

Lysophosphatidylcholine에 의해 유발된 혈관 평활근세포 증식에 대한 Naringin의 작용

김상현¹ · 김인욱^{2,3} · 이해영^{2,3} · 채인호^{2,3} · 김명아¹ · 김효수^{2,3} · 김철호^{2,3}
손대원^{2,3} · 오병희^{2,3} · 이명묵^{2,3} · 박영배^{2,3} · 최윤식^{2,3} · 이영우^{2,3}

The Effect of Naringin on the Lysophosphatidylcholine-induced Proliferation of Vascular Smooth Muscle Cells

Sang Hyun Kim, MD¹, In Wook Kim, MS^{2,3}, Hae Young Lee, MD^{2,3}, In Ho Chae, MD^{2,3},
Myung A Kim, MD¹, Hyo Soo Kim, MD^{2,3}, Cheol Ho Kim, MD^{2,3},
Dae Won Sohn, MD^{2,3}, Byung Hee Oh, MD^{2,3}, Myoung Mook Lee, MD^{2,3},
Young Bae Park, MD^{2,3}, Yun Shik Choi, MD^{2,3} and Young Woo Lee, MD^{2,3}

¹Department of Internal Medicine, Seoul Municipal Boramae Hospital, Seoul, ²Division of Cardiology, Department of Internal Medicine, Seoul National University College of Medicine, Seoul,

³Cardiovascular Laboratory, Clinical Research Institute, Seoul National University Hospital, Seoul, Korea

ABSTRACT

Background and Objectives : Lysophosphatidylcholine (lysoPC), an atherogenic lysophospholipid, is known to induce the proliferation of vascular smooth muscle cells (VSMCs). Naringin is a flavonoid in grapes and grapefruits and has anti-inflammatory as well as antioxidative effects. We investigated whether naringin could protect VSMCs from the effect of lysoPC. Additionally, we investigated the changes of nuclear translocation of nuclear factor-kappa B (NF- κ B). **Materials and Methods :** VSMCs were prepared from the aorta of Sprague-Dawley rats. Near-confluent VSMCs were preincubated in media containing 0, 10, 100 μ M naringin, and incubated with 0, 10, 20, 100 μ M lysoPC. The degree of proliferation of VSMCs was evaluated with [H3]-thymidine incorporation and MTT assay. The changes of nuclear translocation of NF- κ B in VSMCs were investigated with EMSA. **Results :** LysoPC promoted the growth of VSMCs, whereas naringin inhibited the proliferative effect of lysoPC on VSMCs. MTT assay showed a $63 \pm 24\%$ and $89 \pm 17\%$ increase of cellular growth in the 10 and 20 μ M lysoPC groups, respectively, as compared with the control group with media only ($p < 0.01$). [H3]-thymidine incorporation assay also showed $61 \pm 25\%$ and $92 \pm 25\%$ increase in the 10 and 20 μ M lysoPC groups, respectively ($p < 0.01$). However, the growth of VSMCs was suppressed in the 100 μ M lysoPC group ($p < 0.01$). Naringin inhibited the proliferative effects of lysoPC on VSMCs by $34 \pm 5\%$ (MTT assay) and $35 \pm 5\%$ ([H3]-thymidine incorporation assay) in the 100 μ M naringin group ($p = 0.01$). The nuclear translocation of NF- κ B was stimulated by lysoPC and suppressed by naringin. **Conclusion :** LysoPC promoted the growth of VSMCs, whereas naringin inhibited the proliferative effect of lysoPC on VSMCs. These

: 2001 7 4
: 2001 11 14
: , 110 - 744 28
: (02) 760 - 2684 · : (02) 766 - 8904 · E - mail : ihchae@snu.ac.kr

KEY WORDS : Lysophosphatidylcholines ; Naringin ; Muscle, smooth, vascular ; NF-kappa B.

Fig. 1. The structure of naringin (4', 5, 7-trihydroxyflavone-7-hesperidoside).

가 NF- κ B ,
naringin ,
naringin ,
lysoPC
가
NF- κ B (nuc-
lear translocation) DNA 가
가

재료 및 방법

세포배양

Sprague - Dawley rat
, DMEM/F12 (Gibco BRL)
60 mm ,
10% FBS
(Fetal bovine serum, Gibco BRL) /
(penicillin and amphotericin B, Gibco
BRL) 가 . 60 mm
10 가 ,
trypsin (T - 75)
. 5 80%
Naringin(Sigma) lysoPC 가 24
, 0, 10, 20, 100 μ M 가
가 . Naringin 가 24 lyso -
PC(Sigma) 가 0, 10, 100 μ M
가 . LysoPC 가 0
6, 24, 48 [H³] - thymidine incorpora -
tion assay , naringin lysoPC
DNA 가 , 24, 48,
72 MTT (3 - [4,5 - dimethylthiazol - 2 - yl] -
2, 5 - diphenyltetrazolium bromide, Sigma) assay
naringin lysoPC
가 . lyso -
PC 가 2 NF - κ B
EMSA(electrophoretic mobi -
lity shift assay) naringin, lysoPC

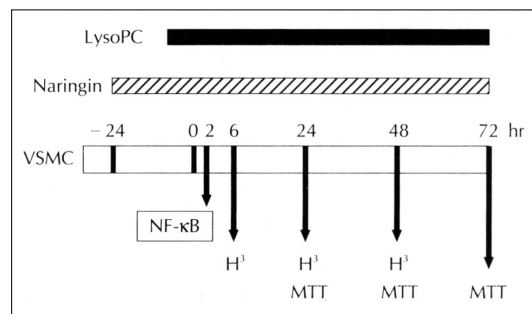


Fig. 2. Experimental protocol (time table). H³ : H³-thymidine incorporation assay at 6, 24, 48 hours after the addition of lysoPC, MTT : MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium-bromide) assay at 24, 48, 72 hours, NF- κ B EMSA : electrophoretic mobility shift assay for nuclear factor kappa B at 2 hours, MTT : methylthiazol, VSMC : vascular smooth muscle cells.

NF- κ B

Fig. 2

세포증식정도 측정

MTT assay
가 naringin lysoPC가
. MTT(Sig -
ma) 2.5 mg/mL 가 3
가 .
96 - well microtiter plate ,
well 200 μ L 10⁴
. 24 FBS 가
0, 10, 100 μ M naringin 가 ,
10% FBS 0, 10, 20, 100 μ M lyso -
PC, 0, 10, 100 μ M naringin 가
. Naringin lysoPC
, well 24
Naringin lysoPC 8 well
LysoPC 24, 48, 72 MTT
assay . Well 2.5 mg/mL MTT 10
 μ L 가 , 37 4
가 well
. 4 well 150 μ L
, 0.04 M HCl 100 μ L
well 가 formazan
(optical density)

ader 595 nm
Naringin lysoPC
ringin lysoPC 가

DNA 합성정도 측정

[H³] - thymidine incorporation assay
DNA
24 - well pl -
ate , well 200 μ L
10⁴ . 24 FBS
가 0, 10, 100 μ M naringin 가
10% FBS 0, 10, 20, 100
 μ M lysoPC, 0, 10, 100 μ M naringin 가
Naringin lysoPC
well 24
Naringin lysoPC 6
well
LysoPC 6, 24, 48 H³ - Thy -
midine incorporation assay
4 1
 μ Ci/mL of [H³] - thymidine 가
lysoPC 6, 24, 48
[H³] - thymidine
PBS(phosphate buffered saline)
cold 10% TCA(trichloroacetic acid)
95% ethanol 2
0.2 M NaOH
[H³] - thymidine , liquid scintillation
counter
naringin lysoPC [H³] - th -
ymidine incorporation

핵단백질 추출과 Electrophoretic mobility shift assay (EMSA)

0, 10, 100 μ M naringin 24
20 μ M lysoPC 2

ice - cold
PBS , 200 μ L ice - cold hypotonic
lysis buffer(0.5 M Hepes/pH 7.9, 1 M KCl, 150 mM
MgCl₂, 250 mM sucrose, 10% Nonidet P - 40, 1 mM
DTT, 100 mM EGTA, 500 mM EDTA, 5 μ g/mL
leupeptin, 0.1 mM PMSF) 가 15
15,000 rpm 5

100 μ L washing buffer(0.5 M Hepes/pH 7.9,
1 M KCl, 150 mM MgCl₂, 100 mM EGTA, 500 mM
EDTA, 5 M NaCl, 10% glycerol, 5 μ g/mL leupep -
tin, 0.1 mM PMSF) 15,000 rpm
5

200 μ L hypertonic extraction buffer
(0.5 M Hepes/pH 7.9, 1 M KCl, 150 mM MgCl₂,
100 mM EGTA, 500 mM EDTA, 5 M NaCl, 10%
glycerol, 1% Nonidet P - 40, 5 μ g/mL leupeptin, 0.
1 mM PMSF) 가 1
15,000 rpm 30

polyacrylamide gel
Bradford

EMSA
NF - B iNOS pr -
omoter (- 107 to - 97) NF - B
oligonucleotide
(Promega) forward : 5 ' - TGGG -
GACTCTCC - 3 ' , complement : 5 ' - AAGGGAGA -
GTCC - 3 ' T4 polynucleotide ki -
nase NF - B
[- 32P]ATP

10 μ g
10 binding buffer (50 mM Tris/pH 7.
5, 250 mM NaCl, 2.5 mM DTT, 2.5 mM EDTA, 5
mM MgCl₂, 20% glycerol and 1 μ g poly(dI - dC))
가 50 fM
가 20 , 1 μ L of loading buffer
(250 mM Tris/pH 7.5, 0.2% Bromophenol Blue,
50% glycerol) 가 (24)25)
4% polyacrylamide gel 5

polyacrylamide gel
- 70 12
NIH image program
densitometry

통계분석
MTT assay 1 naringin ly -
soPC 8 well , [H³] -
thymidine incorporation assay 6
well , naringin, lysoPC가
± p<0.05
Student t - test
ANOVA
PC -
SAS version 6.12

결 과

hill and valley
60 mm 가 , MTT
assay 96 well plate
[H³] - thymidine incorporation assay 24
well plate , NF - B EMSA T - 75

혈관 평활근세포 성장에 대한 LysoPC와 naringin의 작용
LysoPC
lysoPC
10 μM lysoPC
63 ± 24% 가 , 20
μM lysoPC 89 ± 17% 가 (p<0.01).
100 μM lysoPC
57 ± 13% (p<0.01),
lysoPC 10 μM, 20 μM
, 100 μM
(biphasic)
(Fig. 3).
Naringin lysoPC

lysoPC
Naringin
Naringin 10 μM
lysoPC 20 μM 가 , naringin
lysoPC 20 μM 가
21 ± 6% (p=0.01)(Fig. 4).

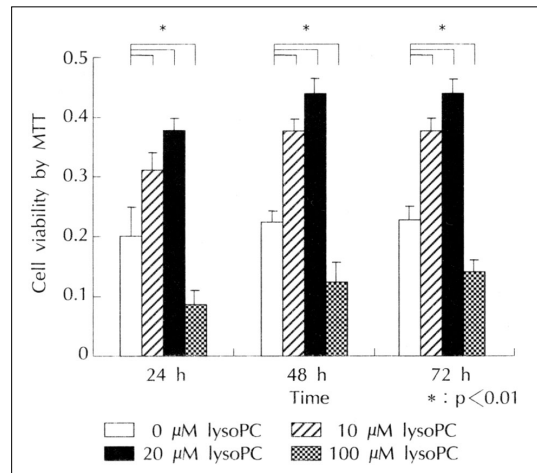


Fig. 3. MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl-tetrazolium bromide) assay-the effect lysophosphatidylcholine (lysoPC) on the proliferation of vascular smooth muscle cells (VSMCs) : The cell viability was increased significantly by lysoPC as compared with control group with media only (p<0.01). But 100 μM lysoPC decreased the cell viability significantly as compared with control group (p<0.01). MTT : methylthiazol.

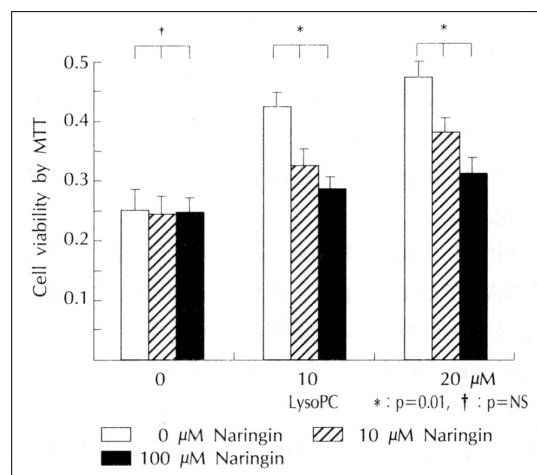


Fig. 4. MTT assay-the effect of naringin on the proliferation of VSMCs at 48 hours : naringin protected VSMCs against the proliferative effect of lysoPC (* : p=0.01). Naringin per se had no cytotoxic effect on VSMCs († : p=NS). MTT : methylthiazol, NS : not significant

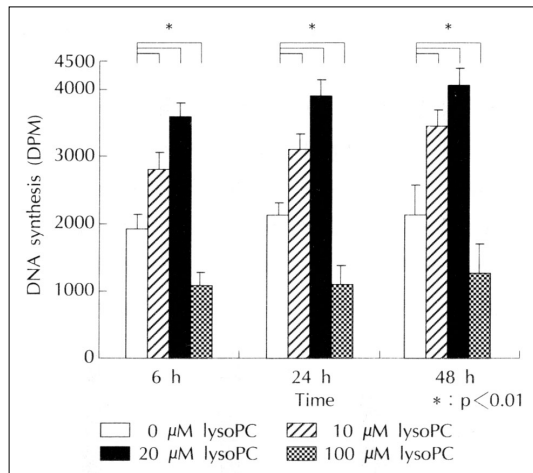


Fig. 5. [H³]-thymidine incorporation assay-the effect of lysoPC on the proliferation of VSMCs : DNA synthesis of VSMCs were increased significantly by lysoPC as compared with control group with media only ($p < 0.01$). But in 100 μM lysoPC group, DNA synthesis was decreased significantly ($p < 0.01$), and this biphasic response of DNA synthesis with the increment of lysoPC dose was similar to that of MTT assay. MTT : methylthiazol, DPM : decay per minute, VSMCs : vascular smooth muscle cells.

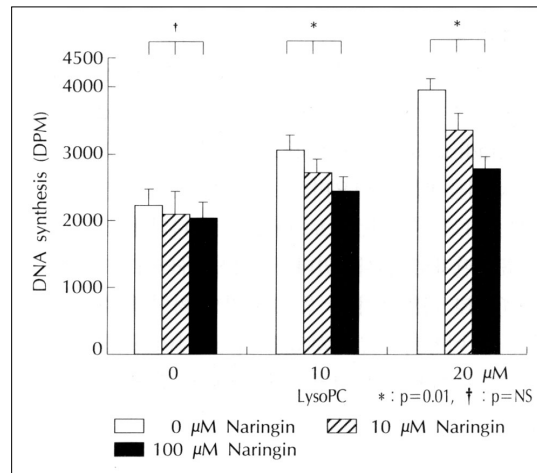


Fig. 6. [H³]-thymidine incorporation assay-the effect of naringin on the proliferation of VSMCs at 24 hours : naringin significantly decreased DNA synthesis of VSMCs induced by lysoPC as compared with that of the group incubated in lysoPC only ($p = 0.01$). NS : not significant, DPM : decay per minute, VSMCs : vascular smooth muscle cells.

DNA 합성에 대한 lysoPC와 naringin의 작용

lysoPC, naringin, DNA, [H³]-thymidine incorporation assay, DNA

5, 6).

DNA
0 μM lysoPC, 10 μM lysoPC, 20 μM lysoPC, 100 μM lysoPC
61 ± 25%
92 ± 25%
($p < 0.01$).
49 ± 5%, MTT assay

lysoPC, DNA, 가, 가
($p < 0.01$)

(Fig. 5).

lysoPC, DNA, naringin, Naringin, lysoPC 20 μM, 가, naringin, 10 μM, lysoPC 20 μM, 가, DNA, 17 ± 4%

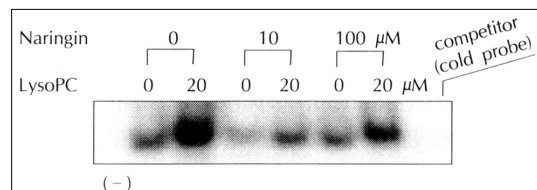


Fig. 7. NF-κB EMSA (electrophoretic mobility shift assay). LysoPC increased the nuclear translocation of NF-κB, and naringin suppressed the nuclear translocation of NF-κB. There was no band in negative control and cold probe group. The band of NF-κB was increased significantly in 20 μM lysoPC group, compared with that in 0 μM lysoPC group. The ratio of band density of 0 μM, 20 μM lysoPC was decreased significantly in 10, 100 μM lysoPC group, compared with that in 0 μM naringin group. (-) : control.

, naringin 100 μM, 35 ± 5%
($p = 0.01$)(Fig. 6).

NF-κB EMSA(electrophoretic mobility shift assay)

NF-κB, 가, lysoPC, 가, naringin, lysoPC, 가, NF-κB, EMSA, LysoPC, NF-κB, , na-

ringin lysoPC NF - B
 . Fig. 7 , cold pr -
 obe NF - B가 probe
 . 0 μ M lysoPC 20 μ M ly -
 soPC NF - B 가 가 , na -
 ringin lysoPC NF - B
 가 , 10, 100 μ M naringin
 20 μ M lysoPC 가 naringin
 20 μ M lysoPC 가 54%
 (Fig. 7).

고 찰

lysoPC
 ,
 naringin , lysoPC
 NF - B 가 naringin
 .
 LysoPC가
 ,¹⁰⁾¹¹⁾
 .¹²⁾
 naringin , lysoPC
 가
 . LysoPC 10 μ M, 20 μ M
 가
 . LysoPC 100 μ M
 . , lysoPC
 naringin . ,
 , NF - B
 , lysoPC
 가 , naringin .
 , naringin , lysoPC
 가
 , NF - B
 .
 lysoPC
 .
 lysoPC가 2가 가 가 .¹⁶⁾

가 ,
 phospholipase A2 phosphati -
 dylcholine lysoPC(lysophosphatidylcholine) 가
 .³⁾ lysoPC
 .⁵⁾ LysoPC
 가 가 lecithin/cholest -
 erol acyltransferase .
 lysoPC
 .²⁶⁾
 LysoPC endothelial NOS(eNOS)
 nitric oxide
 .⁴⁻⁶⁾ lysoPC ICAM - 1,
 VCAM - 1 P - selectin
 가 ,⁷⁻⁹⁾
 가 .⁹⁾
 LysoPC 가
 , lysoPC가 NO
 protein kinase C ,⁵⁾
 P - selectin ,
 protein kinase C ⁸⁾ lysoPC
 protein kinase C . Protein
 kinase C lysoPC
 , prote - in kinase
 C 가 , 100 μ M
 lysoPC protein kinase C
 ,²⁷⁾
 .
 Naringin lysoPC
 . na -
 ringin 가 ,
 , ,
 . Naringin protein ki -
 nase ,¹⁷⁾ lipopol -
 ysaccharide tumor necrosis factor
 ,¹⁸⁾
 naringin ,
 가 ,¹⁵⁾
 naringin
 가 가 가 .¹⁶⁾

B
 NO
 iNOS
 lysoPC
 NF - B
 , naringin
 NF - B
 , naringin, narin-
 genin, resveratrol
 NF - B
 iNOS
 가²³⁾
 3 - hydroxyl - 3 - methyl - glutaryl - coenzyme A
 (HMG - CoA)
 lovastatin, at-
 orvastatin
 NF - B
 , mo-
 nocyte chemoattractant protein - 1 (MCP - 1)
 iNOS
 , naringin
 ,
 ,
 가³⁰⁾
 , lysoPC
 , naringin
 lysoPC
 ,
 lysoPC, nari-
 ngin
 NF - B
 , naringin
 , naringin
 ,

요약

배경 및 목적 :
Lysophosphatidylcholine(lysoPC)
(ICAM - 1, VC - AM - 1)
. Na - ringin(4, 5, 7 - trihydroxy - flavonone - 7 - hesperi - doside)

배경 및 목적 :

AM - 1)

, naringin, lysoPC

가, NF- κ B

가

방 법 :

DMEM/F12

, naringin 0, 10, 100 μ M 24

, lysoPC 0, 10, 20, 100 μ M

. LysoPC

6, 24, 48 H^3 - Thymidine inc -

orporation assay DNA

, 24, 48, 72 MTT (3 - (4, 5 - dimethylth -

iazol - 2 - yl) - 2, 5 - diphenyltetrazolium bromide) as -

say

nu -

clear factor kappa B(NF- κ B)

lysoPC naringin, NF- κ B

electrophoretic mobility shift assay(EMSA)

결 과 :

LysoPC

, naringin lysoPC

. H^3 - Thymidine incorporation

assay, lysoPC ly -

soPC 10 μ M $61 \pm 25\%$, 20 μ M $92 \pm 25\%$

, (p<0.01).

lysoPC

MTT assay, lysoPC

lysoPC 10 μ M $63 \pm 24\%$, 20 μ M 89

$\pm 17\%$,

lysoPC (p<0.01).

, lysoPC 100 μ M

, H^3 - Th -

ymidine incorporation assay $49 \pm 5\%$, MTT ass -

ay $57 \pm 13\%$, naringin

lysoPC

, naringin 100 μ M

$34 \pm 5\%$ (MTT assay), $35 \pm 5\%$ (H^3 - Thymidine in -

corporation assay) 가

(p=0.01). EMSA, NF- κ B

lysoPC naringin

결 론 :

LysoPC, na -

ringin lysoPC

lysoPC naringin NF- κ B

naringin

중심 단어 : Lysophosphatidylcholine ; Naringin ;

; NF- κ B.

(BSKG1420 -)

REFERENCES

- 1) Steinberg D, Parthasarathy S, Carew TE, Khoo JC, Witztum JL. *Beyond cholesterol: modifications of low-density lipoprotein that increase its atherogenicity.* *N Engl J Med* 1989;320:915-24.
- 2) Henriksen T, Mahoney EM, Steinberg D. *Enhanced macrophage degradation of low density lipoprotein previously incubated with cultured endothelial cells: recognition by receptors for acetylated LDLs.* *Proc Natl Acad Sci USA* 1981;78:6499-503.
- 3) Parthasarathy S, Barnett J. *Phospholipase A₂ activity of low density lipoprotein: evidence for an intrinsic phospholipase A₂ activity of apolipoprotein B100.* *Proc Natl Acad Sci USA* 1990;87:9741-5.
- 4) Liao JK, Shin WS, Lee WY, Clark SL. *Oxidized low-density lipoprotein decreases the expression of endothelial nitric oxide synthase.* *J Biol Chem* 1995;270:319-24.
- 5) Kugiyama K, Kerns SA, Morrisett JD, Roberts R, Henry PD. *Impairment of endothelium-dependent arterial relaxation by lysolecithin in modified low-density lipoproteins.* *Nature* 1990;344:160-2.
- 6) Yokoyama M, Hirata K, Miyake R, Akita H, Ishikawa Y, Fukuzaki H. *Lysophosphatidylcholine: essential role in the inhibition of endothelium-dependent vasorelaxation by oxidized low density lipoprotein.* *Biochem Biophys Res Commun* 1990;168:301-8.
- 7) Kume N, Cybulsky MI, Gimbrone MA Jr. *Lysophosphatidyl-choline, a component of atherogenic lipoproteins, induces mono-nuclear leukocyte adhesion molecules in cultured human and rabbit arterial endothelial cells.* *J Clin Invest* 1992;90:1138-44.
- 8) Murohara T, Scalia R, Lefer AM. *Lysophosphatidylcholine promotes P-selectin expression in platelets and endothelial cells.* *Circ Res* 1996;78:780-9.
- 9) Quinn MT, Parthasarathy S, Steinberg D. *Lysophosphatidyl-choline: a chemotactic factor for human monocytes*

- and its potential role in atherogenesis. *Proc Natl Acad Sci USA* 1988;85:2805-9.
- 10) Yui S, Sasaki T, Miyazaki A, Horiuchi S, Yamazaki M. Induction of murine macrophage growth by modified LDLs. *Arterioscler Thromb* 1993;13:331-7.
 - 11) Sakai M, Miyazaki A, Hakamata H, Sato Y, Matsumura T, Kobori S, Shichiri M, Horiuchi S. Lysophosphatidylcholine potentiates the mitogenic activity modified LDL for human monocyte-derived macrophages. *Arterioscler Thromb Vasc Biol* 1996;16:600-5.
 - 12) Yoon BK, Oh WJ, Kessel B, Roh CR, Choi D, Lee JH, Kim DK. 17 β -estradiol inhibits proliferation of cultured vascular smooth cells induced by lysophosphatidylcholine via a nongenomic antioxidant mechanism. *Menopause* 2001;8:58-64.
 - 13) Limasset B, le Douchen C, Dore JC, Ojasoo T, Damon M, Crastes de Paulet A. Effects of flavonoids on the release of reactive oxygen species by stimulated human neutrophils. *Biochem Pharmacol* 1993;46:1257-71.
 - 14) Calomme M, Pieters L, Vlietinck A, vanden Berghe D. Inhibition of bacterial mutagenesis by citrus flavonoids. *Planta Medica* 1996;62:222-6.
 - 15) Le Marchand L, Murphy SP, Hankin JH, Wilkens LR, Kolonel LN. Intake of flavonoids and lung cancer. *J Natl Cancer Inst* 2000;92:154-60.
 - 16) So FV, Guthrie N, Chambers AF, Moussa M, Carroll KK. Inhibition of human breast cancer cell proliferation and delay of mammary tumorigenesis by flavonoids and citrus juices. *Nutr Cancer* 1996;26:167-81.
 - 17) Gordon PB, Holen I, Seglen PO. Protection by naringin and some other flavonoids of hepatocytic autophagy and endocytosis against inhibition by okadaic acid. *J Biol Chem* 1995;270:5830-8.
 - 18) Kawaguchi K, Kikuchi S, Hasegawa H, Maruyama H, Morita H, Kumazawa Y. Suppression of lipopolysaccharide-induced tumor necrosis factor release and liver injury in mice by naringin. *Eur J Pharmacol* 1999;368:245-50.
 - 19) Shin YW, Bok SH, Jeong TS, Bae KH, Jeoung NH, Choi MS, Lee SH, Park YB. Hypocholesterolemic effect of naringin associated with hepatic cholesterol regulating enzyme changes in rats. *Int J Vitam Nutr Res* 1999;69:341-7.
 - 20) Iwashina M, Hirata Y, Imai T, Sato K, Marumo F. Molecular cloning of endothelial, inducible nitric oxide synthase gene from rat aortic endothelial cell. *Eur J Biochem* 1996;237:668-73.
 - 21) Palombella VJ, Rando OJ, Goldberg AL, Maniatis T. The ubiquitin-proteasome pathway is required for processing the NF- κ B precursor protein and the activation of NF- κ B. *Cell* 1994;78:773-85.
 - 22) Katsuyama K, Shichiri M, Marumo F, Hirata Y. NO inhibits cytokine-induced iNOS expression and NF- κ B activation by interfering with phosphorylation and degradation of I κ B- α . *Arterioscler Thromb Vasc Biol* 1998;18:1796-802.
 - 23) Tsai SH, Lin-Shiau SY, Lin JK. Suppression of nitric oxide synthase and the down-regulation of the activation of NF- κ B in macrophages by resveratrol. *Br J Pharmacol* 1999;126:673-80.
 - 24) Zhou J, Struthers AD, Lyles GA. Differential effects of some cell signalling inhibitors upon nitric oxide synthase expression and nuclear factor- κ B activation induced by lipopolysaccharide in rat aortic smooth muscle cells. *Pharmacol Res* 1999;39:363-73.
 - 25) Katsuyama K, Shichiri M, Marumo F, Hirata Y. Role of nuclear factor- κ B activation in cytokine- and sphingomyelinase-stimulated inducible nitric oxide synthase gene expression in vascular smooth muscle cells. *Endocrinology* 1998;139:4506-12.
 - 26) Mohandas N, Wyatt J, Mel SF, Rossi ME, Shohet SB. Lipid translocation across the human erythrocyte membrane: regulatory factors. *J Biol Chem* 1982;257:6537-43.
 - 27) Oishi K, Raynor RL, Charp PA, Kuo JF. Regulation of protein kinase C by lysophospholipids: potential role in signal transduction. *J Biol Chem* 1988;263:6865-71.
 - 28) Miyake Y, Yamamoto K, Tsujihara N, Osawa T. Protective effects of lemon flavonoids on oxidative stress in diabetic rats. *Lipids* 1998;33:689-95.
 - 29) Herrera MD, Marhuenda E. Effect of naringin and naringenin on contractions induced by noradrenaline in rat vas deferens: evidence for postsynaptic α -2 adrenergic receptor. *Gen Pharmacol* 1993;24:739-42.
 - 30) Choi SJ, Kim HS, Jeong TS, Bok SH, Park YB. Antiatherogenic effect of naringin independent of lipid-lowering action in hypercholesterolemic rabbits. *Korean Circ J* 1998;28:1873-81.