Quantitative Scanning of Osteogenic Sarcoma with Nitrogen-13-Labeled L-Glutamate

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N-13 L glutamate was used to image an osteogenic sarcoma in a 9-year-old patient. Serial quantitative measurements of the amount of N-13 taken up by the primary tumor showed a decrease of 40% after 10 wk of chemotherapy. Blood-clearance data obtained from normal subjects indicate that more than 90% of the N-13 activity had left the blood before scanning of the tumor was begun. It appears that the N-13 label concentrated in the soft-tissue portion of this osteogenic sarcoma, whereas Tc-99m diphosphonate uptake was greatest in the regions where calcification was occurring.

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As part of a program of study of the metabolism of biologically significant compounds labeled with cyclotron-produced radionuclides, we have been synthesizing and evaluating nitrogen-13 L-amino acids as tumor-scanning agents. Measurements of selective uptake of nitrogen-13 as L-glutamate and L-glutamine in spontaneous canine tumors (*1,2*) have preceded clinical studies with these compounds. To make the product suitable for human studies, the enzymatic labeling (*3*) of N-13-L glutamate has been modified by immobilizing glutamate dehydrogenase on a solid support of CNBr-activated Sepharose (*4*), and this compound has been regularly produced in our laboratory in large quantities (>100 mCi). Nitrogen-13 is a positron emitter (half-life 10 min); the associated annihilation radiation (0.511 MeV) is readily detected externally so that its distribution in a patient can be determined on an in vivo basis.

The purpose of this note is to describe the use of N-13 L-glutamate for the imaging of osteogenic sarcoma in a 9-year-old patient. Our results suggest that this agent may be useful for the detection of this and other types of cancer and for evaluating the effects of therapy.

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MATERIALS AND METHODS

Approximately 5 mCi of N-13 L-glutamate were administered intravenously to a 9-year-old boy with a histologically proven osteogenic sarcoma in the right distal femur. Quantitative scanning studies were made with this compound before and after he was given a 2 mo course of high-dose methotrexate therapy (*5*). Both studies were performed on our High Energy Gamma (HEG) dual-headed rectilinear scanner, which is equipped with constant-response collimators and digital electronics for computer analysis (*6*). Scanning of the subject's lower extremities was begun 5 min after injection; each scan required approximately 15 min to complete. The digital scan data were analyzed as follows. The operator obtained alphameric printouts of the image matrix, which had been corrected for natural background and the physical decay of the N-13 label. From these printouts, a rectangular region of interest enclosing the entire tumor area was selected, and a boundary-detecting algorithm was applied to

the region to obtain the tumor's silhouette. A second algorithm was used to obtain the total count rate within the silhouette; this quantity was compared with the count rate in a phantom source of similar size, which contained a known amount of activity. In this manner an estimate of the amount of N-13 activity in the tumor was obtained. Because subsequent calculations showed that the activity contained within the tumor's blood volume was small, no correction was made for radioactivity in the blood circulating through the tumor. An approximate attenuation correction was made for the thickness of tissue above and below the tumor; since the size of the patient's leg did not change appreciably during the course of therapy, we could neglect errors in attenuation correction when comparing the pre- and post-therapy count rates.

In subsequent studies with two normal volunteers and a patient with retroperitoneal ganglioneuroma, venous blood samples were drawn at frequent intervals after the administration of labeled glutamate. Aliquots of whole blood were counted in a scintillation counter; the blood volume of each subject was estimated from his body weight, and the percentage of injected dose in the blood compartment was calculated. In one case, the red-cell and plasma fractions were counted separately to determine the distribution of N-13 label in those major blood components.

An attempt was made to determine qualitatively the chemical form of the N-13 label by administering the labeled compound to a mouse (C3H strain) containing an implanted Dunn osteogenic sarcoma. The tumor was excised and homogenized in picric acid. After centrifugation, the aqueous fraction was passed through a high-pressure liquid chromatograph (HPLC) and the fractions assayed for radioactivity. The N-13 content of the precipitable fraction, containing proteinaceous material, was also measured.

RESULTS AND DISCUSSION

As in the reported animal studies (*3*), the blood clearance of N-13 L-glutamate in humans is quite rapid. Approximately 91–95% of the injected activity had left the blood compartment within 4 min after i.v. administration, with the remaining activity clearing more slowly. At 20 min after injection, over 90% of the blood activity was in the plasma fraction.

Figure 1 is a scan of the patient's legs in anterior projection. Figure 2 relates the position of the region of increased N-13 concentration to anatomic structures that are not visualized in the scan. It appears that the highest uptake of this labeled compound is in the soft portion of the tumor, whereas

FIG. 1. Distribution of N-13 in lower extremities of 9-year-old boy after administration of labeled glutamate. Image was formed by computer analysis of digital data from a quantitative scanner and displayed in dot-density format; brighter areas qualitatively represent regions of increased N-13 uptake.

Tc-99m diphosphonate uptake was greatest in the regions where calcification was occurring. From the scan data, the tumor volume was computed to be on the order of 100 ml and to contain about 3.1% of the administered activity. According to the blood-clearance curves obtained in the other human subjects, only about 7% of the administered activity was in the blood compartment at the time of the scan, or 0.28% per 100 ml of blood. The counts contributed by blood in the tumor, therefore, were considerably less than one tenth of the total, thus reducing the importance of blood-activity correction in the calculations.

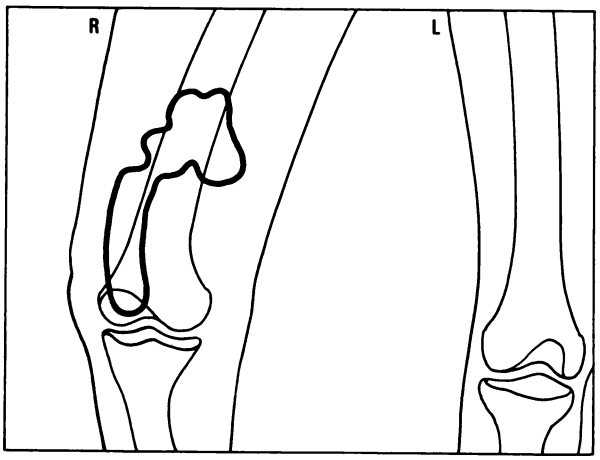

FIG. 2. Position of lower extremities for scan of Fig. 1. Region of increased N-13 uptake is outlined on figure to relate its position to bony structures not visualized in Fig. 1.

Analysis of the posttherapy scan showed that the tumor still concentrated N-13, but that the activity content was only 60% of its pretherapy value. Concurrently, the serum alkaline phosphatase level decreased from 269 units/l to 146 units/l (normal range: 150–250 units/l). Regarding the accuracy of the method, Clarke et al. (6) obtained a good correlation between activity levels measured in vitro (by assay of homogenates of splenic tissue) and in vivo with the HEG system in a series of 29 patients who were administered Cr-51-labeled red cells and then splenectomized. The error in the in vivo Cr-51 quantitation was about 15%; the lower tissue attenuation, better counting statistics, and reduced blood correction afforded by the use of N-13 should allow us to measure serial changes of greater than 5%.

The analysis of the murine osteogenic sarcoma was qualified by the low count rates obtained in the HPLC eluant. It was observed however, that less than 5% of the activity was present in the protein fraction, with insignificant amounts in the fraction containing nucleic acid precursors. About one third of the activity accompanied the fraction containing glutamate; the remainder was present as neutral amino acids or other metabolites.

These initial results are encouraging, and we are continuing to evaluate N-13 L-glutamate as a tumor scanning agent in osteogenic sarcoma. This compound has the following advantages: it clears from the blood rapidly; it provides a high ratio of information to patient radiation dose (the total-body dose is about 6 millirads per millicurie); it appears to be taken up actively in tumor tissue, and hence the extent to which a tumor concentrates the label may be correlated with its metabolic requirements. We are investigating the use of this labeled amino acid as a significant monitor of the progress of chemotherapy, as well as its distribution in other tumor types, to assess further its usefulness in studies of tumor uptake and metabolism.

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