【S-7】

Cerebrovascular Toxicity of Inhalable Particulate Matter

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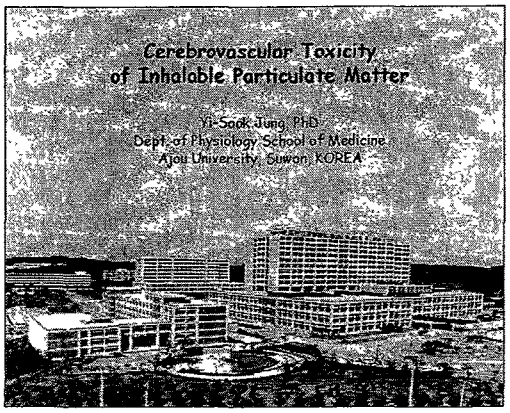
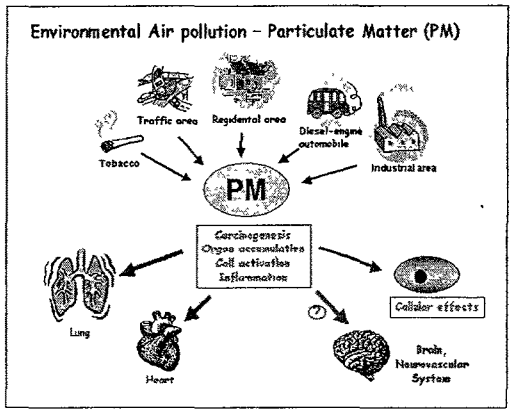
Among adhesion molecules known as inflammatory biomarkers, intercellular adhesion molecule-1 (ICAM-1), expressed on the surface of endothelial cells, and integrins (CD11b/CD18, CD11a/CD18), expressed on the surface of leukocytes, have been extensively studied in various animal models. During inflammatory neurological diseases such as multiple sclerosis, circulating leukocytes transmigrate into the nervous system via leukocyte-endothelial cell interactions triggered by binding of ICAM-1 to integrins, subsequently resulting in brain damage. Epidemiological data show that particulate matter (PM) present in ambient air pollution may underlie increased morbidity and mortality rates related to pulmonary and cardiovascular systems. Although a recent study has suggested that inhaled PM may trigger a proinflammatory response in nervous tissue, few studies have addressed the possibility of PM effects on cerebrovascular system. In our study, we investigated whether PM2.5 and Cd, one of the PM components, can increase inflammatory biomarkers ICAM-1 and CD11b in brain endothelium-derived bEnd.3 cells, and monocyte U937 cells, respectively. We also investigated the mechanisms for the PM2.5- and Cd-induced ICAM-1 expression in bEnd.3 cells.

The treatment with traffic and residential PM2.5 induced ICAM-1 expression in bEnd.3 cells possibly through p38 and ERK signaling pathways, and induced CD11b in U937 cells in a concentration-dependent manner. The treatment with 30 μM Cd, one of the PM components, induced apoptotic bEnd.3 cell death and p38 MAPK activation, and the cell death induced by Cd was blocked by p38 MAPK inhibitor SB202190. The treatment with 3 μM of Cd increased the expression of ICAM-1 at the level of protein and mRNA, and these increases were almost completely inhibited by a specific NF-κB inhibitor SN50. The treatment with Cd induced the translocation of NF-κB from cytosolic to membrane fraction and increased DNA binding activity of NF-κB and tyrosine phosphorylation of IκBα, while Cd did not alter the degradation of IκBα. In summary, this study suggests that traffic and residential PM2.5 may cause cerebrovascular inflammation through p38 and ERK pathways.

Cd has dual effects in bEnd.3 cells according to concentrations; 1) low concentration (3 μM) of Cd stimulates ICAM-1 expression, via NF-κB activation mediated by tyrosine phosphorylation of IκBα, 2) high concentration (30 μM) of Cd induces apoptotic cell death via p38 MAPK activation.





Particulate matter (PM)

- PM is characterised by particle size (aerodynamic diameter)
  - Coarse particles - PM10 : 10μm > particles > 2.5 μm
  - Fine particles - PM2.5 : 2.5 μm > particles
  - Ultrafine particle - 0.1 μm > particles
- particulate mass
- composition
  - polyaromatic hydrocarbons
  - heavy metals
- the presence of biogenic components
  - endotoxins, pollen, bacteria, viruses
- other factors

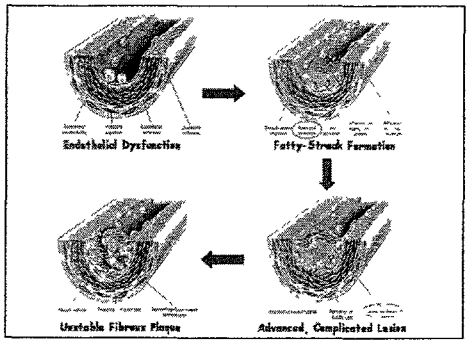
Particulate matter (PM)

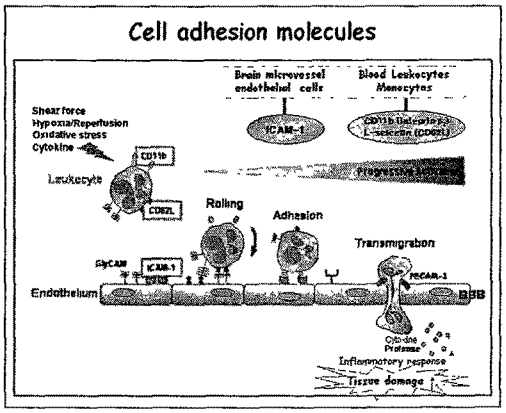
- PM 2.5 induced significant increases (in a concentration- and time-dependent manner) in protein secretion and/or gene expression of inflammatory cytokines, such as TNF-α, IL-1b, GM-CSF in human epithelial lung cells (Dagher Z *et al*, 2005)
- PM increased mortality and morbidity rates related to pulmonary and cardiovascular systems (Pope CA *et al* 2000)
- PM2.5- and PM10-induced oxidative stress in rat lung epithelial cells (Choi JH *et al* 2004)
- Exposed PM2.5 are associated with decreased heart rate variability (HRV) (Park SK *et al* 2005)
- PM exposure leads to local pulmonary vascular inflammation/microvascular thrombosis and systemic endothelial changes resulting in alterd myocardial contractility (Frampton MW *et al* 2001)

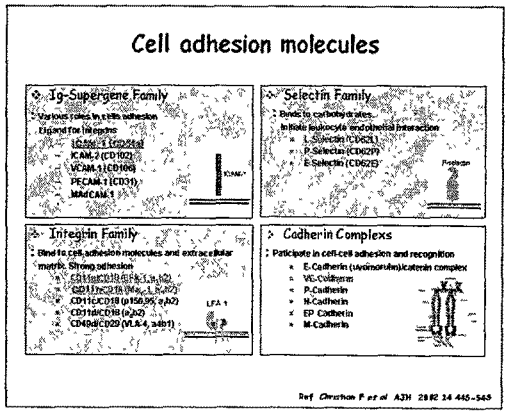
Particulate matter (PM)

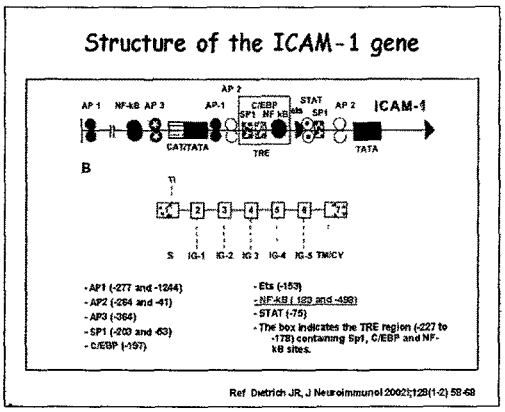
- PM increase biomarkers of inflammation in mouse brain (Campbell A *et al*, 2005)
- Air pollutants are significantly associated with ischemia stroke mortality, which suggests an acute pathology process in the cerebrovascular system induced byair pollution (Hong YC *et al* 2002)
- Association between exposure to air pollutant and hospital admissions for stroke (Tsai SS *et al* 2003)
- Alzheimer's disease may be the sequela of air pollutant exposures and the resulting systemic inflammation (Calderon-Garcidueñas L *et al* 2003)
- A portal of entry into the CNS for solid UTP (< PM0.1μM), circumventing the tight blood-brain berrier (Oberdorster G *et al* 2004)

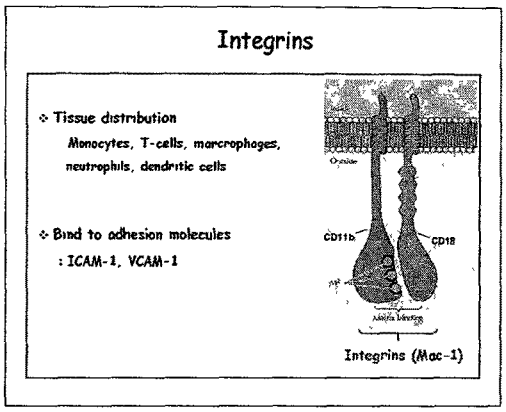
Adhesion molecules & Atherosclerosis

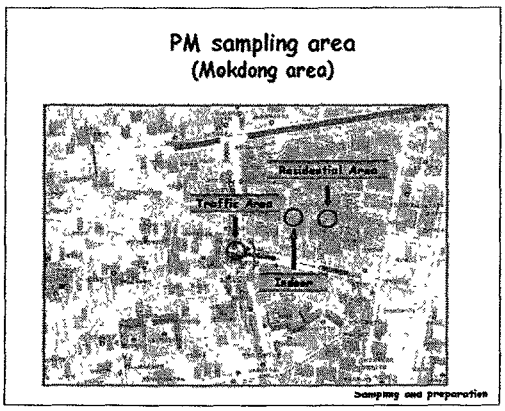


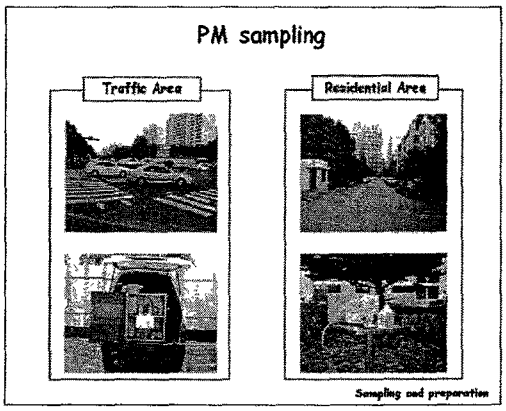

Cell adhesion molecules
Brain microvessel endothelial cells
Blood Leukocytes Monocytes
Shear force
Hypoxia/Reperfusion
Oxidative stress
Cytokine
ICAM-1
CD11b
CD62L
Leukocyte
Rolling
Adhesion
Transmigration
PECAM-1
Endothelium
Cytokine
Protease
Inflammatory response
Tissue damage

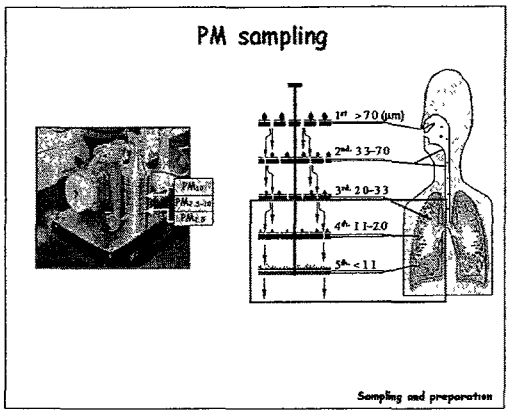

Cell adhesion molecules
Ig-Supergene Family
Various roles in cell adhesion
Ligand for integrins
ICAM-1 (CD54)
ICAM-2 (CD102)
VCAM-1 (CD106)
PECAM-1 (CD31)
MADCAM-1
Selectin Family
Bind to carbohydrates
Initial leukocyte endothelial interaction
L-Selectin (CD62L)
P-Selectin (CD62P)
E-Selectin (CD62E)
Integrin Family
Bind to cell adhesion molecules and extracellular matrix. Strong adhesion
CD11a/CD18 (LFA-1, Ly-62)
CD11b/CD18 (Mac-1, Ly-40)
CD11c/CD18 (p150,95, gp140)
CD49d/CD18 (VLA-4)
CD49d/CD29 (VLA-4, 3a01)
LFA-1
Cadherin Complexes
Participate in cell-cell adhesion and recognition
E-Cadherin (uvomorulin)/catenin complex
VE-Cadherin
P-Cadherin
N-Cadherin
EP Cadherin
M-Cadherin
Ref Christian P et al AJH 2002 14 445-545

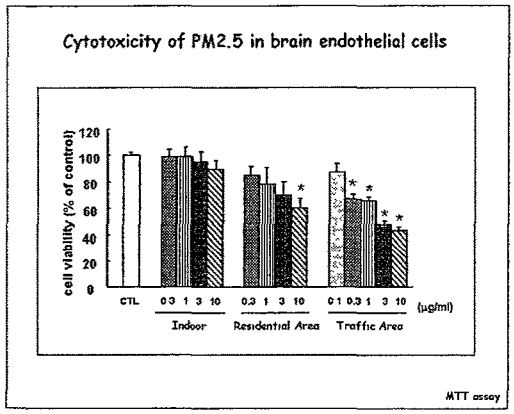

Structure of the ICAM-1 gene
AP 2
AP 1 NF-kB AP 3 AP-1 C/EBP SP1 NF kB STAT kb SP1 AP 2 ICAM-1
CAT/TATA
TRE
TATA
B
S IG-1 IG-2 IG 3 IG-4 IG-5 TM/CY
- AP1 (-277 and -1244)
- AP2 (-264 and -41)
- AP3 (-364)
- SP1 (-203 and -53)
- C/EBP (-197)
- Ets (-153)
- NF-kB (189 and -498)
- STAT (-75)
- The box indicates the TRE region (-227 to -178) containing Sp1, C/EBP and NF-kB sites.
Ref Dietrich JR, J Neuroimmunol 2002;129(1-2) 58-68

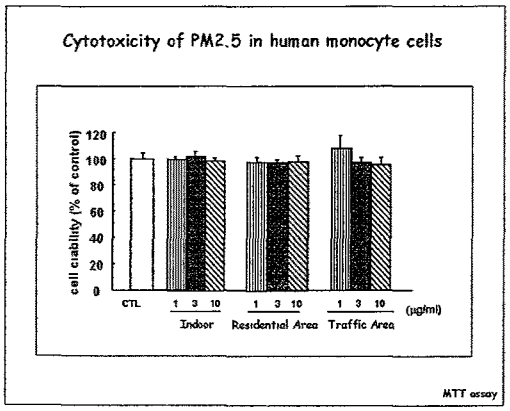

Integrins
Tissue distribution
Monocytes, T-cells, marcrophoges, neutrophils, dendritic cells
Bind to adhesion molecules
: ICAM-1, VCAM-1
CD11b
CD18
Integrins (Mac-1)

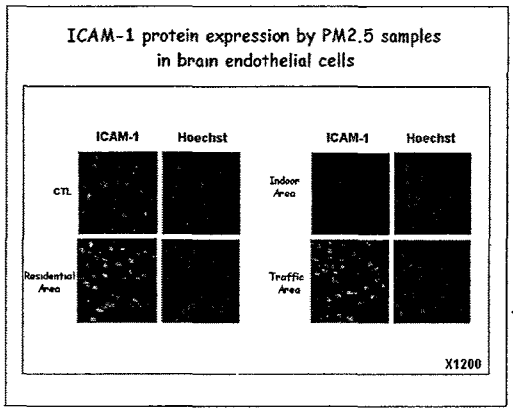

PM sampling area
(Mokdong area)
Residential Area
Traffic Area
Sampling and preparation

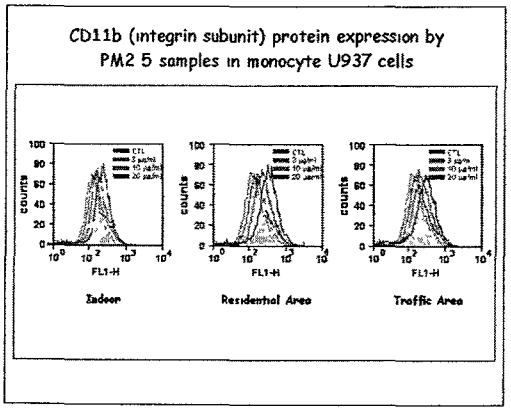

PM sampling
Traffic Area
Residential Area
Sampling and preparation

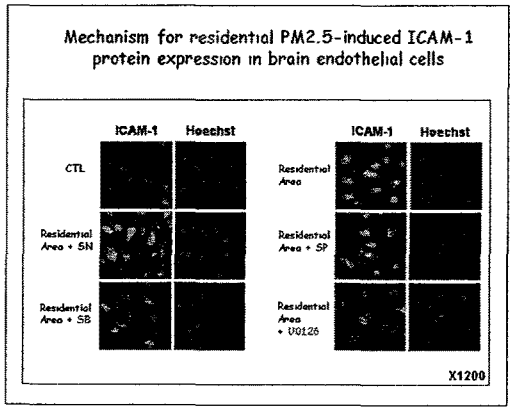

PM sampling
1st >70 (μm)
2nd 33-70
3rd 20-33
4th 11-20
5th <11
Sampling and preparation


Cytotoxicity of PM2.5 in brain endothelial cells
cell viability (% of control)
CTL
0.3 1 3 10
0.3 1 3 10
0.1 0.3 1 3 10
(μg/ml)
Indoor
Residential Area
Traffic Area
MTT assay

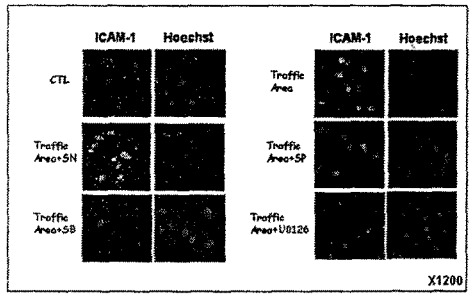

Cytotoxicity of PM2.5 in human monocyte cells
cell viability (% of control)
CTL
1 3 10
1 3 10
1 3 10
(μg/ml)
Indoor
Residential Area
Traffic Area
MTT assay


ICAM-1 protein expression by PM2.5 samples in brain endothelial cells
ICAM-1
Hoechst
CTL
Residential Area
Indoor Area
Traffic Area
X1200


CD11b (integrin subunit) protein expression by PM2 5 samples in monocyte U937 cells
counts
FL1-H
Indoor
Residential Area
Traffic Area


Mechanism for residential PM2.5-induced ICAM-1 protein expression in brain endothelial cells
ICAM-1
Hoechst
CTL
Residential Area + SN
Residential Area + SB
Residential Area + SP
Residential Area + U0126
X1200

Mechanism for traffic PM2.5-induced ICAM-1 protein expression in brain endothelial cells

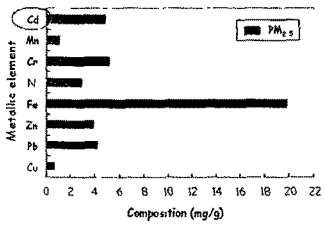


Cerebrovascular toxicity of PM2.5

- Residental and traffic area PM2.5 indicated cytotoxicity in bEnd.3 cells, but not U937 cells.
- Inflammatory factor (ICAM-1, CD11b) expression incresed by residental and traffic area PM2.5 in bEnd.3 cells and U937 cells.
- Residental and traffic area PM2.5-induced ICAM-1 expression in bEnd 3 cells may involved p38 and ERK MAPK signalling pathway.
- Traffic area > Residental area > Indoor

PM2.5 (R,T) → p38, ERK → ICAM-1 → Inflammation

Averaged metallic element composition of PM2.5

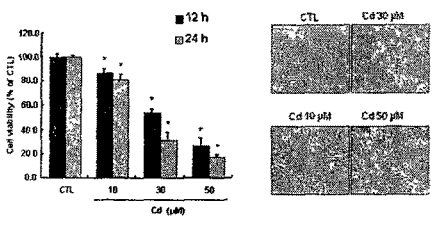


Fang GC et al Chemosphere 51 (2003) 983-91

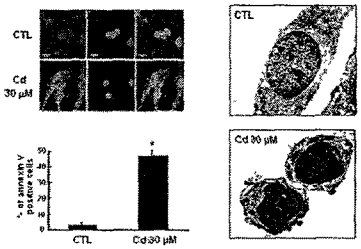
Cadmium (Cd)

- Environmental pollutants including heavy metals are known to cause inflammations in various tissues (Pritchard KA et al *J Environ Pathol Toxicol Oncol* 2000 19 251-260)
- Cadmium plays a pivotal role in inflammatory response (Goering PL et al, *In Vitro Mol Toxicol* 2000 13 125-36)
- Chronic exposure to cadmium causes central nerve system disorders (Murami A et al *Brain reaserch* 2001 894 336-39)
- Chronic exposure to Cadmium has been demonstrated to cause a higher risk of cerebrovascular mortality and stroke (Elliott P et al, *Occup environ med* 2000 57 94-97)
- Cadmium accumulate the out-side of BBB (Takeda A et al *Brain Res Bull* 1999 49(6) 453-7)
- Cadmium increases the BBB permeability, resulting in severe pathologies of the central nervous system (CNS) (Shukla A et al *Hum Exp Toxicol* 1996 15 400-405)

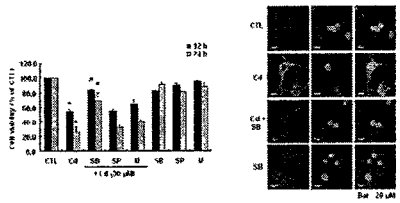
Cytotoxicity of Cd in brain endothelial cells



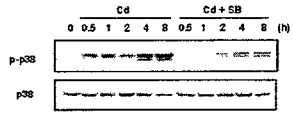
Cytotoxicity of Cd in brain endothelial cells



Mechanism for Cd-cytotoxicity in brain endothelial cells

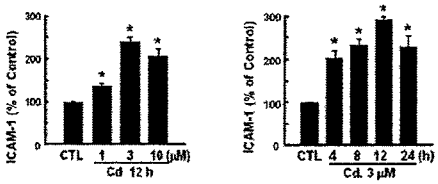


Mechanism for Cd-cytotoxicity in brain endothelial cells



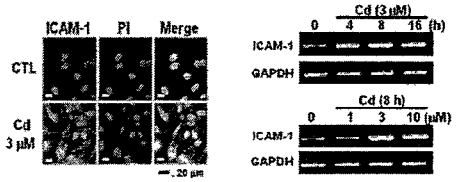
Cd → p38 MAPK → apoptosis

Effects of Cd on the protein expression of ICAM-1



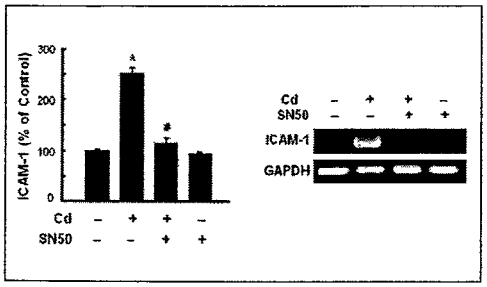
E -M Jeong *et al*, BBRC 320 (2004) 887 892

Effects of Cd on the protein expression of ICAM-1



E -M Jeong *et al*, BBRC 320 (2004) 887 892

Effects of SN50 on the Cd-induced ICAM-1 expression



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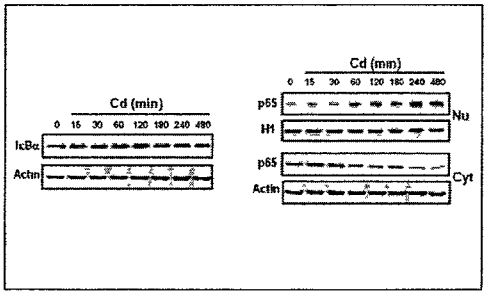
Effects of Cd on IκBα degradation, p65 translocation



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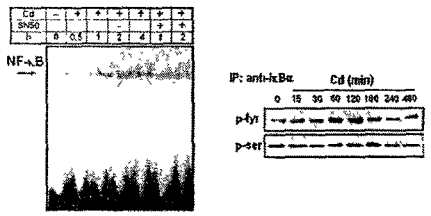
Effects of Cd on NF-κB DNA binding activity and tyrosine phosphorylation of IκBα.



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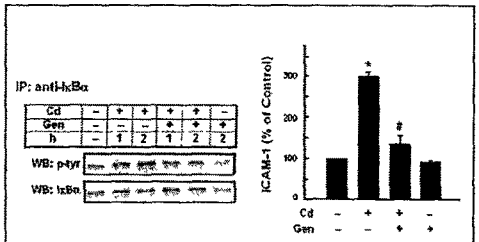
Effects of genistein on Cd-induced tyrosine phosphorylation and ICAM-1 expression



E -M Jeong et al BBRC 320 (2004) 887 892

Cerebrovascular toxicity of Cd - dual effect -

- Low concentration of Cd stimulates the expression of ICAM-1 in bEnd.3 cells, via NF-κB activation that is mediated by the tyrosine phosphorylation of IκBα.
- High concentration of Cd induces apoptotic cell death of bEnd 3 cells, via p38 MAPK.

Low conc Cd → NFκB → ICAM-1 → Inflammation

High conc Cd → p38 MAPK → apoptosis

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