Cytochrome b5 : Specific Inhibition of Cytochrome P450 3A4 by Zinc (II) Ion

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CYP3A4 is the most abundant human CYP and oxidizes a diversity of substrates, including various drugs, steroids, and carcinogens. A variety of metal ions are known to affect microsomal monooxygenase activities. Effects of a series of divalent metal ions on the CYP3A4-catalyzed reaction of reconstituted system containing purified CYP3A4, NADPH-P450 reductase (NPR), and cytochrome b5 (b5) were examined. Only Zn2+ inhibited the activity of testosterone 6값-hydroxylation catalyzed by CYP3A4 with IC50 value of 27 ∂ 9 mM. However, Mg2+, Mn2+, Ca2+, and Co2+ had no apparent effects on the activities of CYP3A4 within the range examined here. Zn2+ decreased the CO-binding spectra of CYP3A4 reduced by NPR, b5, and NADPH. Interestingly, Zn2+ didn···t inhibit the CYP3A4 reduction by NPR and NADPH in the absence of b5. Conformational change of CYP3A4 accompanying the Zn2+-induced inhibition in the enzyme activity was shown by CD, fluorescence spectroscopy, and absorption spectroscopy. The decrease in activity of CYP3A4 occurs concomitantly with the conformational change including decreased 감a-helix content. Intrinsic fluorescence intensity of CYP3A4 is also decreased in the presence of effective ion, Zn2+. The conformational change of CYP3A4 induced by Zn2+ might diminish the enhancing effect of b5 on the CYP3A4 reduction. It is known that the stimulatory effect of b5 on the CYP3A4-catalyzed reactions comes from the protein-protein interaction of CYP3A4 and b5. However Zn2+ didn···t show any apparent effects on the activity and conformation of NPR. Zn2+ can bind to CYP3A4 with a higher affinity (Ks = 24 and 22 mM) with or without the substrate, respectively, indicating that the substrate binding was not affected by Zn2+. It can be suggested that Zn2+ can modulate the CYP3A4 activity by changing the conformation of CYP3A4 and the interaction with b5. Conformation of CYP3A4 seems to be important for the proper protein-protein interaction with b5. These results suggest that the balance of metal ions including zinc and copper present in the cytosol might be important for a functional conformation of CYPs in a monooxygenase system including NPR and b5 . [This work was supported by Korea Research Foundation Grant (KRF-2000-015-FS0002)].

[PA1-52] [04/17/2003 (Thr) 14:00 - 17:00 / Hall P]

Increase of susceptibility against apoptotic stimuli in PC12 cells carrying mutant PS2 : Increase of p53 mRNA level, 8-oxo-dG formation and NF-κB activiation

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Alzheimer's disease (AD) is a neurodegenerative disorder characterized by the progresive deterioration of cognition and memory in association with widespread neuronal loss. AD is supposed to be very often associated with missense mutation located on homologous protein Presenilin (PS1) and (PS2). Up to now, the molecular mechanisms underlying the role of the gene mutation in AD still remain unclear. To compare the response against apoptotic stimuli of the wild PS2 tranfected PC12 (PC12/PS2w) cells and that of the mutant PS2 tranfected PC12 (PC12/PS2m) cells, the acetylcholine esterase activity, 8-oxo-dG levels,p53 expression and activation of transcription factors in the two cell types treated with Aβ and glutamate were compared. We found that the acetylcholine esterase activities, p53 mRNA and 8-oxo-dG levels were higher in PC12/PS2m cells compared to those of PC12/PS2w cells. We also found the levels of NF-κB DNA-binding activity were higher in PC12/PS2m cells than in the PC12/PS2w cells treated with either glutamate or Aβ. Correlated well with the different responses, the induction of apoptosis by apoptotic stimuli was much higher in PC12/PS2m cells than that in

PC12/PS2w cells. These findings indicate the PC12/PS2m is more sensitive than PC12/PS2w cells in response to apoptotic stimuli, L-glutamate and Aβ stimuli.

[PA1-53] [04/17/2003 (Thr) 14:00 - 17:00 / Hall P]

Btg-1 Induction by Oxidative Stress

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B cell translocation gene-1 (Btg-1), originally discovered from chromosomal translocation in chronic B-cell lymphocytic leukemia, belongs to the APRO family. Btg-1 exhibit antiproliferative function, being expressed during the G_0/G_1 transition phase of cell cycle. Btg-1 is fully expressed in quiescent and differentiated cells, while the protein expression decreases as the cell progresses through the cell cycle. Previous studies from this laboratory have shown that Btg-1 is induced by protein calorie malnutrition, a condition of *in vivo* oxidative stress. In the present study, we investigated the effects of oxidative stress on the activation of Btg-1, the role of Btg-1 in the expression of inflammatory mediators and the responsible signaling pathway(s) in Raw264.7 cells. Btg-1 was induced by sulfur amino acid deprivation and other oxidative stress (i.e. *t*-BHQ, SIN-1 and BSO) in the cells. Btg-1 induction was controlled by the signaling pathways involving PI3-kinase and p70S6 kinase, but not MAP kinases. Confocal microscopic analysis using pEGFP-Btg1, which encodes GFP-Btg-1, revealed that oxidative stress caused cytoplasmic Btg-1 to translocate into the nucleus. Oxidative stress reduced the expression of iNOS in macrophages, Overexpression of Btg-1 also inhibited iNOS induction, suggesting that the induction of Btg-1 by oxidative stress may affect expression of the gene. These results provide evidence that oxidative stress induces Btg-1 in macrophages and leads to the inhibition of iNOS expression. The approaches to the modulation of cell function in association with Btg-1 may allow us to identify the pharmacological targets for immune modulation or therapeutic advantages. (Supported by the fund of Ministry of Welfare and Public Health, 02-PJ1-PG3-21403-0003)

[PA1-54] [04/17/2003 (Thr) 14:00 - 17:00 / Hall P]

Preventive effect of whole bee venom on arthritis and its mechanism: inhibition of COX-2 and iNOS expression through inactivation of $NF-_kB$

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Bee venom (BV) has been utilized to relieve pain and to treat inflammatory diseases such as rheumatoid arthritis (RA). BV contains a variety of different peptides including melittin, apamin, adolapin and mast cell degranulating (MCD) peptide. In addition, it also contains enzyme (i.e. phospholipase A2), biologically active amines and non-peptide components. However, it is likely that a complex stimulation effect of individual components of BV may be responsible for anti-inflammatory effect. Although the treatment of bee venom (BV) has been reported to show an anti-arthritis effect in vivo, the mechanism by which BV-induced anti-arthritis effect has been