# $^{99m}$Tc-MDP extraction in relation to $^{81m}$Kr perfusion following a partial osteotomy in the dog tibia

R. A. A. Khan[1]

*University of Texas Medical Branch, Division of Nuclear Medicine, Galveston (Texas 77550, USA), 12 December 1979*

*Summary.* The bone mineral extraction of technetium-99m-methylene diphosphonate ($^{99m}$Tc-MDP), and blood supply of Krypton-81m ($^{81m}$Kr) have been compared in normal and osteotomy regions of the canine tibia. A partial osteotomy was carried out under aseptic conditions, and isotopic measurements made over a period of 123 days. Both blood flow and bone mineral extraction increase after a partial osteotomy.

Several factors have been put forward which could influence the skeletal distribution of a bone seeking radiopharmaceutical. Among these are local alterations in blood flow, osteogenesis, metabolic activity, capillary permeability, and surface area for tracer exchange[2-5]. Increased mineral uptake has been demonstrated in a fractured bone[6,7] and it has been suggested that this might be due to an increase in vascularity which occurs at the fracture site during the healing process[8].

These experiments were carried out to study the change in bone mineral extraction of $^{99m}$Tc-MDP and the alteration in blood supply of $^{81m}$Kr following a partial osteotomy in the canine tibia. $^{99m}$Tc-MDP is one of the bone seeking diphosphonate compounds which has been shown to clear very rapidly from the blood and soft tissue compartments[9,10] thus permitting bone scanning to be carried out as early as 2 h after i.v. injection. Its preparation in kit form and subsequent labelling with pertechnetate ($^{99m}TcO^{-}_{4}$) has been described previously[11].

$^{81m}$Kr is a metabolically inert, radioactive gas with an ultrashort half-life of 13 sec. It was eluted in aqueous solution from a $^{81}$Rb-$^{81m}$Kr generator system. The preparation of such a generator for clinical use has been described previously[12]. When infused into an artery at a constant rate, $^{81m}$Kr distributes by penetrating the extracapillary compartments. Its distribution reflects regional blood supply which was monitored in these experiments by external recording over normal and osteotomy regions of the tibia.

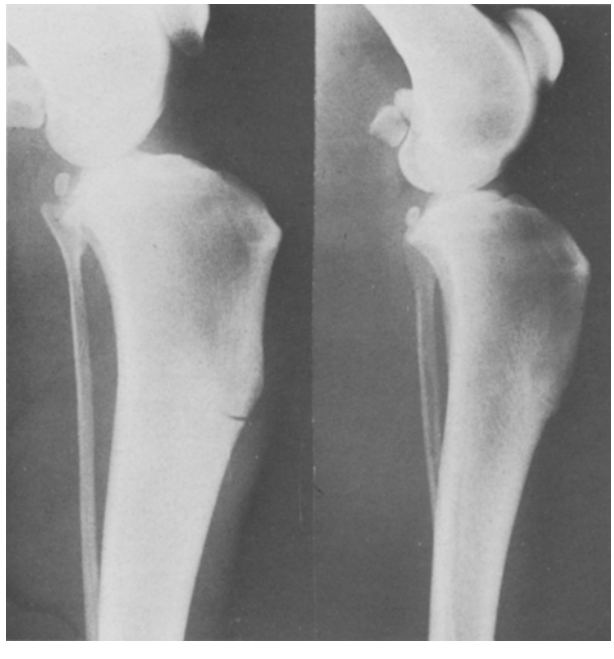

Fig. 1. X-ray of the dog leg to show (left) partial osteotomy below the anterior tibial tubercle and (right) healing 3 weeks after the osteotomy was performed.

*Materials and methods.* These experiments were carried out in 3 greyhound dogs (25–32 kg in weight). a) Osteotomy. Each dog was anaesthetised and intubated using an endotracheal tube. Under sterile conditions, a partial osteotomy was carried out about 1 cm below the anterior tibial tubercle (figure 1). To do this, a small incision was made in this region and the skin and periosteal covering of the bone retracted. The bone was then cut transversely to a depth of about 1 cm using a hack saw. The wound was closed, and a dressing applied. The dog was allowed free activity in its kennel, and fed on 0.5 g tetracycline daily in its diet for a period of 7 days.

b) Experimental. The following $^{81m}$Kr and $^{99m}$Tc-MDP studies were carried out before the osteotomy to obtain a baseline measurement, and then at 24, 40, 47, 61, 68, 75, 96, 103, 110, 117, and 123 days after the osteotomy.

On each occasion, a dose of 400–500 µCi $^{99m}$Tc-MDP was administered i.v. into a vein in the fore-leg, and 2 h later the dog was anaesthetised and intubated. A femoral arterial puncture was made and the needle connected to the $^{81}$Rb-$^{81m}$Kr generator system by a manometer line provided with Luer fittings.

A 2.5-cm diameter collimated sodium iodide scintillation probe was placed over the region of the osteotomy and in contact with the bone. The generator pump was switched on, and when infusion reached equilibrium the count rate of $^{81m}$Kr over the osteotomy region was recorded on a scaler-ratemeter unit for a pre-set time of 300 sec. The pump was switched off, and after 3 min (10 half-lives for $^{81m}$Kr) the count-rate of $^{99m}$Tc-MDP was next recorded under the same geometrical conditions. The probe was then positioned over a normal region of the same bone, and the 2 measurements repeated as above for $^{81m}$Kr and $^{99m}$Tc-MDP.

At the end of each experiment, the dog was returned to the animal house where it was kept under observation until it recovered from the anaesthesia. It was important that the needle be withdrawn very carefully from the femoral artery in order to prevent formation of a haematoma. The ex-

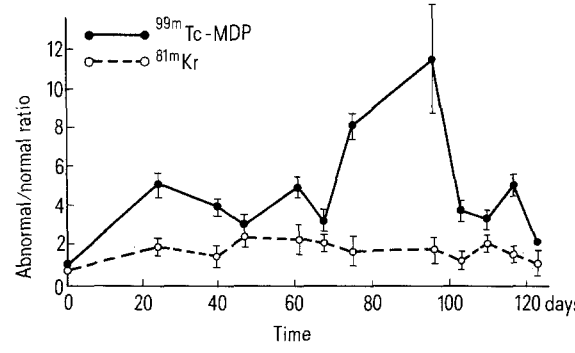


Fig. 2. Abnormal to normal ratio $^{99m}$Tc-MDP and $^{81m}$Kr shown as a function of time. Measurements were made with a NaI (Tl) scintillation probe placed first over the osteotomy region and then over a normal region of the same bone.

periment was terminated after the last observation which was made at 123 days.

*Results.* Each measurement was corrected for background and, in the case of $^{81m}$Kr and $^{99m}$Tc-MDP. The results are shown in figure 2. It can be seen that this ratio increases for both agents, the increase being greater in the case of $^{99m}$Tc-MDP. It reaches a maximum at about 14 weeks after the osteotomy, thereafter returning to baseline levels. The increase in $^{81m}$Kr is more or less constant throughout except that it shows a slight diminution towards the end of the experimental period.

*Discussion.* Bone blood supply has been shown to increase following a complete osteotomy[13]. Bone blood flow also increases and reaches a maximum of 4 times the control value at 10 days following an experimentally induced tibial fracture[14]. This increase in flow produces an increase in capillary surface area from recruitment and dilatation of new capillary beds. The result of the capillary recruitment is that the radioactive tracers are distributed over a larger surface area, with a corresponding increase in the number of bone mineral binding sites which are available for exchange.

The mechanism of uptake of isotopes in bone containing tumour has been examined and it was suggested that there are 2 stages of uptake, a rapid vascular stage and a selective concentration in immature new bone[15]. In an osteotomy at 10 days, although there is a great deal of osteoblastic activity with periosteal new bone formation, there is also proliferation of blood vessels with re-establishment of the medullary circulation.

These experiments have confirmed the findings that following an osteotomy blood flow to bone increases. This increase however, is shown to be proportionately less than the increase in extraction of $^{99m}$Tc-MDP. The high increase in extraction of the latter agent following the osteotomy supports the two-stage theory of uptake mentioned previously[15], i.e. an increase in flow followed by an increase in extraction by newly formed bone. The extraction reaches a peak at about 14 weeks by which time healing and bone remodelling has neared completion.

It can be concluded therefore, that the effect of a partial osteotomy is to produce both an increase in bone blood flow as shown by $^{81m}$Kr, as well as an increase in mineral extraction of the bone-seeking agent, $^{99m}$Tc-MDP. These results have also shown that it is the increased efficiency for extraction of $^{99m}$Tc-MDP by the newly formed bone which is the more important of the 2 factors.

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## Rapid development of acoustic trauma-induced audiogenic seizure risk in 3 strains of seizure-resistant mice[1]

C.-S. Chen

*Department of Psychology, Monash University, Clayton (Victoria 3168, Australia), 17 December 1979*

*Summary.* Audiogenic seizure risk can be induced in genetically seizure resistant mice by exposure to an intense noise a few days prior to testing for seizure. This experiment demonstrates that the priming induced seizure risk can develop within 6–16 h after priming. It was argued that this finding suggested an alternative hypothesis of priming involving peripheral auditory mechanisms.

Certain strains of inbred mice, such as C57BL/6J or BALB/c mice are genetically non-susceptible to audiogenic seizure in the sense that they rarely exhibit seizure responses on the very first exposure to an intense noise. However, audiogenic seizure risk can be induced in these so-called seizure-resistant mice by exposure to an intense noise a few days before testing for audiogenic seizure[2,3]. It has been suggested that the seizure risk develops because the priming exposure has triggered development of a hyper-reactive state similar to the well-known physiological phenomenon of disuse or denervation supersensitivity in the higher auditory system[4]. This hypothesis argues that the priming exposure causes stimulation damage to the mouse cochlea. Consequent to this damage the afferent input to the higher auditory structures is reduced leading to development of a supersensitive state in these structures. When the primed mice are re-exposed to an intense noise, the supersensitive structures are stimulated and audiogenic seizure responses are precipitated.

The disuse hypothesis of priming implies that susceptibility to audiogenic seizure should take time to develop. Indeed, available evidence indicates that the priming induced seizure risk generally takes 1.5–3 days to develop[2,3,5,6]. However, results showing unusually rapid development of seizure risk have occasionally been reported[5,7]. Since the developmental rate of the seizure risk could be an important limiting factor for the credibility of the disuse hypothesis of priming, the present study was designed to obtain additional information regarding this temporal dimension of the priming phenomenon.

The animals used were BALB/c mice, C57BL/6J mice and priming prone mice selectively bred in this laboratory. The acoustic stimulus used for priming and testing for audiogenic seizures was a repeated impulse noise with a repeti-