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# COMPARATIVE STUDIES ON TUMOR AFFINITY OF $^{111}$IN-BLEOMYCIN WITH THOSE OF $^{67}$GA-CITRATE AND $^{111}$IN-CHLORIDE

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## Abstract

**The tumor affinity of $^{111}$In-Bleomycin was studied with rabbits bearing VX-2 cancer and cases of lung cancer, and the results are briefly summarized as follows.**

**1) Basic study**

**The clearance of $^{111}$In-Bleomycin from blood was about the same as that of $^{111}InCl_3$. The tumor affinity of $^{111}$In-Bleomycin was confirmed. However, in comparing $^{111}InCl_3$ and $^{67}$Ga, the ratio of its accumulation in tumor to tissue, in most cases the accumulation of $^{111}$In-Bleomycin was less, whereas its excretion into urine and feces was greater.**

**2) Clinical study**

**The radioactivity in term of the ratio between tumor and surrounding normal tissue was greater with $^{67}$Ga than with $^{111}$In-Bleomycin.**

**From the above findings it seems that $^{111}$In-Bleomycin has an excellent tumor affinity but it is not so good as to replace $^{67}$Ga.**

## INTRODUCTION

Clinical evaluation of $^{67}$Ga-citrate has come to be practically established and its excellent tumor affinity has been confirmed, but several demerits have been also pointed out about it[1-3]. However, several tumorophilic radiopharmaceuticals that have been developed since $^{67}$Ga do not surpass it, yet the demerits of $^{67}$Ga still remain as they are.[4] Therefore, it is urgently necessary to develop still superior tumorophilic radiopharmaceutical. $^{111}$In-Bleomycin (BLM) was reported by Thakur et

al.[5], in 1972, since then reports about it have appeared, especially about its clinical significance[5,6,7].

Therefore we conducted a series of clinical studies on the efficacy of various tumorophilic radiopharmaceuticals including $^{67}Ga$ centering around VX-2 cancer as well as lung cancer, and on the basis of such observations we have attempted to evaluate the role of BLM among the available other similar agents and report here our findings.

## MATERIALS AND METHODS

I. Basic Study

As experimental animals normal rabbits (weighing 2-3 kg) and VX-2 cancer-bearing rabbits were used. VX-2 cancer was made into a 20 % cell suspension and 1 ml of it was transplanted into the femoral muscle, and 14 days after transplantation radiopharmaceutical was administered. The radiopharmaceuticals used were $^{111}In$-Bleomycin (supplied from Kaken Radiopharmaceuticals), $^{67}Ga$-citrate (The Daiichi Radioisotope Labs.), and $^{111}In$-chloride (Nihon Mediphysics Laboratory).

1. The blood clearance

To 3 normal rabbits 100 $\mu$Ci of BLM was administered, and to other 3 normal rabbits 100 $\mu$Ci of $^{111}InCl_3$ was given and blood was aspirated from the animals by the heart puncture 1, 3, 6, 24 and 30 hours after the administration. Then the activity per 1 g blood was plotted on the semilogarithmic phase table and T 1/2 of the first phase was calculated.

2. The tissue distribution of BLM and $^{111}InCl_3$

Two days after the intravenous injection of 100 $\mu$Ci BLM and 100 $\mu$Ci $^{111}InCl_3$ to two groups (each consisting of 3 rabbits) the activity in main tissues was determined and the activity per gram of tissue was estimated.

3. Comparative distribution of BLM and $^{67}Ga$ in tissue

By the radioassay of main tissues 2 and 3 days after the simultaneous administration of 100 $\mu$Ci of BLM and 100 $\mu$Ci of $^{67}Ga$ to VX-2 cancer-bearing rabbits, the radioactivity per gram of tissue was determined.

4. Comparison of BLM and $^{67}Ga$ activities in the urine and feces excreted

BLM and $^{67}Ga$ were administered simultaneously in dose of 100 $\mu$Ci each to normal and VX-2 cancer-bearing rabbits, and the radioactivity in the urine and feces excreted 1, 2, 3 and 4 days later was measured,

and it was expressed in the percentage of the activity against the dose administered.

5. Comparative accumulation of BLM and $^{67}$Ga in the inflammatory tissue

At first 0.2 ml of turpentine oil was administered to the left femoral muscle of normal rabbits, two days later BLM and $^{111}InCl_3$ 100 $\mu$Ci each were injected intravenously, and still two days later the radioassays of the inflammatory tissue and the normal contralateral femoral muscle were conducted. The degree of accumulation of both agents in the inflammatory tissue was represented by the ratio of radioactivity in the inflammatory focal tissue/g to that in normal tissue/g.

II. Clinical Study of Cases with Lung Cancer

To seven cases with primary or metastatic lung cancer 2-2.5 mCi of BLM was administered and two days later the scanning was done with 5-inch scanner (Elscint, Israel). During the period of 1-2 week after the scanning 2-2.5 mCi of $^{67}$Ga was administered, and the scanning was similarly performed 2-3 days later. In addition, the region of interest (ROI) was set on the image recorded by a color display apparatus (CDP-1, Elscint) and the radioactivity ratios of BLM and $^{67}$Ga in the lesion to the surrounding normal tissue of the lung were calculated.

## RESULTS

I. Basic Study

When the clearance of BLM from blood (Fig. 1) is expressed by T 1/2 of the first phase, it took the time of 14.5 hours and that of $^{111}InCl_3$ was 12 hours, both being about the same.

The tumor affinity of BLM is compared with that of $^{111}InCl_3$ (Table 1), the ratio of BLM in tumor to blood is 2.68, and that of $^{111}InCl_3$ is 3.58, and the ratio of BLM in tumor to muscle is 7.81, while that of $^{111}InCl_3$ is 15.94, with both ratios that of $^{111}InCl_3$ is greater. The ratio $^{111}InCl_3$ in tumor to other tissues was also greater with $^{111}InCl_3$.

The tumor-accumulation of BLM as compared with that of $^{67}$Ga administered simultaneously is shown in Table 2. First, the ratio of BLM in tumor to blood is 4.94 two days after administration, and it is 3.91 3 days later, showing a greater ratio on the second day. Such a tendency was also observed in the small intestine, lung, and spleen. The ratio of tumor to muscle was 10.40 two days after administration and it was 10.42 on the third day, both being approximately the same.

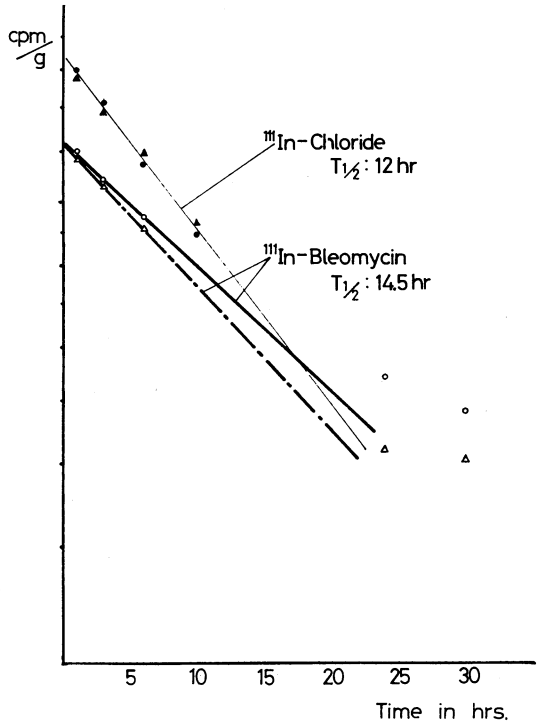


Fig. 1. Blood Clearance of $^{111}$In-Bleomycin and $^{111}$In-Chloride in Normal Rabbits.

TABLE 1

Comparison of Distribution of $^{111}$In-Bleomycin with That of $^{111}$In-Chloride in V2 Rabbits

| | Ratio Tumor Concentration to Tissue | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | $^{111}$In-Bleomycin | | | | $^{111}$In-Chloride | | | |
| | No. 1 | No. 2 | No. 3 | Aver. | No. 1 | No. 2 | No. 3 | Aver. |
| Blood | 3.12 | 2.27 | 2.66 | 2.68 | 3.65 | 4.59 | 2.50 | 3.58 |
| Liver | 0.96 | 0.91 | 0.78 | 0.88 | 0.82 | 0.92 | 1.05 | 0.93 |
| Small Intestine | 3.32 | 2.98 | 3.14 | 3.15 | 4.34 | 3.53 | 1.88 | 3.25 |
| Lung | 2.25 | 2.16 | 1.70 | 2.04 | 2.26 | 2.45 | 2.00 | 2.24 |
| Spleen | 0.79 | 0.69 | 0.68 | 0.72 | 0.64 | 0.80 | 0.69 | 0.71 |
| Kidney | 0.25 | 0.27 | 0.30 | 0.27 | 0.54 | 0.70 | 0.46 | 0.57 |
| Bone | 5.59 | 6.61 | 5.26 | 5.94 | 9.91 | 13.39 | 21.95 | 15.08 |
| Marrow | 0.43 | 0.52 | 0.43 | 0.46 | 0.62 | 0.67 | 1.33 | 0.87 |
| Muscle | 8.00 | 7.44 | 8.00 | 7.81 | 13.05 | 22.30 | 12.47 | 15.94 |
| Necrotic Tumor | 1.65 | 1.00 | 1.15 | 1.30 | 1.02 | 1.61 | 1.23 | 1.29 |

TABLE 2

Comparison of Distribution of $^{67}$Ga with That of $^{111}$In-Bleomycin in V2 Rabbits

| | Ratio Tumor Concentration to Tissue Post injection days | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 2 | | | | | | | |
| | Ga | BLM | Ga | BLM | Ga | BLM | Average Ga | Average BLM |
| Blood | 5.95 | 5.05 | 7.35 | 7.11 | 3.15 | 2.65 | 5.48 | 4.94 |
| Liver | 0.81 | 0.83 | 0.71 | 0.77 | 0.79 | 0.90 | 0.77 | 0.83 |
| Small intestine | 2.87 | 2.98 | 49.59 | 5.48 | 18.57 | 3.74 | 23.68 | 4.07 |
| Lung | 2.76 | 1.80 | 3.33 | 2.04 | 2.73 | 2.06 | 2.94 | 1.97 |
| Spleen | 0.85 | 0.57 | 0.90 | 0.80 | 0.92 | 0.72 | 0.89 | 0.70 |
| Kidney | 0.94 | 0.37 | 0.75 | 0.28 | 0.76 | 0.46 | 0.82 | 0.37 |
| Bone | 4.46 | 4.74 | 9.18 | 6.77 | 5.16 | 10.08 | 6.27 | 5.76 |
| Marrow | 1.00 | 0.56 | 0.81 | 0.56 | 0.94 | 0.63 | 0.92 | 0.58 |
| Muscle | 53.1 | 9.86 | 56.9 | 10.93 | 41.7 | 14.5 | 50.57 | 10.40 |
| Necrotic tumor | 1.33 | 1.48 | 1.57 | 1.27 | 1.11 | 1.26 | 1.34 | 1.34 |
| | Ratio Tumor Concentration to Tissue Post injection days | | | | | | | |
| | 3 | | | | | | | |
| | Ga | BLM | Ga | BLM | Ga | BLM | Average Ga | Average BLM |
| Blood | 2.66 | 1.72 | 7.42 | 4.53 | 6.93 | 5.48 | 5.67 | 3.91 |
| Liver | 0.80 | 0.62 | 0.84 | 0.84 | 1.00 | 1.01 | 0.88 | 0.82 |
| Small intestine | 1.96 | 1.02 | 14.74 | 3.97 | 12.53 | 3.70 | 9.74 | 2.90 |
| Lung | 2.21 | 1.34 | 3.47 | 2.26 | 2.85 | 2.14 | 2.84 | 1.91 |
| Spleen | 0.51 | 0.28 | 0.95 | 0.67 | 0.62 | 0.54 | 0.69 | 0.50 |
| Kidney | 0.52 | 0.25 | 0.99 | 0.49 | 0.99 | 0.55 | 0.83 | 0.43 |
| Bone | 6.09 | 4.15 | 7.33 | 10.98 | 5.88 | 8.06 | 6.43 | 7.73 |
| Marrow | 0.83 | 0.37 | 2.49 | 1.13 | 1.05 | 0.60 | 1.46 | 0.70 |
| Muscle | 15.67 | 5.89 | 60.00 | 19.05 | 17.41 | 6.33 | 31.03 | 10.42 |
| Necrotic tumor | 1.24 | 1.33 | 1.29 | 1.61 | 1.13 | 1.56 | 1.22 | 1.50 |
| Urine | 2.79 | 2.09 | 9.78 | 1.60 | 10.07 | 7.24 | 7.55 | 3.64 |

Next, when the ratio of BLM in tumor to tissue is compared with that of $^{67}$Ga, on both second and third days the $^{67}$Ga level was clearly greater in blood and muscle, and in other tissues also there was no tissue in which BLM showed any remarkable ratio.

BLM and $^{67}$Ga excreted in feces and urine are shown in Fig. 2. The

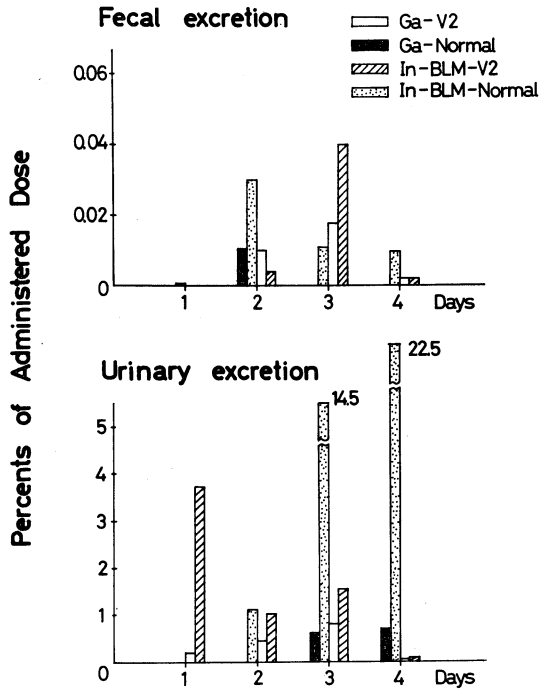


Fig. 2. Comparison of Fecal and Urinary Excretion of $^{441}$In-Bleomycin with That of $^{67}$Ga in Normal and V2 Rabbits.

amounts of BLM excreted in urine and feces were greater than those of $^{67}$Ga with both normal rabbits and VX-2 cancer bearing rabbits. On looking at the accumulation of BLM and $^{67}$Ga in the inflammatory lesion induced in the femoral muscle by turpentine oil, the accumulation of BLM is less than in the case of $^{67}$Ga (Table 3).

TABLE 3

Deposition of $^{67}$Ga and $^{111}$In-Bleomycin in Turpentine-induced Inflammation

| Inflamed tissue to muscle ratio | |
|---|---|
| $^{67}$Ga | 21.70~77.48 |
| $^{111}$In-BLM | 7.36~21.83 |

turpentine (0.2ml) $\xrightarrow[\text{i.m.}]{2d}$ $^{67}$Ga, $^{111}$In-BLM $\xrightarrow{2d}$ radioassay

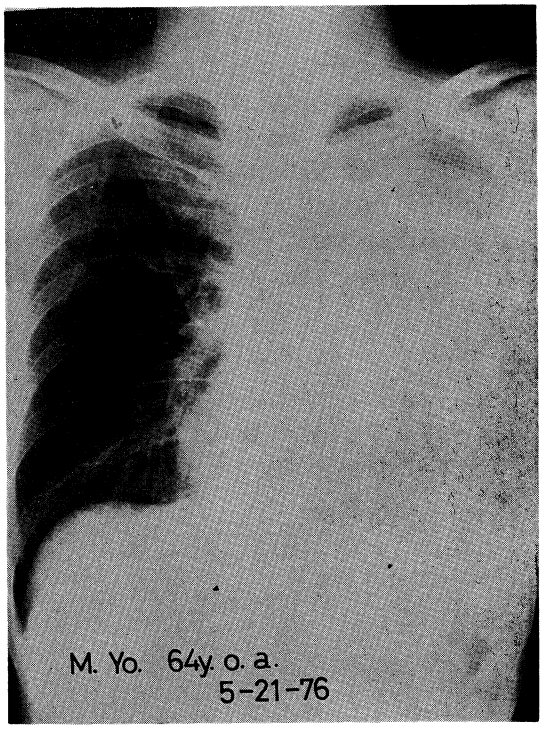


Fig. 3. Case 1 in Table 4. Posteroanterior roentgenogram reveals a pattern of collapse of the left lung.

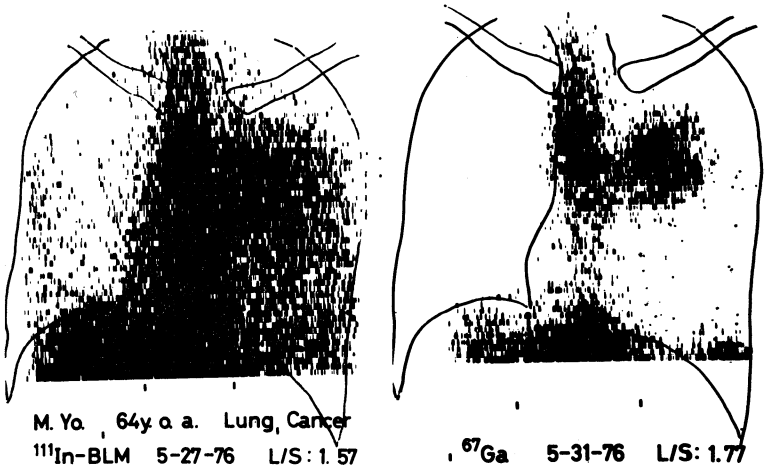


Fig. 4. $^{111}$In-BLM and $^{67}$Ga Scans of Case 1.
L/S: Ratio tumor concentration to normal lung region.

II. Clinical Study

A case of lung cancer is shown in Figs. 3 and 4. This is a case whose left main bronchus is occupied by tumor, showing atelectasis of the left lung, and both BLM and $^{67}$Ga display the tumor. However, $^{67}$Ga displays the tumor more distinctly and the ratio of radioactivity in the tumor to the surrounding normal tissue with $^{67}$Ga is greater by ROI. The summary of observation results of cases with lung cancer is illustrated in Table 4. It shows the accumulation of $^{67}$Ga to be greater than BLM.

TABLE 4

Comparison of $^{111}$In-Bleomycin with $^{67}$Ga Citrate in Lung Cancer

| Patient | Histology | Ratio Lesion to Normal | |
|---|---|---|---|
| | | $^{111}$In-BLM | $^{67}$Ga |
| M. Yo. | Epidermoid cell ca. | 1.57 | 1.77 |
| Y. Ku. | | 1.25 | 1.56 |
| H. Sa. | Metastatic (Salivary gland tumor) | 1.29 | 1.63 |
| N. Ko. | Epidermoid cell ca. | 1.33 | 2.20 |
| S. Ak. | Epidermoid cell ca. | 1.57 | 1.61 |
| H. In. | Epidermoid cell ca. | 1.21 | 1.61 |
| T. Ra. | | 1.32 | 2.15 |

## DISCUSSION

The labeling of Bleomycin with $^{99m}$Tc or $^{57}$Co has already been used for tumor diagnosis, and its tumor affinity has been recognized. However, in the case with $^{99m}$Tc-Bleomycin there are problems of the yield of labeling and the stability of labeling, and as for $^{57}$Co-Bleomycin there is a difficulty in its handling because of $^{57}$Co is a nuclide with a long half life. Moreover, both of these chemicals tend to accumulate more in necrotic tumor than in viable tumor[4].

Nonetheless, it seems to be a reasonable approach to make attempts at developing a tumorophilic radiopharmaceutical with Bleomycin taking advantage of its tumor affinity as it is to be used for the tumor diagnosis.

From the results of our attempts at improving several disadvantages

of $^{99m}$Tc and $^{57}$Co-Bleomycin with $^{111}$In-Bleomycin, we can point out that the latter is superior to the other two kinds of radionuclides.

One of the reasons is the fact that the physical property of $^{111}$In resembles closely to $^{67}$Ga and has less difficulty in handling than in the case with $^{57}$Co. The second is that differing from $^{99m}$Tc- and $^{57}$Co-Bleomycin[4], its accumulation in viable tumor is greater. This property is, needless to say, one of the essential requirements possessed to tumorophilic radiopharmaceuticals.

By the comparison with $^{111}InCl_3$ since the accumulation in tissue differs between the two,[8] in the case of $^{111}$In-Bleomycin it is difficult to imagine that $^{111}$In dissociates itself and conjugates with transferrin and ultimately behaves exactly like $^{111}InCl_3$. However, it is possible to presume that $^{111}$In may dissociate from $^{111}$In-Bleomycin even partially.

$^{67}$Ga is an excellent one among the tumorophilic pharmaceuticals so far reported, but from the present experimental results as well as from clinical results $^{111}$In-Bleomycin cannot be considered to surpass $^{67}$Ga. There is a report that $^{111}$In-Bleomycin is superior to $^{67}$Ga in the detection of abdominal tumors[7], but from our basic study it did not necessarily yield satisfactory results. Furthermore, we obtained a still lower level of $^{111}$In-Bleomycin accumulation in the inflammatory lesion than $^{67}$Ga, it cannot necessarily be taken as a merit of $^{111}$In-Bleomycin, because the agents with high tumor affinity would also deposit greater in the inflammation[4].

$^{111}$In-Bleomycin scintigraphy of lung cancer patients reflected well the results of basic study. Namely, the tumor display was good, but when compared with $^{67}$Ga, $^{111}$In-Bleomycin gererally presented a greater tissue background and a less ratio of tumor to surrounding lung tissue by ROI. For this reason it may be presumed that in the detection of mediastinal lesion it would be inferior to $^{67}$Ga.

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