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$^{99}Tc^{m}$-DP ACCUMULATION IN RABBIT SKULL BONES AFTER ^{60}Co GAMMA IRRADIATION

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Mandibular necrosis as a complication following radiation therapy of intraoral carcinoma was first described by REGAUD (1922). In the literature, the incidence of mandibular radiation necrosis ranges from 4.6 per cent (WILDERMUTH & CANTRIL 1953) to 37 per cent (MACCOMB 1962). Trauma followed by infection considerably increases the risk of osteonecrosis after radiation therapy (WATSON & SCARBOROUGH 1938, RUBIN & CASARETT 1968).

The total dose, the quality of the radiation, the over-all time of irradiation, and the condition of the tissues in the radiation field, including the dental state (PARKER 1972), are factors influencing the radiation tolerance of the matuie bone. Dental caries is particularly ill-reputed in eliciting secondary infection and osteonecrosis in the mandible or the maxilla and may result in tooth extraction after irradiation. The onset of osteonecrosis is often insidious and may occur months to years after completion of the radiation therapy (RUBIN & CASARETT, REGEZI et coll. 1976). Ablative and reconstructive surgery of the mandible is thus rendered hazardous. For the time being, there are no reliable methods for detecting radiation-induced osteonecrosis at an early stage.

Experiments were performed to determine whether $^{99}Tc^{m}$-diphosphonate could be used to detect early and discrete bone abnormalities following ^{60}Co irradiation. In case irradiation of normal bone should be shown to cause an abnormal uptake of

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$^{99}Tc^{m}$-DP, the intention was also to analyse if a scintigraphic differentiation between radiation-induced abnormalities and neoplastic or inflammatory lesions was possible.

Material and Methods

Ten adult rabbits with a mean weight of 4.2 kg, aged 1.5 to 3 years, of different breed and sex, were exposed to single doses of ^{60}Co radiation. The distance between the radiation source and the skin was 75 cm and the radiation field was 40 mm × 40 mm. The dose rate was 1.15 to 1.25 Gy/min. During irradiation the rabbits, in general anesthesia, were positioned on the side, the median plane of the head and body coinciding with the horizontal plane. Irradiation was performed with a horizontal beam directed to the centre of the mandible. One side of the facial skeleton was shielded with a lead block 50 mm thick, that side serving as a control. The lead block was positioned so that the upper surface coincided with the median plane of the rabbit. The radiation field extended from the symphysis of the mandible 40 mm dorsally. Five rabbits were given 10 Gy and another 5 rabbits 20 Gy as single doses. According to Kirk's formula (ELLIS 1967, KIRK et coll. 1971), these doses correspond to a total dose of 24 Gy and 74 Gy, respectively, if fractionated with a dose of 2 Gy a day for 5 days a week. At present, the latter schedule is applied in radiation therapy of intraoral carcinoma at Radiumhemmet, Karolinska sjukhuset. For detailed information concerning the dosimetry the reader is referred to NATHANSON & BÄCKSTRÖM (to be published).

Scintigraphy of the rabbit head was carried out 5 to 7 weeks after irradiation, using a gamma camera (Pho Gamma IV Nuclear Chicago). The scintigraphy was performed 6 to 7 hours after intravenous injection of 4 mCi $^{99}Tc^{m}$-DP. The nuclide batch was discarded if the impurity exceeded 5 per cent. The rabbits were kept still manually 8 cm under a pin-hole collimator with an aperture of 20 mm. In each image 200 000 counts were collected. Great care was taken to avoid asymmetric projections, and at least 2 images were obtained. Eight weeks after irradiation, the rabbits were operated under general anesthesia and a piece of bone sized about 0.5 cm × 1.5 cm was resected from the irradiated half of the mandible. An autologous transplant from the humerus replaced the defect in the mandible. The resected piece was decalcified, embedded in paraffin wax and sectioned in slices 5 to 10 μm thick. Serial sections were stained either with hematoxylin-eosin or Mallory's Azan method for examination in light microscopy. Twelve weeks after irradiation 10 mCi $^{99}Tc^{m}$-DP was given intravenously and 6 to 7 hours later the rabbits were decapitated after an overdose of barbiturate. All soft tissues were carefully dissected from the skeleton of the heads, with the exception of the mandible, which was removed for histologic evaluation of the incorporation of the humeral graft in the irradiated mandible and the state of the contralateral non-irradiated half of the mandible (the results will be published separately). No other part of the face skeleton was submitted to microscopy. An image of the dissected skull without the mandible was recorded by the

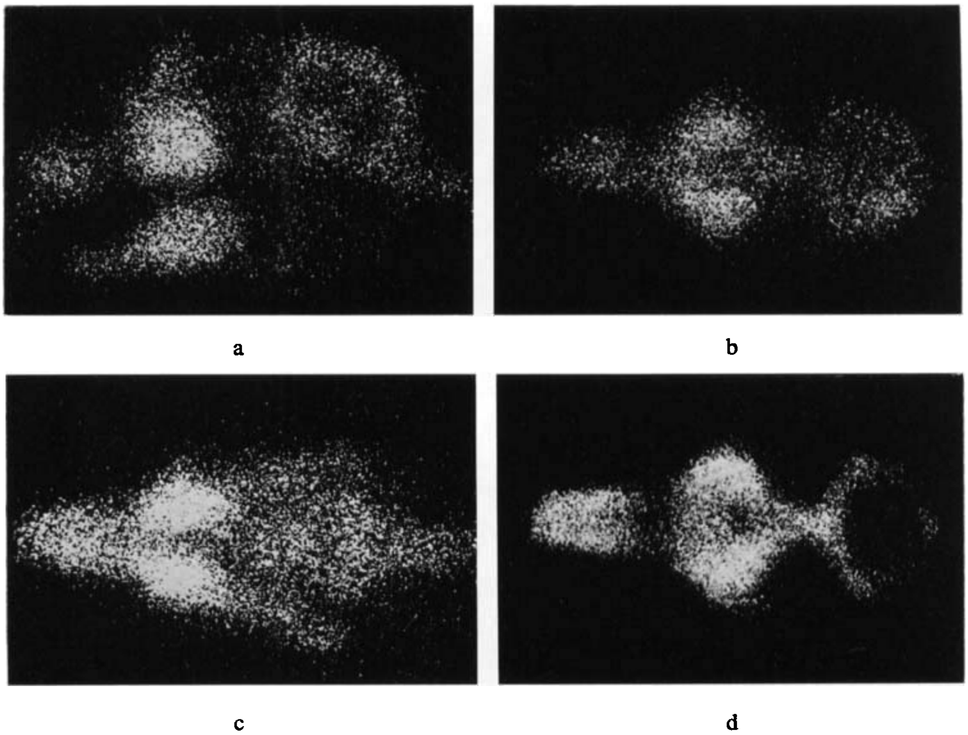


Fig. 1. Gamma camera images of rabbit heads after administration of $^{99}Tc^m$-DP. a) Left lateral, b) a.p. projection of a normal head. c) A.p. projection 5 weeks after unilateral irradiation with 20 Gy. d) A.p. projection of a dissected skull 12 weeks after unilateral irradiation with 20 Gy.

gamma camera with care taken to obtain exactly symmetric projections. The skulls were then split in the median plane and each half was measured in a well counter in 4 different positions in order to avoid errors caused by different measuring geometry. The background count was subtracted from the mean value of these 4 measurements. Finally, the skull halves were weighed within half an hour and the activity per gram tissue was calculated. Two rabbits died before the end of the observation period and were therefore excluded from analyses.

Results

Scintigraphy of the skull did not reveal abnormal distribution of $^{99}Tc^m$-DP, which was perfectly symmetric in all 4 rabbits irradiated with 10 Gy, as well as in the 4 rabbits which were irradiated 5 to 7 weeks before the examination with 20 Gy (Fig. 1). Neither could any asymmetry in the $^{99}Tc^m$-DP distribution in the skeleton of the head be demonstrated by the gamma camera after decapitation and dissection 12 weeks after irradiation, nor by the calculated activity per gram tissue (Fig. 1). The

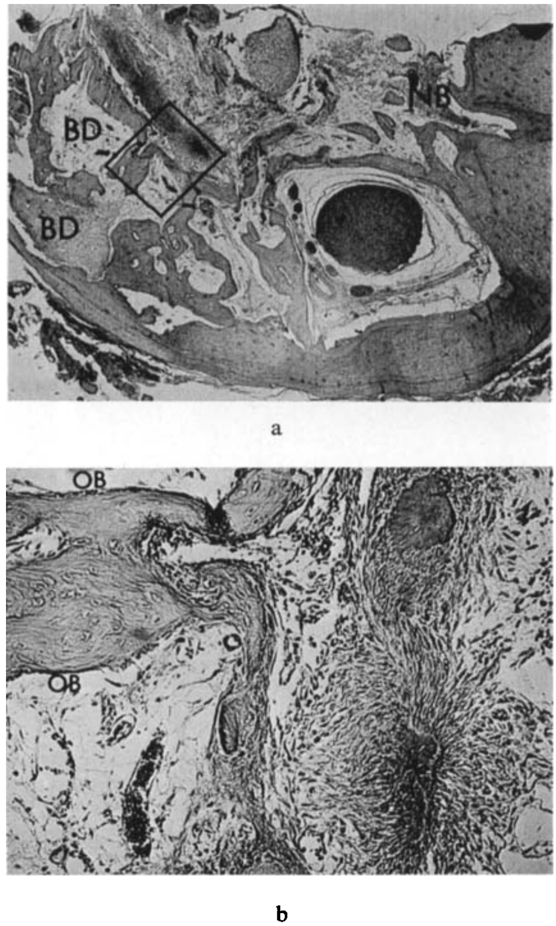


Fig. 2. Cross-section of mandible 8 weeks after irradiation with 20 Gy. a) Macrophotograph (×14) of a resected piece. Areas with bone destruction (BD) and with new bone formation (NB). b) ×70. Flat osteoblasts (OB) coating the newly formed bone within square indicated in (a).

difference in activity per gram tissue between the irradiated and the non-irradiated side varied between 1 and 8 per cent in all 8 rabbits, without any correlation to the dose given or to the small difference in time interval between irradiation and examination. The background subtraction was small and the differences caused by different measuring geometry in the well counter were negligible.

Histology. No abnormality was found in any of the bone pieces resected from the side of the mandible irradiated with 10 Gy 8 weeks previously. On the other hand, abnormalities ranging from new bone formation to bone destruction were present in 3 of the 4 bone pieces resected from the mandible that had been irradiated 8 weeks previously with 20 Gy (Fig. 2). The new bone had been formed, particularly at the fundus of the alveolus (Fig. 3), but many osteoblasts lining the newly formed bone were flat, with an abnormal appearance (Fig. 2 b). The fourth specimen in this group had advanced bone destruction without any formation of new bone. In all

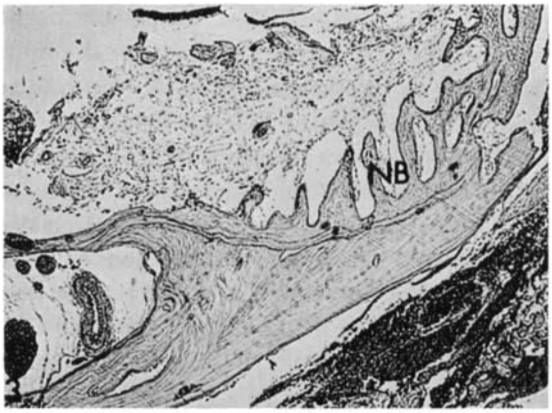
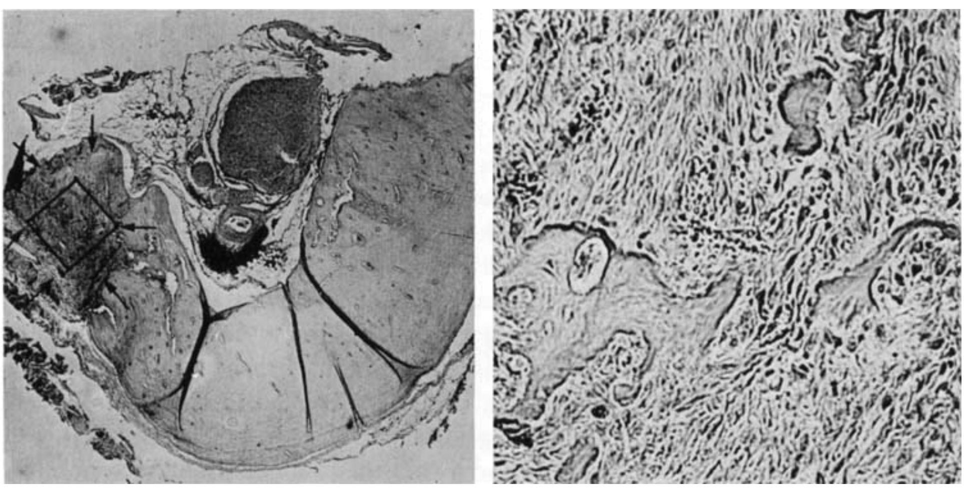


Fig. 3. Macrophotograph of specimen 8 weeks after irradiation with 20 Gy. Intense new bone formation (NB) at fundus of alveolus.

4 specimens large resorption cavities were observed inside the cortical bone (Fig. 4). In 2 animals these cavities occupied as much as 1/4 of the pieces resected. Evidence of high osteoclastic activity with concomitant fibrosis was observed within the resorption cavities (Fig. 4 b). No osteoblasts of normal appearance coated these cavities and almost no new bone formation was observed inside the cavities. The intracortical vessels and the alveolar artery and vein were without demonstrable lesion in all 8 specimens from the mandibular halves irradiated with 10 or 20 Gy. No new bone formation and no bone destruction were found in the specimens from the non-irradiated sides of the mandibles at 12 weeks after the irradiation.



a b

Fig. 4. a) Macrophotograph of specimen 8 weeks after irradiation with 20 Gy. Great resorption cavity (→) occupying about 1/4 of the area of the resected piece. b) ×210. Centre of the cavity as indicated by the square in (a) with fragments of destroyed bone surrounded by fibrous tissue.

Discussion

The observations made by light microscopy corresponded completely to previous morphologic observations of the effects of single doses of ^{60}Co irradiation on the mandible of rabbits (NATHANSON & BÄCKSTRÖM). In that investigation the irradiated bone was examined by radiography, light and fluorescence microscopy and by a microangiographic method using an Indian ink infusion technique. No effects on the mandible or on the intracortical vessels were found with any of these methods 6 weeks after a single dose of 10 Gy. Only a possible bone destruction in 2 of 7 rabbits was demonstrated at radiography 6 weeks after irradiation with a single dose of 20 Gy. However, all specimens from mandibles irradiated with 20 Gy by fluorescence or by light microscopy gave evidence of new bone formation, particularly in the fundus of the alveolus, while in the cortical bone, osteoclasia was present, creating large resorption cavities filled with fibroblasts and lined by normal osteoblasts. It was also observed that newly formed bone degenerated with disintegrating osteoblasts 6 to 12 weeks after irradiation with 20 Gy, and 24 weeks after the same dose bone destruction was predominant and new bone, if formed at all, had an abnormal appearance. The number of Indian ink-filled intracortical vessels was slightly reduced and observed only in 3 of 6 rabbits examined 6 weeks after a single dose of 20 Gy. Nor could roentgen spectrophotometry reveal any relevant difference between irradiated and non-irradiated bone (NATHANSON, unpublished data). It was also found that irradiation of the continuously growing teeth in rabbits led to a stunted growth and a retarded eruption of the teeth after as short a time as 2 weeks. This could result in an asymmetric set of teeth. The asymmetry gives rise to changed pressure directions on the teeth when the rabbit is chewing, which in turn might stimulate the osteogenic cells lining the alveolus to formation of new bone.

In the present experiments the irradiated bone was examined 8 weeks instead of 6 weeks after irradiation with 20 Gy, and many of the osteoblasts did not appear normal, indicating degeneration of the newly formed bone.

Despite these histologically demonstrable osteogenic and bone destructive reactions, no change or asymmetry in the ^{99}Tc^{m}-DP uptake was demonstrable at examination 5 to 7 weeks and 12 weeks after the irradiation.

The exact mechanisms of ^{99}Tc^{m}-DP uptake in bone are not established. Increased uptake is correlated to trauma, inflammation and neoplasia of bone as well as to different metabolic disorders such as hyperparathyroidism, osteomalacia and Paget's disease (GENANT et coll. 1974, KAYE et coll. 1975, ROSENTHALL & KAYE 1975, GARCIA et coll. 1976). The bone circulation, osteogenesis, osteolysis, osteoid collagen, as well as the total bone-crystal surface have been suggested as important factors influencing the uptake of bone-seeking technetium compounds (TILDEN et coll. 1973, GENANT et coll., KAYE et coll., ROSENTHALL & KAYE, GARCIA et coll.). Both technetium and the phosphates have been suggested as responsible for the binding to the bone substance (KAYE et coll., ROSENTHALL & KAYE). Most explanations are based on different aspects of an increase in bone metabolism. Autoradiography is reported to demonstrate that at the microscopic level the deposit of technetium polyphosphates is related to different stages of bone maturity, proximity to bone marrow and to osteocytes (TILDEN et coll.). Decreased accumulation of $^{99}Tc^m$-DP in normal bone has been reported in some patients as a consequence of radiation therapy and reduced bone circulation was suggested as a possible explanation (Cox 1974). It cannot be excluded that such decrease of $^{99}Tc^m$-DP uptake in the irradiated rabbit mandible was balanced by a locally increased uptake in the reactive zone at the fundus of the alveolus in the present series. Nor can it be excluded that radiation injury to the rabbit skull bones may be demonstrable by scintigraphy if the time interval between the irradiation and the examination is prolonged beyond 12 weeks, as the morphologic appearance changes with time and is dominated by bone destruction at 24 weeks after irradiation of the mandible with 20 Gy (NATHANSON & BÄCKSTRÖM).

However, it may be concluded that a scintigraphy with $^{99}Tc^m$-DP is not effective in demonstrating early radiation injury to the bone tissue in the rabbit. These results are probably also relevant for man, despite the differences in the radiation pathology of continuously growing teeth in rabbits and on permanent teeth in adult human beings. Furthermore, the results obtained suggest that radiation therapy does not cause an increased uptake in the facial skeleton.

Acknowledgements

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SUMMARY

Histology demonstrated new bone formation and bone destruction in rabbit mandibles irradiated with 20 Gy in a single exposure, but no abnormality following 10 Gy in a single exposure. Gamma camera examination of the $^{99}Tc^m$-DP distribution could not demonstrate any abnormalities, and is concluded not to be effective in demonstrating early radiation injury to bone tissue. Radiation therapy is considered a negligible source of false positive findings in scintigraphy of the facial skeleton.

ZUSAMMENFASSUNG

Nach einer einzelnen Dosis von 20 Gy wurde im Unterkiefer von Kaninchen Neubildung von Knochengewebe und Knochendestruktion bei histologischer Untersuchung festgestellt, dagegen wurden keine Veränderungen nach 10 Gy beobachtet. Keine Veränderungen konnten bei Untersuchung mit der Gamma-Kamera der $^{99}Tc^m$-DP Verteilung festgestellt werden und daraus wurde der Schluss gezogen, dass diese Untersuchungsmethode nicht effektiv ist um frühzeitige Strahlenschäden im Knochengewebe nachzuweisen. Strahlentherapie wird als keine Ursache zu falschen positiven Ergebnissen bei Szintigraphie des Gesichtsschädels betrachtet.

RÉSUMÉ

L'histologie a mis en évidence une formation d'os nouveaux et une destruction osseuse sur les mandibules de lapins irradiés par 20 Gy en une seule exposition mais pas d'anomalie après 10 Gy en une exposition unique. L'examen à la gamma caméra de la distribution du $^{99}Tc^{m}$-DP n'a mis en évidence aucune anomalie et les auteurs concluent qu'elle n'est pas efficace pour mettre en évidence les lésions précoces du tissu osseux dues aux radiations. Le traitement par les radiations est considéré comme une source négligeable de résultats faussement positifs dans la scintigraphie du squelette de la face.

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