

increase of the formation of thiobarbituric acid-reactive substances (TBARS) as a measurement of lipid peroxidation in the hepatic cytosol fractions. The activities of cytosolic glutathione S-transferase (GST), glutathione reductase (GR), and copper/zinc-superoxide dismutase (Cu/Zn-SOD) were also significantly induced. However, The catalase and glutathione peroxidase activities in hepatic cytosol were no changed in TCDD treated group compared with the control group. Treatment of TCDD caused the overall increase of the Cu/Zn-SOD activity in the all brain regions, especially showing the marked increase in hippocampus, cerebellum, and striatum. On the other hand Mn-SOD activity showed no characteristic change in the brain regions by TCDD administration. When compared with the control, administration of TCDD led to the regional specific increase of GR activity especially in cortex and striatum. Our result further showed that lipid peroxidation was increase in all the brain regions, especially statistic increase in thalamus. These results provide strong evidence that, even though the vulnerability to oxidative stress by TCDD is non-specific to brain region, exposure to TCDD induce an oxidative stress in the hepatic and brain tissues.

E108

Induction of Oxidative Stress and Cell Death in Neuronal SK-N-MC Cells by Stimulation with 2,2',5,5'-Tetrachlorobiphenyl (PCB 52)

Sang-Gu Hwang¹, Hyung-Chul Lee¹,
Woo-Hong Joo², Yong-Kweon Cho³ and
Ja-Young Moon³

Institute of Genetic Engineering, Changwon National University, Changwon 641-773¹; Dept. of Biology², and Dept. of Biochemistry and Health Sciences, College of Natural Sciences, Changwon National University, Changwon 641-773³

Polychlorinated biphenyls (PCBs) are large scale industrial chemicals which are using in diverse applications, such as in dielectric fluids, in transformers and capacitors, in hydraulic fluids, and as sealants. The goal of this study was to determine if exposure to 2,2',5,5'-Tetrachlorobiphenyl (PCB 52) leads to an increase in the production of active oxidants, and subsequently promotes apoptosis of neuronal SK-N-MC cells. Upon treatments with PCB 52, the time- and concentration-dependent inhibition of cell viability were observed. The capability of PCB 52 to induce apoptosis was associated with proteolytic cleavage of specific target proteins such as poly (ADP-ribose) polymerase and beta-catenin proteins suggesting the possible involvement of caspases. Reactive oxygen species (ROS) formation was examined in SK-N-MC cells after treatment of PCB 52 by concentrations (5, 10, 15, and 20 $\mu\text{g}/\text{ml}$) and incubation times (15, 30, 45, 60, 75, 90, 120 min), respectively. It showed that the rate of ROS production in the cells was increased in a dose-dependent manner to 45 min, followed by a return towards control levels after 120 min treatment. ROS formation was also measured in the presence of superoxide dismutase (inhibitor of oxygen free radical production) and mannitol (hydroxyl radical scavenger). Mannitol significantly inhibited ROS generation in PCB 52-treated group. We examined the association of PCB-induced apoptosis with the modulation of biomarkers of oxidative damage to lipids (malondialdehyde [MDA]) in SK-N-MC cells. Increased MDA was observed in cytosol treated with 10, 15, and 20 $\mu\text{g}/\text{ml}$ of PCB 52 for 12 h and in media treated with 10, 15, and 20 $\mu\text{g}/\text{ml}$ of PCB 52 for 24 h. The activities of antioxidant enzymes, catalase, CuZn-Superoxide Dismutase (CuZn-SOD), were also examined. The cells did not show any significant increase in the rate in CuZn-SOD activity. On the other hand, when

treated with 10 $\mu\text{g}/\text{ml}$ of PCB 52 for 24 h, the cells had a two-fold greater rate of change in catalase activity when compared to control group.

E109

Purification and Characterization of Lysozyme from Hemolymph of Sweet Potato Hornworm, *Agrius convolvuli* Larvae

Jong-Wan Kim^{*} and Sung Moon Yoo
Dept. of Biology, Dankook University, Cheonan
330-714

Lysozyme plays a central role in initiating and maintaining the antibacterial defense response of insect. A new family member of insect lysozyme, an antibacterial peptide, has been isolated from fifth instar *Agrius convolvuli* larvae. Larvae was vaccinated with *E. coli* K12 D21 (4×10^6 cells of log phase) into abdomen. After 24 hours, immunized hemolymph was collected and stored at -70°C . *Agrius* lysozyme was isolated by an cation-exchange chromatography and reversed-phase fast performance liquid chromatography, and sequenced by HPLC system. The purified *Agrius* lysozyme was heat-stable and had a molecular weight of about 15 KD by SDS-PAGE. *Agrius* lysozyme had specific antibacterial activity against Gram-positive bacteria (*Micrococcus luteus*) but no activity against Gram-negative bacteria (*E. coli*). N-terminal sequence of *Agrius* lysozyme was similar with lysozyme from *Heliothis virescens* larvae (about 70% homology in first 17 sequence). Western blot patterns of *Agrius* lysozyme with anti body from *Artogeia rapae* lysozyme showed that *Agrius* lysozyme was similar with *Artogeia* lysozyme.

E110

Cloning and Expression of *Galleria*

***mellonella* Ferritin That Encodes Two Major Subunits**

Beom Su Kim¹, Chi Young Yun² and Hak R. Kim¹

Dept. of Biology, Korea University, Seoul 136-701¹;
Dept. of Biology, Taejon University, Taejeon
300-120²

Ferritin 26kDa and 32kDa subunit cDNAs were obtained from RT-PCR using primer designed from N-terminal sequence analysis and degenerate primers. RACE was used to obtain the complete protein coding sequence. The 26 kDa subunit encodes a 211 amino acid polypeptide including a 20 amino acid leader peptide whereas the 32 kDa subunit encodes a 232 amino acid polypeptide containing a 19 leader peptide. An IRE (iron-responsive element) sequence with a predicted stem-loop structure were present in the 5'-untranslated region of the wax moth ferritin mRNA 26 kDa and 32 kDa, respectively. The 26 kDa sequence alignment has a 74% homology with *Calpodes ethlius* (S), 50% with *Drosophila melanogaster* and 39% with *Aedes aegypti*. The seven residues were associated with the metal-binding site in mature polypeptide. Northern blot analysis indicated that there was 1.5 and 1.75 fold increases in the expression of ferritin mRNA 26kDa after iron-fed fat body and midgut, respectively. Also, we confirmed that the ferritin mRNA is not expressed in adult ovary and testis. The 32 kDa sequence alignment has a 78% homology with *Manduca sexta*, 69% with *C. ethlius* (G). The *G. mellonella* ferritin subunits showed little resemblance to each other (19%). The glycosylation site (Asn-X-Ser/Thr) was found in 32 kDa subunit but not in 26 kDa.

E111

Effect of Some Natural Products from Herbs on Cell Proliferation in Cultured Mammalian Cells