PROTECTION OF PARAOXONASE BY LIPIDS

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Effect of lipids on the oxidative inactivation of paraoxonasel (PON1), a HDL-associated antioxidant protein, was investigated, based on the hydrolysis of phenyl acetate. First, various fatty acids were examined for the prevention against the inactivation of PON1 by ascorbate/Cu2+ system, which caused a remarkable (≥ 90%) inactivation of PON1, accompanied by a partial loss of histidine residues. Compared to saturated fatty acid (C6-C18) exhibiting a modest protection (9-40 %), monounsaturated fatty acids showed a greater protection (Emax, 70-82%). In addition, a remarkable protection was also expressed by conjugated linoleic acid, but not a-linoleic acid. The most protective was oleic acid (EC50, 2.7 mM), which fully prevented the oxidative loss of histidine residues of PON1. Noteworthy, either the introduction of hydroxyl group at C12 of oleic acid or the derivatization of carboxylic group caused the loss of protective action, emphasizing the importance of both acyl chain and anionic charge. Consistent with this, dioleoylphosphatidylglycerol, negatively-charged, was more protective than other phospholipids including dioleoylphosphatidylcholine. Similarly, such a protective action of lipids was also observed in different types of inactivation of PON1, where ascorbate/Fe2+, peroxides, or p-hydroxymercuribenzoate were employed. The same pattern of protection by lipids was also observed when PON1 activity was determined using paraoxon as substrate. These results suggest that some lipids play a beneficial role in maintaining PON1 activity from oxidative stress in vivo.

[PC1-25] [04/18/2003 (Fri) 09:30 - 12:30 / Hall P]

Proteomic analysis of proteins Secreted by Human Bronchial Epithelial Cells in Response to Pathogenic Bacterial Infections

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Bacterial infection is a very complex process in which both pathogens and host cells play crucial roles, and the host cells undergo drastic changes in their physiology, releasing various proteins in response to the pathogenic infection. Human airway epithelial surface serves as a first line of defense against microorganisms and the external environment. It is well known that bronchial epithelial cells secrete various chemokines and cytokines such as IL-6 and IL-8 to cope with various respiratory pathogens. Under infectious conditions, cultured mammalian cells release a number of proteins to its culture medium. While most of these proteins localized in intracellular compartments result from cell death, some of the proteins are known to serve as immunologically responsive proteins, such as interferons, interleukins, colony stimulating factors (CSFs), transforming growth factor-b (TGF-b). Although many kinds of these cytokine proteins are identified and characterized for their biological roles, such cytokine-like proteins as a functionally unknown protein could be found through high-throughput identification of the proteins in the extracellular space. In this study, we used a combined technology of column chromatography and display proteomics to identify such proteins from pathogenic bacteria-infected human bronchial epithelial (BEAS-2B) cells. Using some of ion-exchange column chromatographies, two-dimensional gel electrophoresis, we found at least twenty different proteins that specifically secreted to the culture medium in the response to the bacteria infection. While some of these proteins were revealed as known cytokines and other functional

protein as a result of MALDI-TOF mass analysis, several proteins were revealed as novel proteins to be functionally studied.

[PC1-26] [04/18/2003 (Fri) 09:30 - 12:30 / Hall P]

A Study on the Effects of Brassica oleracea L. Fractions on the Membrane Fluidity of the Liposomal Phospholipid Membranes

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This research was designed to investigate the effects of Bassica oleracea L.(BO) fractions on the membrane fluidity of the liposomal phospholipid membranes. The sample BO was extracted and fractionated to six different types, methanol(BOM), hexane(BOMH), ethylether(BOMEE), etylacetate(BOMEA), butanol(BOMB) and aqueous(BOMA) fractions. The fluidity of dipalmitoylphosphatidylcholine(DPPC) liposomal membranes incorporated with BO fractions was measured by means of high-sensitivity differential scanning calorimetry(DSC). Compared to the other fractions of Brassica oleracea L., the BOMEE and BOMEA fractions markedly affected the thermotropic properties of DPPC liposomes, broadened and shifted the thermogrames of the transition to lower temperatures. The incorporation of the BOMEE and BOMEA in DPPC liposomes was preferentially located in the hydrophobic core of DPPC bilayers, where it reduced the lipid packing orderness in the gel state compared to it in the liquid-crystalline state.

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Alteration of Cellular Adhesion Molecules during Aging and Their Modulation by Calorie Restriction

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Expressions of cellular adhesion molecules (CAMs) are closely related to the formation of early atherosclerosis, an age-dependent vascular disorder. However, previous research provided only limited and conflicted reports on age-related alterations of CAMs'expressions and even much less is known the modulation of CAMs by calorie restriction (CR). In this study, expression of vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), E-selectin, P-selectin and platelet/endothelial cell adhesion molecule-1 (PECAM-1) in aorta and kidney were investigated by western blot and immuno-histochemical stain utilizing *ad libitum* (AL) and CR rats. mRNA levels were detected by RT-PCR. Current data demonstrated that expressions of VCAM-1, ICAM-1, E-selectin and P-selectin were significantly increased during aging and suppressed by CR. RT-PCR data showed increased expression of VCAM-1 and P-selectin during aging and blunted by CR, while ICAM-1 mRNA level kept no change. In addition, mechanism of these alteration were investigated. The up-regulated expressions of CAMs stimuli, TNFα and IL-1β, were found in old AL but not in old CR rats. Our study further explored the effect of aging and CR on common promoter binding sites of CAMs. Result showed that DNA-binding activity of NFκB, AP-1 and CREB increased during aging which was blunted by CR. In conclusion, our data documented that most of the inflammatory CAMs increased expression