F. Tumor Diagnosis

Effect of Membrane Active Agents on the Uptake of Tumor Scanning Reagents into Tumor Cells

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Polyenes, like amphotericin B, and vitamin A are reported to cause an increase in the uptake of antitumor agents into animal cells by acting on cellular membranes. We are studying on the effect of these membrane active agents on the uptake of Ga-67 citrate and Co-57 bleomycin into Ehrlich ascites tumor cells in vitro. The cells were harvested 6 to 8 days after intraperitoneal transplantation in ddk mice, washed 3 times by centrifugation with 0.9% NaCl solution and resuspended in 2 m*l* of the washing solution (5×10^7 cells in each tube). 0.05 μCi of Ga-67 citrate or Co-57 bleomycin was added to each tube. As membrane active agents, amphotericin B (E R Squibb and Sons Inc.), vitamin A (Chocola A of Eisai KK) or lysozym (Neuzym of Eisai KK) was tested. The final concentration adopted was 2.5–50 μg/m*l* for amphotericin B, 2.5–50 IU/m*l* for vitamin A, and 100–1,000 μg/m*l* for lysozym respectively. After incubation at 37° C for 1 hour, the suspensions were centrufuged and the cells were washed 3 times with 0.9% NaCl solution. The radioactivity retained in the cell pellet was then measured in a well scintillation counter. The radioactivity in the cell pellet thus prepared was about 10% and 45% of the added dose of Ga-67 citrate and Co-57 bleomycin respectively. Amphotericin B increased the uptake of Ga-67 citrate into the cells upto about 2.5 times as much as the control. But a part of the increase may be due to cell swelling observed on phasecontrast microscopy. Lysozym at the concentration of more than 600 μg/m*l* also increased Ga-67 uptake without any cell swelling. No other combination of isotope and membrane active agents increased the uptake.

An-aproach to the Chelating Structure of Co-bleomycin

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With the intention of studying the imaging efficiency of the components in Co-BLM complexes, the stability and the structure of each complex has been planed to examine.

Commercial Bleomycin (BLM) consists of A! (67%), B_2 (25%), Demethyl A_2 (8%), and some minor components. BLM-A_2, -B_2 and DMA_2 were separated on CM-Sephadex C-25 column. To this was added 1, 2 equivalent (mole), $CoCl_2$: $6H_2O$ respectively. Obtained each complex gave two spots in Silicagel TLC and also showed two peaks in C-25 column chromatography and high speed liquid chromatography. These phenomena were considered that each BLM component formed two complexes. These two were separated preparatively with high speed liquid chromatography (column : μ-Bondapack C-18 4 mm i,d, $\times$ 90 cm), to determine the molar ratio of cobalt to BLM. Quantitative analysis of cobalt and BLM were carried out with the atomic absorption spectropholometry and ultra violet absorption. Consequently about 1 equivalent of cobalt to BLM was included in A_2 and B_2 complexes, DMA_2 was contained 0.6 equivalent cobalt and no difference was found in two types of complexes.

The ratio of two type complexes were invariable with reference to both BLM and cobalt concentrations. This, however, changed with the lapse

of time. One of two types was considerably less stable, especially that of DMA_2(70h : CoA_2 50/50 CoB_2 56/44 $CoDMA_2$ 91/9) was unstable.

^1H NMR spectrum of the Co-$BLMA_2$ (solvent : D_2O 60MHZ)was compared with that of $BLMA_2$.

Intracellular State of Tumor-Radionuclides: Determination of Intracellular Gallium with X-ray Microanalyzer (EDAX). First Report

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Intracellular distribution of radionuclides with tumor affinity, sich as gallium, was detected, identified and semiquantitatively determined with energy-dispersive X-ray microanalyzer (EDAX). The first attempt was to obtain ultrathin samples containing gallium in native state, i.e., without any artificial changes which occures during fixation with liquid agents such as uranyl acetate or glutaraldehyde. To overcome these drawbacks, liver and tumor was dissected in frozen state under liquid nitrogen with LKB 8800 ultramicrotome. The thin sections obtained were vacuum-dried in cupper meshes before placing in scanning electron-microscope Hitachi S-500. In SEM, samples were scanned to obtain transmission images, and electron-microbeam was irradiated to the selected sites, to obtain characteristic X-ray spectrum with EDAX model 711. The spectrum was displayed on CRT, and underwent computer processing to EDAX program. The program includes smoothing, subtraction of special peaks of a certain element (interfering co-existing elements) and quantitative estimation of element in problem.

Our system revealed, however, its sensitivity inadequate to demonstrate elements injected at sublethal level and, experiments in such a condition inevitably lead to enrichment of elements to toxic levels. To overcome these drawbacks, an attemp to increase system sensitivity was carried out by putting silicon detector at shorter distance from sample. At present, estimation of sensitivity increment is in progress.

Experimental Studies on Mechanism of ^{201}Tl Uptake

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The tumor affinity of ^{201}Tl was studied with normal and VX-2 cancer-bearing rabbits. As a result it was found that ^{201}Tl-clearance from blood was quite rapid, showing a similar tendency as ^{42}K.

^{201}Tl distribution in normal rabbit tissues was greatest in the kidney and heart muscle followed by the thyroid gland, small intestine, spleen, lung, liver, bone marrow, bone, skeletal muscle, and blood in the order mentioned. The accumulation into the thyroid varied greatly according to individuals, generally the taller was the height of follicular cells, the greater was the affinity.

The accumulation of ^{201}Tl into the tumor transplanted into femoral muscle reached its maximum within one hour after its daministration, therer after it decreased gradually.

When the tumor affinity was compared with that of ^{67}Ga, the ratio of ^{67}Ga accumulation into tissues except blood was greater than that of ^{201}Tl.

The accumulation of ^{201}Tl was significantly correlated to that of ^{42}K, and the mechanism of ^{201}Tl-tumor affinity seemed to be triggered by the acceleration of potassium metabolism of tumor.

As the reasons why ^{201}Tl is an excellent agent in clinical diagnosis of thyroid cancer, a marked