Fundamental and Clinical Studies on ^{99m}Tc labeled Bleomycin, a new Tumor Imaging Agent

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Bleomycin, an anticancer drug, was labeled with ^{99m}Tc, and this compound was found very useful for tumor imaging.

^{99m}Tc labeling was performed by using Stannous Chloride according to our previously reported procedures. The labeling efficiencies were around 50 to 75%, and high specific activities of 1 to 3 mCi/mg Eq. were achieved. After passing through Millipore filter (0.22 μ) the material was proved non-toxic, steril and pyrogen free.

In Normal rodents after intravenous administration ^{99m}Tc Blepmycin was found to accumulate rapidly into kidney and further to bladder having a character of GFR substance.

However, when injected into fibrosarcoma bearing mouse, in spite of very rapid decrease of radioactivities in blood, various organs and whole body (whole body retained radioactivity fell down to 15% level of the initial by 24 hours), approximately 1% of the dose was observed in the tumor and 0.58% was recovered even after 24 hours, indicating strong affinity to the tumor.

Actually ^{99m}Tc Bleomycin gave sharp tumor image within 15 min. after intravenous injection in a case with small squamous cell cancer in the right neck region, and plasma radioactivity disappearances were so rapid in the early stadium as only one third of the 5 min. values were recovered at 1 hour and the decline thereafter tended much slower.

From these observations, scintiphotography within 1 hour seemed favorable and whole body irradiation after 3 to 5 mCi dose was calculated as only 2 mrads according to MIRD's formula.

Clinically 107 cases were examined, and out of 76 cases with malignant tumors 56 cases (74%) gave positive and 17 cases (22%) gave suggestive findings, and only 3 cases (4%) remained negative. On the other hand, in 14 control cases mainly with various inflammations 79% gave negative results, but 2 cases with lung abscess and fungus ball of Aspergillosis revealed positive accumulation and a case with stone disease of parotid gland was also suggestive.

Remainings were benign tumors and some of them gave positive results. Especially in brain tumors, ^{99m}Tc Bleomycin was quite compatible with $^{99m}TcO_4$ and was found favorable in tumors located in brain basis. There were 3 cases in the series who were found to have brain metastases occasionally.

Comparing the demonstrability if malignant tumors in various organs and tissues between ^{99m}Tc Bleomycin and ^{67}Ga citrate, the results did not differ so much each other as a whole, however, ^{99m}Tc Bleomycin gave superior results in gastric, thyroid, breast and even lung cancers and tumors in soft tissues. On the other hand, malignant lymphomas and hepatomas were better domonstrated by ^{67}Ga citrate.

In quite a few instances there observed certain descrepancies between the images obtained from these 2 agents.

Accumulation of ^{99m}Tc Bleomycin were found to match better with the primary involved lesions, but ^{67}Ga citrate favored to accumulate in the secondary involved areas such as lymph nodes.

One more definite advantage for ^{99m}Tc Bleomycin was much less accumulation into the inflammatory changes, especially to active tuberculosis. None of 3 cases showed any significant accumulation, but 2 of them revealed apparent accumulation of ^{67}Ga citrate.

False positive and suggestive results in 13 control cases were high as 54 and 15%, respectively.

From these results, ^{99m}Tc Bleomycin was considered to be the best RI tumor imaging agent we ever had.

The only disadvantage of this method in rather high back ground in kidneys and bladder as well as in blood streams, especially in cardiac

pool.

Further improvement will be achieved by pursuing investigation in the same category.

Studies on the Accumulation Mechanism of Radioisotopes Used for Tumor Diagnostic [^{67}Ga-citrate, ^{169}Yb-citrate and ^{51}Cr-Bleomycin]

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As reported previously [H. Orii; Tumor scanning with Ga-67 and its mechanism studied on rats. Strahlentherapie 144 192–200 (1972)], the accumulation of Ga-67 citrate in the rat experimental tumor was not influenced by the tumor growth rate. Also the incorporation of Ga in regenerating rat liver after partial hepatectomy was not increased. Further it was observed that the rate of Ga incorporation in the tumor remained fairely unchanged after the treatment with chemotherapeutic agent and X-irradiation. However, when human patinents are treated in the same way, a decrease in scan-image was observed when treated effectively.

The author pointed out that even when the uptake per unit mass of the tumor remains unchanged after treatment, the discrepancy may result from the difference in the assessment, i.e., cpm/g of animal experiments v. scan density expressing cpm/g × depth. As to the mechanism of Ga transport in the blood, the author and T. Hishizawa found that Ga binds transferrin, as indicated by the fractionation study of $^{59}FeCl_3$ and ^{67}Ga labelled (*in vitro*) mouse serum on Sephadex G-200.

Ga-protein binding *in vivo* of liver supernatant and blood serum was found very labile, and when filtered through Sephadex G-50 repeatedly, a dessociation of Ga from protein was observed.

The lability of the binding many influence the localization study of Ga in the cell, in which most radioactivity was shifted in 105,000 g supernatant fraction during centrifugation, as a result of its dissociation from the protein, and the absence of a firmly bound Ga-protein complex in liver supernatant may indicate the absence of the much discussed Ga-specific protein receptor in cells as well as in the tumor.

The alternative hypothesis of Ga localization in tumor, the adsorption of Ga to bone matrix (hydroxyapatite) as proposed by Anghileri (1971), was in turn examined by in vitro adsorption experiment and expressed in adsorption isotherm, which indicated that no more affinity exist between Ga/Yb and hydroxyapatite as compared to other none-tumoraffin radionuclides (Cr, Fe, Sr, Hg, PO_4 and TcO_4).

The tumor localization of radionuclide by way of tumor-specific chemotherapeutic agent was investigated on ^{51}Cr-labelled Bleomycin. The preparation of ^{51}Cr(III)-Bleomycin was carried out in acid range with excess of ligand and the chelated ligand was separated on Sephadex G-10. The preparation was identified on TLC by Umezawa's method.

Tumor localization by this agent was studied *in vivo* on rabbits inoculated with Dr. Rous' V 2 squamous cell carcinoma and on mice inoculated with Ehrlich's carcinoma. A positive scan was obtained in rabbits within 60 min after intravenous injection of 200 μCi of ^{51}Cr-Bleomycin and the positive image persisted for 48 hrs. In mice higher radioactivity was found in solid tumor and bone. Our further study on ^{169}Yb- and ^{85}Sr-Bleomycin is in progress.