Radionuclide Imaging of Hard and Soft Tissues of Dog Jaws under Heavy Orthodontic Forces

DANIEL A. GARCIA, D.D.S., M.D.S., M.S.
DAVID J. HIGGINBOTHAM, D.M.D.
KRISHAN K. KAPUR, D.M.D., M.S.

Radionuclide imaging provides information of a functional nature that complements and enhances the morphologic information of radiography. A number of radiopharmaceuticals are available for imaging the thyroid, liver, brain, skeleton, kidney and other tissues.[1] Clinical applications of this diagnostic procedure to pathology of the oral cavity have been limited primarily to salivary gland function testing.[2,3] Recent experimental applications of skeletal imaging, however, have shown its potential usefulness for the detection of tooth eruption in rabbits,[4] periodontal disease,[5] mandibular bone grafts,[6,7] periapical pathology,[8,9] and orthodontic forces[10] in dogs.

In the last-mentioned study[10] we reported the increased uptake of a bone-seeking radionuclide in the jaws of dogs resulting from the application of forces to mandibular teeth. The results of that study indicated that bone-seekers were accumulated in amounts directly related to the magnitude of the applied force. The present study was undertaken to identify the specific tissues in which skeletal agents are incorporated as a result of these forces. This was done by comparing skeletal images to measurements of radioactivity in autopsy samples of dog jaws. In addition, two other types of agents were used to examine the effects of orthodontic forces on the imaging of soft tissues and the regional vasculature.

From the Dental and Nuclear Medicine Services, Veterans Administration Hospital, West Roxbury, Mass.

MATERIALS AND METHODS

Animals

Young beagles with complete dentitions, clinically healthy gingiva, and radiographically normal alveolar bone were used for the study. They were housed individually in cages and fed regular dog chow and water ad libitum. They weighed 10 to 12 kg.

Orthodontic Appliance

A separating force was applied between the lower right first molar and fourth premolar of each dog by means of a double helical coil, shown in Figure 1. Shallow grooves were cut into the occlusal surfaces of both teeth to receive the arms of the coil, and the appliance was cemented with a composite resin which covered and retained the arms within the grooves. The coils were made from 0.036 inch hard-tempered stainless steel wire. Two dogs received coils delivering a 300 gram force and two received 600 gram coils.

Imaging Agents

Three agents containing the radionuclide technetium-99m (140 KEV gamma, 6 hour physical half-life) were used. The radionuclide was obtained from a molybdenum-99 generator as pertechnetate (TcO_4), and this was combined with other substances to form soft tissue, vascular and bone imaging agents. The vascular agent, Tc-99m-albumin, was prepared from dog serum albumin using the labelling procedures of Eckelman and colleagues.[11] The soft tissue agent, Tc-99m-Sn-diethylenetriaminepentaacetic acid (Tc-DTPA), was prepared by adding pertechnetate to a commercial prepara-

Fig. 1 The appliance used for separating the first molar and fourth premolar consisted of a double helical coil on the lingual with extension arms which encircled the crowns of both teeth and engaged shallow grooves cut into the occlusal surfaces. The device was retained by cementation of the arms within the grooves with a composite resin.

tion of DTPA and stannous chloride. The bone imaging agent, Tc-99m-Sn-polyphosphate (Tc-PP), was prepared by combining pertechnetate with a commercial preparation of polyphosphate and stannous chloride. The labelling efficiency for all three agents, determined by descending paper chromatography with 85% methanol, was 95% or greater. All three agents were administered intravenously immediately after preparation at doses of 1 mCi/kg of body weight.

Imaging

Right and left lateral head images were made on X-ray film by means of a rectilinear scanner containing a 5 inch diameter NaI scintillation detector and a multihole focusing collimator. Imaging was performed under nembutal anesthesia four hours after administration of the agents.

Three to four weeks after cementation of the coils, each animal was imaged on separate occasions with two different agents, first with either Tc-DTPA or Tc-albumin, and three days later with Tc-PP. Because of both the short physical half-life of Tc-99m and the breakdown and excretion of the compounds, no radioactivity of any consequence was retained in the tissues from one scan to affect the results of the second scan made 72 hours later.

Tc-PP Tissue Distributions

Immediately after the second or skeletal imaging the animals were killed by an excess of intravenously administered KCl. Right and left samples of skin, buccal mucosa, tongue and attached gingiva were removed, and the mandible disarticulated and freed of soft tissues. The gingival samples were full thickness flaps from buccal and lingual surfaces. All posterior teeth were extracted and buccolingual blocks of alveolar bone extending inferiorly from the alveolar crest to the superior border of the mandibular canal were excised. Slices were made through the root sockets to obtain contiguous blocks of alternating interdental and interradicular alveolar bone.

All samples were rinsed lightly in saline to remove excess blood then sponged and weighed. Radioactivity in the samples was measured with a NaI well-counter to determine Tc-99m counts per minute per gram of wet tissue. Tc-PP uptake in samples from the *right* or treated mandibles was compared with that of their counterparts from the *left* or control mandible in the form of right-to-left specific activity ratios.

Results

Radiographic Changes

Serial intraoral radiographs revealed

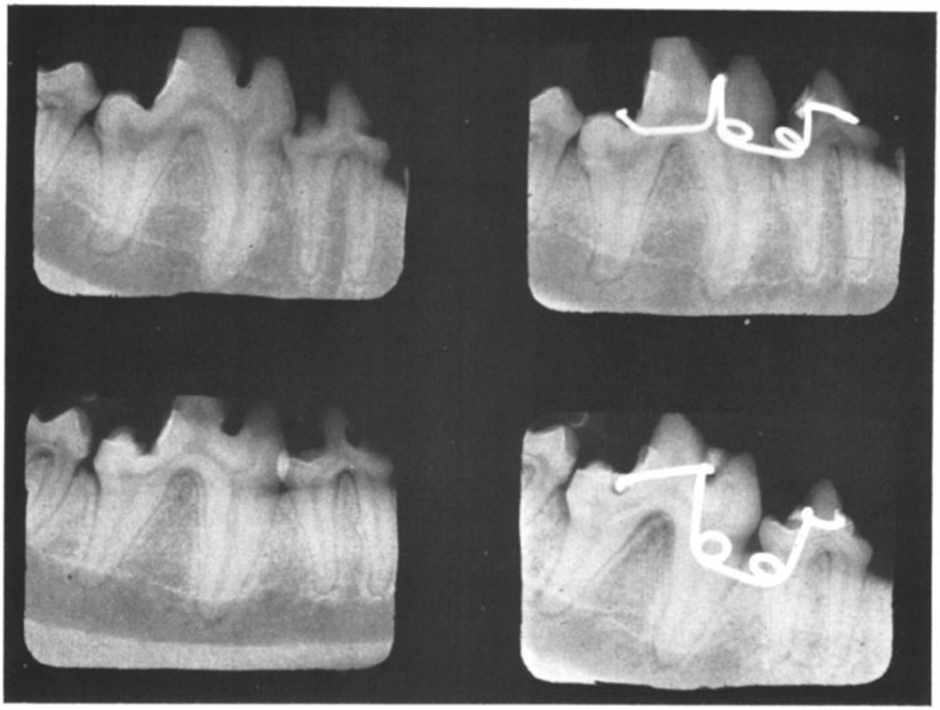

Fig. 2 Intraoral radiographs of the lower right first molar and fourth premolar of two dogs. *Top left and right:* Immediately before, and four weeks after insertion of a 300 gram appliance. The interproximal contacting surfaces between the first and second molars were relieved to allow distal movement of the first molar. A widening of the PDM space can be seen on the tension side of the distal root of the first molar and the mesial root of the fourth premolar. *Bottom left and right:* Immediately before and three weeks after insertion of a 600 gram coil. In addition to a widening of the PDM space, periapical radiolucencies can be seen at the root tips of the first molar.

alveolar bone changes in the furcations and apices of the coil-bearing teeth consisting of a widening of the PDM space with forces of 300 grams and focal resorption with 600 gram forces (Fig. 2). No changes were observed in the supporting bone of the remaining teeth of either mandible.

Radionuclide Images

All Tc-PP images contained zones of concentrated radioactivity surrounding the stressed teeth (Fig. 3). Coils delivering 600 gram forces produced larger zones than those of 300 grams (compare Figs. 3R and 4A). Figure 4 contains images of the same animal injected first with Tc-DTPA and three days later with Tc-PP. Tc-DTPA images showed that radioactivity was distributed throughout the head and neck, but more of it was concentrated in the neck region. In contrast, the Tc-PP image showed an intense accumulation of the radionuclide in the area of the appliance. No differences were observed between right and left Tc-DTPA images. Similar results were obtained with the vascular agent (Fig. 5). There were no selective accumulations of Tc-albumin in the coil area, nor were there differences in uptake between right and left mandibles.

Tc-PP Tissue Distributions

All tissue samples removed from Tc-

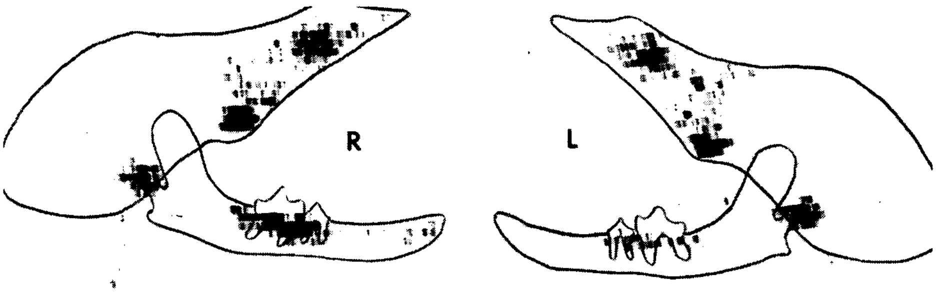

Fig. 3 Right and left lateral head skeletal images made with a rectilinear scanner four hours after intravenous administration of Tc-PP. The skeletal outlines were drawn with the images superimposed on lateral head radiographs. A separating force of 300 grams was applied continuously to the right first molar and fourth premolar for a four week period prior to imaging. A high concentration of Tc-99m activity can be seen adjacent to the right or treated teeth, but not in the left or control teeth.

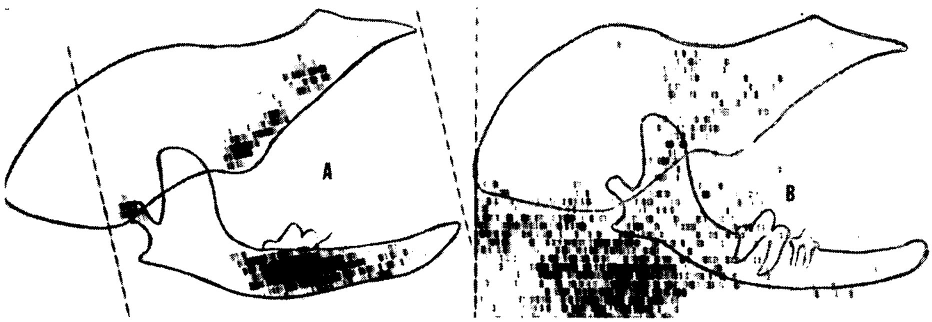

Fig. 4 Right lateral head images made with intravenously administered Tc-PP (A) and, three days earlier, with Tc-DTPA (B). The dog wore a 600 gram coil for three weeks prior to imaging. Tc-DTPA imaged the soft tissues of the head and neck, but concentrated primarily in tissues posterior and inferior to the mandible. In contrast, Tc-PP images showed an intense accumulation of radioactivity in an area surrounding the treated teeth. There were no differences in radionuclide distributions between right and left Tc-DTPA images.

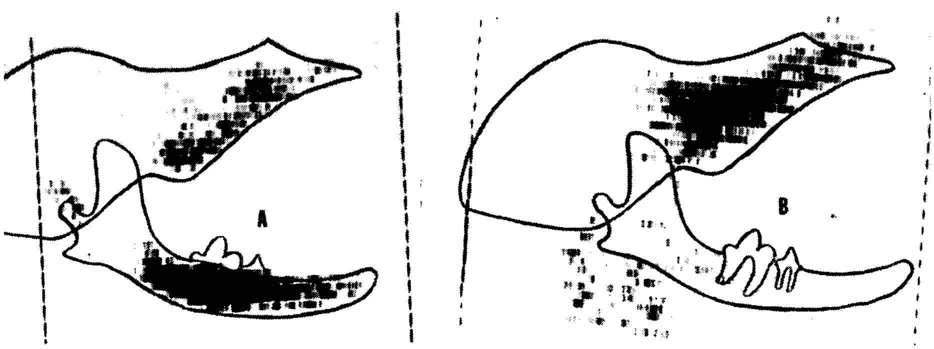

Fig. 5 Right lateral head images made with intravenously administered Tc-PP (A) and, three days earlier, with Tc-albumin (B). The animal wore a 600 gram coil for four weeks prior to imaging. Radioactivity was concentrated in an extensive area mesial and distal to the coil-bearing teeth on images made with the skeletal agent, Tc-PP, but not with the vascular agent, Tc-albumin. There were no differences in radionuclide distributions between right and left Tc-albumin images.

TABLE I

Relative Tc-PP Concentrations in Tissues of the Dog Mandible Six Hours After Intravenous Administration.

Tissue	Relative Concentration*
Tongue	1.0
Skin	1.30
Buccal Mucosa	1.34
Gingiva	2.83
Teeth	3.35
Crown	1.46
Root	5.02
Alveolar Bone	7.36
Condylar Bone	11.31

*The data were derived from Tc-99m cpm/gram (wet weight) of tissues removed from the normal left mandibles of four dogs. The specific Tc-99m activities of the tissues of each dog were normalized to the tongue, and the values of the four dogs were combined and averaged.

PP injected dogs contained Tc-99m activity. The lowest concentrations were measured in tongue samples and the highest in condylar bone. Table I contains the relative concentrations of radioactivity in tissues from the left or normal jaws. The data of each dog were normalized to the specific activity of the tongue samples, and the relative numbers of all four dogs were combined and averaged. The gingiva contained twice as much radioactivity on a per gram basis than buccal mucosa, and uptake in teeth was more than three times higher in the root than in the crown.

Table II contains right-to-left Tc-99m specific activity ratios for alveolar bone and tooth roots of two dogs with 300 or 600 gram appliances. Ratios for the soft tissues showed no differences in Tc-99m activity between treated and control sides and were omitted from the table. Alveolar bone samples showed the largest differences in Tc-PP uptake. Some of the roots of the coil-bearing teeth showed increased uptake but, in general, these increases were much smaller than those for alveolar bone. The results of Table II show that the alveolar bone segments of abnormally high Tc-PP uptake included areas of pressure (mesial of second molar and fourth premolar) and tension (mesial of first molar). Furthermore, the mesiodistal extension of the high uptake region was greater for the 600 than the 300 gram coil.

Alveolar bone ratios for the two remaining dogs are plotted in Figure 6. The area of high Tc-PP uptake with a

Table II. Right-to-left Tc-99m Specific Activity Ratios of Mandibular Alveolar Bone and Tooth Roots from Tc-PP Injected Dogs*.

Tooth Root	Dog 1 (300 g)	Dog 2 (600 g)	Alveolar Bone	Dog 1 (300 g)	Dog 2 (600 g)
1st premolar	0.93	0.99	1st premolar, mesial	1.11	0.98
2nd premolar, mesial	1.06	0.94	2nd premolar, mesial	1.05	1.12
2nd premolar, distal	0.97	1.04	2nd premolar, furcation	0.91	1.23
3rd premolar, mesial	1.12	1.11	3rd premolar, mesial	0.89	1.51
3rd premolar, distal	1.12	0.96	3rd premolar, furcation	1.17	1.40
4th premolar, mesial	1.28	1.09	4th premolar, mesial (P)**	1.69	2.10
4th premolar, distal	0.98	1.29	4th premolar, furcation	1.59	1.87
1st molar, mesial	0.91	0.93	1st molar, mesial (T)**	1.77	2.75
1st molar, distal	1.08	1.38	1st molar, furcation	1.70	1.83
2nd molar, mesial	1.06	0.97	2nd molar, mesial (P)**	1.48	2.34
2nd molar, distal	0.90	1.01	2nd molar, furcation	1.20	1.93
3rd molar	0.97	1.07	3rd molar, mesial	0.92	1.89
			3rd molar, distal	0.98	1.64

* Tissues removed six hours after intravenous administration of Tc-PP were weighed and then counted in a NaI well counter to obtain Tc-99m cpm/gram (wet weight). The data shown are ratios of cpm/gram of right or treated to left or normal mandibular autopsy samples.

** A separating force of either 300 or 600 grams was applied with a coil to the lower right fourth premolar and first molar of each dog, creating areas of pressure (P) at the mesial of the fourth premolar and second molar, and tension (T) at the mesial of the first molar.

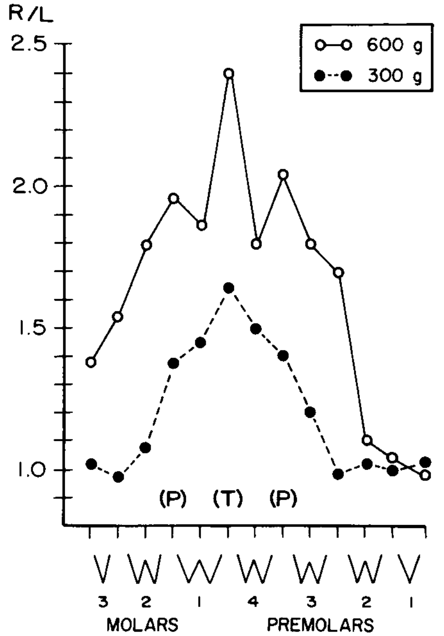

Fig. 6 Right-to-left (R/L) alveolar bone ratios were derived from Tc-99m specific activity measurements of contiguous interdental and interradicular samples removed six hours after intravenous administration of Tc-PP. The ratios are plotted in relation to the mandibular posterior teeth. Data are shown for two dogs that wore 600 or 300 gram coils for four weeks. The device applied a reciprocal separating force between the lower right first molar and fourth premolar which created areas of pressure (P) and tension (T). Larger increases in Tc-PP uptake were observed with the 600 gram coil. The maximum increase for both coils was measured in the tension area (T). The data also show that the mesiodistal extension of the affected mandibular alveolar bone was greater with the stronger coil.

300 gram coil extended from the mesial of the second molar to the furcation of the third premolar. The 600 gram coil produced an area extending from the distal of the third molar to the mesial of the third premolar. In both instances the highest ratios corresponded to the area of tension. The anatomic location and extension of the darkened zones on right bone images (Figs. 3R, 4A, 5A) were determined by superimposing the images on lateral head radiographs using the temporomandibular joints and other skeletal structures as landmarks. The dimensions of these zones were compared with those derived from alveolar bone ratios (Table II, Fig. 6) showing an increase in uptake between right and left of 20% or greater. The images were found to be from 5 to 8 mm larger, mesiodistally. This relatively small discrepancy between images and tissue concentrations was due primarily to the spatial resolution limitations of the collimated rectilinear scanner. The over-all anatomic location of the image zones, however, closely paralleled the data derived from the autopsy samples.

Discussion

The results of this study showed that the application of heavy orthodontic forces produced changes in tooth-supporting alveolar bone resulting in an abnormally high uptake of a bone-seeking imaging agent, Tc-PP. The role of the phosphate component of this agent was made clear by the results obtained with the two other agents, Tc-DTPA and Tc-albumin. For radioactivity to concentrate in the tissues adjacent to the coil, it was necessary that the radionuclide, technetium-99m, be bound to the mineral metabolite, polyphosphate. Therefore, it is reasonable to conclude that the distributions of radioactivity on Tc-PP images reflected the regional changes in mineral metabolism triggered by the applied forces.

Images made with a vascular agent, Tc-albumin, showed no selective concentrations of radioactivity in the area of stress comparable to Tc-PP images (Fig. 5). This finding suggested that heavy orthodontic forces do not alter regional patterns of circulation to the extent of being externally detectable by imaging. Microscopic dye perfusion studies reported in the literature have shown that vessels of the periodontal ligament become more numerous and dilated when 50 to 250 gram forces are applied to dog teeth.[12-13] These findings suggested that an increase in vascularity was associated with tooth movement. It would appear from our results with Tc-albumin that these reported changes may be so small in magnitude as to escape detection by radionuclide imaging.

Our finding that the concentration of Tc-PP in alveolar bone was approximately 2.2 times that of intact teeth (Table I) is in agreement with comparable data reported by Stover and colleagues[14] who found that, 24 hours after injecting calcium-45 in a young beagle, the average skeletal uptake was approximately 2.4 times that of the teeth.

The notable difference in Tc-PP uptake between the roots and crowns of teeth is best understood from the autoradiographic findings of Jee and Arnold[15] in their studies of beagle teeth. They found a preferential localization of several bone-seeking radionuclides tested on newly-formed dentinal surfaces of pulp chambers and the cemental surfaces of roots, while deposition on enamel surfaces was negligible. It is likely then that the higher specific activity of roots observed in the present study (Table I) reflected the larger total surface area (dentinal and cemental) available for Tc-PP incorporation relative to that of the coronal portion. These authors also reported a marked uptake in the periosteal surfaces of bone. This observation may explain the differences in Tc-PP concentrations between the full thickness gingival samples containing periosteum and the buccal mucosal samples which did not.

The data of Table II show that the force-induced increases in mineral metabolism occurred exclusively in alveolar bone, and that the coil-bearing teeth served only as transmitters of these forces. Regionally, the metabolic changes measured in areas of tension were greater than those of pressure (Fig. 6). It should be noted that, since the appliance delivered a tipping as well as a separatory force, the areas we have arbitrarily identified as tension and pressure may have been a mixture of both. For this reason it is not possible with the data at hand to conclude that metabolism is enhanced to a greater degree on the side of tension than of pressure. This matter is currently under investigation in a study of bodily tooth movement.

The close anatomic agreement between imaging with Tc-PP and the regional distributions of radioactivity measured in autopsy samples indicated the accuracy with which the rectilinear scanner can record alterations in mineral metabolism within the jaws. This technique holds promise as a valuable research tool in metabolic studies of orthodontic forces.

Summary

Rectilinear scanning with soft tissue, vascular and skeletal imaging agents was used to study the effects of heavy orthodontic forces on the tissues of dog jaws. A coil delivering either a 300 or 600 gram separating force to two adjacent lower right posterior teeth was inserted in each of four dogs. The animals were scanned with the three imaging agents three to four weeks after insertion of the coils. Only the skeletal agent, Tc-PP, was selectively concentrated in tissues adjacent to the stressed teeth. Tissue distributions of this agent, determined from autopsy samples, revealed that, although all the hard and soft tissues examined contained measurable amounts of Tc-PP, only alveolar bone demonstrated increased uptake of the agent as a result of the applied

forces. Tc-PP concentrations were highest in areas of pressure and tension. The total area of alveolar bone showing increased uptake of Tc-PP was larger with 600 than with 300 gram coils. There was close agreement between rectilinear scanning and the tissue distributions of Tc-PP with respect to both the size and anatomic location of the alveolar bone regions affected by the separating forces. This technique may have useful applications in orthodontic research.

1400 VFW Parkway
West Roxbury, Mass. 02132

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