

NO	가	bovine serum (FBS)	(penicillin 100 U/ml, amphotericin 2.5µg/ml, streptomycin 100µg/l)	Dulbecco's Modified Eagle Medium (DMEM)	5 ml	가
superoxide				(95% O ₂ /5% CO ₂)	24	10%
peroxynitrite (ONOO ⁻)		FBS	DMEM		3~4 ml	
NO	peroxynitrite					
protein tyrosine residue	nitro-					
tyrosine	(8-10)				5~7	
tyrosine					가	3~4 1
Cu, Zn-SOD (superoxide dismutase), aconitase, Mn-SOD						
cytochrome oxidase					2)	
DNA						
NO						cover glass
	가					3.7% paraformaldehyde 10
						0.01% Triton X-100 3
Glutathione (GSH)	glutathione peroxidase					peroxidase
						0.5% H ₂ O ₂ 30
	가					0.5% bovine serum
tocopherol)	(11) vitamin E (alpha-					myosin
		albumin (BSA)				37°C
		1 : 500			100%	가
						1
Vitamin C (ascorbic acid)						1 : 100
vitamin E	(13) Lipoic acid					anti-
dihydropyridine (DHLA)	peroxynitrite					rabbit IgG (Fab) 2 peroxidase conjugate
	(14-16)					1 : 400
	가					rabbit PAP
C, vitamin E, GSH	DHLA					Tris
	vitamin					3,3'-diaminobenzidine tetrahydrochloride
						H ₂ O ₂
						10
	NFκB (nuclear factor κB)					Harris's hematoxylin 1
cytokine	iNOS					DePeX (BDH)
	(17)					3)
	가					
	가 NO					N ₂ 95%
						CO ₂ 5%
						O ₂ 95% CO ₂ 5%
NO						4) nitrite
NOS	가					nitrite (NO ₂ ⁻)
						12,000 g, 5
						Griess (0.1% NEDD
						2% phosphoric acid 1% sulfanilamide
						10 550 nm
1)						
	(Sprague-Dawley rat, 250					sodium nitrite
g)	4°C Hank's Bal-					5) PO ₂ , PCO ₂ , SO ₂ , HCO ₃ ⁻
	anced Salt Solution (HBSS)					PO ₂ , PCO ₂ , SO ₂ ,
가						HCO ₃ ⁻ 12000 g, 5
	50% fetal					가 AVL

OMNI™ system (AVL Medical Division, Austria)

6) iNOS, NFκB p65 Western blot

가 가 2

(Vibra Cell, SONICS and MATERIALS INC., USA)

12,000 g, 4°C

(Kit, BIO-RAD, USA)

. 80 µg

2×

resolving gel stacking gel SDS-PAGE gel

nitrocellulose (NC membrane,

Protran, Schleicher & Schuell, Germany)

NC membrane 5% non

fat dry milk

1

10

3

iNOS, NFκ

B

0.1% BSA, 0.05% Tween 20

1:2000

NC membrane

1

10

3

anti-rabbit HRP 0.1% BSA,

0.05% Tween 20

1:4000

1

10

3

NC membrane

electrochemiluminescence (Renaissance Western Blot Chemiluminescence reagent, NEN life science products, USA)

1

X

iNOS, NF

κB band

, NIH Image program

(Version 1.61/ppc, NIH, USA)

7)

lipic acid, DHLA, vitamin C,

vitamin E, GSH, N^G-nitro-L-arginine (L-NAME), HBSS, DMEM, sulfanilamide, NEDD Sigma (St. Louis, USA)

, fetal bovine serum, antibiotics- antimy-

cotics, trypsin-EDTA GibcoBRL (New York, USA)

10⁻⁷~10⁻⁵ M

10⁻⁶ M

Western blot

iNOS, NFκB p65

Santa Cruz

(Santa Cruz, USA)

kit,

protein size marker,

, SDS BIO-RAD

(Hercules, USA)

±

Student's t-test (QuickTTest, Version 1.1, Steve

Ashcroft, UK)

P value가 0.05

1)	PO ₂ , PCO ₂ , SO ₂ , HCO ₃ ⁻
	(PO ₂ , mmHg) 151±1.7
24	PO ₂
80±2.4	
24	3
159±1.4	가 , 6
	(Fig. 1).
	(PCO ₂ , mmHg) 38±3.9
24	PCO ₂
27±0.8	
24	(Fig. 1).
	SO ₂ (%) 99±0.1
24	
SO ₂ 98±0.3	, 24
	(Fig. 1).
	HCO ₃ ⁻ (mg/dl)
26±1.1	. 24

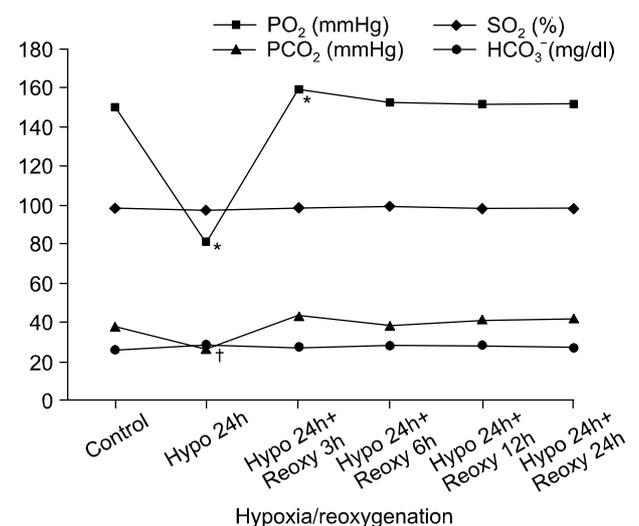


Fig. 1. Effects of hypoxia and/or reoxygenation on the PO₂, PCO₂, SO₂ and HCO₃⁻ level in vascular smooth muscle cell culture media. Values express mean±S.E.M. (n=4). Hypo = Hypoxia; Reoxy = Reoxygenation. *P<0.05 significantly different from control on the PO₂ level. †P <0.05 significantly different from control on the PCO₂ level.

HCO₃⁻ 28±1.3 가
 , 24
 (Fig. 1).

2) - nitrite
 nitrite , 24
 nitrite 가
 (%) 133±6.0
 24
 nitrite 가 가 , nitrite
 (%) 3 150±6.0, 6
 157±8.0, 12 160±7.0, 24
 165±18.0 가 (Fig. 2).
 24 24
 6~12 nitrite 가 가
 (Fig. 2).

3) 24 nitrite
 1
 , 24
 nitrite
 Nitrite (%) L-NAME 10⁻⁶ M 83±4.9,
 vitamin C 10⁻⁶ M 70±2.5, vitamin E 10⁻¹ IU 균

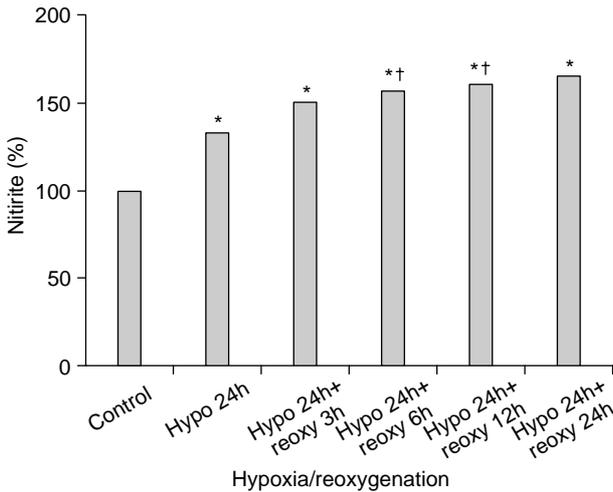


Fig. 2. Effects of hypoxia and/or reoxygenation on the nitrite level in vascular smooth muscle cell culture media. Values express mean±S.E.M. (n=8). Hypo = hypoxia; Reoxy = reoxygenation. *P<0.05 significantly different from control. †P<0.05 significantly different from Hypo 24 h.

77±4.6, GSH 10⁻⁶ M 85±3.6, lipoic acid 10⁻⁶ M
 73±0.7, DHLA 10⁻⁶ M 72±5.0
 nitrite 가 (Fig. 3).

4) 24 6
 nitrite 1

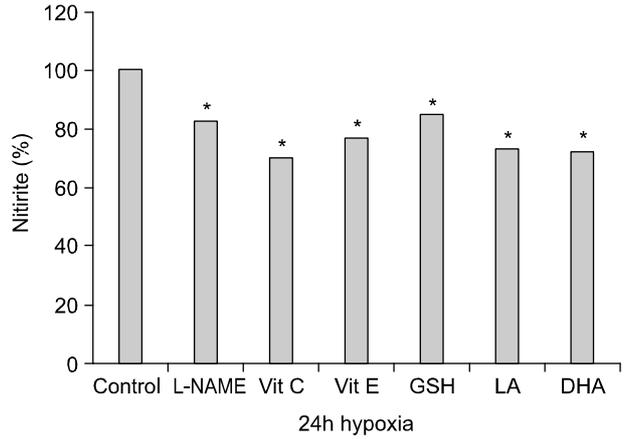


Fig. 3. Effects of L-NAME and antioxidants on the nitrite level in vascular smooth muscle cell culture media of 24 hours hypoxia. Values express mean±S.E.M. (n=6). L-NAME = N^G-nitro-L-arginine; Vit C = vitamin C; Vit E = vitamin E; GSH = glutathione; LA = lipoic acid; DHLA = dihydrolipoic acid. *P<0.05 significantly different from control.

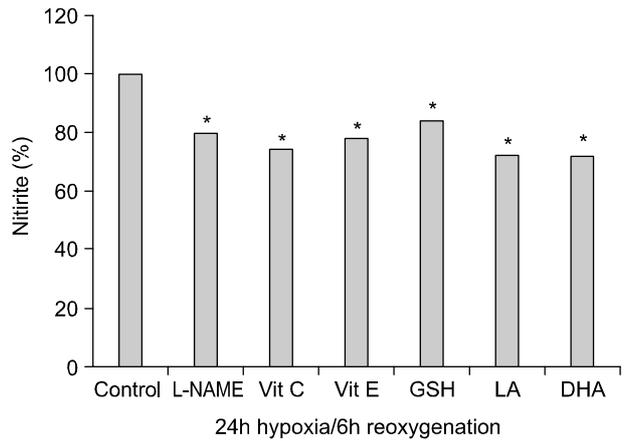
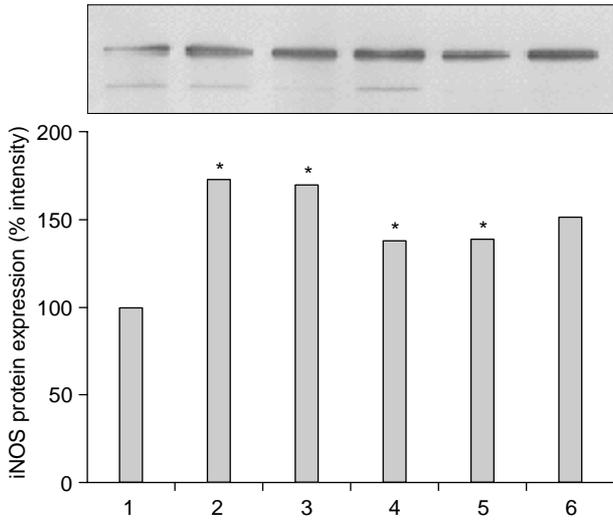
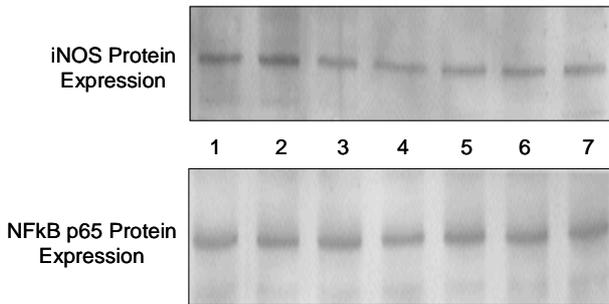


Fig. 4. Effects of L-NAME and antioxidants on the nitrite level in vascular smooth muscle cell culture media of 24 hours hypoxia and 6 hours reoxygenation. Values express mean±S.E.M. (n=6). L-NAME = N^G-nitro-L-arginine; Vit C = vitamin C; Vit E = vitamin E; GSH = glutathione; LA = lipoic acid; DHLA = dihydrolipoic acid. *P<0.05 significantly different from control.



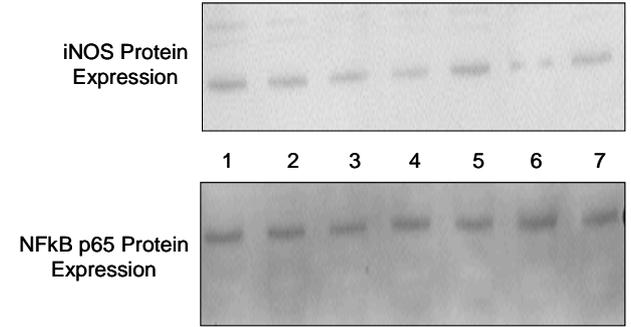
Lane 1: Control
 Lane 2: Hypoxia 24 h
 Lane 3: Hypoxia 24 h+Reoxygenation 3 h
 Lane 4: Hypoxia 24 h+Reoxygenation 6 h
 Lane 5: Hypoxia 24 h+Reoxygenation 12 h
 Lane 6: Hypoxia 24 h+Reoxygenation 24 h

Fig. 5. Effects of hypoxia-reoxygenation on the iNOS protein expression in vascular smooth muscle cell. Values express mean±S.E.M. (n=4). *P<0.05 significantly different from control.



Lane 1: Hypoxia 24 h
 Lane 2: Hypoxia 24 h+DHHLA 10⁻⁶ M
 Lane 3: Hypoxia 24 h+GSH 10⁻⁶ M
 Lane 4: Hypoxia 24 h+Lipoic acid 10⁻⁶ M
 Lane 5: Hypoxia 24 h+L-NAME 10⁻⁶ M
 Lane 6: Hypoxia 24 h+Vit C 10⁻⁶ M
 Lane 7: Hypoxia 24 h+Vit E 10⁻¹ IU

Fig. 6. Effects of L-NAME and antioxidants on the hypoxia induced iNOS, NFkB p65 protein expression in vascular smooth muscle cell. DHHLA = dihydrolipoic acid; GSH = glutathione; L-NAME = N^G-nitro-L-arginine; Vit C = vitamin C; Vit E = vitamin E.



Lane 1: Hypoxia 24 h/Reoxygenation 6 h
 Lane 2: Hypoxia 24 h/Reoxygenation 6 h+DHHLA 10⁻⁶ M
 Lane 3: Hypoxia 24 h/Reoxygenation 6 h+GSH 10⁻⁶ M
 Lane 4: Hypoxia 24 h/Reoxygenation 6 h+Lipoic acid 10⁻⁶ M
 Lane 5: Hypoxia 24 h/Reoxygenation 6 h+L-NAME 10⁻⁶ M
 Lane 6: Hypoxia 24 h/Reoxygenation 6 h+Vit C 10⁻⁶ M
 Lane 7: Hypoxia 24 h/Reoxygenation 6 h+Vit E 10⁻¹ IU

Fig. 7. Effects of L-NAME and antioxidants on the hypoxia 24 hours-reoxygenation 6 hours induced iNOS, NFkB p65 protein expression in vascular smooth muscle cell. DHHLA = dihydrolipoic acid; GSH = glutathione; L-NAME = N^G-nitro- L-arginine; Vit C = vitamin C; Vit E = vitamin E.

, 24 6
 nitrite
 Nitrite (%) L-NAME 10⁻⁶ M 80±5.0,
 vitamin C 10⁻⁶ M 74±4.2, vitamin E 10⁻¹ IU
 78±4.4, GSH 10⁻⁶ M 84±4.8, lipoic acid 10⁻⁶ M
 72±5.2, DHHLA 10⁻⁶ M 72±4.4
 nitrite 가 (Fig. 4).

5) Western blot

iNOS , 24
 iNOS (% of intensity)
 172.5±9.1
 24 3 , 6 12
 169.7±9.7, 137.7±9.9 139.1±9.7
 가 (Fig. 5).
 24 NFkB
 p65, iNOS 24 6
 NFkB p65, iNOS DHHLA
 10⁻⁶ M, GSH 10⁻⁶ M, lipoic acid 10⁻⁶ M, vitamin C 10⁻⁶ M,
 vitamin E 10⁻¹ IU NOS L-NAME 10⁻⁶ M
 (Fig. 6, 7).

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