in JNK1(-)-transfected cells. Conclusions: DA-125 potently inhibited topoisomerase II activity and induced apoptosis with the high rate of prooxidant production. DA-125 exhibited high- affinity DNA binding with improved cellular drug accumulation. Apoptosis induced by DA-125 involved the pathway of JNK1, but not ERK1/2 or p38 kinase

[OA-6] [04/20/2001 (Fri) 14:45 - 15:00 / Room 1]

Altered Expression of Ferritin Light Chain (FLC) by Sulfur Amino Acid Deprivation in Hepa1c1c7 and Raw264.7 Cells: The Role of Cellular Ca2+ and Free Iron for Prooxidant Production

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Ferritin expression is induced by oxidative stress, which confers resistance to oxidative insults. Sulfur amino acid deprivation (SAAD) induces oxidative stress through a decrease in a GSH content. The molecular mechanisms of cell-type specific ferritin light chain (FLC) expression in association with increases in intracellular Ca2+ and free iron pools were investigated in Hepa1c1c7 and Raw264.7 cells exposed to SAAD. Intracellular Ca2+ level was rapidly increased by SAAD, followed by returning to control at later times. Sulfhydryl-containing compounds prevented the increase in intracellular Ca2+ by SAAD, supporting the role of redox-state in the regulation of Ca2+. Thapsigargin or Ca2+-free medium inhibited the increase in intracellular Ca2+, showing that Ca2+ originated from endoplasmic reticulum as well as from extracellular source. Inhibition of Ca2+ mobilization decreased fluorescence of free iron pool inside cells and inhibited dichlorofluorescein oxidation. Deferoxamine also inhibited dichlorofluorescein oxidation. Hence, the increase in cellular Ca2+ content coupled with elevation in intracellular free iron pool and subsequent prooxidant production. FLC protein level was detected by Western blotting in Raw264.7 cells, but not in Hepa1c1c7 cells. SAAD alone or in combination with FeSO4, however, down-regulated FLC expression. On the contrary, the FLC mRNA level was increased by SAAD in both Hepa1c1c7 and Raw264.7 cells. Calcium or iron chelators prevented increases in the FLC mRNA. These results provided evidence that oxidative stress by SAAD inhibited FLC protein expression but increased the mRNA level through intracellular Ca2+ and subsequent release of iron.

[OA-7] [04/20/2001 (Fri) 15:00 - 15:15 / Room 1]

DNA Adduct Formation, Induction of Apoptosis and Cell Cycle Arrest by N-Nitroso Metabolite of Carbofuran

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Carbofuran (CF) is one of the most widely used carbamate pesticide in the world applied to insect and nematode control. Due to its widespread use in agriculture and households, contamination of food, water, and air has become imminent, and consequently adverse health effects are inevitable in humans, animals, wildlife and fish. It has been reported that CF alone or in combination with other carbamate insecticides influences the level of reproductive and metabolic hormones such as thyroxine and corticosterone, and results in impairment of endocrine, immune and behavioral functions. In this study, we evaluated the effects of CF and its N-nitroso derivative N-nitrosocarbofuran (NOCF) on DNA adduct formation, genotoxicity, cell growth and apoptosis of CHL cells. NOCF, but not CF, induced the formation of O6- and N7-methylguanine DNA adducts in calf thymus DNA and induced genotoxicity determined by Ames test. NOCF inhibited the growth of Chinese hamster lung fibroblast

(CHL) cells with IC50 of 12.8 mM. NOCF induced apoptosis of CHL cells, which was demonstrated by morphological changes, DNA fragmentation and flow cytometric analysis. Treatment of CHL cells with NOCF induced significant G2/M cell cycle arrest without any change in the level of p53 protein. Caspase-3, an executioner of apoptosis was also activated by the treatment of CHL cells with NOCF. The concentration of NOCF inducing apoptosis was so low that we speculate about the environmental significance of these pesticides.

[OA-8] [04/20/2001 (Fri) 15:15 - 15:30 / Room 1]

Hypoxia-induced Neuronal Cell Death Is Caused by Increase in the de novo Synthesis of Ceramide Linked to Caspase Activation

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Ceramide is known to be a lipid-derived second messenger in the cell signaling pathway involved in a variety of cellular responses ranging from cell differentiation, cell cycle arrest, cellular senescence, apoptosis to cell survival and cell proliferation in a number of cells. To examine the role of ceramide in hypoxia-induced neuronal cell death, ceramide generation was measured in SH-SY5Y human neuroblastoma cells metabolically labeled with [3H]palmitic acid or [3H] serine. The chemical hypoxia resulted in a rapid increase in ceramide production with subsequent evidence of cell death in SH-SY5Y cells. The inhibitor of ceramide synthase, fumonisin B1, but not L-cycloserine, a serine palmitoyltransferase inhibitor, reduced the hypoxia-induced enhancement of ceramide. Cobalt chloride also upregulated hypoxia-inducible factor 1a (HIF-1a) known to stimulate the transcription of provoked apoptosis followed by elevation of ceramide levels, but did not induce a concurrent decrease in sphingomyelin. C6-ceramide also induced apoptosis in SH-SY5Y cells in a similar kinetic frame. NOE (N-oleoylethanolamine), an inhibitor of ceramidase, and PDMP (DL-threo-1-phenyl-2-decanoylamino-3-morpholino-1-propanol), an inhibitor of glucosyl ceramide synthase, increased the ceramide level and induced DNA fragmentation in SH-SY5Y cells. Cobalt chloride-induced cell death and ceramide production were significantly potentiated by both NOE and PDMP. A bacterial Sphingomyelinase increased ceramide level, but did not induce cell death. This hypoxia-induced neuronal cell death was potently inhibited by an inhibitor of caspases, z-vad-fmk (z-vad-fluoromethylketone). Our results suggest that hypoxia-induced neuronal cell death may be caused by increase in the de novo synthesis of ceramide pathway and subsequent activation of caspase.

Oral Presentations - Field B

[B1. Physiology] [B2. Pathology] [B3. Neuroscience] [B4. Immunology]

[OB-1] [04/20/2001 (Fri) 13:30 - 13:45 / Room 2]

Role of intracellular calcium release in asiatic acid-induced apoptosis in HepG2 human hepatoma cells

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