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A convenient bioassay of nerve growth factor(NGF) is essential for assessing its potency during the course of product development and quality controls afterwards. We have set up a cell-based bioassay for determining the potency of recombinant NGF using rat pheochromocytoma (PC12) cells. Cell survival was measured by monitoring the reduction of the alamarBlue^{TM} dye by living cells. The assay is simple and dose not require collagen-coated culture plates and shows reproducible dose-response growth responses after 2 or 3 days incubation under serum free conditions. We tested three different types of NGF which were recombinant human β-NGF, WHO reference reagent(93/556) and murine NGF. The effective ranges were 1-100 ng/ml for human β-NGF and 5-500 ng/ml for the others. The curve patterns of first two NGFs were steeper than that of murine NGF, the slops of them dramatically increased around EC_{50}. This cell survival assay determined the NGF potency, indirectly by using its downstream cellular response as an end-point, therefore we have tried a direct method at upstream level by measuring NGF-induced tyrosine kinase receptor TrkA combined with ELISA in terms of receptor phosphorylation using endogenously expressed NGF receptor in PC12 cells. We compared the result with antiphosphotyrosine Western blot analysis and they showed good correlation.

[PC1-47] [10/17/2002 (Thr) 13:30 - 16:30 / Hall C]

Alternation of Adhesion Molecules during Aging and Modulation by Calorie Restriction

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Expressions of adhesion molecules (AMs) are closely related to the formation of early atherosclerosis, an age-dependent process. However, previous research only provided limited and conflicted reports about alternated AMs' expressions during aging and even much less is known about modulation of AMs by calorie restriction (CR), the only established anti-aging experimental paradigm.
In this study, expression of inflammatory AMs: vascular adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), E-selectin, P-selectin and plate/endothelial cell adhesion molecule-1 (PECAM-1) and non-inflammatory AM: vascular adhesion protein1 (VAP-1) in aorta and kidney were investigated by western blot and immuno-histochemistry stain in Ad libitum (AL) and CR rats. Their mRNA level were detected by RT-PCR. Current data demonstrated that: (1) in the aorta, expression of VCAM-1 significantly increased during aging. (2) in the kidney, expression of VCAM-1, E-selectin, PECAM-1 in kidney increased during aging; VCAM-1, E-selectin expression were down-regulated by CR. (3) RT-PCR data shown increased expression of VACM-1 and PECAM-1 during aging and blunted by CR, while ICAM-1 mRNA level kept no change during aging.
In conclusion, our data demonstrated that most of the inflammatory AMs increased expression during aging and down-regulated by CR. Increased AMs contribute to pathological process of vascular aging.

[PC1-48] [10/17/2002 (Thr) 13:30 - 16:30 / Hall C]

Studies on standardization and characterization of recombinant interferon alfa

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This study was intended to establish test methods equivalent to those of "Interferon alfa-2 concentrated solution" monograph in European Pharmacophoeia(EP). Two recombinant interferon alfa concentrated solutions manufactured in Korea were tested according to the monograph of EP. Tests of identification(biological activity, isoelectric focusing, SDS-PAGE under reducing condition, peptide mapping), related proteins, impurities of molecular masses differing from that of interferon alfa-2 (SDS-PAGE under reducing and non-reducing condition), bacterial endotoxin, protein, potency, host-cell-derived proteins, and host- cell-derived DNA were performed in the laboratories of manufactures and division of biotechnology, KFDA. The results of this study showed that specifications of interferon alfa concentrated solutions manufactured in Korea were within the acceptance criteria of EP. Based on this study, specifications and test methods for interferon alfa concentrated solution can be established according to the monograph of EP suggesting the revision of 「Minimum requirements

for biological products.

[PC1-49] [10/17/2002 (Thr) 13:30 - 16:30 / Hall C]

Methylation by Protein Arginine Methyltransferase

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Arginine methylation is a common post-translation protein modification in eukaryotic cells. Protein-arginine N-methyltransferase transfer methyl groups from S-adenosyl-L-methionine to the guanidino group of arginine residues. However, The significant of this modification has been questionable, because it occurs rarely and is present at very low abundance. Recently, the discovery of two protein arginine methyltransferase, PRMT1 and CARM1, as cofactors required for responses to nuclear hormone receptors provided an indication that arginine methylation have an important role in transcriptional regulation. Substrate for protein-arginine methyltransferase include many RNA-binding protein, RNA-transporting protein, transcription factor, nuclear matrix protein, and cytokines. To expand our knowledge on the regulation and role of PRMT1 and CARM1 in cells, we used the yeast two-hybrid system to identify proteins that interact with PRMT1 and CARM1. Bait plasmid pGBKT7-PRMT1 and pGBKT7-CARM1 were used to screen the pACT cDNA library from the human fetal brain poly(A+) mRNA. Vectors encoding pGBKT7-PRMT1 and pGBKT7-CARM1 were constructed by inserting an EcoRI-BamHI fragment of pM-PRMT1 and an EcoRI-BglII fragment of pSG5 • HA-CARM1 into the EcoRI-BamHI site of pGBKT7. We will identify proteins that interact with protein-arginine methyltransferase, and elucidate the biological role of these proteins and protein-arginine methyltransferase in vivo.

[PC1-50] [10/17/2002 (Thr) 13:30 - 16:30 / Hall C]

Conformational Study of Cyclic Ac-Cys-Pro-Xaa-Cys-NHMe Peptides: a Model for Chain Reversal and Active Site of Disulfide Oxidoreductase

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The conformational study on cyclic Ac-Cys-Pro-Xaa-Cys-NHMe (Ac-CPXC-NHMe; X = Ala, Val, Leu, Aib, Gly, His, Phe, Tyr, Asn, and Ser) peptides has been carried out using the ECEPP/3 force field and the hydration shell model in the unhydrated and hydrated states. This work has been undertaken to investigate structural implications of the CPXC sequence as the chain reversal for the initiation of protein folding and as the motif for active site of disulfide oxidoreductases. The backbone conformation DAAA is in common the most feasible for cyclic CPXC peptides in the hydrated state, which has a type I β-turn at the Pro-Xaa sequence. The proline residue and the hydrogen bond between backbones of two cystines appear to play a role in stabilizing this preferred conformation of cyclic CPXC peptides. However, the distributions of backbone conformations and β-turns may indicate that the cyclic CPXC peptide seems to exist as an ensemble of β-turns and coiled conformations. The intrinsic stability of the cyclic CPXC motif itself for the active conformation appears to play a role in determining electrochemical properties of disulfide oxidoreductases.

Poster Presentations - Field C2. Microbiology

[PC2-1] [10/17/2002 (Thr) 13:30 - 16:30 / Hall C]