Enhancement of Skeletal Uptake of Bone-seeking Agents in Rat Models by Dietary Manipulation

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Summary

The use of bone-seeking agents, such as the calcium analog, Sr-89 chloride, or the radiolabeled phosphate compound, Sm-158 EDTMP provides effective means of dilivering systemic radiation therapy for osseous metastases. Bone marrow suppression, however, remains a main side effect. Enhancement of skeletal uptake of these bone-seeking agents is, therefore, required.

Three rat models with different bone metabolism were established by means of dietary manipulation ; normal control (C), low-calcium group (A), and low-phosphate group (B). Ca-47 chloride and Tc-99 m MDP were injected at the second week and 24-h global skeletal uptake (GSU) was measured. Rats were, then, sacrificed. Each femur was weighed and counted for Ca-47. The values for 24-h GSU (%) of Tc-99 m MDP were as follows ; C group : 51.7±2.7, A group : 49.6±3.5, B group : 69.3±5.6 ($p<0.01$). The values for 24-h GSU (%) of Ca-47 chloride were as follows ; C group : 80.2±2.7, A group : 93.7±3.1 ($p<0.01$), B group : 72.3±4.3 ($p<0.01$). The values for femoral uptake (% dose/g) of Ca-47 were as follows : C group : 3.76±0.33, A group : 4.27±0.15 ($p<0.01$), B group : 3.09±0.18 ($p<0.01$). In conclusion, a 34.0% increase in GSU of Tc-99 m MDP in the low-phosphate group and a 16.3% increase in GSU of Ca-47 in the low-calcium group were observed. Enhancement of skeletal uptake of Tc-99 m MDP and Ca-47 chloride was achived by the dietary manipulation.

Introduction

Bone metastases are common in patients with breast and prostate cancer[1)2)]. Once these cancers have involved in bony tissues, the management of intractable bone pain and improving the quality of life is often the primary management problem facing the physician. The use of bone-seeking agents, such as calcium analog, strontium-89 (Sr-89)[3)] or the phosphate compounds labeled with rhenium-186 (Re-186 HEDP)[4)] or samarium-153 (Sm-153 EDTMP)[5)] provides effective means of dilivering systemic radiation therapy for osseous metastases. Bone marrow suppression, however, remains a main side effect in clinical trials. Enhancement of skeletal uptake of these bone-seeking agents is, therefore, required to provide effective irradiation to the osseous metastases.

The purpose of this study is to enhance the

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skeletal uptake of bone-seeking agents in rat models by dietary manipulation.

Material and Methods

Animal preparations

Eight-week old Wistar male rats (n=18) were housed in a dark room for two weeks. Three models with different bone metabolism was established by means of dietary manipulation ; normal control (C), low-calcium diet group (A), and low-phosphate diet group (B). Low-phosphate diet group (B) was feeded by a vitamin D_3-free diet, which contained 75.5% ground corn meal, 20% wheat gluten, 3.5% $CaCO_3$, and 1% NaCl. Low-calcium diet group (A) was feeded by the same diet, but with a low level of calcium and a high level of phosphate (0.018% $CaCO_3$, 1.5% equimolar (K_2HPO_4). Supplementary vitamin D_3 (50,000 IU) was added to 100 g of the diet to prevent the concomitant development of rickets. A control group (C) was created by feeding the same rachitogenic diet, but with addition of vitamin D_3 as described above.

Radiopharmaceuticals

Approximately 10 μCi of Ca-47 chloride (Amersham, U.K.) instead of Sr-89 chloride was injected into the tail vein of the rats at the 2 nd week, and 100 μCi of Tc-99 m MDP (Dai-ichi Radioisotope Lab., Japan) was also used instead of Sm-153 EDTMP.

In vivo counting

Early (5-min) and late (24-h) quantitative *in vivo* counting was performed using a thyroid uptake probe without collimator[6]. A rat in a plastic immobilization device was placed 40 cm beneath the detector, and the 24-h global skeletal uptake (GSU) of the bone-seeking agents was calculated for Tc-99 m MDP and Ca-47 chloride, respectively by taking the 5-min count as 100% and corrected for radioactive decay.

In vitro counting

Rats were sacrificed at the 2 nd week after the measurement of 24-h GSU of Ca-47. Each distal femur was dissected from the surrounding soft-tissues, weighed and counted in a well-type scintillation counter for the percentage dose of radioactivity per gram of bone tissue.

Results

Bone scintigram and radiograph

A whole-body scintigram of rat was taken 24-h after injection of Tc-99 m MDP. Most of the radiotracer was observed in the skeleton (Fig. 1). 24-h whole-body retention of the radiotracer, therefore, represents global skeletal uptake.

Bone radiographs of femurs in each group were taken at the 2 nd week. No significant difference was observed among femurs in three groups (Fig. 2).

Global skeletal uptake (GSU) and femoral uptake

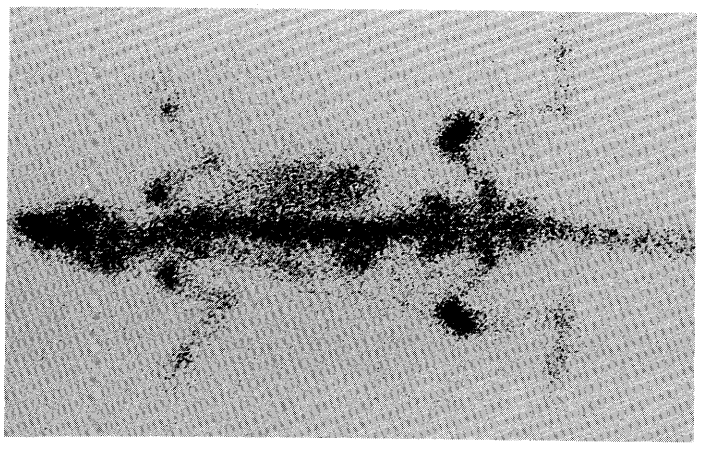

Fig. 1 A whole-body scintigram of rat with Tc-99 m MDP at 24-h after injection reveals most of the tracer in the skeleton.

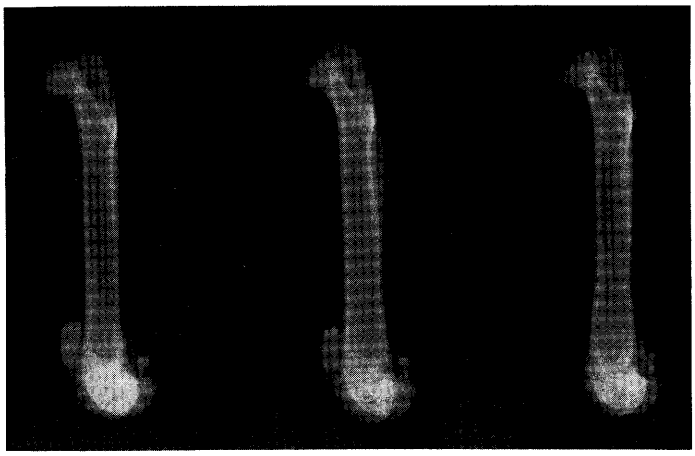

Fig. 2 Bone radiograph of femurs in three groups of rat shows no significant difference.
Left : control (C),
Middle : low-calcium (A),
Right : low-phosphate (B)

Table 1 24-h global skeletal uptake of radiopharmaceuticals in three groups of rats (%)

Pharmaceutical	Control (C) (n=5)	Low-calcium (A) (n=5)	Low-phosphate (B) (n=5)
Ca-47 chloride	80.2±2.2	93.7±3.1*	72.3±4.3*
Tc-99m MDP	51.7±2.7	49.6±3.5	69.3±5.6*

*$p<0.01$

Table 2 24-h percent femoral uptake of Ca-47 in three groups of rats (% dose/g)

Pharmaceutical	Control (C) (n=5)	Low-calcium (A) (n=5)	Low-phosphate (B) (n=5)
Ca-47 chloride	3.76±0.33	4.27±0.15*	3.09±0.18

*$p<0.01$

The values for 24-h GSU of Tc-99 m MDP and Ca-47 chloride were shown in Table 1. 24-h GSU (%) of Tc-99 m MDP in each group was as follows ; C group : 51.7±2.7, A group : 49.6±3.5, B group : 69.3±5.6 ($p<0.01$). 24-h GSU (%) of Ca-47 chloride was as follows ; C group : 80.2±2.7, A group : 93.7±3.1 ($p<0.01$), B group : 72.3±4.3 ($p<0.01$). The values for femoral uptake of Ca-47 chloride were shown in Table 2. Femoral uptake (% dose/g) of Ca-47 in each group was as follows ; C group : 3.76±0.33, A group : 4.27±0.15 ($p<0.01$), B group : 3.09±0.18. There are a 34.0% increase in GSU of Tc-99 m MDP in the low-phosphate diet (B) group and a 16.3% increase in GSU of Ca-47 in the low-calcium diet (A) group compared to those of normal control (C) group.

Discussion

In internal radiation therapy with bone-seeking agents for osseous metastases, phosphorus-32 (P-32) has been used as systemic radiation therapy for the management of bone pain[7)8)]. However, significant bone marrow suppression prevents us from its routine clinical use. Recently, the bone-seeking radiopharmaceuticals Sr-89 chloride, Sm-153 EDTMP, and Re-186 HEDP have been used as palliative treatment for patients with painful osseous metastases[3)~5)]. Excellent responses with acceptable hematological toxity in the clinical trials were observed.

To achive more effective irradiation to the osseous metastases, enhancement of skeletal uptake of these bone-seeking agents is required. Until now, androgen[9)] and PTH[10)] have been used with radiophophorus to reduce bone pain in can-

cer patients. However, no good response was observed at that time. In our study of rat models. We used two-week special dietary manipulation to enhance skeletal uptake of bone-seeking agents such as Tc-99 m MDP and Ca-47 chloride. A 34.0% increase of global skeletal uptake (GSU) of Tc-99 m MDP in the low phosphate group and a 16.3% increase in GSU of Ca-47 in the low-calcium group were observed as compared to those of control group.

This dietary manipulation is simple and safe to achive a significant increase in skeletal uptake of bone-seeking agents. Our method, therefore, might be applicable for both Sr-89 chloride or phosphate compounds labeled with Sm-153 or the Re-186 therapy in clinical studies.

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