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혈관평활근세포에서 산화에너지대사 억제에 의한 아밀로이드전구단백질 대사의 변화

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Inhibition of Oxidative Energy Metabolism Alters Amyloid Precursor Protein Metabolism in Cultured Vascular Smooth Muscle Cells

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Background : A reduction in the activity of cytochrome c oxidase (COX) has been recently identified in mitochondria from platelets and postmortem brain tissue of AD patients. Sodium azide (NaN_3), a COX inhibitor, is an effective chemical agent producing energy shortage and oxidative stress both *in vitro* and *in vivo* system. Furthermore, it has been suggested that vascular compromise could be either directly involved in AD pathogenesis or indirectly associated with triggering pathogenetic events leading to AD. This study was performed to investigate amyloid precursor protein (APP) metabolism by inhibition of mitochondrial energy metabolism in cultured vascular smooth muslce cells (VSMCs). Materials and Methods : VSMCs isolated from the aorta of seven weeks old Sprague-Dawley rat were treated with NaN_3 in a low concentration (100-500 μM) or in a high concentration (1-100 mM). Cellular proliferation and viability were determined by MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) assay. Cellular APP was detected with N-terminal specific antibody 22C11. Cell death was determined by observation of morphology and terminal deoxynucleotidyl transferase-mediated dUTP-fluorescein nick end labeling stain (TUNEL). We used ginkgo biloba extract (EGb761) and melatonin as anti-oxidants to investigate the mechanism of latered APP metabolism. Results : The viability of VSMCs was increased after treatment with 1 mM and 10 mM NaN_3 ($p<0.05$) until 3 hr and then diminished . Many TUNEL positive cells were found in 10 mM and 100 mM treatment group, but were not apoptotic in nature. 22C11 immunoreactivity was not changed at 3 hr, 6 hr, 12 hr. Anti-oxidants reduced cellular proliferation ($p<0.05$), but did not block TUNEL positivities and did not influence the 22C11 immunoreactivity. In a low concentraion NaN_3 treatment group, the viability of VSMCs was increased concentration dependently ($p<0.05$). Immunoblot with 22C11 showed the concentration dependent decrease at 145 kDa, 125 kDa, and high molecular weight range (>160 kDa). TUNEL staining showed DNA fragmentations and condensations of nuclear chromatin suggesting apoptosis. After treatment with anti-oxidants, the cellular proliferation was more decreased ($p<0.05$), and TUNEL positive cell deaths were blocked. Immunoreactivities of 125 kDa (immature APP), 145 kDa (mature APP), and higher molecular weight bands were recovered below 400 μM of NaN_3. Immunoreactivity of 145 kDa was recovered in only 100 μM NaN_3 treated group. Conclusions : The presumed mechanism of low concentration COX inhibitor is the overproduction of reactive oxygen species resulting from a depression of the mitochondiral electron transport chain, whereas potential consequence of high concentration COX inhibitor might be related to depression of ATP synthesis and bioenergetic impairment. Reactive oxygen radicals in response to low concentration COX inhibitor alters the processing of APP in VSMCs. This investigation demonstrated an altered APP metabolism as a peripheral marker of AD. Therefore VSMCs treated with low concentration COX inhibitor could be considered as a novel *in vitro* model of AD.

Key Words: *Vascular smooth muscle cells, Sodium azide, Reactive oxygen species, Amyloid precursor protein, Alzheimer's disease*

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서 론

(ÃŽ¥)Bm{ifjnfsjtfbtf-!BE*| ww© xÔÃœ ,5§ -!•v,£‰ ¼Ã-!ÔWŽÿ †‰ •ÿ½ åß} •Ï ~z¹-!MÈ €Ä½zâœ MÈ†- œO à¥ •m)tfojmf qmbrvMÈ!†- ×‰ MÈü€q Ü¸)ofvspgjcsjmmbszwub-o× BQQ!Åó‰ ý_Ñ †-ó‰ ,¾} Ïœ hmf*-! w€Ï`âŽ}ëÞ u)bn zmpje!bohjpqbuiz*! Ã¾‰! Å ,8‰ BE!°X¥w Vâ óï_4 ÄÒ MÈ† åß½• œÁ} Æ0Ù³ \2^/!å©-•!mÜ ëÞ u‰ Vè - ÅM¥ wŽv BE‰ \\–}€ ,£Žÿ °X¥wœ X„ „ • ï„‰ ñ0 `âŽ}) bnzmpje!-!B *!¶yz KM`½Ž >œ ëÞ(by†- )wbtdvmbs!tnppui!nvtdmf | BE!o ,¾Ü —ð `"Ã¶ Þ×_4 ˜4-!ÒyŽv dfmmt-!WTNŽDÃ¸*¥! BE!o ,¾} }¿ÌVœ ƒ§ _èÅ Ä}â •$_4 Ø ³)bnzmpje!izqpuiftjt*/!ÿØÃÆ B ƒ „4¶4 ,¾zâ MÈN„Ü ëÞ u} qoÃ¹ 4 ¶ [—‰ • ÿuÿÜ °Þ_œv¥ ÅÃ-œ w `OÅ ÃäŽ ÆÄv Ï ˜³\3^/! Å BE| MÈÊŠ} aŒÃœ za‘ ßŠŽ ©I ¾Mzazâw-!MÈÊ6wŽà‰ ‹| •Æ6w¥w `âŽ}¾X¶yz)bnzmpje!qsfdvstps!qspufjo-!BQQ!Ü¥ óïÅ ëÞ(by†-œ-!8V` Tqsbhvf.Ebxmfz ‰ ý_ƒ o Å³/!ŽÃÅ BQQÑ B ‰ ý_•| BE‰ • Sbu‰ ÅW˜¥w €Å WTNDtxŽïÃ¸³/!óï` x- Æv™âw •$_Ï ˜Ï-!BE¥w ŽÃÅ •Æ6w‰ ý_œ | ENFN!x- ¥-!61!nM‰ ð: ë·)gfubm!cpwjof _ÛMÈÊ‰ ý_Æ œ¸‰ xÔÜ °Þ„} •Ï ˜•Ž tfsvn-!GCT*-!6!nM‰ Ë &)qfojdjmmjoÜ tusfqupn u¿_Ï ˜³ \4^/! QT*-!3!nM‰ 2!N!IFQFT-!6!nM‰ 311!nN!hmvubnjof} Òy¥ BEaœ¥w \\–}€ ¥ævÅó‰ Ã-Ñ ö_ y@³/!†- x-| 6&!DP ₃-!:6&!Ú,ƒ Ú~_œ 48‰ x ½ œx)pyjebujwf!tusftt*¥ÿ‰ÌBQQ‰ ¶y Ì -,x óïÃ¸³/! DPY!b„ 5&x iÃ- ObO ₄} óïÃ Ü$Ž ýð_4 ‹| B ´,w½} -ÈÅ ? ƒï„ `â ¸³/!OÜKœ 211! N¥w 211!nN!Žv‰ Mâ 45 u Ž}¾X¶yz)j otpmvcmf!BQQ* †‰ Åó@)nfubcpmjuÀ¥!£xÃ- m-Ã¸³/!Ëö_&âw FHc872Ü nfmbupojo _…¸)joufsnfejbuf*•Ž œ ¯Ž pè3³/!ŽÃÅ \ } óïÃ¸³/!FH c872} 1/6!nh0nM‰ MâÃ¸Ï \–}€ ¥ævÅó Žÿ‰ _Ä½• ý_ƒ BE‰ o Nfmbupojo| 211!mNÃâ³/! BQQ!Åó‰ ý_x Þ9 ¥ ˜4w , ½• o ,¾‘ ¯ŽIœ ƒ}¥ ÅÅ u§•Ž Ã, iÌw ‘5Ë¸âw Bouj!Bm{ifjnfs!Q sfdvstps!Qspufjo o™_Ï ˜œ=-!Å™½• ¯Ž dzupdispnf!d!pyjebujwf!Ebt!f)33D22ïÃ¸Ï Ž5Ë¸âœ bouj .npvtf!JhH-)DPY*‰ŽÿŽ³/!BEaœ‰ ëœý¥w Q bslfs!†Ž 2::1 ipstfsbejti!qfspyjdÃ¸³/!†-óx Þ9Ã, iÅ þ ¯•zâ DPY!bW„ ÃŸ} oÁÃ¸Ï-! 2::3þ Ljti!† UVOFM!´ | Jo!Tjuv!Dfmm!Efbui!Efufdujpo!Lju-!QP Ž BEaœ 6w¥w DPY!bW„ ÃŸ} oÁÃ¸³\5- óïÃ¸³/! 6^/!BaÃœ•‰ 6w _-!å© ¾fµ-!f$µ w€Ï Ì^ i¥w \\–}€ ¾œ¾¸Ê‰ ,£Ž ¾¸½zâ ¼Ã_ 4 ˜Ï-!‹| ¾œ¾¸Ê‰ qœ _ å© DPY!bW„Ž 61& Žÿ 'ÛÃ¶ Šœ_@•Ž pè3³\5^/!Žv -Ã °Xœ• ¥ ‰Ì DPY!bW„ ÃŸÜ BE!o ,¾Ü‰ ÞÊx t¿Ã , iÅ °Xƒ KÔ_4 Ø³\7.9^/!4!nN!Žÿ‰ Ï M Obq è| K…)3K…*!m-Ãœ v•zâ DPY‰ ,£} 5¶Å °Xƒ ˜@zÆ-!Ž v•zâœ †-ƒ ó“ ÿ:¥w †-×‰ ~¿Å ý_x -V¶ _Wâ-!§,…¥ «] †- Ÿÿzâ o Ãœ BE‰ •¸ •ä¥ ½ïÆ À ˜œ Â@ } Š• À >@³/

°X¥wœ \\–}€ ‰ qœ _ ö_¥ævÅó)pyjebujwf!fofshz!nfubcpmjtn!ÈÅ qœŽ» w-!BEaœ¥w ,£ ŽÿŽ pè. ˜œ DPY‰ ,£ 5&& • tpejvn!b{jef!)Ø!kO-Å Mâ óïÃ- BE‰ • ¸•ä¥ ƒ§ y"Å \\–}€ ,£Žÿ Â@} Š•Ï- ö_ ¥ævÅó Žÿ †¥ ‰Å †- × BQQ!Åó‰ ý_- †-ó-!†-uL $Mx Þ9Ã¸³\:-!21^/!w€Ï Ëö_&x m-Å v Æ0Æœ ObO ₄ qÜ‰ 5¶ œ ý_x Þ9Èzâ wub-o× BQQ!Åó‰ ý_Ñ †-ó‰ ,¾} Ïœ - ÅM¥ wŽv BE‰ \\–}€ ,£Žÿ °X¥wœ KM`½Ž >œ ëÞ(by†-)wbtdvmbs!tnppui!nvtdmf dfmmt-!WTNŽDÃ¸*¥!

방 법

1. 실험재료

2. 실험방법

1) 세포의 생존력(viability) 및 증식(proliferation) 관찰

†-‰ 8ÖÜ uL x} iÃ- :7!x fmm!qmbuf¥ †-À x 6 •⁵2d1mmt0nMâ 3:-ÃÏ 35K…W 1/3&!GCT ENFN!x- ¥w tubswbujpoÃ¸³/!Žv ³-Å M‰ ObQ m- Mþâ „„ 9•‰ xfmm•¥ ¶N œ Ë ö_&x ¾¯[v m-Ã¸Ï K… ý_¥ “I NUT!v•z â †-uL $Mx ô$Ã¸³/!†-uL)dfmm!qspmjgfsbujpo* œ _Ä½^Š„)difnptfotjujwjuz!*!Ø ˜œ †- ‰ Çœx Ã$ÌVœ ? x•)dpmpsjnfusjd!nfuipe*• NUTv•|-!? ƒï„• NUTƒ Åóƒ boÅ †-‰ efizesphfobtf¥ ‰Ì„‰ gpsnb{boŽIœ @zâ ¾

a_Ï-!¾a` gpsnb{bo| 5:1!on‰DM¥w ô$_¹-
ô$` ŸDMœ †- x- •¥ ø ˜œ †-‰ ÇœÑ w
"½zâ ?ÞÃ- ™K`³/! 211! N¥w 611! NŽv ¼
M m-Kœ ObO₄!m- v 8'...} x-Ã»w —' †-uL
$Mx ô$Ìw K...ÈÜ¥ "w ý_x Þ9Ã¸³/!2!nN
Žÿ Ï Mâ ¯€Å Èð¥œ ObO ₄!m- v 35K...W Þ9
Ã»w †- 8ÖÜ uL} Þ9Ã¸³/!iÑ'| v•zâ
x-Ã»w K...ÈÜ¥ "w †-uL ý_x Þ9Å v-!„„
‰ O₄!MZ¥w †-uLŽ ŠœÃ, K•Ãœ K,x Þ9
Ã- BQQ‰ ý_Ñ †-ó‰ ý_x Þ9Ãœ ,X K!zâ
$Ã¸³/

2) 세포내 APP의 변화

WTNDtƒ 71.81&!$M_@} ý 35K...W 1/3&
GCT!ENFN¥w tubswbujpoK*v 211! N¥w 611!
N‰ ¼ M ObO₄}-!2!nN!Žÿ Ï M ObO₄} ¶ N œ
FHc872¾¯[Å v i‰ †-uL Þ9 OÜ¥ "w $Ì
x ,...W x-Ã¸³/!2!nN!Žÿ Ï M m-Z¥wœ
1/3&!GCT!ENFN¥w qvÃ»w x-Ã¸³/!$Ìx
,... x-v $ÂÅ ¶yz 61! h¥ ÌÁÃœ Kõx çÃ--
21&!TET!hfm¾¥W} OKÃ¸³/! 33D22!Ë¸x '5
Ë¸âw ŽïÃ- pwfsojhiu!m,} K* v-!Ž5Ë¸ bouj.
npvtf!JhH-!ipstfsbejti!qfsóÃ¸-2!K.f} W O
Æ¥w m,} K'³/!³K 51 ... UCTUâ †°Å v FDM
m,} K* v ý_x Þ9Ã¸³/

3) 세포사 관찰

Å6Z} ,Xzâ Ï •ÃÏ xÃ¶ ‡ zâ ´ _œ †-
x †-‰ ð:¥ ÞÊ>Ž UVOFM!´ -„zâ ý¶Ã¸
³/!†-ƒ •Ã¶ ´ _»w Í Ü)qzloptjt*!œÁ} Ž§
Æ-!ÍŽ •| -M¥Ž•¯Ç -Ã •â ¿)gsbhnfoubujpo*b„öœ)sfbdujwf!pyzhfo!tqfdjft*Ñ '| ö_½ œx¥ ‰
_4 Žœ ¯| †-Ïó)bqpquptjKÃœ œÁzâ $ Ìw WTNDt‰ „§Ü uL- x Þ9Ã, iÌ I ₃P₃ x m -
‰Ã¸³\22^/ •ÃÏ xÃ¶ ´ ` ÍŽ-!$ÿ Í ³ !¶ Æ Ã¸³/!I ₃P₃x 41! N-!61! N-!211! N-!311! N-!411! N-
0ÆÏ Í Â-Ž û„ð)usjbohvmbs!tibqf*-!0üð-! œ ö 511! N‰ Mâ w"m-ÃÏ †-uL $Mx Þ9Ã¸³/
ÿ)ejtqfstfe!tibqf*Ž§Æ-{$ÿ$Mx Žœ ¯Ü y- 411! NMŽv Å6Z¥ ?Ìw †-uL $Mƒ uƒÃ¸
w "ÿð)sfujdvmbs!tibqf*‰ ð:â ' ÿ-Kó)ofdsp. ³/!41! N¥w 211! NŽvœ M¥?ÞÌw uƒÃ¸Ï-
tjt*‰œÁzâ $‰Ã¸³\ 23.25^/ 211! N}$!zâ †-uL $M‰ uƒ.Ž ŠœÃœ -
†-Í UVOFM!´ Ü Þ9} iÌw †-x 35!xfmm!qmbuf x ¸³/!Ëö_&x m-Ã¸} ý-!I ₃P₃ m- v¥ Æ0Æ
¥ †-Àx 3•21 ⁵ dfmmt0nMâ Ãû $-MÃÏ-!35K...W œ †-uL‰ uƒ.Ž ŠœÃœ ð:x ¸z¹-!ŽÃÅ œ
1/3&!GCT!ENFN¥w tubswbujpo} KÔÅ₄} v ¶ ObO Á| ObO₄} m-Ó} ýÑ '| -ÿ‰ †-uL‰ ý_x
N œ FHc872¾¯[Å v m-Ã¸³/!w€Ï m-Ãœ ¸³/
ObO₄‰ M¥ "Iw i‰ †-uL Þ9 OÜ¥ "w $Ìx
,...W x-v „„ ´ } KÔÃ¸³/!´ K }•Çx,z 2) 고농도 NaN₃ 투여 후 효과
)nfubm!fobiodfe!tvctusbuf*âw EBCƒ!fƒÃçtusbufxObO₄} 2!nN-!21!nN-!211!nÏNMâ m-Å v K
³/!†- Þ9} iÌw iÿ5ê\È• Pmznqvt!JW61.22}..pâ †-uL $Mx Þ9Ã¸³/!4K... Þ¥w 2!nNÜ
óïÃ¸Ï-!Ž ê\È¥ KWD!UL2491V!)879e•5x5*! 21!nN!m-Z¥w †-uLŽ Å6ZÜ ?SÃ- „„ 34&-
°ÃÃÏ Jnbhf.Qsp!Qmvt!4W1µâw¹} ŽïÃ- †-ó
x Þ9ÃÏ Ð¹Ã¸³/! EOB! ¿_)gsbhnfoubujpo*x -ó
Ã, iÃ- ÏL½• Tpvuifso!cmKÔÅ₄³/

5) 자료 통계분석

ObO₄m-ZÜ Å6Z ?SÑ FHc872‰¾v qÜx
?SÃ, iÌw BOPWB-!Nboo.Xijuofz!V-!Xjmdpypo
tjhofe!sbol!-$KÔÃ¸³/

결 과

1. 세포 생존력(viability)과 증식(proliferation) 분석

1) 저농도 NaN_3 투여 후 변화

ObO₄} 211! N-!311! N-!411! N-!511! N-!611! N‰
¼ Mâ m-Å v K...pâ †-uL $Mx NUT!bttbzâ
ô$Ã¸³/!3'î E Å6ZÜ ?SÃ- Â• M¥w †-
uLŽ uƒÃ, K•Ã¸Ï-!4'î‰ †-uL $Mœ 611! N
)ƒ1/16*!m-Z} &àÅ 211! N-!311! N-!411! N-!511
N!m-Z¥w Å6Z¥ ?Ì „„ 23&-!58&-!54&-!52&
uƒÃ¸³) ƒ1/16*/!6'îÑ'î¥œ 411! N! M Žÿ¥
w Å6Z¥ ?Ìw †-uL $Mƒ ŠœÃœ -ÿ} Ž,
K•Ó³/!8'îœ 311! N! M Žÿ Â• MZ¥w Å6Z
¥ ?Ìw †-uL $Mƒ ŠœÃœ -ÿ} ¸³/
Ëö_&• FHc872} 1/6!nh0n¾¯[ÃÏ 4'î¥
Þ9Ó} ý-!Å6Z³ †-uLŽ 51.61&&_œ -ÿ}
¸³) ƒ1/12*/!Nfmbu¾¯[Å}Èð¥œ 411! N!Ž
ÿ‰ m-Z¥wœ FHc872!ZÜ '| $M‰ †-uL 5
&qÜx ¸³) ƒ1/16*/!
Ìw WTNDt‰ „§Ü uL- x Þ9Ã, iÌ I ₃P₃ x m -
Ã¸³/!I ₃P₃x 41! N-!61! N-!211! N-!311! N-!411! N-
511! N‰ Mâ w"m-ÃÏ †-uL $Mx Þ9Ã¸³/
411! NMŽv Å6Z¥ ?Ìw †-uL $Mƒ uƒÃ¸
³/!41! N¥w 211! NŽvœ M¥?ÞÌw uƒÃ¸Ï-
211! N}$!zâ †-uL $M‰ uƒ.Ž ŠœÃœ -
x ¸³/!Ëö_&x m-Ã¸} ý-!I ₃P₃ m- v¥ Æ0Æ
œ †-uL‰ uƒ.Ž ŠœÃœ ð:x ¸z¹-!ŽÃÅ œ
Á| ObO₄} m-Ó} ýÑ '| -ÿ‰ †-uL‰ ý_x
¸³/

2) 고농도 NaN_3 투여 후 효과

ObO₄} 2!nN-!21!nN-!211!nÏNMâ m-Å v K
Mâ m-Å v K...pâ †-uL $Mx Þ9Ã¸³/!4K... Þ¥w 2!nNÜ
21!nN!m-Z¥w †-uLŽ Å6ZÜ ?SÃ- „„ 34&-

4:& uƒÃ¸Ï) ¢‹1/16*-!211!m-ÅZ¥wœ †-uL ‰ Šœƒ Þ9_@³/!23K… Žv E 35K… ÈÜ óŽ¥ † -uL $Mœ Â• M¥w Å6Z¥ ?Ã- †-uL Šœƒ Þ9_@³/!

Ëö_& FHc872Ü nfmbuƒ¾o]Ã}} Èð-!4K… ÈÜK!¥w 2!nN-!21!nN¥w †-uL uƒ $Mƒ ObO 4 x ¶N m-Ó} ý ³ 5&_@³/!wÃÆ 211!nN¥w † -uL $Mœ Ä©Õ Å6ZŽÆ ³w MZ• ³ 31&Žÿ uƒÃ¸³) ¢‹1/16*/

2. 세포내 APP의 변화

1) 저농도 NaN_3 투여 후 효과

¼ Mâ m-Å v 4'î Ë¸ 33D22‰®m„} Þ9 Ã¸³/!271!IEb!Žÿ Ï œÂ ¶yz•‰ »®m„ŠŽ Þ 9_@Ï-! Mƒ }Àã m?ÞÌw !5½zâ ŠœÃ¸³/ Å6Z¥w »®m„Ž 'ÛÅ cboeâ ŽÒ 236!IEbÜ 256!IEb¶yz‰ »®m„| Â• M¥w œO_@³/!

FHc872} m-Å v 211! N¥w 411! NŽv‰¥w 236!IEb‰ ¶yz cboe-!271!IEbÜ 361!IEbÂ Žÿ ¶yz•‰ »®m„ •M•Ž Å6ZÜ 5Žƒ >} $Mâ j_@³/!wÌvŠ j‰ $M¥w 5Žx ¸œ=-!236 IEbÜ 256!IEb‰ ¶yz cboe‰®m„ •Mœ 211! N ‰ M¥wœ Å6ZÜ '| $Mâ j_@Ï-! 311! NÜ 411 N¥wœ 236!IEbŽ &Ã¶@z¹-! 256!IEb| 311 NŽÿ¥wœ »®m„‰ jŽ 'ÛÃv ³)Gjh/!2*/

2) 고농도 NaN_3 투여 후 효과

ObO 2!nN-!21!nN‰ Ï Mâ m-Å v 4-!7-!23K… ÈÜK Ë¸ 33D22‰®m„} Þ9Ã¸³/!K…þâ »® m„ •M‰ 5Žœ >@³/!2!nN-!21!nN-!211!nN} m -ÃÏ 4K… v Â• M¥w Å6ZÜ 5Žƒ >œ »®m, „ •Mx Æ0ÞÏ-!Ëö_&• FHc872} 'Ž m-Å v¥M m-¾Ü 5Žƒ >@³)Gjh/!3*/

3. 세포사 관찰

1) 저농도 NaN_3 투여 후 효과

ObO m- v 4'î¥ „ m- MZ} 311•xt¥w Þ 9Ó} ý (u 4&-!4&-!5&-!7&-!21&‰ UVOFM!-„ † -•} ¸³/!611 N!m-Z} &àÅ ³w Z¥wœ UVOFM!-„†-•| Í ÜÜ Í ¿ †‰ †-Ïóx K óÃœ œÁ} Þ9Æ À ˜@Ï-!†-Kóx KóÃœ œÁ| Þ9_v ³/! 611! Nm-Z‰ 21&!UVOFM!-„ †- • _¥w Í ¿ †‰ †-Ïóƒ ‰Q_œ Èðƒ ¿m$M¸

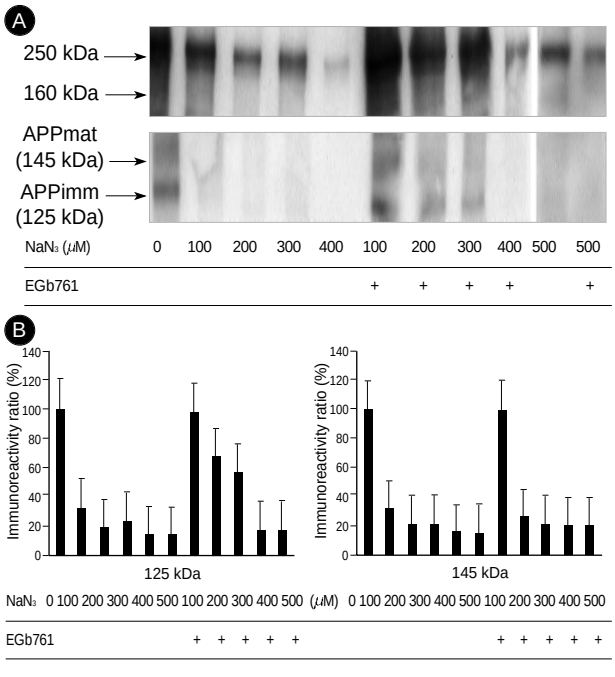

Fig. 1. The alteration of cellular APP metabolism in cultures of vascular smooth muslce cells treated with different low concentration NaN_3 for 3 days. (A) Immunoblot analysis of altered celluar APP metabolism in presence of increasing NaN_3 concentrations. Immunoprecipitations were performed with N-terminal APP specific antibody 22C11. After treatment with NaN_3, immunoreactivities of high molecular weight were decreased in the presence of increasing NaN_3 concentration. Immunoreactivities of 145 kDa and 125 kDa N-terminal derivatives were disappeared. After treatment of NaN_3 and EGb761, immunoreactivity of 145 kDa N-terminal was not detected and 125 kDa N-terminal derivative was precipitated in presence of 100 μM to 300 μM NaN_3. (B) Results of densitometrical quantitative analysis are mean ~S.D. Celluar APP was expressed as % of control.

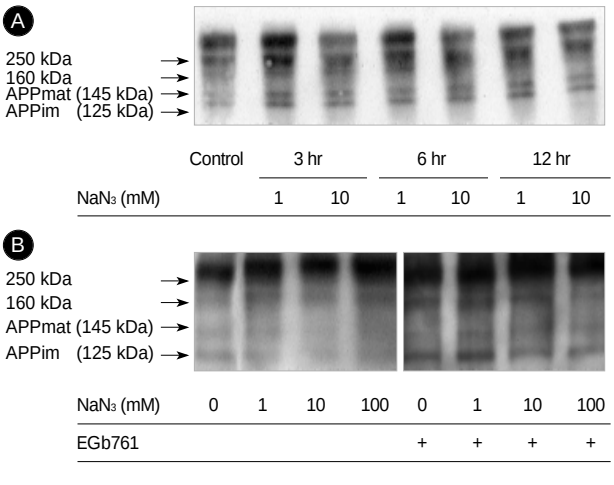

Fig. 2. The alteration of cellular APP metabolism in cultures of vascular smooth muslce cells treated with different high concentration NaN_3 for 3 hr. 22C11 immunoreactivity was not changed in the presence of 1 mM and 10 mM NaN_3 at 3 hr, 6 hr, 12 hr (A) and in the presence of increasing NaN_3 concentrations pre- and post-treatment with EGB761 (B).

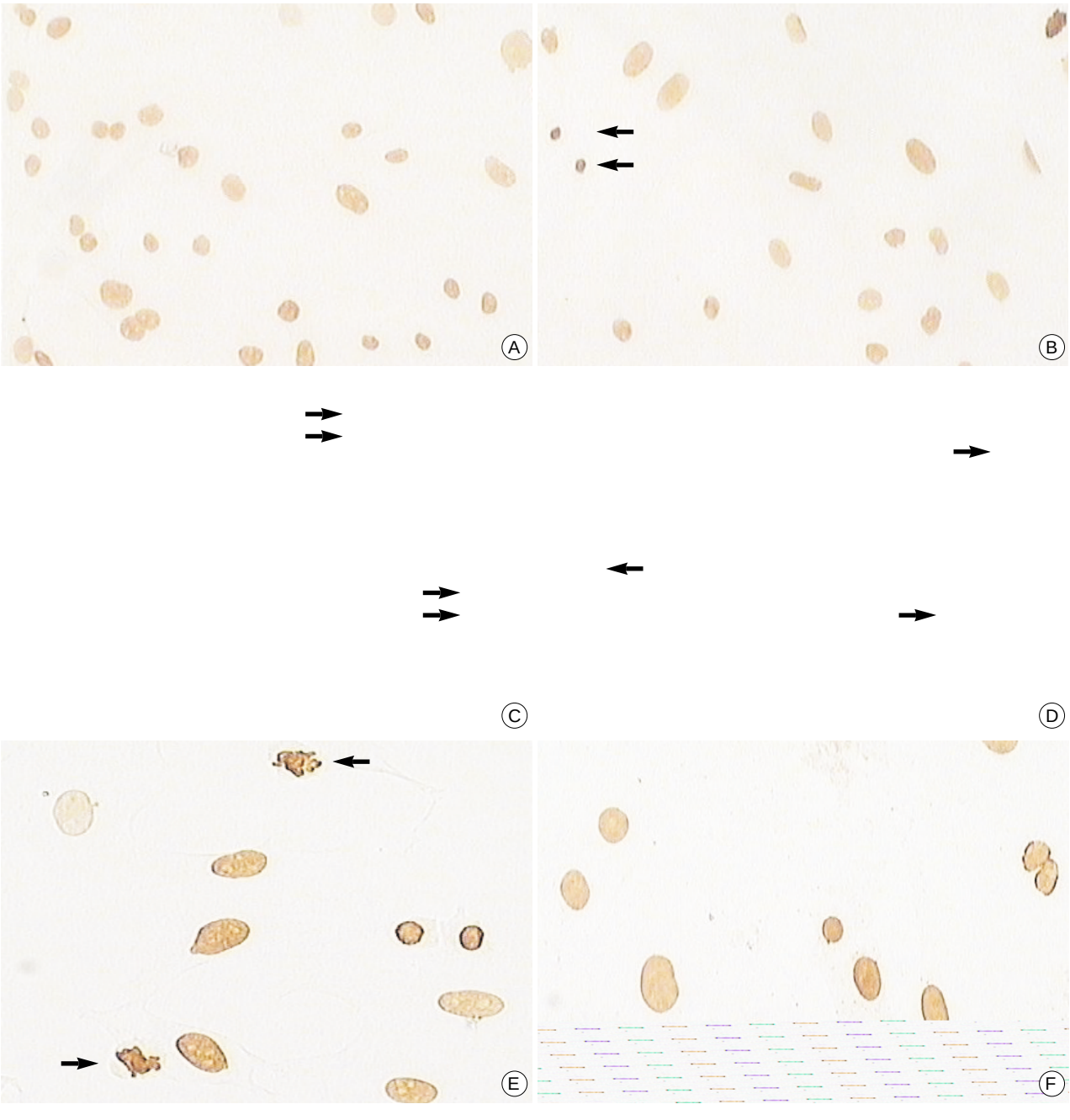

Fig. 3. TUNEL staining of vascular smooth muscle cells after treatment with low concentration NaN_3 and EGb761 (0.5 mg/mL). Control (A), 100 μM (B), 200 μM (C), 200 μM (D), 400 μM (E), 400 μM+EGb761 (F). TUNEL-positivite cells were disappeared after treatment with EGb761. Single arrow: nuclear fragmentation. Double arrows: nuclear condensation. Magnification: A and B; 100 • . C, D, E, F; 200 • .

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2) 고농도 NaN_3 투여 후 효과

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Fig. 4. TUNEL staining of vascular smooth muscle cells after treatment with high concentration NaN_3 and EGb761. 10 mM (A), 100 mM (B), A+EGb761 (C), B+EGb761 (D). Necrotic cells showed a large, dispersed, dark brown TUNEL positivity. TUNEL positive cells can be seen after treatment with EGb761. Arrow: large, dark brown nucleus. Magnification: • 100.

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4. DNA 분절화(fragmentation)

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| EOBÿ:x ¸³/

고 찰

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