DYNAMIC STUDIES WITH ^{99m}Tc-HEDP IN NORMAL SUBJECTS AND IN PATIENTS WITH BONE TUMORS

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Blood clearance, urinary excretion, and spinal uptake of ^{99m}Tc-HEDP have been measured in ten normal control subjects. Blood clearance from 15 min is biexponential. Approximately 70% of the administered activity was excreted in the urine within 6 hr of injection. Spine-to-background ratios rise sequentially with time. Net normal bone uptake rises little after 2 hr and improved visualization of the normal skeleton thereafter is due to reduction of blood background activity. In 13 patients with bone tumors, direct measurement of diphosphonate uptake by the tumor-involved and corresponding normal bone showed a continued rise in diphosphonate levels in the tumor-involved bone due to increased metabolic activity.

Four different ^{99m}Tc-labeled phosphates have now been described and evaluated as bone-scanning radiopharmaceuticals; long chain polyphosphates (*1*), pyrophosphate (*2*), diphosphonate (*3–5*), and monofluorophosphate (*6*). The diphosphonates are the most suitable of these compounds, combining a high degree of chemical stability (*7*) with the most favorable biologic properties (*6,8*). Ethane-hydroxy-diphosphonate (HEDP) is the most widely available diphosphonate and we have studied in detail its blood clearance, urinary excretion, and uptake by normal bone in healthy young male subjects. We have also directly measured serial uptake of ^{99m}Tc-HEDP by tumor-involved bone in patients with known bony tumors.

MATERIALS AND METHODS

Blood clearance and urinary excretion of ^{99m}Tc-labeled HEDP were measured in ten healthy young male volunteer subjects (age 22–25) with no evidence of bone disease. Each subject was given 5 mCi of ^{99m}Tc-labeled HEDP by intravenous injection. Whole-blood radioactivity levels were measured at 15-min intervals for 1 hr, and hourly thereafter until 6 hr after injection; 5-ml blood samples were collected by venipuncture, and whole-blood radioactivity was measured in a well scintillation counter. A standard sample obtained from the injected material was also counted. Results were expressed as a percentage of injected activity per liter of whole blood.

Before injection, the subject emptied his bladder. The voided urine was discarded. Urine was collected at regular intervals during the 6-hr study period. The radioactivity in each sample was later counted in a bulk sample scintillation counter and in this way the cumulative urinary excretion from 0 to 6 hr after injection was derived.

Serial uptake of diphosphonate in the lower dorsal spine was measured using a scintillation camera interfaced to a multi-channel analyzer (*9*). A profile was drawn transversely across the scintillation camera field of view just above the kidneys to include the lower dorsal spine and surrounding area (Fig. 1). The gross counts from the region of interest corresponding to the spine and the gross counts from the same number of channels in the surrounding soft-tissue background were calculated from the teletype printout of the multi-channel analyzer. Counts from the two areas were recorded serially from 15 min until 6 hr after injection and their ratio (spine-to-background ratio) was calculated for each time of study.

In the second related study serial bone uptake of ^{99m}Tc-diphosphonate was recorded in 13 patients with known bone tumors. There were 11 women and 2 men (age 34–72). Ten patients had bone metastases (nine breast, one prostate, one lung, and one occult primary) and the other patient had a Ewing's sarcoma of pelvis. In all but one patient the

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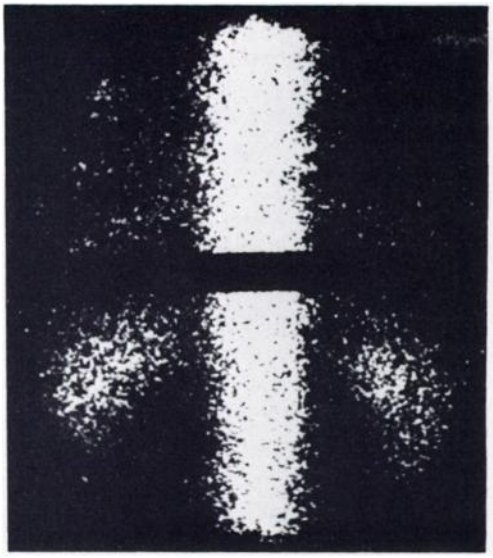

FIG. 1. Control study: scintigram of lower dorsal and lumbar spine. Horizontal strip shows region of interest for activity profile.

TABLE 1. WHOLE BLOOD AND URINARY LEVELS OF ^{99m}Tc-HEDP

Time	Blood activity* (% injected activity/liter of whole blood)	Cumulative urinary excretion* (% of injected activity)
5 min	7.22 ± 0.64	—
15 min	4.74 ± 0.26	13.0 ± 1.13
30 min	3.66 ± 0.20	21.9 ± 1.42
45 min	2.74 ± 0.24	29.2 ± 1.80
1 hr	2.36 ± 0.20	34.5 ± 1.98
2 hr	1.38 ± 0.12	48.1 ± 2.58
3 hr	0.99 ± 0.10	55.8 ± 2.96
4 hr	0.73 ± 0.06	60.1 ± 3.25
5 hr	0.59 ± 0.04	68.4 ± 1.65
6 hr	0.43 ± 0.03	70.6 ± 1.80

* Mean ± s.e.m. (mean of ten studies).

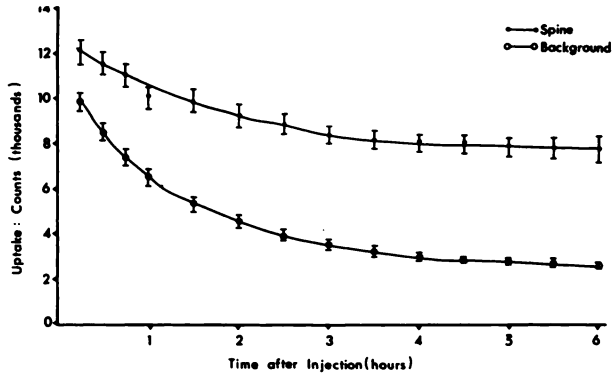

FIG. 2. Gross counts from spine and background (mean ± s.e.m.) in ten normal control subjects.

tumor-involved areas studied were radiologically visible. In the remaining patient (carcinoma of breast) a previous scan had demonstrated a lesion in the lumbar spine and confirmation from postmortem examination was obtained within 2 months of the present study.

Scintillation camera scintigrams were obtained serially from 30 min until 4 hr after the intravenous injection of 15 mCi of ^{99m}Tc-labeled HEDP. By means of the scintillation camera multi-channel analyzer system, we quantitatively measured serial ^{99m}Tc-diphosphonate activity from 30 min until 4 hr after injection in 24 pathologic areas of the skeleton and 24 corresponding areas of normal bone in the 13 patients studied. The ratio between the gross counts from the tumor-involved bone and those from the corresponding normal bone areas (tumor-to-bone ratios) were calculated for all times of study.

RESULTS

Blood clearance. Initial clearance of ^{99m}Tc-HEDP from the blood was extremely fast; the whole blood levels fell to 7.22 ± 0.64%/liter of the injected activity at 5 min after injection (Table 1). From 15 min until the end of the 6-hr period of study the blood clearance is well described by a biexponential function, A(t) (percentage of injected activity per liter) $= 4.36e^{-0.032t} + 2.19e^{-0.0045t}$, where the decay constants are in minutes^{-1} and correspond to half-lives of 21.8 and 155 min, respectively. The parameters were obtained from an iterative least-squares fit to the biexponential expression itself and the correlation coefficient between observed results and those calculated from the biexponential expression is 0.9996 with a regression coefficient of 0.9995.

Urinary excretion. Six hours after injection a total of 70.6 ± 1.8% (mean ± s.e.m.) of the injected activity had been excreted in the urine (Table 1).

Normal bone utpake. In the control subjects, when the total number of counts (corrected for the physical decay of the nuclide) from the regions of the profile corresponding to the spine and to the soft-tissue background were plotted separately against time, a regular fall in activity was observed in both areas. The gross activity in the spine area fell more slowly than that in the nonspine background area (Fig. 2). Because of this, the spine-to-background activity ratio rose continuously throughout the 6 hr of the study (Fig. 3).

The gross activity recorded from the spine included activity in the nonosseous tissue in the area under study. If it is assumed that the soft-tissue background across the profile is uniform, then subtraction of this background activity from the gross counts measured in the spine area gives a measure

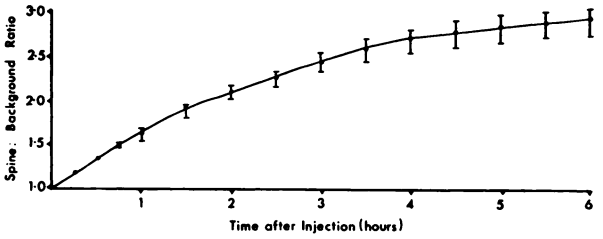

FIG. 3. Spine-to-background ratios (mean ± s.e.m.) in ten normal control subjects.

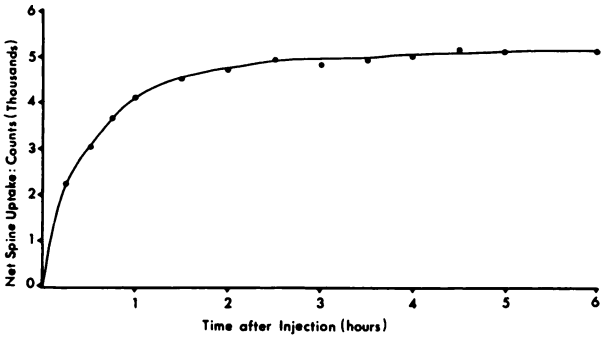

FIG. 4. Net spine uptake (mean) in ten normal control subjects.

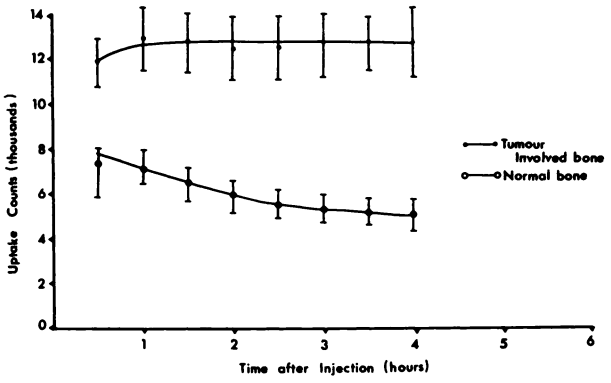

FIG. 5. Gross counts from tumor-involved and corresponding normal bone (mean ± s.e.m.) in 24 tumors in 13 patients.

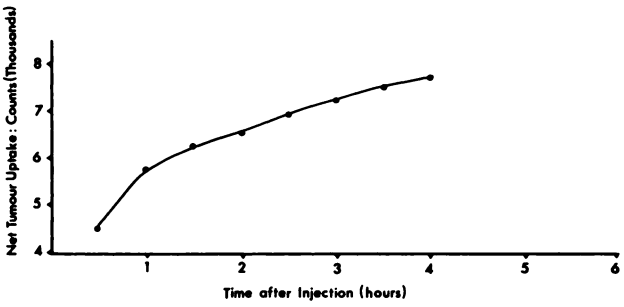

FIG. 6. Net tumor uptake (mean) in 24 tumors in 13 patients.

of net uptake by the spine. Net spine uptake rose rapidly and by 60 min had attained 80% of its value at 6 hr, when the study was terminated (Fig. 4). This dynamic uptake can be described by a biexponential function ($T_{1/2}$ of Exponent I = 11.8 min, $T_{1/2}$ of Exponent II = 53.0 min).

Uptake by tumor-involved bone. When the gross counts from the scintillation camera image of the tumor-involved bone were plotted against time (after correcting for the physical decay of the nuclide) a rapid rise was noted for the first hour of the study, after which a constant level was maintained throughout the remainder of the 4-hr study. In the corresponding normal bone there was a regular decrease in gross activity, similar to that observed in the spine of the control subjects (Fig. 5). The gross counts recorded from the tumor-involved bone and corresponding normal bone were obtained from equal areas of the skeleton. A measure of the increased uptake of diphosphonate in tumor-involved areas due to the tumor involvement can be obtained by subtracting the two sets of data (i.e., net increase in diphosphonate uptake in tumor-involved area A is gross recorded counts in A minus gross recorded counts in area of normal bone corresponding to area A). To simplify the nomenclature, we have called this increase in activity due to tumor involvement "net tumor uptake." Net tumor uptake rose throughout the 4 hr of the study (Fig. 6) in contrast to the net normal bone uptake in the control subjects, which reached a maximum value within 120 min of injection.

Because of the continued increase in activity in the tumor-involved bone and the regular decrease in gross activity in the normal bone, the tumor-to-bone ratio rose steadily throughout the 4 hr of the study (Fig. 7).

DISCUSSION

The blood kinetic data for HEDP reported here are very similar to those collected by other workers using diphosphonate obtained from different sources (*10,11*). The compound clearly provides reliable, highly reproducible results. The biexponential blood clearance is thought to represent early rapid clearance into bone followed by a slower renal excretion (*10*). Although this statement is generally true, part of the rapid clearance of diphosphonate from the blood is also due to renal excretion; within 30 min of intravenous injection over 20% of the administered dose was excreted in the urine. In our control subjects (healthy young men with normal renal function), 70% of the administered activity was excreted within 6 hr without the use of diuretics or a water load.

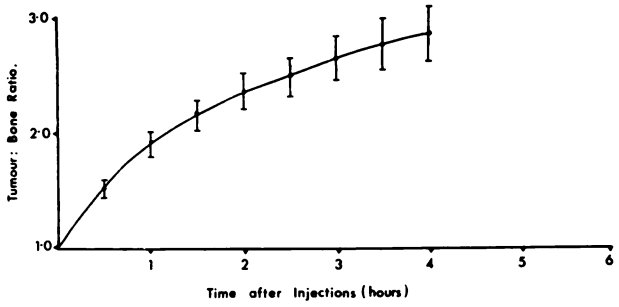

FIG. 7. Tumor-to-bone ratios (mean ± s.e.m.) for 24 tumors in 13 patients (^{99m}Tc-HEDP).

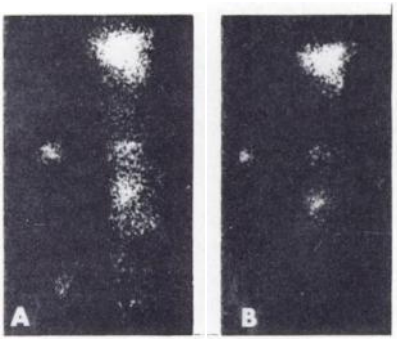

FIG. 8. Metastases in dorsal spine and ribs. (A) 90 min and (B) 120 min after injection of ^{99m}Tc-HEDP.

The rise in the observed spine-to-background ratio with time confirms what is apparent visually; the longer the delay between injection and scanning, the greater the improvement in scan quality. This increase in spine-to-background ratio with time is due to two factors: a continuous rise in bone uptake of diphosphonate and a continuous decrease in soft-tissue background activity due to renal excretion of the diphosphonate not taken up by bone. The second of these factors is by far the most important. This is in accord with our observation that HEDP, which has the fastest blood clearance of the ^{99m}Tc-phosphates (*8*), provides the highest spine-to-background ratios, although its net bone uptake is no higher than that of the alternative phosphate compounds (*12*).

A normal bone uptake pattern similar to that previously described has been observed in rabbits. There was no significant difference between bone levels of ^{99m}Tc-HEDP at 1 hr and 4 hr after injection (*4*). In the same study, absolute skeletal uptake of ^{99m}Tc-HEDP was no higher than that of ^{85}Sr, the higher target-to-background ratios observed being due entirely to more rapid excretion of the diphosphonate (*4*).

The gross activity recorded from the tumor-involved bone includes a proportion of activity in the blood and soft tissues within the image of the "hot spot." Gross recorded activity remains constant because the activity lost due to decreasing blood levels is compensated by the continuous uptake in the tumor-involved bone. In both normal and abnormal bone the rapid rise in activity represents rapid clearance from blood, and the absence of any short-term fall in net activity as the blood activity drops rapidly is in accord with the concept of chemisorption of a stable, nonhydrolysable compound (*11*). In normal bone, net activity increases little after 120 min (by which time only 6% of the injected activity remains in the blood). This observation is in accord with the recent suggestion that blood flow may be more important than metabolic activity in determining diphosphonate uptake in normal bone (*13*). By contrast, the net uptake in tumor-involved bone continues to increase despite falling blood levels, and this is presumably a consequence of the increased metabolic activity of the tumor-involved bone.

The tumor-to-bone ratio as defined is of practical value as a direct quantitative expression of the contrast between tumor-involved and normal bone in the scintillation camera image. Clearly the higher the tumor-to-bone ratio the easier is the detection of the tumor on inspection of the image. Tumor-to-bone ratios were higher 4 hr after injection than at any earlier time during the study although ratios obtained within 120 min of injection were generally high enough to allow clear visualization of most tumors (Fig. 8).

There are two important practical considerations that follow from the work described here; first, the demonstration that rapid blood clearance of radiopharmaceutical is more important than absolute bone uptake is relevant to the development of new bone-scanning techniques and radiopharmaceuticals. It is possible in this context that methylene-diphosphonate, which apparently has a marginally more rapid blood clearance than ethane-hydroxy-diphosphonate, will prove to be a superior agent (*14*). Second, the measurement of a quantitative difference between the dynamic uptake of diphosphonate by normal and tumor-involved bone suggests that the measurement of dynamic uptake of diphosphonate by a bone tumor, when compared with the uptake of normal bone, may provide information regarding the biologic activity of the tumor and may be used as a method of assessing therapeutic response.

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REFERENCES

1. SUBRAMANIAN G, MCAFEE JG, BELL EG, et al: ^{99m}Tc-labeled Polyphosphate as a skeletal imaging agent. *Radiology* 102: 701–704, 1972

2. PEREZ R, COHEN Y, HENRY R, et al: A new radiopharmaceutical for ^{99m}Tc bone scanning. *J Nucl Med* 13: 788–789, 1972

3. CASTRONOVO FP, CALLAHAN RJ: New bone scanning agent: ^{99m}Tc-labeled 1-hydroxy-ethylidene-1, 1-disodium phosphonate. *J Nucl Med* 13: 823–827, 1972

4. SUBRAMANIAN G, MCAFEE JG, BLAIR RJ, et al: ^{99m}Tc-EHDP: a potential radiopharmaceutical for skeletal imaging. *J Nucl Med* 13: 947–950, 1972

5. YANO Y, MCRAE J, VAN DYKE DC, et al: Technetium-99m-labeled stannous ethane-1-hydroxy-1 1-diphosphonate: a new bone scanning agent. *J Nucl Med* 14: 73–78, 1973

6. CITRIN DL, BESSENT RG, GREIG WR: Clinical evaluation of ^{99m}Tc-labeled monofluorophosphate: a comparison with ethane hydroxy diphosphonate. *J Nucl Med* 15: 1110–1112, 1974

7. DUNSON GL, STEVENSON JS, COLE CM, et al: Preparation and comparison of technetium-99m diphosphonate, polyphosphate and pyrophosphate nuclear bone imaging radiopharmaceuticals. *Drug Intell Clin Pharm* 7: 470–474, 1973

8. CITRIN DL, BESSENT RG, TUOHY JB, et al: A comparison of phosphate bone scanning agents in normal subjects and patients with malignant disease. *Br J Radiol* 48: 118–121, 1975

9. CITRIN DL, BESSENT RG, TUOHY JB, et al: Quantitative bone scanning: a method for assessing response of bone metastases to treatment. *Lancet* 1: 1132–1133, 1974

10. KRISHNAMURTHY GT, TUBIS M, ENDOW JS, et al: Clinical comparison of the kinetics of ^{99m}Tc-labeled polyphosphate and diphosphonate. *J Nucl Med* 15: 848–855, 1974

11. BERG G, CASTRONOVO F, MCKUSICK K, et al: Normal criteria for early bone dynamics of ^{99m}Tc-diphosphonate. *J Nucl Med* 15: 477–478, 1974

12. CITRIN DL, BESSENT RG, GREIG WR, et al: Pharmacological studies with ^{99m}Tc labelled Polyphosphate, Pyrophosphate and E.H.D.P. in patients and normal subjects. Presented at the Annual Meeting of the British Nuclear Medicine Society, London, March 1974

13. GENANT HK, SINGH M, BAUTOVICH GJ, et al: Bone-seeking radionuclides: an in vivo study of factors affecting their uptake. *Radiology* 113: 373–382, 1975

14. SUBRAMANIAN G, BLAIR RJ, MCAFEE JG, et al: ^{99m}Tc-labeled methylene diphosphonate: a superior agent for skeletal imaging. Presented at the First World Congress of Nuclear Medicine, Tokyo, October 1974