Bone-Seeking Radiopharmaceutical Uptake

as an Indicator of Mandibular Growth in Rats

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Uptake of a bone-seeking radiopharmaceutical is correlated with growth changes in the rat mandible. Uptake was highest in newborn rats, decreasing with increasing age.

KEY WORDS: • GROWTH • RADIOPHARMACEUTICAL • SCINTIGRAPHY •

Accurate assessment of the dynamics of growth is essential in treating children with facial deformity. Cephalometric analysis is the primary method currently available to assess facial skeletal growth. An individual cephalograph, however, documents only completed growth, requiring serial radiographs over a period of months or years to assess growth as a physiologic process (BJÖRK 1963). A technique that directly measures growth dynamics in a single examination could greatly improve the clinician's ability to evaluate the growing child, and bone seeking radiopharmaceutical uptake quantitated by skeletal scintigraphy is such a technique (KABAN ET AL. 1982).

It has been shown that bone-seeking radiopharmaceutical uptake is a sensitive indicator of bone pathology, and that regions of pathology can be detected earlier by this means than with standard radiography (BARRETT AND SMITH 1974, SILBERSTEIN ET AL. 1974 AND 1975). Skeletal scintigraphy can also be used to predict changes in bony architecture that occur with skeletal metastasis and periodontal disease (KAPLAN ET AL. 1978A AND 1978B, AND JEFFCOAT ET AL. 1980).

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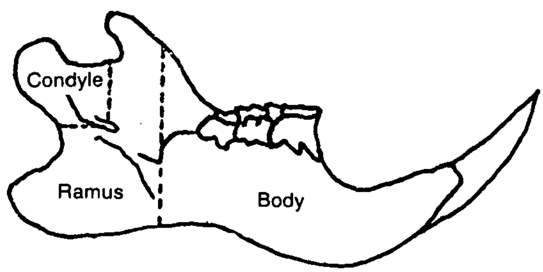

Fig. 1 **Division of the rat mandible for analysis.**

Bone-seeking radiopharmaceutical uptake, using Technetium-99m Methylene Diphosphonate, has been used to assess mandibular growth in humans (Kaban et al. 1982). However, the specificity of isotope uptake as an indicator of rate of bone growth as not been established.

This report presents data which demonstrates that bone-seeking radiopharmaceutical uptake quantitated by skeletal scintigraphy reflects the rate of mandibular growth in developing rats.

— Materials and Methods —

Fifty Charles River male rats were used in this study, distributed by age as follows: birth (n=6), 3 days (n=6), 7 days (n=5), 12 days (n=5), 14 days (n=5), 21 days (n=6), 35 days (n=5), 56 days (n=4), and 224 days (n=4). The bone-seeking radiopharmaceutical uptake and size of the mandible were measured.

Radiopharmaceutical Preparation and Injection

An intraperitoneal injection of Technetium-99m Methylene Diphosphonate solution (1.5mCi per kilogram body weight) was administered to each animal. The radiopharmaceutical (^{99m}TcMDP) was prepared by reconstituting a lyophilized kit of Osteolite™ with a 5ml saline solution of pertechnetate ($Na^{99m}TCO_4$), so that a specific activity of approximately 8mCi/mg was obtained. The compound was administered within one hour of preparation.

Labeling efficiency of the kit was determined with ascending paper chromatography on Whatman No. 1 paper strips, using 85% methanol as the eluant. To determine the number of counts of radioactivity injected, quadruplicate (1µl pipette) standards of ^{99m}TcMDP solution were prepared and measured in a Squibb Medotopes Radiometer. After a two-hour waiting period to allow for blood and soft tissue clearance of radiopharmaceutical, each animal was sacrificed.

Measurement of Mandibular Size

Right and left sides of the mandibles were removed. They were sectioned into condyle, ramus and body (Fig. 1). The teeth were not removed. The specimens were weighed (mg) and measured in width and length (mm) with vernier calipers.

Condyle width and length were determined by measuring the greatest transverse and anteroposterior convexities. Body and ramus width were measured at the transverse midsection of each region.

Body length was defined as the distance from the cut edge to the junction of the incisor with the alveolar crest. Ramus length was defined as the distance from the cut edge to gonial angle.

Radioactivity Determination

Each mandibular segment was placed in a counting tube and bone-seeking radiopharmaceutical uptake measured using a Squibb Medotopes Radiometer. Radiation counts were corrected for background, and the percent of injected dose for each region was computed.

Statistical Analysis

The mean ($\pm$standard deviation) was determined for weight (mg), length and width (mm) for each condyle, ramus and body specimen. Total and per mg radiopharmaceutical uptake were measured for each mandibular region. These parameters were plotted against age and a regression analysis performed correlating bone-seeking radiopharmaceutical uptake versus rate of growth of condyle, ramus and body. Rate of growth was defined as change in weight, length and width of the mandibular regions per day.

— Results —

Bone-seeking radiopharmaceutical uptake in percent of injected dose per mg for the condyle, ramus and body regions was plotted against age (Fig. 2). Total uptake for each region is shown in Fig. 3.

At birth all mandibular regions (condyle, ramus and body) demonstrated high uptake of the radiopharmaceutical. A rapid decrease in uptake was evident during the first 12–14 days. The decrease then continued at a lessening rate to 224 days. The condyle exhibited the highest uptake per milligram of bone (Fig. 2). The largest segment studied, the body of mandible, exhibited the highest total uptake (Fig. 3).

A linear regression analysis was performed comparing uptake with rate of growth of condyle, ramus and body. Rate of growth per day was determined by changes in length and weight of each region from one examination period to the next (Figs. 4 and 5).

A positive correlation was found between bone-seeking radiopharmaceutical uptake and growth changes. Smaller changes in length and weight manifested a lower uptake and greater rates of growth correlated with higher uptake. The correlation coefficient between bone-seeking radiopharmaceutical uptake and rate of linear growth for the entire mandible was significant at 0.86 ($p<0.01$), and with rate of weight gain for the entire mandible it was 0.71 ($p<.05$). The correlation between uptake and changes in weight and length was significantly less in condylar and ramus regions than in the body.

— Discussion —

Bone-seeking radiopharmaceutical uptake in the rat mandible decreases with increasing age. This is in agreement with human clinical observations at Children's Hospital Medical Center, Boston (Kaban et al. 1982).

The rapid decrease in uptake from 1 to 12 days of age should be interpreted with consideration of the rapidly maturing rat kidney. Aperia and Herin (1975) have observed that the renal function in Sprague-Dawley rats does not reach full maturity until 17 days of age. In the present study, the rapid change in bone-seeking radiopharmaceutical uptake from day 0 to 12 probably reflects improving renal clearance.

Even with the rapid increase in renal function, condylar uptake is high in this growth period, which is consistent with increased condylar metabolism (Fig. 2). Durkin, Heeley and Irving (1979) have

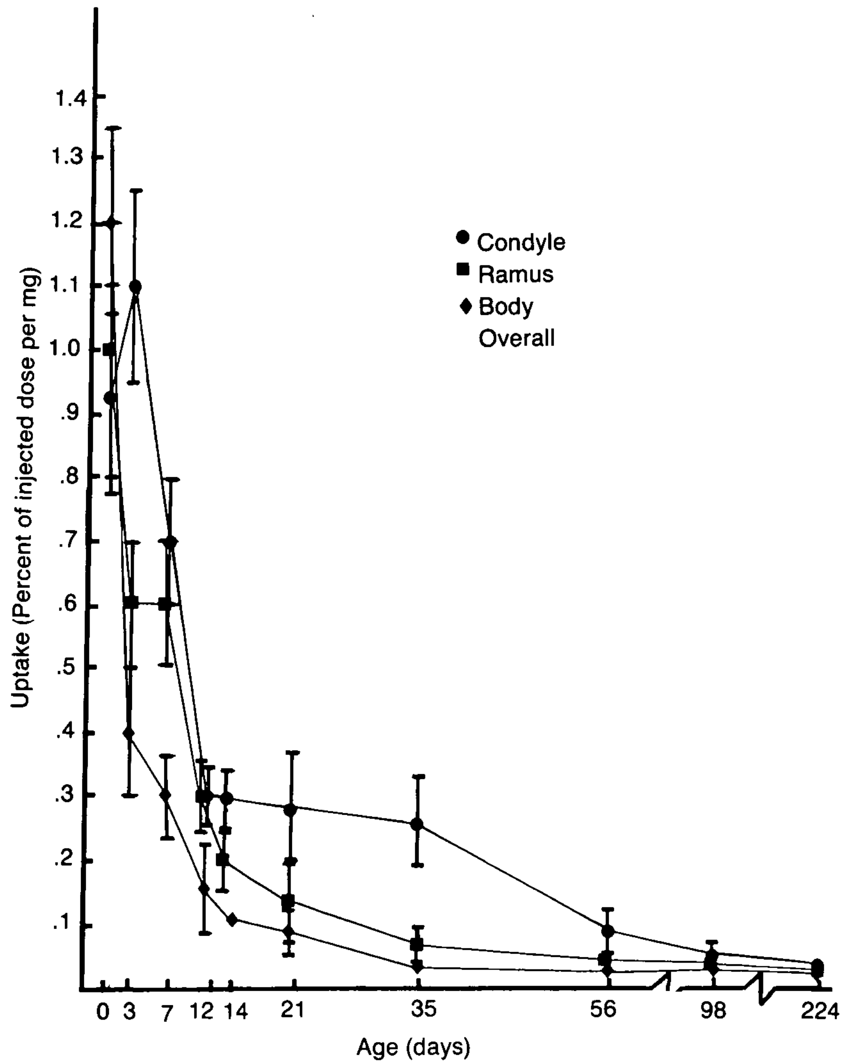

Fig. 2 Uptake per mg vs. age in maturing rats

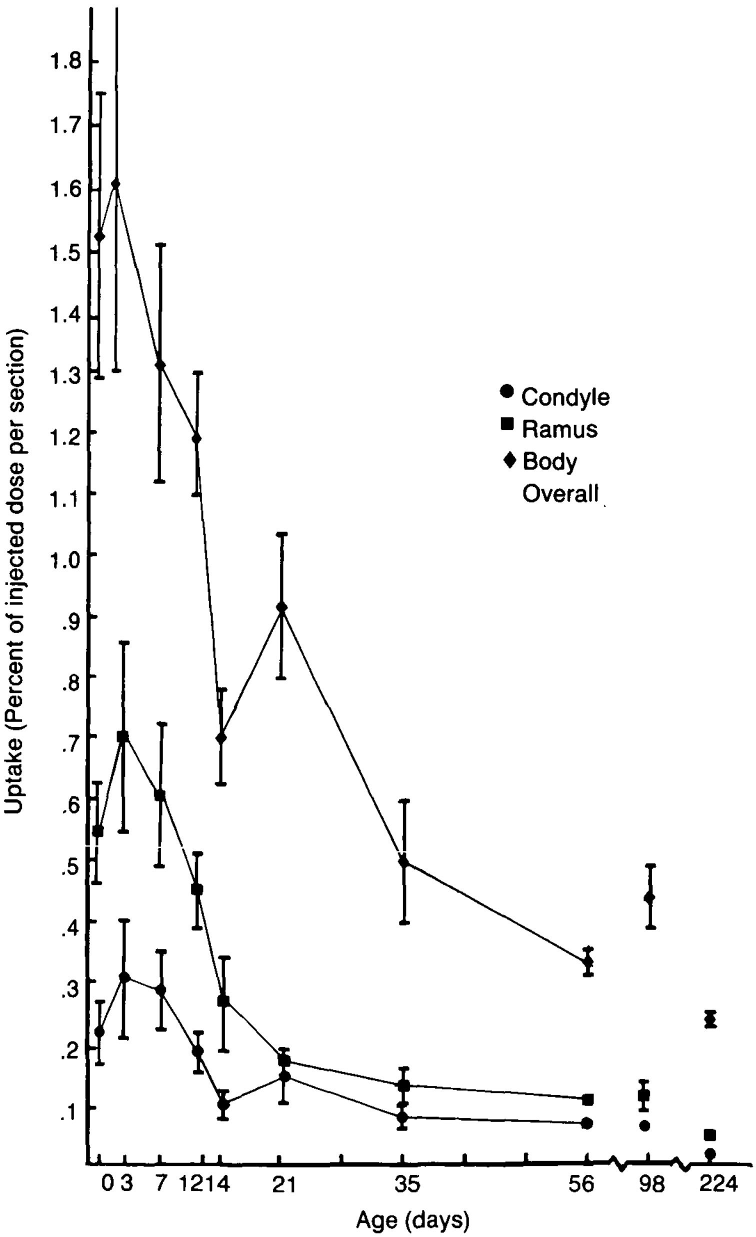

Fig. 3 Uptake per section vs. age in maturing rats

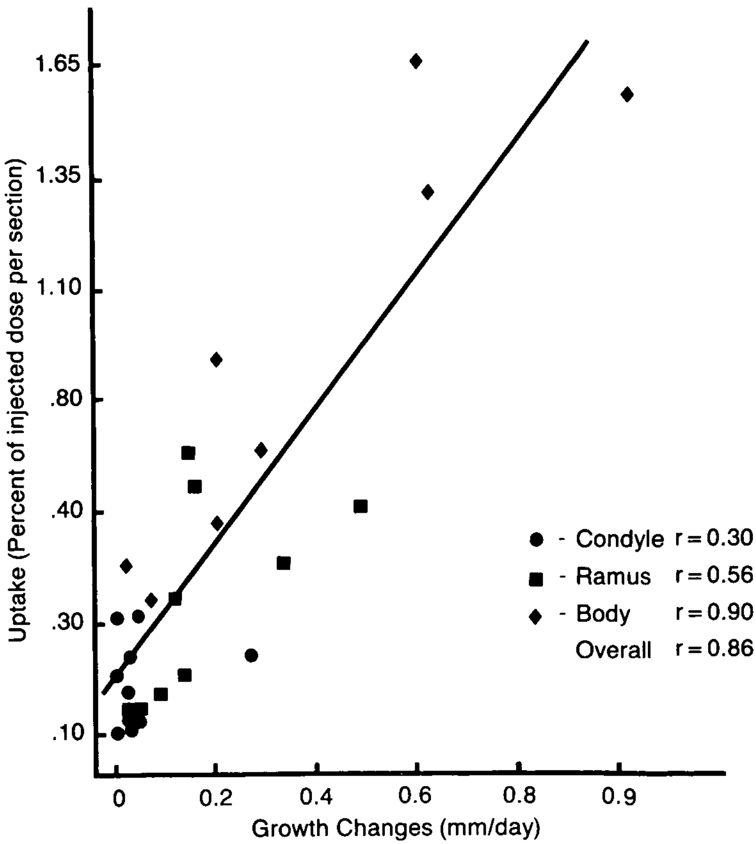

Fig. 4 **Uptake per section vs. rate of linear growth in rat mandible**

shown that the condylar cartilage proliferates rapidly during these twelve days. Furthermore, Kuftinec and Miller (1972, 1973) have shown that overall mandibular growth is high as measured by protein synthesis and phosphatase levels. ^{99m}TcMDP uptake remains high from 12 to 35 days, and then falls off as the rats mature and growth ceases (Figs. 2 and 3).

In this study, ^{99m}TcMDP uptake showed no correlation with dental development. In fact, at the time of incisor and molar eruption (7 and 21 days), radiopharmaceutical uptake was decreasing. Admittedly, it would have been ideal to remove the teeth prior to calculating uptake, but his would have been technically impossible without destroying bone.

The large standard deviations found within the 0–12 day age groups may be predominantly due to the dissection technique used. The mandible, particularly

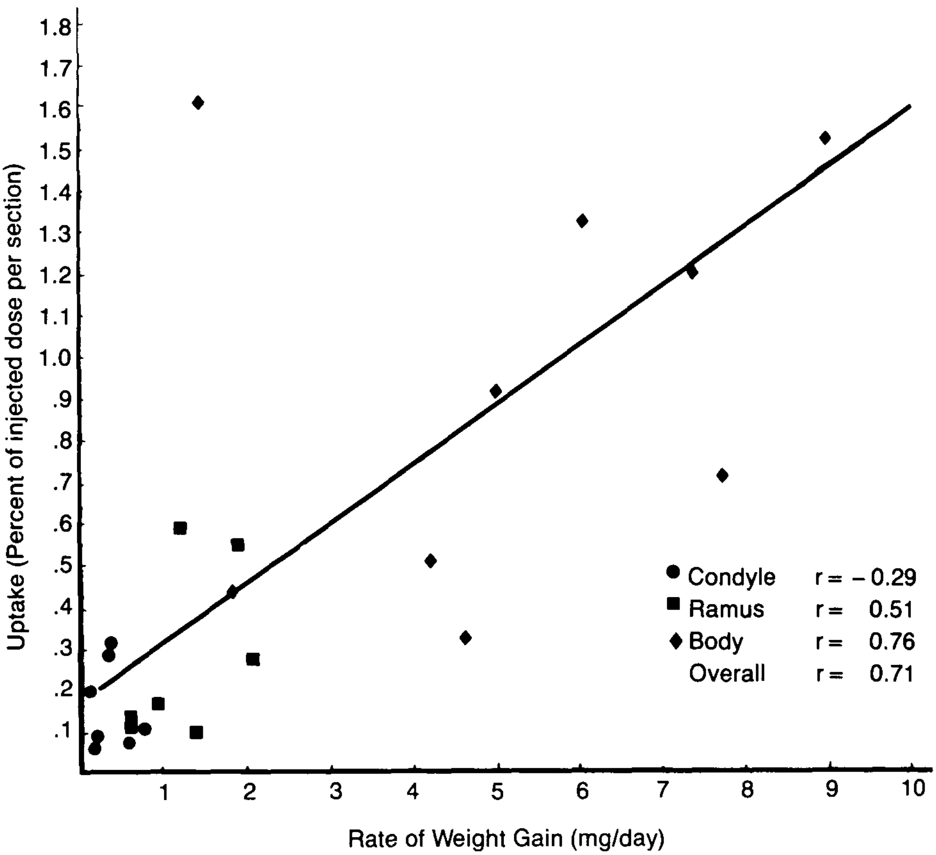

Fig. 5 Uptake per section vs. rate of weight gain in rat mandible

the condyle, is extremely soft and cartilaginous at these ages. Gross soft tissue dissection with sharp instruments in such areas may cause damage to the condyle and/or loss of tissue.

After 14 days, the condylar uptake pattern in rats was similar to that in humans. This observation is in agreement with the findings of Durkin, Heeley and Irving (1979) who have shown that the 14-day-old rat condyle is histologically similar to the human newborn condyle, and they are then parallel in development.

From 98 to 224 days of age, bone-seeking radiopharmaceutical uptake measurements for all three mandibular regions were approximately equal. This observation probably reflects a decrease in metabolic activity with age. The condyle, ramus and body exhibit similar mineralization patterns in older rats.

The linear regression analyses of bone-seeking radiopharmaceutical uptake versus rate of linear growth and weight gain should be interpreted with respect to overall dimensional changes. In the rat condyle, as defined in this study, relatively small changes in length and weight occurred with time. The body segment, however, showed the greatest changes in weight and length. The correlation coefficients shown in Figs. 4 and 5 are therefore consistent with the actual changes that occurred in the rat mandible with time. More sophisticated measurements of condylar length and weight might have produced a somewhat higher correlation.

These data have far-reaching and significant implications. All previous reports on the use of skeletal scintigraphy for facial deformity utilize standard scintigraphic methods, which are totally subjective and qualitative. DONOFF ET AL. (1978) showed that areas of increased uptake in patients with suspected condylar hyperplasia were associated with histologic evidence of hyperplasia. Studies in progress in this institution also indicate that areas of increased active growth may be detected using skeletal scintigraphy (KABAN ET AL. 1982, CISNEROS AND KABAN 1982).

We have established normal standards of bone-seeking radiopharmaceutical uptake in humans, so that scintigraphy can be used to objectively assess facial skeletal maturity in the same way as hand-wrist radiographs are used (CISNEROS AND KABAN 1984 AND 1985). The question of whether growth has ceased in a symmetrical deformity such as mandibular prognathism can be answered with this technique. Furthermore, if there is asymmetric uptake, the normal side can now be clearly documented based on objective data (CISNEROS AND KABAN 1984).

The advantages of skeletal scintigraphy are that one can obtain physiologic information on growth dynamics with one observation. The technique is reproducible by the same examiner and between different examiners.

Scintigraphy could become a useful technique to solve difficult diagnostic problems involving growth, and to help make decisions for timing of treatment in the growing child. It can be used to assess the effects of surgical and functional therapy, and it is an excellent research tool.

It is important, however, to place the technique in perspective. Scintigraphy should never be used routinely for all patients. The procedure is too expensive, and the radiation exposure is too high. Exposure is equivalent to a full skeletal radiographic survey, and the greatest radiation exposure is to the gonads and kidneys. In order to counter this, the patients are kept well hydrated and instructed to void frequently to clear the radiopharmaceutical as quickly as possible.

— Summary

^{99m}TcMDP uptake has been previously shown to correlate with mandibular growth in humans. this study is the first attempt to demonstrate the specificity of radiopharmaceutical uptake for incremental growth changes. In male Charles River rats, bone-seeking radiopharmaceutical uptake was indicative of the rate of growth (change in length and weight per day) of the mandible.

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