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大會議程及論文摘要
Programs & Abstracts

中國生理學會 The Chinese Physiological Society
中華藥理學會 The Pharmacological Society in Taiwan
中華民國解剖學學會 The Association of Anatomists of the Republic of China
中國生物化學及分子生物學會 The Chinese Society for Biochemistry and Molecular Biology, Taipei
中華民國細胞及分子生物學學會 The Chinese Society of Cell and Molecular Biology
中華民國臨床生化學會 Chinese Association for Clinical Biochemistry
中華民國毒物學學會 Toxicology Society of Taiwan

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P 5

THE EFFECT OF VITAMINS AND MELATONIN ON DIGOXIN CYTOTOXICITY

C.Y. Kao*, Y.T. Chou and J.S. Wei*

Graduate Institute of Basic Medical Sciences and School of Medical Technology, Chang Gung University, Taoyuan, Taiwan, R.O.C.

Upon digoxin treatment, increase of fluorescent intensity from hepatoma cells loaded with non-fluorescent compound dichloro-fluorescein diacetate was observed under fluorescent microscope. This technique allows us to visualize the "oxidative stress" in hepatoma cells when induced by digoxin. Alterations to isoforms of nitric oxide synthase (both eNOS and iNOS) gene expression in SK-Hep-1 cells in response to digoxin treatment were confirmed by RT-PCR. Expression of Ca^{-}-dependent eNOS gene was up-regulated by digoxin treatment whereas Ca^{-}-independent iNOS gene was not induced under the same experimental conditions. Taken together, we believe that excess generation of NO in cells by eNOS upon digoxin treatment is the primary cause of its cytotoxicity. We therefore tested the effects of several antioxidants on the digoxin cytotoxicity and found that vitamin C is the most effective agent to prevent cell death caused by digoxin. Both vitamin E and melatonin have moderate effectiveness in comparison whereas beta-carotene has no effect at all.

P 6

DETERMINATION OF DIGOXIN-LIKE IMMUNOREACTIVE FACTORS FROM BIOLOGICAL SAMPLES BY IMMUNOPRECIPITATION/ ENZYME IMMUNO ASSAY

Z.W. Zhuang*, M.H. Lin and J.S. Wei*. School of Medical Technology, Chang Gung University, Taoyuan, Taiwan, R.O.C.

Numerous interferences have been described for many digoxin assays. Among them, digoxin-like immunoreactive factor (DLIF) is an endogenous substance that cross-reacts with antibody to digoxin. Presence of DLIF is a source of interference that makes interpretation of digoxin concentrations difficult. In this report, we used anti-digoxigenin superparamagnetic polystyrene particles to isolate endogenous DLIFs from human and chicken blood samples. The bound DLIFs were subsequently eluted, then measured by a highly specific enzyme immunoassay which bearing with very low cross-reactivity against the proposed DLIF candidates such as ouabain, bufalin, and fatty acids, etc. In summary, we describe in this report a simple and efficient immunoprecipitation/EIA method to quantitate the DLIF in the biological samples. This method has also been proposed to analyze digoxin accurately in the clinical laboratory without the interference from heterofhilic antibodies when existed in the samples which may generate false positivity.

P 7

MUTATION ANALYSIS OF MUT GENE IN CHINESE METHYLMALONIC ACIDEMIA CAUSED BY METHYLMALONYL CoA MUTASE DEFICIENCY

S.-F. Lee[1*], T.-T. Liu[2], S.-J. Wu[2], K.-J. Hsiao[1,2*]. Institutes of Genetics[1], National Yang-Ming University; Department of Medical Research and Education[2], Veterans General Hospital-Taipei, Taipei, Taiwan, R.O.C

Methylmalonic acidemia (MMA, MIM 251000) is a common autosomal recessive disease of organic acid metabolism. It may be caused by methylmalonyl CoA mutase (MCM, EC 5.4.99.2) deficiency, or by adenosylcobalamin, the MCM cofactor, deficiency. To investigate the molecular defect of Chinese MMA caused by MCM deficiency, the exon 2-13 encoding MCM and its leader peptide were PCR amplified and sequenced from the genomic DNA isolated from 5 Chinese MCM deficient families. A missense (1280G>A) and a nonsense (682C>T) mutation were identified. The 682C>T transition caused an Arg to termination change at codon 228 (R228X) and has been reported in a Caucasian patient previously. The novel 1280G>A transition caused a Gly to Asp change at codon 427 (G427D). None of 56 normal Chinese were found to have the 1280G>A transition by restriction analysis of the genomic PCR products. On the other hand, the 1280G>A allele was detected in another unrelated Chinese MMA family. These data indicated the 1280G>A might be a disease causing mutation in MCM deficient patients.

P 8

PROPERTIES OF HYPOOSMOTICALLY INDUCED POTASSIUM TRANSPORT IN LEUKEMIA CELLS

H.-B. Wang* and C.-C. Huang

Department of Physiology, Chung Shan Medical and Dental College, Taichung, Taiwan, R.O.C.

The properties of potassium transport have been examined in leukemia cell exposed to hypoosmotic condition. Hypoosmotic cell swelling resulted in a dramatically increased potassium efflux. The hypoosmotically induced potassium efflux was occurred in an osmolarity-dependent manner. Addition of ouabain and barium significantly reduced this hypoosmotic induced potassium efflux but not completely inhibition. The hypoosmotically induced potassium efflux was significantly affected by Cl^- replacement by sulfamate, suggesting this efflux may occur via a Cl^--dependent pathway. These results suggest that hypoosmotically swelling leukemia cells result in the activation of a Cl^--dependent transport pathway that is capable of mediating net potassium efflux.

B194

P 177

IMMUNOLOCALIZATION OF SCAVENGER RECEPTOR CLASS B TYPE I IN ARTERIAL WALLS OF RATS

Y.-P. Liu*, V.-C. Yang#. Department of Biology, Tunghai University, Taichung, Taiwan, R.O.C.

The scavenger receptor class B, type I (SR-BI) has been identified to binds high-density lipoprotein (HDL) and mediates the selective uptake of HDL cholesteryl ester in liver and steroidogenic tissues. However, recent studies suggested that a potentially important role of SR-BI is in the initial steps of cholesterol efflux in the Chinese hamster ovary cells. This may be crucial in preventing the accumulation of cholesterol in the arterial wall as occurs in atherosclerosis. Our previous studies have demonstrated that SR-BI was expressed in the endothelial and the smooth muscle cells *in vitro*. In this study, specific antibodies for the SR-BI were used to immunolocalize this protein in the arterial walls of rats after normal or high-cholesterol diet treatment.

Cryosections of fixed tissue were incubated with antibodies, which were subsequently labeled with anti-rabbit IgG conjugated with HRP and examined under light microscope. For immunoelectron microscopy, the post-embedded thin sections were incubated with antibodies follow by anti-rabbit IgG with colloidal gold. Under high-cholesterol diet treatment, more SR-BI labeling was observed in the endothelial cells as well as in the smooth muscle cells compared with those of normal diet-treated group. Furthermore, labelings were predominantly in the endothelial layer in both groups. The electron microscopic observation showed that the most labelings were found at the cell surface. Some of the labelings were clustered in the caveoli-like structure. Very few labelings were observed in the extracellular matrix.

P 178

COLOCALIZATION OF HIGH DENSITY LIPOPROTEIN AND CELLULAR CHOLESTEROL IN RAT ENDOTHELIAL CELLS BY FLUORESCENCE MICROSCOPY

W.-T. Chao*, T.-F. Wang, V.-C. Yang#. Department of Biology, Tunghai University, Taichung, Taiwan, R. O. C.

The plasma concentration of high-density lipoprotein (HDL) is inversely correlated with atherosclerotic coronary artery disease. Although the role of HDL in reverse cholesterol transport has been defined, there is still considerable debate on how HDL removes excess cholesterol from cells. This process is crucial in the prevention of arterial plaque formation and atherosclerosis.

In the present study, we performed double labeling fluorescence microscopy to directly visualize the uptake of HDL and the distribution pattern of HDL and cholesterol in the aortic endothelial cells. The results showed that when cells were preloaded with fluorescence labeled cholesterol for 48 hrs and incubated with HDL-DiI for 2 hr at 4℃, HDL-DiI was found on the cell surface. After 5 to 30 min incubation at 37℃, HDL-DiI appeared in the cytoplasm. In addition. HDL-DiI was found to colocalize with the fluorescence labeled cholesterol in the cytoplasm. The results provide evidence in that the HDL could enter the cells by plasmalemmal vesicles and interact with cellular cholesterol. Further significance of colocalization is to be investigated.

P 179

IDENTIFICATION OF THREE NOVEL α-GALACTOSIDASE A MUTATIONS (274G>T, 781G>A, AND EXON 2 DELETION) IN PATIENTS WITH FABRY DISEASE

M.-Y. Liu[1]*, C.-H. Chen[3], S.-J. Wu[1], Y.-T. Hsieh[4], T.-T. Liu[1], K.-J. Hsiao[1,2]#. Dept. of Med. Res. & Edu.[1], Veterans General Hospital-Taipei, Taipei; Inst. of Genetics[2], Natl. Yang-Ming Univ., Taipei; Inst. of Human Genetics[3], Tzu-Chi Collage of Medicine, Hualien; Dept. of Obs. and Gyn.[4], Veterans General Hospital-Taichung, Taichung, Taiwan.

Fabry disease (MIN 301500) is an X-linked inborn error of sphingolipid catabolism caused by α-galactosidase A (α-Gal A, E.C. 3.2.1.22) deficiency. To characterize the molecular defects of Chinese Fabry patients, the α-Gal A transcripts and genomic sequence from three unrelated patients were sequenced after PCR amplification. A G to T transversion at nucleotide 274 and a G to A transition at nucleotide 781 of α-Gal A cDNA were identified in two unrelated patients without other nucleotide alteration in the whole coding sequence. These alterations resulted in the amino acid from substitution of Asp to Tyr and Gly to Ser at codon 92 and 261, respectively. These substitutions were tightly linked with the heterozygotes diagnosed by low plasma activity of α-Gal A in the patient's family. None of 100 alleles (50 females) of unrelated normal Chinese were found to have these 274G>T and 781G>A alterations by sequencing of the corresponding PCR products of exon 2 and exon 5. These data indicated these two alterations might be disease-causing mutations. For the third patient, the exon 2 was found to be skipped with two abnormal transcripts of α-Gal A by RT-PCR analysis and sequencing. These deleted transcripts introduced a termination at codon 64. Southern hybridization for this patient revealed a gross deletion, about 2.6 kb in length, comprising partial intron 1, exon 2, and partial intron 2. To our knowledge, these alterations and deletion were novel mutations found in the human α-Gal A gene.

B195

P 180

IDENTIFICATION OF A NOVEL GLUCOSE 6-PHOSPHATE DEHYDROGENASE (G6PD) 551C>T MUTATION IN A CHINESE FAMILY WITH G6PD DEFICIENCY

H.-L. Liu[1]*, T.-T Liu[3], S.-J Wu[3], L.-C. Toung[4], X.-B.Qu[4], K.-J. Hsiao[2,3]#. Department of Life Science[1], Institute of Genetics[2], National Yang-Ming University, Taipei; Department of Medical Research and education[3], Veterans General Hospital-Taipei ; Guangzhou Children Hospital[4], Guangzhou.

Glucose 6-phosphate dehydrogenase (G6PD) deficiency is a common genetic disease in southern Chinese population. The incidences of G6PD deficiency are estimated to be around 2% in Taiwan and 3.7% in Canton, respectively. A PCR-based single strand conformation polymorphism (SSCP) analysis with nonradioisotopic detection by silver stain was developed to screen unknown mutation from dried blood spot specimens collected on filter paper. A novel mutation 551C>T causing a Ser to Phe change at codon 184 on G6PD gene was identified in a Cantoness G6PD deficiency family. Besides this 551C>T transition and a 33C/G polymorphism, no other neucleotide alter ation was found in this patient's G6PD coding region and exon/intron boundaries. None of 100 and 60 normal alleles of Taiwaness and Cantoness, respectively, was found to have this 551 C>T transition by SSCP. On the other hand, the 551 C>T transition was found in the maternal family and tightly linked to the heterzygotes diagnosed by low activity of blood G6PD. The 551 C>T was found in only one of 102 Cantonese patients, while it was not found in 208 Taiwanese male patients. These data highly suggested that 551 C>T alterlation might be a rare disease-causing mutation in southern Chinese.

B196