ; ; ;

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