# A Comparison of Scintigraphy with Tumor-seeking Radiopharmaceuticals to Detect an Experimental Bone Tumors in the Rabbits

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**ABSTRACT.** A comparative study on the accumulation of $^{99m}$Tc-phosphorous compound, $^{99m}$Tc-hexakis-2-methoxy isobutyl-isonitrile (MIBI), and $^{99m}$Tc-tetrofosmin (TF) in the experimental bone tumors using the VX-2 cell was performed. In the group of the femoral metastatic bone tumor, $^{99m}$Tc-MIBI showed no accumulation in the femur at 12 days after the transplantation despite the presence of a bone marrow tumor.

In the group of the iliac metastatic bone tumor, a bone scintigraphy showed decreased accumulation in the ileum at 16 days, but hot lesions were observed in same sites at 18 days after the transplantation on $^{99m}$Tc-MIBI and $^{99m}$Tc-TF scintigrams. The tumor to soft tissue accumulation ratio was higher for $^{99m}$Tc-MIBI ($3.03 \pm 1.03$) than for $^{99m}$Tc-TF ($2.55 \pm 0.80$) ($P < 0.05$).

This study showed that $^{99m}$Tc-MIBI is less satisfactory for the early diagnosis of tumors than bone scintigraphy, and a combined study with both $^{99m}$Tc-phosphorous compounds and $^{99m}$Tc-MIBI is useful for the evaluation and diagnosis of lesions.

**Key words : $^{99m}$Tc-MIBI — $^{99m}$Tc-HMDP — Bone tumor — VX-2 cancer**

Bone scintigraphy using the $^{99m}$Tc-phosphorous compound is widely recognized as an excellent method for the detection of bone metastasis.[1] However, the accumulation of the $^{99m}$Tc-phosphorous compound in bone lesions does not directly indicate the presence of tumor cells, but rather the accelerated bone formation following bone remodeling.[2] Therefore, bone scintigraphy frequently results in negative findings when tumor growth is not accompanied by bone formation.[3,4]

However, $^{99m}$Tc-hexakis-2-methoxy isobutyl-isonitrile ($^{99m}$Tc-MIBI), which was originally developed as a myocardial perfusion imaging agent, has been found to be a tumor-seeking radiopharmaceutical.[5]

In the present study, we evaluated the usefulness of bone and $^{99m}$Tc-MIBI scintigraphies for the detection of experimental bone tumors, consisting of VX-2 cancers implanted into the bone marrow of rabbits, and compared the effectiveness of $^{99m}$Tc-MIBI with $^{99m}$Tc-tetrofosmin (TF), another tumor seeking radiopharmaceutical.

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## MATERIALS AND METHODS

### Animal model

Albino rabbits weighing 2.5-3.5 kg were used as experimental animals.

### Transplantation

The VX-2 tumors, an epidermoid carcinoma derived from the Shope virus, were transplanted into the thigh muscles. After the tumors were excised from the rabbits and washed, a phosphate buffer containing penicillin 2,000 U/ml was added to the suspension. Then, the suspension was filtered through gauze, and the filtrated fluid was centrifuged. After the tumor cells were isolated and prepared in a 20% cell suspension, 0.1 ml of this cell suspension was transplanted into the iliac or femoral marrows.

### Detection of femoral tumor

Scintigraphy was done with the rabbits prone position. Bone-marrow scintigraphy with $^{99m}$Tc-phytate was carried out at 7 and 10 days after tumor cell transplantation into the femoral marrow or until the scintigraphy became positive. At this time, in addition to the bone marrow scintigraphy performed after 10 days, tumor imaging with $^{99m}$Tc-MIBI was started and followed up for two weeks. Both $^{99m}$Tc-phytate and $^{99m}$Tc-MIBI were administered in a dose of 148 MBq each into an ear vein. Using a scintillation camera equipped with a high-resolution collimator, a marrow scintigraphy with $^{99m}$Tc-phytate was done 30 min. after the injection, and a $^{99m}$Tc-MIBI scintigraphy was performed 15 min. later.

### Detection of iliac tumor

Bone scintigraphy with $^{99m}$Tc-hydroxymethylene diphosphonate (HMDP) was done at 14 and 17 days after the tumor cell transplantation into the iliac marrow, and until the scintigraphy became positive. At that time, tumor imaging with $^{99m}$Tc-MIBI or $^{99m}$Tc-TF was started and followed up for three weeks. Both $^{99m}$Tc-MIBI and $^{99m}$Tc-TF were administered into an ear vein in a dose of 148 MBq each, and scintigraphies were done 15 min. later.

### Bone radiography

Bone radiography was done with nonscreen films under the condition of 65 Kvp, 300 mA, and 0.3 sec.

### Data analysis

The regions of interest (ROIs) in both the iliac tumor and the contralateral side were set, and the tumor to soft tissue accumulation ratios of $^{99m}$Tc-MIBI and $^{99m}$Tc-TF were calculated.

These experiments were approved by the Animal Research Committee of Kawasaki Medical School (No. 01-013, 2001), and conducted according to the Guide for the Care and Use of Laboratory Animals of Kawasaki Medical School.

## RESULTS

VX-2 cell suspension was transplanted into the femoral marrows of five rabbits, and the tumor growth was followed with bone marrow scintigraphies with $^{99m}$Tc-phytate. The bone marrow scintigraphy changed to be positive, wich lesions showed a photon deficiency at 10 days after transplantation. On the other hand, the tumor scintigraphy with $^{99m}$Tc-MIBI showed no accumulation of the radionuclide in the femur at 12 days. At the same time, bone radiography showed no abnormality in the femur (Table 1, Fig 1).

VX-2 cell suspension was transplanted into the marrow of the iliac crest of five rabbits, and the tumor growth was followed with bone scintigraphy with $^{99m}$Tc-HMDP. The bone scintigraphy changed to be positive, which lesions showed the decreased accumulation of the radionuclide at an average of 16 days after transplantation. On the other hand, the tumor scintigraphies with $^{99m}$Tc-MIBI and $^{99m}$Tc-TF showed the increased accumulation of the radionuclide in the ilium at an average of 18 days. At the same time, bone radiography showed osteolytic change in the ileum (Fig 2). The tumor to soft tissue accumulation ratio was higher for $^{99m}$Tc-MIBI

TABLE 1. Findings of bone marrow, $^{99m}$Tc-MIBI and bone scintigraphies in rabbits after transplantation of VX-2 cell suspension in the femoral marrow

| | Bone marrow | | $^{99m}$Tc-MIBI | | Bone | |
|---|---|---|---|---|---|---|
| | day | findings | day | findings | day | findings |
| 1 | 10 | defect | 12 | no accumulation | 14 | decreased accumulation |
| 2 | 10 | defect | 12 | no accumulation | 14 | decreased accumulation |
| 3 | 10 | defect | 12 | no accumulation | 14 | decreased accumulation |
| 4 | 10 | defect | 12 | no accumulation | 14 | decreased accumulation |
| 5 | 10 | defect | 12 | no accumulation | 14 | decreased accumulation |

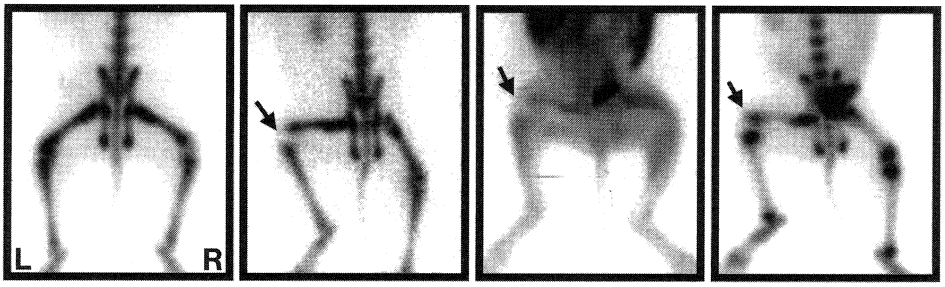


Fig 1. a. Bone marrow scintigraphy at 7 days after transplantation. No abnormality.
b. Bone marrow scintigraphy at 10 days after transplantation. Decreased accumulation in the left femur.
c. $^{99m}$Tc-MIBI scintigraphy 12 days after transplantation. No hot area in the left femur.
d. Bone scintigraphy at 14 days after transplantation. Decreased accumulation in the left femur.

(3.03±1.03) than for $^{99m}$Tc-TF(2.55±0.80) ($P<0.05$) (Table 2).

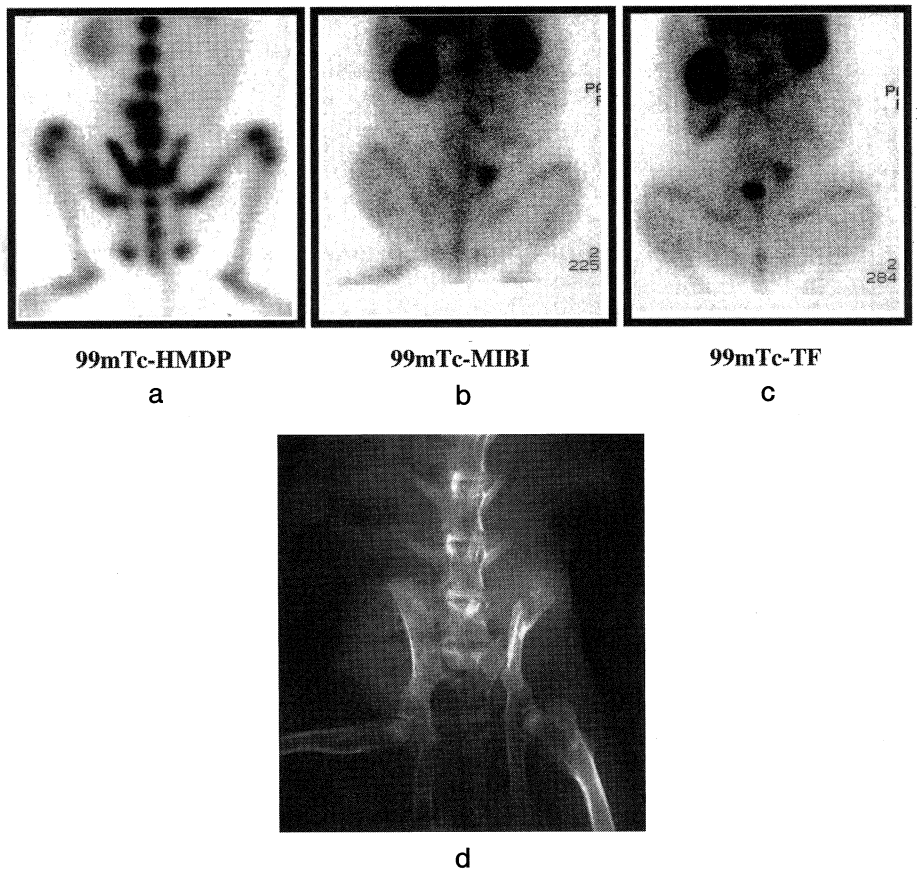


Fig 2. Case No. 7 in Table 2.
a. Decreased accumulation of $^{99m}$Tc-HMDP in the right ileum.
b. c. Tumor scintigraphies with $^{99m}$Tc-MIBI (b) and $^{99m}$Tc-TF (c) showed increased accumulation in the right ileum.
d. Bone radiography showed osteolytic change in the right ileum.

TABLE 2. Comparison of $^{99m}$Tc-MIBI and $^{99m}$Tc-TF scintigraphies in tumor to soft tissue uptake ratio in rabbits after transplantation of VX-2 in the iliac marrow

| | Bone | | Tumor | | |
|---|---|---|---|---|---|
| | day | findings | day | MIBI | TF |
| 6 | 17 | decreased accumulation | 19 | 2.90 | 2.88 |
| 7 | 17 | decreased accumulation | 19 | 3.52 | 2.95 |
| 8 | 17 | decreased accumulation | 19 | 4.50 | 3.60 |
| 9 | 14 | decreased accumulation | 16 | 1.84 | 1.60 |
| 10 | 14 | decreased accumulation | 16 | 2.41 | 1.74 |
| | 15.8 | | 17.8 | 3.03 | 2.55 |
| | ±1.6 | | ±1.6 | ±1.03 | ±0.80 |

## DISCUSSION

A diagnosis of metastatic bone tumor by bone scintigraphy greatly facilitates an early diagnosis. However, $^{99m}$Tc-phosphorous compounds accumulate specifically not in malignant bone tumors but in areas of rapid bone formation, which is part of the process of repair of bone destruction due directly to the tumor or indirectly to the activation of osteoclasts.[6] When bone formation occurs, resulting later in osteosclerotic changes in a metastatic lesion on skeletal radiography, there is strong accumulation of the radionuclide. However, when there is only minimal bone formation in malignant tumors such as in hepatocellular carcinoma[7] or renal cancer,[4] little accumulation of the radionuclide is usually evident on bone scintigraphy, even though tumor cells are present. This results in an appearance of osteolytic change on skeletal radiography. Thus, normal or photon deficient images are often obtained on bone scintigraphies.[8] Therefore, bone scintigraphy alone is not a reliable technique for the detection and the diagnosis of metastasis. In recent years, $^{99m}$Tc-MIBI, developed as a drug for myocardial perfusion imaging, has shown the increased accumulation in tumors, and has been applied to tumor imaging.[9] We evaluated the usefulness of bone and $^{99m}$Tc-MIBI scintigraphies for the detection of experimental bone tumors.

Bone metastasis is usually initiated via the arterial system, and the primary foci are formed in the marrow. Therefore, the early diagnosis of tumor metastasis requires the detection of bone marrow metastasis. Wakasugi *et al*[10] pointed out that bone marrow scintigraphy with $^{99m}$Tc-MIBI is a useful method for the diagnosis of metastasis, because bone marrow uptake of $^{99m}$Tc-MIBI is significantly higher than that of a conventional bone tracer. The results of our experiments with intramedullary implantation of VX-2 revealed that $^{99m}$Tc-MIBI scintigraphies presented a negative image in bone marrow tumor when the bone marrow scintigraphy showed abnormality. Although, when bone destruction was confirmed by soft X-ray, the scintigraphy became positive for $^{99m}$Tc-MIBI.

This study showed that the usefulness of $^{99m}$Tc-MIBI scintigraphy for the early diagnosis of bone tumor is less satisfactory than that of a bone marrow scintigraphy with $^{99m}$Tc-phytate. However, bone marrow imaging is not always applicable to the marrow of the whole body because the majority of radioactive colloids accumulates in the liver.[11] Results obtained from our clinical study of bone metastasis from thyroid cancer showed that the $^{99m}$Tc-MIBI scintigraphy is positive even though bone scintigraphy was negative.[12] On the basis of these experimental and clinical results, it is reasonable to conclude that $^{99m}$Tc-MIBI is deposited in the tumor itself rather than in the bone invaded in the case of osteolytic change. $^{99m}$Tc-MIBI and $^{99m}$Tc-TF were developed as myocardial perfusion imaging agents. With this in mind, we carried out a comparative study of $^{99m}$Tc-MIBI and $^{99m}$Tc-TF as tumor-seeking radiopharmaceuticals in VX-2 tumor-bearing rabbits. As a result, it was found that the accumulation of $^{99m}$Tc-MIBI in the bone tumor was more significant than that of $^{99m}$Tc-TF. It is considered that the accumulation of $^{99m}$Tc-MIBI depends not only on the metabolic activity of the tumor tissue but also on mitochondria content

and such factors as blood flow and enhanced vascular permeability. $^{99m}$Tc-MIBI accumulates in cells, and is distributed in cytoplasm, primarily mitochondria, depending upon the potential inside and outside the cell or mitochondrial membrane. Reflecting the highly active metabolic activity in malignant tumors, the potential differences between mitochondria membrane might be high, and this may be the cause of the increased accumulation of $^{99m}$Tc-MIBI in tumors.[13-15]

In conclusion, this study showed that it is necessary to pay careful attention to photon-deficient areas in the detection of bone metastasis when using bone scintigraphy because these could be areas of tumor cells. In addition, $^{99m}$Tc-MIBI is less satisfactory for the early diagnosis of tumors than bone scintigraphy, and a combined study with both $^{99m}$Tc-phosphorous compounds and $^{99m}$Tc-MIBI is useful for the evaluation and diagnosis of lesions.

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