INVESTIGATIVE NUCLEAR MEDICINE

Bone Healing in Rabbits after Compression Osteosynthesis, Studied by Tc-99m(Sn)Polyphosphate Scintimetry and Autoradiography

Jørgen Greiff

University of Copenhagen and Hvidovre Hospital, Hvidovre, Denmark

The purpose of the present study was to determine the scintimetric time course (STC) for Tc-99m(Sn)polyphosphate in rabbit tibias after various osteosynthetic procedures, and to correlate the findings with those from serial radiographs and with autoradiographic and histologic evaluation of the bone. The STC was similar for all treatment groups, with a peak value within the second week after surgery. Significantly different levels of the STC were found after subperiosteal exploration, plate insertion, osteotomy and compression plating, or osteotomy and medullary nailing. The radiological, autoradiographic, and histological findings revealed that Tc-99m scintimetry monitors callus formation. The STC thus appears to be a valuable tool for the quantitative study of bone healing.

J Nucl Med 22: 693–698, 1981

Tc-99m(Sn)polyphosphate accumulates in newly formed mineralizing bone tissue (*1*,*2*), and the sequence of events during fracture healing has been demonstrated autoradiographically in rat fractures (*2*).

The aim of this study was to determine the Tc-99m(Sn)polyphosphate scintimetric time course (STC) in a series of osteotomies and experimental fractures of the rabbit's tibia and to evaluate the ability of scintimetry to demonstrate differences and variations of callus formation by comparison of the STC with previously published macroscopical, radiological, and histological findings (*3*).

MATERIAL AND METHODS

Thirty-five white rabbits with an average weight of 3.4 kg (range 2.4–4.5 kg), divided into eight groups according to the operation performed (Table 1), were anesthetized once a week during 4 wk for scintimetric examination of the tibia, with the exception of four of the animals in Group C (details in Table 1). One animal in Group C was excluded due to a persistent large hematoma. Details of the anesthesia and operation have been published earlier (*3*).

The rabbits were placed on a special examining table (Fig. 1). The location of the osteotomy or fracture was determined by radiography before each study. A dose of 1 mCi of Tc-99m mixed with 10 mg polyphosphate was given by ear vein.

The scintimetry was performed with a lead-shielded scintillation detector (NaI, 1.75 × 2 in.) connected to a pulse-height discriminator with ratemeter and linear recorder. The collimator had a cylindrical aperture, 3.6 cm in diam and 7.2 cm long. During the examination the scintillation detector was placed with the collimator centered over the lesion, touching the skin, thus counting over an area of 10.18 cm^2. The radioactivity was recorded continuously (Fig. 2). When the count rate was reasonably constant for 2 min, the scintillation detector was moved to the corresponding site on the other leg. The procedure was repeated three times on each leg (Fig. 2). The background radiation was measured before each study (Fig. 2B) but was so small that it could be ignored.

From the chart the count rate (av. ~ 2500 cps) was read and corrected for the physical decay. Corresponding

Received July 18, 1980; revision accepted March 13, 1981.

For reprints contact: Jørgen Greiff, Ahornvej 40, DK-2970 Hørsholm, Denmark.

TABLE 1.

Group	Type of operative procedure	No. of animals
A	Subperiosteal exploration of the tibia.	4
B	Subperiosteal application of a plate to the tibia.	4
C	Osteotomy, plate osteosynthesis, intended longitudinal compression.	7
D	Osteotomy, plate osteosynthesis, intended longitudinal compression, reaming of the medullary canal.	4
E	Osteotomy, plate osteosynthesis, intended longitudinal compression, interposition of a polystan disc.	4
F	Osteotomy, plate osteosynthesis, no compression intended.	4
G	Fracture, plate osteosynthesis, longitudinal compression intended.	3
H	Osteotomy, medullary nailing.	4
Total		34

to the three successive measurements, ratios (R_1, R_2, and R_3) were calculated for the count rate on the operated side over the contralateral cps. The coefficient of variation for the triplicate measurement was calculated to be about 5% for all levels of the ratio.

The systematic creep during a measuring session revealed that on the average the ratio increased by 0.098 ($p < 0.01$) from R_1 to R_2 (average $R_1 = 2.47$), and by 0.061 ($p < 0.01$) from R_2 to R_3. On the average the first measurement was accomplished after $T_1 = 13.8 \pm 5.8$ (1 s.d.) min after administration of the tracer, and all measurements were finished after 28.5 ± 7.1 min. However, on the average this increase of the ratio amounts to only 10% of R_1 over the time period ($T_1 \pm 2$ s.d.) during which R_1 was measured. This scintimetric ratio was used as an index of the bone formation around the fracture and for the further statistical analysis of the results.

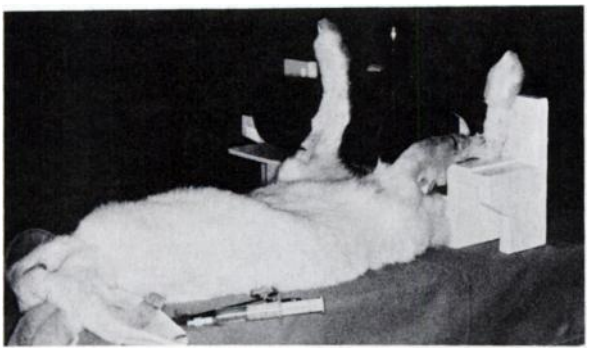

FIG. 1. Scintimetry on rabbit, utilizing a scintillation detector and specially designed examining table.

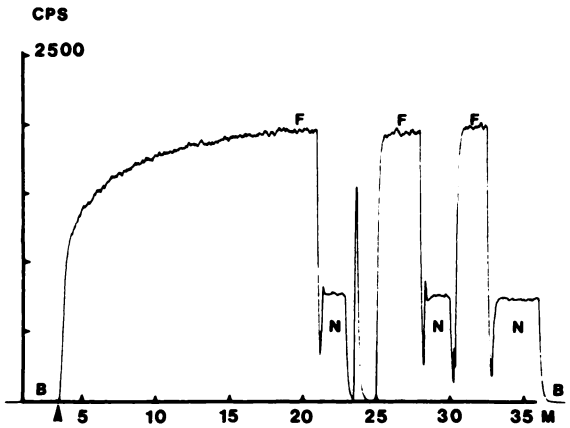

FIG. 2. Recording of triplicate scintimetry, showing maximum counts per second over surgical lesion (F) to be approached at ~15 min after the administration of tracer (arrow). Measurements appear reproducible over surgical lesion (F,F,F) as well as over nonoperated leg (N,N,N). Background activity (B,B) is negligible. All measurements were completed in about 30 min. Abscissa = time in min; ordinate = counts per second.

To ensure sufficient radioactivity for the autoradiograms (*2*), an additional intravenous injection of 10 mCi of Tc-99m mixed with 100 mg of polyphosphate was given 30 min before the animals were killed. The tibia was removed, denuded, and divided along its central sagittal plane for contact autoradiography and histological examination (*3*).

RESULTS

Higher radioactivity was always found on the operated side compared with the nonoperated side, with one exception where the R_1 was 0.97 (Table 2, Group A, No. 75047, 4th wk).

The mean STCs for Groups A, B, C, and H are plotted in Fig. 3, and for Groups C, D, E, F, and G in Fig. 4.

An extensive statistical analysis of the STC has been performed.* This was based on the hypothesis that a given scintimetric ratio (R) is composed of the sum of the level of the ratio, the time effect (weeks), and the biological variation. This hypothesis was validated by a fairly detailed analysis of the deviations.

A nested two-way analysis of variance showed that the time effect may be assumed equal for all eight treatment groups, and that the average ratio of all groups does vary significantly between the weeks, with a peak value in the second week. Since the biological variations must be taken into account, the further comparison between the treatment groups must be made on the basis of group averages, which are given in Table 3. A general test for identity of all eight groups was very clearly rejected ($p < 0.0005$), and so was the hypothesis of homogeneity of the four fundamentally different treatment groups (A, B, C, and H). To compare two groups at a time, one should use the ordinary t-test, with 0.125 as the esti-

TABLE 2. SCINTIMETRIC RATIO (R_1)

Group, * animal no.	Week No. 1	2	3	4	8	12
A 75031	1.64	1.68	1.52	1.50	—	—
A 75053	1.44	1.47	1.09	1.12	—	—
A 75047	1.73	1.61	1.22	0.97	—	—
A 75042	1.92	2.01	1.29	1.39		
B 75029	2.02	2.04	1.65	1.68	—	—
B 75103	2.27	1.78	1.94	1.64	—	—
B 75098	2.32	1.84	1.74	1.67	—	—
B 75062	2.55	3.11	2.12	1.94	—	—
C 75050	1.97	2.69	2.16	1.85	—	—
C 75102	2.66	2.50	2.05	1.93	—	—
C 75097	2.92	2.78	1.89	2.12	—	—
C 75137	—	—	—	1.50	1.44	1.41
C 75141	—	—	—	1.61	1.63	—
C 75140	—	—	—	1.98	1.55	—
C 75138	—	—	—	2.41	1.83	1.77
D 75051	1.96	2.79	2.75	2.78	—	—
D 75105	2.30	2.30	1.83	1.84	—	—
D 75104	2.64	2.43	1.78	1.99	—	—
D 75045	3.01	2.44	3.26	2.60	—	—
E 75063	1.85	2.20	2.43	2.72	—	—
E 75048	2.67	3.15	2.64	2.31	—	—
E 75049	3.26	4.19	3.14	2.55	—	—
E 75041	3.50	3.14	3.41	3.79	—	—
F 75056	2.38	2.94	3.14	2.74	—	—
F 75064	2.50	3.55	2.45	2.31	—	—
F 75023	2.83	2.26	1.91	1.65	—	—
F 75095	3.25	3.15	2.07	2.53	—	—
G 75133	2.81	3.54	2.96	2.55	—	—
G 75134	3.02	2.16	1.82	2.38	—	—
G 75136	2.98	2.85	2.74	2.37	—	—
H 75120	2.81	2.97	2.51	2.10	—	—
H 75119	2.84	3.90	3.45	3.85	—	—
H 75122	3.29	4.34	3.35	3.48	—	—
H 75121	3.39	4.44	3.63	2.94	—	—

* For groups see Table 1.

mated basic variance of a rabbit mean. The comparison of Group C with the modifications of surgical technique in Groups D, E, F, and G showed only Group E to be statistically different ($p < 0.025$).

The autoradiograms made after 4 wk showed a high uptake of the tracer in areas showing new bone formation by macroscopic, radiological, and histological criteria (*3*).

The autoradiograms of Group A showed a small but distinct periosteal accumulation of radioactivity, corresponding to the periosteal callus discernible only by microscopy. The autoradiograms of the remaining groups showed a varying amount of periosteal tracer accumulation corresponding to the varying amount of macroscopically evaluated periosteal callus formation found on the divided bone (Figs. 5 and 6). Endosteal tracer accumulation was substantial in Groups C–H and was comparable to the histologically evaluated endosteal callus. The cortical bone displayed very little, if any, radioactivity. However, the autoradiograms demon-

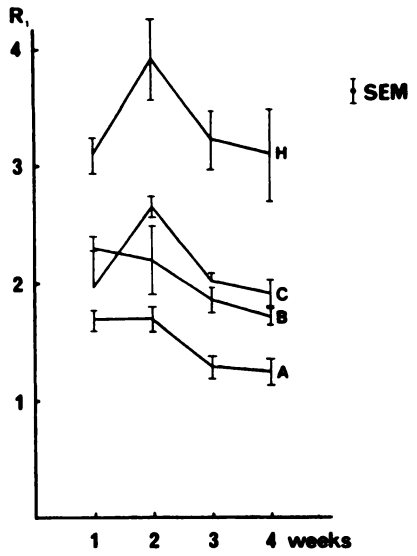

FIG. 3. Mean scintimetric time course (STC), based on mean scintimetric ratio (cps from operated leg/contralateral cps) for the first measurement in each study (R_1) in Groups A, B, C, and H (surgical procedures listed in Table 1). Abscissa = time in weeks after operation; ordinate = mean scintimetric ratio; SEM = standard error of the mean.

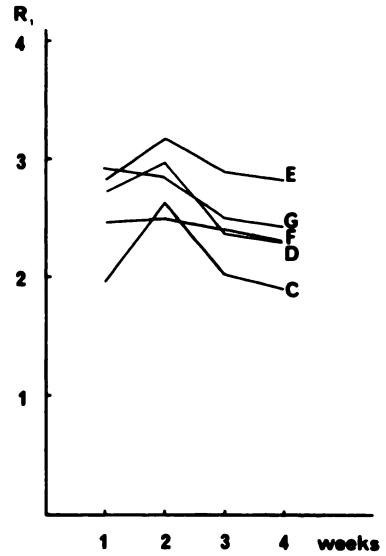

FIG. 4. Scintimetric time courses for average R_1 in groups indicated. For surgical procedures see Table 1. Abscissa = time in weeks after operation; ordinate = mean scintimetric ratio.

strated radioactivity in only a small section of the bone and thus were unsuitable for quantitative evaluation.

DISCUSSION

Radioactive tracers have been used previously in the study of bone healing (for surveys see Nilsonne (*10*) and Falkenberg (*5*)).

Subramanian and McAfee (*1*) showed that Tc-99m(Sn)polyphosphate accumulates in callus to a far greater extent than in mature cortical bone. This finding was confirmed in a previous autoradiographic study of bone healing in rats (*2*), and it substantiates the use of scintimetry for the study of callus formation.

An increased accumulation of the tracer followed the surgical procedure in all animals where new bone formation was clearly visible histologically (*3*). On the average, maximum count rates for practical purposes were found 14 min after the administration of the tracer, a finding compatible with those of Subramanian and McAfee.

The majority (75% or more) of the Tc-99m(Sn)-polyphosphate is cleared from the blood within a few minutes (*6,7*). The decrease of tracer content in the blood, the hyperemia around the surgical lesion (*8,9*), and the stable union between the tracer complex and the callus (*2*) explain the increase of the ratio R with time. It appears from this study, however, that the increase of the ratio within the period of measurement is rather small. This systematic error, and the error involved in reproducing the position of the detector, are included in the estimate of the maximum error of measurement: coefficient of variation ~5%.

The STC was parallel in all groups, with a peak value not later than the second week. These findings are statistically significant and agree with the findings of MacDonald (*4*) and Falkenberg (*5*), who used Sr-85 in studies of fracture healing in rabbits.

The STC indicates that the intensity of the calcification process diminishes with increasing maturity of the callus; this parallels the autoradiologically determined variations that occur during fracture healing in rats (*2*).

Scintimetry disclosed the callus formation earlier than the radiological examinations (*3*). This demonstrates that the mineralization process has to be present for some time before radiological methods can detect new bone formation. Scintimetry shows the accretion process, whereas radiology shows the accumulated accretion.

Statistically different levels of the STC were found in the fundamentally different treatment groups (A, B, C, and H). The radiological and histological analyses, previously published (*3*), explain these findings. When

TABLE 3. AVERAGE GROUP SCINTIMETRIC RATIOS (WEEKS 1, 2, 3, and 4)

Group	A	B	C	D	E	F	G	H
No. of rabbits	4	4	3	4	4	4	3	4
Average R	1.47	2.01	2.29	2.41	2.93	2.60	2.68	3.33

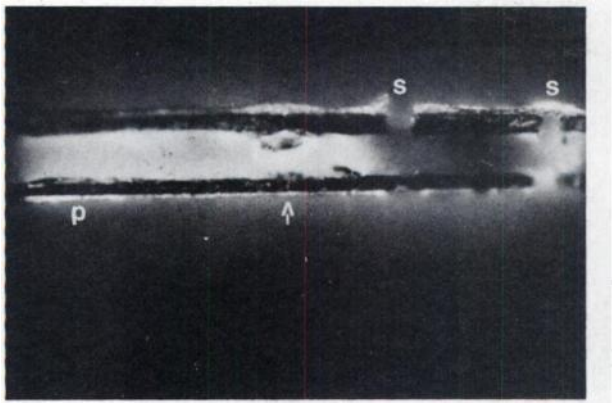

FIG. 5. Autoradiogram of tibia (No. 75102, Group C) 4 wk after osteotomy. Light area indicates concentration of tracer. Arrow indicates site of osteotomy; P indicates cortical bone beneath plate; S indicates sites of screws. Proximal fragment is seen at right (X3).

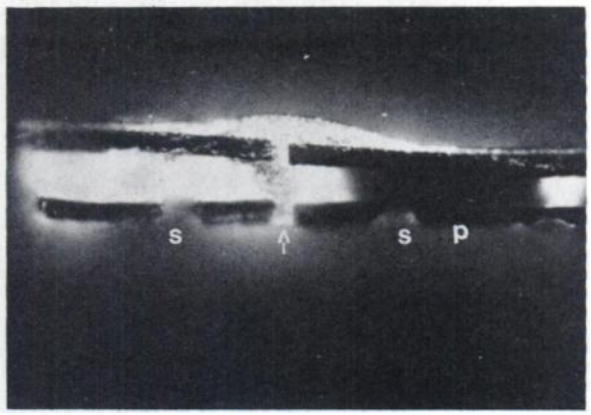

FIG. 6. Autoradiogram of tibia (No. 75064, Group F) 4 wk of observation. Symbols as in Fig. 5.

only a subperiosteal exposure of the tibia was performed (Group A) only histologically detectable periosteal callus developed. When a plate was applied to the intact tibia (Group B), radiologically visible periosteal callus developed at the edge of the plate and at the tips of the screws. An osteotomy and compression-plate osteosynthesis (Group C) induced the development of additional endosteal callus, and medullary nailing (Group H) led to the formation of large amounts of radiologically visible periosteal callus.

From the comparisons between the STCs for the standard surgical procedure (Group C: osteotomy and compression-plate osteosynthesis) and the surgical modifications in Groups D, E, F, and G, it was found that neither destruction of the vessels of the medullary canal (Group D), nor the size of the osteotomy or fracture gap (Groups F and G) led to a statistically significant change in STC. This is in agreement with the autoradiological, radiological, and histological findings, where only minor differences in periosteal and endosteal callus formation could be found.

The STC of Group E was significantly higher than that of Group C. The slower decrease of the ratio in that group could be explained as resulting from a prolonged stimulus to callus formation, since no bridging callus added to the stability of the osteosynthesis. None of the radiological, histological, or autoradiological findings explained the deviation of the scintimetry.

In the present study the autoradiographic technique was used mainly to locate new bone formation, and it confirmed the histological findings (*3*). The formation of new bone in the cortical fragments was negligible.

This study and the previously published data show that the factors promoting callus formation appear to be the elevation of the periost, the application of implant, and the discontinuity of the long bone. The degree of callus formation appears to depend on the degree of stabilization of the fragments and their ability to obtain contact with each other. The local vascularity of the cortical bone, the distance between the fragments (within certain limits), and the extent of the osteotomy/fracture line appear to be of less significance for the development of callus.

In conclusion, sequential Tc-99m(Sn)polyphosphate scintimetry appears to provide a method, with an acceptable error, for the assessment of new bone formation, since the size of the ratio R correlated well with callus formation, as evaluated radiologically, histologically, and autoradiographically.

Since atrophic pseudarthrosis is characterized by a lack of callus formation, and hypertrophic pseudarthrosis by an increased amount of callus formation, scintimetry might provide a diagnostic tool to predict the likelihood of fracture healing in human long bones (*11*).

FOOTNOTE

* Details can be obtained from the author.

ACKNOWLEDGMENTS

I am grateful to N. Keiding, Institute of Mathematical Statistics, University of Copenhagen (now at the Statistical Research Unit, Danish Medical and Social Sciences Research Councils) for performing the statistical analyses. This study was supported by Grants from Novo Foundation and Danish Medical Research Council (No. 512-9066).

REFERENCES

1. SUBRAMANIAN G, MCAFEE JG: A new complex of ^{99m}Tc for skeletal imaging. *Radiology* 99:192-196, 1971
2. GREIFF J: Autoradiographic studies of fracture healing using ^{99}Tc^{m}-Sn-polyphosphate. *Injury* 9:271-277, 1978
3. GREIFF J: Bone healing in rabbits after compression osteosynthesis: a comparative study between the radiological and histological findings. *Injury* 10:257-267, 1979
4. MACDONALD NS: Kinetic studies of skeletal metabolism by external counting of injected radioisotopes: The radioisotope osteogram. *J Lab Clin Med* 52:541-558, 1958
5. FALKENBERG J: An experimental study of the rate of fracture

healing as assessed from the tensile strength and S^{85}-activity of the callus with special reference to the effect of intramedullary nailing. *Acta Orthop Scand Suppl* 50:7-18, 1961

6. SUBRAMANIAN G, MCAFEE JG, BLAIR RJ, et al: Technetium-99m-methylene diphosphonate—A superior agent for skeletal imaging: Comparison with other technetium complexes. *J Nucl Med* 16:744-755, 1975
7. KRISHNAMURTHY GT, HUEBOTTER RJ, WALSH CF, et al: Kinetics of ^{99m}Tc-labeled pyrophosphate and polyphosphate in man. *J Nucl Med* 16:109-115, 1975
8. RHINELANDER FW, BARAGRY RA: Microangiography in bone healing. I. Undisplaced closed fractures. *J Bone Joint Surg* 44A:1273-1298, 1962
9. RHINELANDER FW, PHILLIPS RS, STEEL WM, et al: Microangiography in bone healing. II. Displaced closed fractures. *J Bone Joint Surg* 50A:643-662, 1968
10. NILSONNE U: Biophysical investigations of the mineral phase in healing fractures. *Acta Orthop Scand Suppl* 37:5-81, 1959
11. GREIFF J: The time course of ^{99m}Tc-Sn-polyphosphate scintimetry in healing tibial fracture in man. *Injury*, 1981, in press