

## signaling

**Hyun Mo Shin**<sup>o</sup>, Sang Hun Jung, Kyung Rak Min, Youngsoo Kim

*College of Pharmacy, Chungbuk National University, College of Pharmacy, Chungnam National University*

Aromatic diamine JSH-21 showed an IC<sub>50</sub> value of 9.2  $\mu$ M with 74.5% inhibition at 30  $\mu$ M, 53.5% at 10  $\mu$ M and 24.5% at 3  $\mu$ M on LPS-induced NO production in murine macrophages Raw 264.7. To examine whether inhibitory effect on NO production by JSH-21 was attributed to influence on iNOS expression, iNOS transcript and protein were analyzed by sequantitative RT-PCR and immunoblot analysis. Consistent with previous result on NO production, treatment of the Raw 264.7 cells with JSH-21 decreased the LPS-induced expression of iNOS transcript and protein in a dose-dependent manner with IC<sub>50</sub> values of about 10  $\mu$ M. However, JSH-21 at 30  $\mu$ M showed only 39.6% inhibition on iNOS activity. To further investigate the mechanism responsible for the suppression of iNOS gene expression by JSH-21, we examined the effect of JSH-21 on LPS-induced activation of transcription factors. JSH-21 inhibited NF- $\kappa$ B, AP-1 or OCT-1 binding activity to DNA but not CREB or SP-1 binding activity. Furthermore, JSH-21 inhibited NF- $\kappa$ B transcriptional activity with an IC<sub>50</sub> value of 9.1  $\mu$ M. The aromatic amine JSH-21 seems to target the nuclear translocation of NF- $\kappa$ B without affecting I $\kappa$ B degradation.

[PC1-28] [ 2003-10-10 09:00 - 13:00 / Grand Ballroom Pre-function ]

### **The effects of *C. annuum* L. var. *angulosum* Mill on cancer cell lines and each organ of the mouse**

**Chung Yong Za**<sup>o</sup>, Lee Jeong Hee

*Kyungsung University*

Under the vigorous search for active novel agents for cancer prevention and treatment, some agents have been found from plants and animals which are easily available. Our review of literature on them revealed that *C. annuum* L. var. *angulosum* Mill had high antiproliferating effect on cancer cells. Thus we investigated the efficacy of *C. annuum* L. var. *angulosum* Mill on cancer cell lines and to examined its effect on the mouse to detect other side effect and mechnism by which the extrat of *C. annuum* L. var. *angulosum* Mill had the anti-cancer efficacy on cancer. We observed the morphologic change and aptosis 48hr after treatment with the extract of *C. annuum* L. var. *angulosum* Mill on MCF-7 mammary gland adenocarcinoma cells and Hepatoma cells. We also count cancer cells by trypan blue stain method and MTT method, respectively, to check the cytotoxicity. We also observed the change in hepatic enzyme, morphological changes of liver and spleen of mouse, and effect on lymphocytes of the mouse. Using MTT method we observed the anticancer effect of *C. annuum* L. var. *angulosum* Mill: 35.3%, 42.9% and 94.80% reduction in the number of cancer cells at 10 $\mu$ g/ml, 25 $\mu$ g/ml and 75 $\mu$ g/ml, respectively. It is more than 2 times as potent as 5-fluorouracil (5-FU). We also report the effect of *C. annuum* L. var. *angulosum* Mill on the mouse in terms of the change in hepatic enzyme, morphological change of liver and spleen of mouse, and effect on lymphocytes.

[PC1-29] [ 2003-10-10 09:00 - 13:00 / Grand Ballroom Pre-function ]

### **Expression of p21<sup>WAF1/Cip1</sup> by TGF- $\beta$ Requires ERK Signaling Pathway**

**Kim Yong Kee**<sup>o</sup>, Bae Gyu-Un, Cho Eun-Jung, Lee Hoi Young, Lee Hyang Woo, Han Jeung-Whan

*Dept of Pharmacology, College of Medicine, Kwandong University, Dept of Biochem & Mol Biol, Colledge of Pharmacy, Sungkyunkwan University, Dept of Pharmacology, College of Medicine, Konyang University, Dept of Biochem & Mol Biol, College of Pharmacy, Sungkyunkwan University*

$\beta$ Although it has been demonstrated that p21<sup>WAF1/Cip1</sup>, a well known cell cycle inhibitor, could be induced by TGF- $\beta$  in a p53-independent manner, the detailed signal transduction pathways still remain poorly understood. In this study, we show that ERK is required for TGF- $\beta$  induction of p21<sup>WAF1/Cip1</sup>, but JNK or p38 MAPK is not. ERK activation by TGF- $\beta$  significantly attenuated by treatment with ROS scavenger such as NAC or catalase, indicating that ROS, mainly H<sub>2</sub>O<sub>2</sub>, generation by TGF- $\beta$  might stimulate ERK signaling pathway to require the induction of p21<sup>WAF1/Cip1</sup>. In support of this, treatment of cells with TGF- $\beta$  caused the increase of intracellular ROS

level, which was completely abolished by pretreatment with catalase. However, this activation of ERK does not appear to be attributed to nuclear translocation of Smads, because nuclear translocation of Smads in response to TGF- $\beta$  was not affected by inhibiting ERK signaling pathway, and also treatment with H<sub>2</sub>O<sub>2</sub> alone did not cause the nuclear translocation of Smads. On the other hand, ERK inhibition caused the disruption of interaction between Smad3 and Sp1 induced by TGF- $\beta$ , suggesting that ERK signaling pathway might be necessary for their interaction and essential for the TGF- $\beta$  induction of p21<sup>WAF1/Cip1</sup>. Taken together, these results suggest that H<sub>2</sub>O<sub>2</sub>-mediated ERK signaling pathway might be required for p21<sup>WAF1/Cip1</sup> expression by TGF- $\beta$  and play as a key determinant for interaction between Smads and Sp1 transcription factor.

[PC1-30] [ 2003-10-10 09:00 - 13:00 / Grand Ballroom Pre-function ]

### **15-DEoxy-d<sup>12,14</sup> Prostaglandin J<sub>2</sub> Rescues Pc12 Cells From Hydrogen Peroxide-induced Apoptosis Through Upregulation Of Heme Oxygenase-1**

**Kim Ji-Woo**<sup>o</sup>, Jang Jung-Hee, Surh Young-Joon

*Laboratory of Biochemistry and Molecular Toxicology, College of Pharmacy, Seoul National University, Seoul 151-742, Korea*

Oxidative stress induced by reactive oxygen intermediates (ROIs) has been implicated in a variety of human diseases including cancer, diabetes, rheumatoid arthritis and neurodegenerative disorders. Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), a representative ROI which is produced during the cellular redox process, can cause cell death via apoptosis and/or necrosis depending on its concentrations. 15-Deoxy-D<sup>12,14</sup> prostaglandin J<sub>2</sub> (15d-PGJ<sub>2</sub>), a dehydration product of prostaglandin D<sub>2</sub>, has been reported to possess a number of biological activities such as anti-inflammatory, anticarcinogenic, and antioxidative properties. In this study, we have investigated the protective effect of 15d-PGJ<sub>2</sub> on H<sub>2</sub>O<sub>2</sub>-induced oxidative stress in rat pheochromocytoma (PC12) cells. H<sub>2</sub>O<sub>2</sub> treatment caused oxidative PC12 cell death in a concentration dependent manner. PC12 cells treated with H<sub>2</sub>O<sub>2</sub> exhibited apoptotic cell death as determined by morphological features, internucleosomal DNA fragmentation, cleavage of poly (ADP-ribose)polymerase, an increased Bax/Bcl-X<sub>L</sub> ratio and decreased mitochondrial membrane potential, all of which were inhibited or restored by relatively low concentration of 15d-PGJ<sub>2</sub> pretreatment. In another experiment, PC12 cells treated with 15d-PGJ<sub>2</sub> exhibited transient activation of Akt/protein kinase B as well as extracellular signal-regulated kinase 1/2 and induction of heme oxygenase-1 (HO-1) expression and nuclear translocation of Nrf-2 as an adaptive response to oxidative insult. In conclusion, H<sub>2</sub>O<sub>2</sub> caused apoptosis in PC12 cells by inducing oxidative stress, which was effectively protected by 15d-PGJ<sub>2</sub> through augmentation of the cellular antioxidant defence involving HO-1 and Nrf-2.

[PC1-31] [ 2003-10-10 09:00 - 13:00 / Grand Ballroom Pre-function ]

### **Eupatilin, a Pharmacologically Active Flavone Derived from Artemisia Plants, Induces Cell Cycle Arrest in Ras-Transformed Human Mammary Epithelial Cells**

**Kim Doheeo**, Na Hye-kyung, Surh Young-joon

*College of Pharmacy, Seoul National University, Seoul 151-742*

Extracts of *Artemisia asiatica* Nakai (Asteraceae) possess anti-inflammatory and anti-oxidative activities. Eupatilin (5,7-dihydroxy-3,4,6-tri-methoxy-flavone), one of the pharmacologically active ingredients derived from *Artemisia asiatica*, was shown to induce apoptosis in human promyelocytic leukemia (HL-60) cells (H.-J. Seo and Y.-J. Surh, *Mutat. Res.*, 496, 191-198, 2001). In the present study, we examined the cytostatic effects of eupatilin in H-ras-transformed human breast epithelial (MCF10A-ras) cells. Eupatilin inhibited the growth of MCF10A-ras cells in a concentration-dependent and time-related manner as determined by MTT reduction and [<sup>3</sup>H]thymidine incorporation assays. To determine whether the antiproliferative effects of eupatilin are mediated through dysruption of the cell cycle in MCF 10A-ras, DNA contents were analyzed by the flow cytometry. The area of the peak corresponding to a hypodiploid or apoptotic DNA content didn't change by eupatilin treatment. However, eupatilin (100  $\mu$ M) blocked the cell cycle progression in both G1/S and G2/M phase. Moreover, eupatilin inhibited the expression of Cdk2, Cdc2, cyclin B1 and cyclin D1, which are responsible for mediating