ANNUAL RESEARCH MEETING

FOR

GRADUATE STUDENTS

Nagoya, December 1993

Abstracts

SERUM CHOLESTEROL AND CANCER MORTALITY IN JAPANESE CIVIL SERVICE WORKERS: FINDING FROM A NESTED CASE-CONTROL STUDY

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To examine an association between serum cholesterol level and cancer mortality, a nested case-control study was conducted among 5,796 civil service workers in Japan who underwent periodic health examination. One-hundred and thirty-one deaths (cases), including 73 cancer deaths, were identified in the study period from 1980/1981 to September 1, 1991. Two controls were randomly selected for each case, matched for age, sex, year of examination and job status.

As a major result, an increase in serum cholesterol of 10 mg/dl significantly reduced cancer risk by 0.91 times in men, but not in women. This reduction of cancer risk in men was found not to be confounded by body mass index (BMI), smoking habits, and drinking habits. No significant association could be found between serum cholesterol level and specific sites of cancer. Separate analysis by follow-up period significantly revealed an inverse association between serum cholesterol and cancer deaths in men in 6 years or later after serum measurement. This inverse association was believed not to be ascribable to an effect of a preclinical cancer.

INFERIOR OBLIQUE UNDERACTION

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We have investigated the etiology and incidence of 100 cases of inferior oblique underaction. The difference between congenital and acquired underaction was discussed using Awaya's New Cyclo Tests (NCT) to examine cyclodeviation qualitatively and quantitatively. Inferior oblique underaction had been considered more frequently congenital than acquired. However, 63.0% of our cases was proved to be acquired. The congenital cases are Brown's syndrome and of unknown causes and the acquired cases are mainly of myasthenic or traumatic origin. The results of the NCT examination were as follows: incyclodeviation or no cyclodeviation was encountered in all of congenital underaction except for one postoperative case, but excyclodeviation rather than incyclodeviation occurred in cases of acquired underaction, which showed ocular torticollis to the side of the paretic eye. This result suggested that some other muscles were involved in acquired cases of seemingly isolated inferior oblique underaction, especially in myasthenia gravis. A diagnosis of myasthenia gravis should be considered first when an acquired inferior oblique underaction occurs with association of excyclodeviation demonstrated by the NCT as well as Cogan's lid twitch sign.

EFFECT OF CROMAKALIM ON THE SLOW WAVE IN THE CIRCULAR MUSCLE OF GUINEA-PIG ANTRUM

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In circular muscle strips of the antrum of guinea-pig stomach, the effects of cromakalim were studied on mechanical activity and intracellular membrane potential.

Cromakalim inhibited mechanical activity at concentrations higher than 1 μM, accompanied by membrane hyperpolarization and a decrease in membrane resistance. The hyperpolarization was markedly potentiated in K^+-free solution and was still observed in the absence of Na^+.

Electrical activity, the slow wave, was relatively resistant to cromakalim. Changes in amplitude and frequency were not consistent, but blockade of slow waves was never observed. In many preparations cromakalim induced spike-like potentials at the top of slow wave, or when they already existed they were potentiated, but mechanical activity was always inhibited.

Inhibition by cromakalim of phasic contractions, associated with the slow waves, could not be restored by increasing the external K^+ concentration (12—30 mM).

The results suggest that in the guinea-pig stomach muscle the mechanical supression by cromakalim does not simply result from membrane hyperpolarization or from inhibition of slow waves. A clear dissociation was found between the mechanical and electrical activities. Slow waves, particularly their frequency, are relatively insensitive to membrane hyperpolarization.

THE NEURAL ORIGIN OF THE TRANSCORNEAL ELECTRICALLY EVOKED RESPONSE (EER) OF THE VISUAL SYSTEM; AN INVESTIGATION OF AREAS 17 AND 18 IN CATS

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The transcorneal electrically evoked response (EER) is the evoked visual potential originating in the visual cortex which is elicited by electrical pulse stimulation of the eyeball. In this study, the neuronal responses to transcorneal electrical stimuli in areas 17 and 18 were recorded extracellularly and analyzed in acutely prepared cats. In the visual cortex, neuronal firings were correlated with negative components of EER as noted in the analysis of the lateral geniculate neurons. In particular, some cells in area 17 responded in latencies which correlated to those of both negative components of EER, N1 (9 ms latency) and N2 (20 ms latency). In area 18, fewer neurons fired in correlation with N1 than with N2. We found that excitation of longer latencies was suppressed and the occurrence of periodic firings was unlikely to be found. These findings suggest the existence of inhibitory mechanisms of the visual cortex, and that differences in the electrical thresholds of X and Y axons may affect the EER.

FORWARD MASKING OF TRANSIENTLY EVOKED OTOACOUSTIC EMISSIONS

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To explore mechanisms of cochlear regulation system, two tone phenomena of transiently evoked otoacoustic emissions (EOAEs) were measured using a forward masking paradigm in normal human ears. EOAEs were elicited by probe tone (1 kHz, 25 dBnHL, 3 msec duration) with or without preceding masker tone (841 Hz, 25–85 dBnHL, 10–400 msec duration, 5–160 msec interval). In intensity study, EOAEs showed decrement of amplitude according to the masker intensity. To evaluate effects of stapedial reflex (SR), the forward masking in facial palsy cases with or without SR were measured. For the higher intensity masker, SR effects were observed. Duration study showed a little decrement with lower intensity below SR threshold and over 20 msec duration masker. Interval study showed the decrement even with low and middle (35/55 dBnHL) intensity masker when the intervals were short. These results suggested that not only olivo-cochlear bundle but also other mechanisms, e.g. lateral inhibition and short-term adaptation, may affect the forward masking of EOAEs.

IMMUNOHISTOCHEMICAL INVESTIGATION OF TYPE I, III, IV, V AND VI COLLAGENS IN THE HUMAN KIDNEY VIEWED FROM RESIDUAL RENAL FUNCTION

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[Background] Our knowledge of relationship between intrarenal localization of type I, III, IV, V, and VI collagens and residual renal function is still rudimentary.
[Purpose] The present study was designed to investigate the above issue.
[Methods] Kidney sections were stained with periodic acid-Schiff (PAS) and type-specific antibodies (Abs) against collagens. The following groups were formed to compare the staining patterns. Group A; Eleven patients on maintenance hemodialysis, Group B; Twelve patients with moderate to severe glomerulosclerosis whose serum creatinine levels were below 1.5 mg/dl, Group C; Eight normal controls.
[Results] In group A, most of the glomeruli had thickened Bowman's capsule that was intensely stained with Abs against type I and III collagens (but not intensely with Abs against type V and VI collagens). In group B, as well as in group A, glomerulosclerotic lesions were intensely stained with Abs against type V and VI collagens (but not with Abs against type I and III collagens). Fibrotic lesions of the inter-stitium were stained with all the Abs. Type-specific differences in stainings, however, were not observed.

[Conclusion] If most of the glomeruli of a kidney have thickened Bowman's capsule, rich in type I and III collagens, residual renal function will be totally lost.

VESTIBULAR CHANGES DUE TO BAROTRAUMA

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Morphological vestibular changes caused by barotrauma were studied in guinea pigs. Animals were exposed to rapid decompression from 2 absolute atmospheric pressures (ATA) to 1 ATA, caused inner ear barotrauma in ginea pigs. Induring decompression, spontaneous nystagmus was recorded, which consisted of irritative symptoms initially, followed by paralytic nystagmus. After pressure loading and observation to confirm the abscense of Preyer's reflex with vertigo, the animals were tested for caloric nystagmus using ice water and then sacrificed at varying intervals. Then, morphological changes in vestibular organs and the organ of Corti were studied.

Half of the experimental animals showed canal paresis with the caloric test.

Damage to the organ of Corti was severe but that to the vestibular organs was very slight. Under light microscopy, damage to the sensory cells of the vestibular organs was not clear, in spite of a partial collapse of the labyrinthine membrans. Under scanning electron microscopy, local damage was observed in a portion of the crista ampularis of the semicircular canals. In this area, incomplete or complete disappearance of kinocillium and stereocilia, similar to that seen after rotatostimulation, was observed. However, damage to sensory hairs in the utricles and saccules was not seen.

The observed vestibular organ damage due to inner ear barotrauma suggested potential effects on endolymphatic flow.

A STRESS-INDUCIBLE 40 KDA PROTEIN (HSP40): PURIFICATION BY MODIFIED TWO-DIMENSIONAL GEL ELECTROPHORESIS AND CO-LOCALIZATION WITH HSC70 (P73) IN HEAT-SHOCKED HELA CELLS

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Recently, a novel heart- or stress-inducible protein with apparent molecular size of 40kDa (hsp40) has been found in mammalian and avian cells. Here we report the methods of purification of hsp40 using modified two-dimensional gel electrophoresis, partial amino acid sequence of the N-terminal and intracellular localization of hsp40.

The purified hsp40 was blotted onto a PVDF membrane and amino acid sequence was analyzed with protein sequencer. The N-terminal sequence of hsp40 has a homology to bacterial heat shock protein DnaJ (24 amino acid residues of 48 residues are identical, 50% identity). We also have generated a polyclonal antibody against hsp40. Indirect immunofluorescence revealed that the hsp40 in HeLa cells accumulates in the nucleus and especially in the nucleolus during heat shock and returns to the cytoplasm during the recovery period from heat shock. The kinetics of the accumulation in the nucleoli and subsequent return to the cytoplasm of hsp40 was similar to those of hsp70. In addition, hsp40 was co-localized with hsc70 (p73) in heat-shocked HeLa cells as demonstrated by double immunofluorescence staining.

These results suggest that hsp40 and hsp70 may function together to repair heat-induced denatured proteins in the nuclei and nucleoli by analogy with the function of bacterial DnaJ (hsp40 homologue) and DnaK (hsp70 homologue) proteins.

DEVELOPMENT OF THERMOTOLERANCE IN HSP70 INDUCTION-DEFECTIVE MUTANT OF NRK CELLS

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We investigated the relation between the synthesis of inducible form of heat-shock protein 70 (hsp70) and the development of thermotolerance using NRK (normal rat kidney) cells and their mutant cell line (39-1 cells). In NRK cells, hsp70 was clearly induced by conditioning treatments (42°C for 2h, 45°C for 15 min or 100 μM sodium arsenite for 1h). On the other hand, the induction of hsp70 in 39-1 cells was hardly detectable by these treatments. Other high molecular weight hsps, hsc70 (constitutive form), hsp90 and hsp110 were induced in both cell lines. However, thermotolerance as defined by clonogenic survival was induced in both cell lines to a similar extent by the conditioning treatments. When cells were made thermotolerant by conditioning heating at 45°C for 15 min, the inhibition of protein synthesis after challenge (second) heating was less in NRK cells than that in 39-1 cells. This indicated that the extent of 'translational thermotolerance' was much higher in NRK cells than in 39-1 cells. From these results, it is suggested that the synthesis of inducible hsp70 is involved in the translational thermotolerance rather than the development of thermotolerance as defined by clonogenic survival.

VARIABLE REGION GENE ANALYSIS OF TWO HUMAN MONOCLONAL ANTIBODIES TO GD3

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We analyzed the genetic origins of anti-GD3 antibodies by comparing nucleotide sequences of the heavy and light chain variable (VH and VL) regions from the human monoclonal antibody (mAb), 27-26 (μ, κ), established from a patient with leukemia, and another human anti-GD3 mAb, HJM-1 (μ, λ) derived from a patient with melanoma. The VH segment of 27-26 was remarkably similar to the germ-line gene J00239 (98.0%). The VH segment of HJM-1 shared 94.8% homology to that of the fetal liver cDNA clone 60P2, which is represents germ-line sequence. Deduced amino acid sequence of the VH segment of 27-26 was 85.7% identical to that of HJM-1. Tyr-Ala-Asp-Ser-Val-Lys-Gly residues were present at the 60-66 amino acid region in the CDR2s of both mAbs. However, the VL region gene encoding 27-26 showed little homology with that encoding HJM-1.

These data suggested that anti-GD3 B-cell clone arose from a nearly germ-line repertoire. The mAb 27-26 was thought to be derived from such B cells expanded throughout our experiment. HJM-1 was derived from lymphocytes stimulated by GD3 abundantly expressed on melanoma cells.

CHARACTERIZATION OF GRANZYME A IN INTRAEPITHELIAL LYMPHOCYTES OF MURINE SMALL INTESTINE

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We examined serine proteases in intraepithelial lymphocytes of murine small intestine (iIEL). Protease activity was detected only against synthetic substrate BLT and exclusively resided in the cytoplasmic granules of iIEL as reported on murine CTL cell line. Protease to BLT was partially purified from unfractionated iIEL of an order of 10^8 cells by gel filtration and precipitation at pH 6.0. SDS-PAGE analysis of the protease showed 3 protein bands with molecular size of 58kDa, 30kDa and 28kDa, respectively, under non-reducing condition. Under reducing condition, the 58kDa protein was reduced to 30kDa monomer. However, SDS-PAGE of the protease labeled with [^{3}H]-DFP revealed that only the 58kDa band under non-reducing condition and the 30kDa band under reducing condition were radio-labeled, suggesting that the 58kDa protease is a homodimer of 30kDa serine protease. As reported by others, transcripts of granzyme A were detected by Northern blot analysis using an oligonucleotide of granzyme A of murine CTL and shown to be abundant in iIEL. In addition, messages of granzyme B, C, D, E and F were detected in iIEL by reverse transcription polymerase chain reaction. Monoclonal antibody raised against a synthetic peptide deduced from CTL-granzyme A showed a strong reaction with

the partially purified protease by ELISA but not by western blotting. Granzyme A was also detected in the cytoplasmic granules of a population of iIEL by immunostaining using the mAb but not in spleen T cells. These results indicated that functionally active granzyme A with a molecular weight of 58kDa was rich in the granules of resident iIEL.