THE EFFECT ON CORTICAL BONE OF REAMING AND FILLING OF CANINE TIBIAL DIAPHYSIS WITH INERT BONE WAX

J. STÜRUP[1], L. NIMB[1], J. S. JENSEN[1]

In canine studies the effect of intramedullary reaming on tubular bone was investigated in 4 dogs. Intramedullary reaming was further compared with reaming and intramedullary filling with bone wax in 6 dogs. Bone blood perfusion was measured by a microsphere technique and bone remodelling activity by ^{99m}Tc-MDP uptake. From histological sections bone necrosis and remodelling activity were estimated. The biological response increased with the surgical trauma. If the medullary cavity was only reamed, endosteal apposition was the predominant reaction. Obturation of the medullary cavity resulted in more vigorous subperiosteal and cortical reaction. It is concluded that the remodelling processes differ significantly between reamed bone and bone where the medullary cavity is reamed and blocked. Thus when testing bone cement the studies should include a control operation with obturation of the medullary canal in a way simulating bone cement.

Keywords : canine tibia ; diaphysis ; reaming ; inert bone wax.

Mots-clés : diaphyse ; tibia du chien ; alésage ; cire à os inerte.

INTRODUCTION

Bone damage due to destruction of medullary blood supply to diaphyseal bone after reaming of the medullary cavity has been investigated by Danckwardt-Lillieström (2) and Rhinelander *et al.* (6), who found severe necrosis, but revascularization within 4 weeks (2). After filling of the medullary cavity with PMMA bone cement, necrotic areas were still present after 1 year (6). Sund & Rosenquist (10) believed that the obturation of the medullary cavity prevented revascularization from the bone marrow and might be more deleterious to bone than monomer leakage and curing from PMMA bone cement.

In the present study reaming of the medullary canal was compared to contralateral untouched bone and to obturation of the medullary canal with an inert filter, bone wax. Parameters recorded were bone blood perfusion estimated by a microsphere technic and vital staining with disulphine blue, bone remodelling activity estimated by ^{99m}Tc-MDP uptake, and an evaluation of histological sections.

MATERIAL AND METHODS

In four adult mongrel dogs the one tibia was reamed with hand-driven reamers to a diameter of 8-9 mm, and the medullary cavity curetted, flushed with saline and sucked dry. The contralateral tibia was left untouched. Two dogs were followed for 4 weeks, and 2 dogs for 3 months.

In 6 dogs both tibias were reamed, but the intramedullary canal was filled on one side with inert bone wax (3, 7) containing zirconium oxide as contrast material. Postoperative radiographs confirmed adequate filling of the tibial diaphysis. These dogs were followed for 4 weeks.

For estimation of bone avascularity the dogs were intravitally stained by injecting 30 ml of 7.5% disulphine

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[1] Department of Orthopedics U, Rigshospitalet, University of Copenhagen, Denmark.

Correspondence and reprints : J. Stürup, Department of Orthopedics O, Centralsygehuset i Hillerød, Helsevej 2, 3400 Hillerød, Denmark.

blue (9) 30 minutes before the dogs were killed by an overdose of mebumal sodium. The tibias were collected, stripped of soft tissue and divided longitudinally in half. The grading of unstained areas in the disulphine-stained sections was based on visual impression (table I).

With the purpose of determining the bone blood perfusion (9, 11), Sc-46 labeled 15-micron microspheres (Nen-Track®) were injected, before killing, into the left ventricle of the heart simultaneously with collection of reference blood samples from the abdominal aorta with a suction pump.

The bone remodelling activity was estimated as ^{99m}Tc-MDP uptake (1, 8), given in a dose of 30 mCi intravenously 2 hours before the dogs were killed.

The ^{99m}Tc activity and a week later the Sc-46 activity in reference blood samples and bone specimens were determined. The bone specimens consisted of 5.1-cm long pieces of middiaphyseal bone, alternately harvested from the medial or lateral aspect of the tibia.

The other half of the tibias were freeze-cut on a hard tissue cryostat, photographed and then placed on photographic film for 12 hours.

Autoradiograms were analyzed in a computerized TV-based image analyzer (Leitz TAS-plus) for blackening an area of the cortex, the subperiosteal apposition and/or endosteal bone formation in the central 5 cm of the diaphysis (8, 9).

To measure the amount of blackening contributed by the microspheres, a tourniquet was applied to one front leg of each pair in 9 dogs 2 hours after the administration of ^{99m}Tc-MDP, just prior to injecting the microspheres. Blackening in autoradiograms in a 5-cm middiaphyseal segment from pairs of radii were then compared.

Following the autoradiography, tranverse segments were cut from the diaphysis and prepared for histological examination without decalcification (4). The sections were immersed in 10% neutral buffered formalin for 3 weeks and then dehydrated in ethanol. The bone segments were embedded in epoxy resin, and sections were cut and ground to a thickness of 50-80 µm (Exakt Apparatebau GbmH, Germany). Staining was performed with Stevenels blue/van Gieson picrofuchsin or a modified hematoxylin-eosin (4). The sections were evaluated using transmitting light microscopy, and the amount of cortical remodelling was quantified. A grid-counting method was employed, using a 125 × magnification and counting 100 points in a 100-µm grid. Counting was performed in 5 random locations in each of the 3 cortical zones, inner, middle and outer one-third.

Calculations

Blood flow rates were calculated as activity in bone specimen* reference pump flow/activity in reference samples/weight of bone specimen (9, 11). The blood flow rates given were calculated as the mean value of the 5 diaphyseal specimens.

The ^{99m} Tc-MDP counts, blackening in subperisteal apposition and original cortex were calculated as ratios between the two sides in one animal, whereas periosteal apposition and endosteal bone formation were measured in cm^2 for the central 5 cm of the diaphysis.

For statistical analysis the Wilcoxon two-sided paired rank sum test was employed.

RESULTS

Disulphine-stained sections (fig. 1) revealed larger unstained areas on the side filled with wax in 4/6 cases (table I). After 3 months no unstained areas following reaming could be found.

Table I. — Blood perfusion in canine tibial diaphysis, in untouched bone, in reamed bone and in bone which had been reamed and filled with inert material (bone wax), estimated by microsphere technic and vital staining with disulphine blue

Identification	Part[a]	Disulfinestaining Wax/reaming Unstained areas	Perfusion (Ml/100g/min) Wax	Reaming
54	L	+/+	6.9	3.5
55	M	++/+	22.0	8.2
62	M	+/+	31.2	17.7
64	L	++/+	9.9	6.9
65	L	++/+	11.1	5.9
72	L	++/+	10.2	9.8
Median			10.7	7.6
4 weeks		Reaming/–opr	Reaming	–opr
29	M	++/–	18.9	1.5
30	M	+/–	9.8	0.4
3 months				
20	L	–/–	2.1	1.0
21	M	–/–	0.3	0.2

[a] M = medial, L = lateral part of tibia used for perfusion measurements. Unstained areas following vital staining with disulphine blue, possibly indicating necrosis, were evaluated visually on a 4-step scale : – no unstained areas, + just visible unstained areas, ++, +++ increasing unstained areas extending further through the cortex.

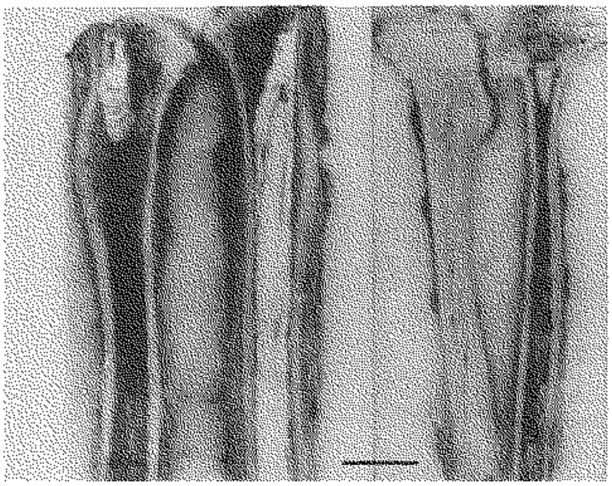

Fig. 1. — Disulphine-stained sections (bar = 2 cm). Left pair of bones (54), left bone reamed, right bone wax filled. The reamed bone demonstrates extensive endosteal bone formation but only very little subperiosteal apposition in contrast to the opposite wax-filled bone where the subperiosteal bone formation is abundant. Right pair of bones (30), left bone untouched, right bone reamed. Note the high degree of staining of all parts of the reamed bone compared to the untouched bone.

Blood perfusion was higher in bone filled with wax than in bone that had only been reamed ($p = 0.03$). However, reaming resulted in an increased blood perfusion compared to untouched bone.

The bone remodelling activity as measured by ^{99m}Tc counts or assessed by blackening in the cortex and periosteal apposition on autoradiograms (table II) was higher in 5/6 cases filled with wax as compared to reaming. Bone remodelling activity in reamed bones was higher than in untouched bones.

There were no measurable differences in blackening between the autoradiograms from radii with and without microspheres, the median ratio between the radii being 1.02 (range 0.82-1.19), indicating that the Sc-46 microspheres did not contribute significantly to the blackening.

Autoradiography (fig. 2) showed larger areas of subperiosteal apposition (table II) in bones filled with wax than in reamed bones ($p = 0.03$). In the dogs followed 3 months the Tc activity in sub-

Table II. — Bone remodelling activity in untouched (-opr) canine tibial diaphysis, or 4 weeks after reaming (R), or filling with bone wax (Wax), investigated by ^{99m}Tc-MDP uptake, measured directly in a scintillation counter, or as blackening on autoradiograms
Areas of subperiosteal (Periost)/endosteal (Endost) apposition and of original cortex were also measured on the autoradiograms

Identification	^{99m}Tc	Area (cm^2)				Blackening			
		Periost		Endost	Cortex	Periost	Cortex	Endost	
	W/R ratio	Wax	R	R		W/R ratio		E/PR	E/PW
54	1.72	3.21	0.29	4.16	1.04	1.26	1.17	1.14	0.96
55	1.52	2.9	1.34	2.06	1.02	1.28	1.32	1.17	0.81
62	1.65	2.45	2.01	0.63	1.06	1.30	1.32	1.14	0.78
64	1.5	1.00	0.83	0.79	1.03	1.35	1.16	1.26	0.93
65	2.01	2.23	1.45	1.23	0.98	1.27	1.28	1.19	0.85
72	0.98	2.38	2.53	1.94	0.96	0.94	0.69	1.25	1.42
Median	1.59	2.42	1.40	1.59	1.03	1.28	1.23	1.18	0.89
4 weeks	R/–opr	R	–opr	R		R/–opr ratio		E/PR	
29	6.74	1.53	0	2.4	1.00	–	1.77	1.24	–
30	5.97	1.68	0	2.45	0.99	–	2.10	1.20	–
3 months									
20	–	–	0	3.83	0.92	–	1.04	–	–
21	–	–	0	4.65	0.95	–	1.10	–	–

W/R = ratio between values on wax-filled and reamed bones. E/PR = ratio between values from endosteal apposition and subperiosteal apposition on reamed bones. E/PW = ratio between values from endosteal apposition on reamed bones and subperiosteal apposition on the opposite wax-filled bone. R/– = ratio between values from reamed and untouched bone.

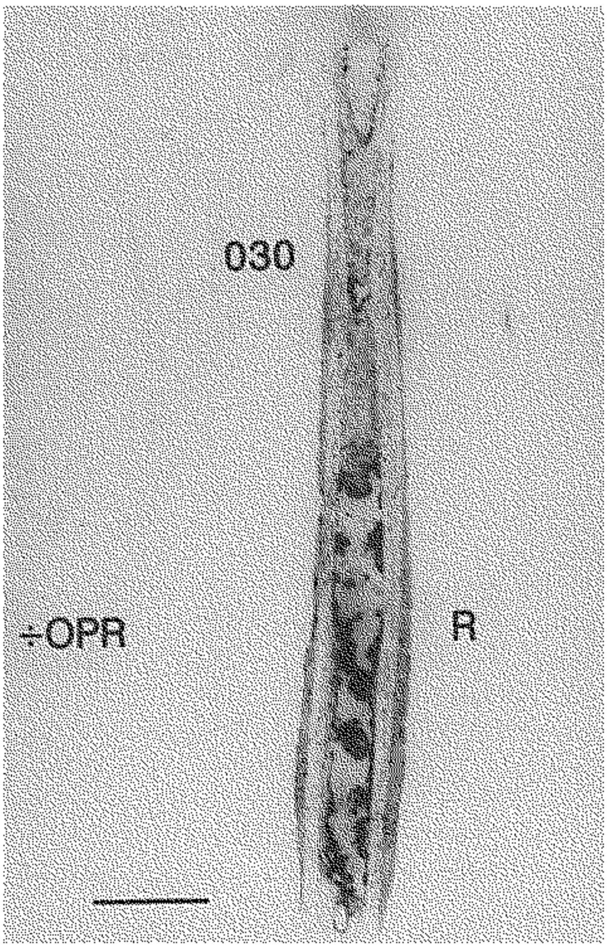

Fig. 2a

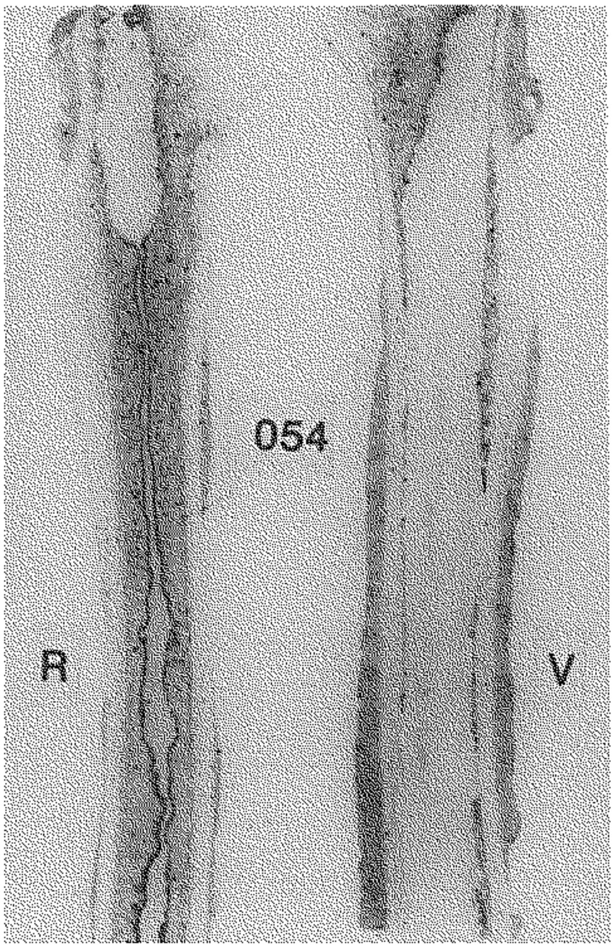

Fig. 2b

Fig. 2. — Autoradiograms of the same bones as in fig. 1 (bar = 2 cm). The contours of the untouched bone (÷OPR) are only just visible, whereas the reamed bones (R) demonstrate heavy activity especially endosteally. The wax-filled bone (V) shows extensive subperiosteal bone formation.

periosteal apposition was weak and not discernible from cortical activity, making measurements of area and blackening technically impossible. The cortical area did not differ significantly within the paired tibias studied, indicating that the sections were anatomically comparable.

Blackening in endosteal bone formation (table II) on reamed bones was consistently greater than blackening in subperiosteal apposition (E/PR ratio), on the same bone in all cases ($p = 0.03$). However, blackening in the endosteal bone formation of reamed bone was smaller in all but one case (72) than in the subperiosteal apposition on the contralateral side filled with wax (E/PW ratio).

The histological sections from the reamed bones showed only very little bone necrosis expressed as empty osteocyte lacunae whereas some necrosis was present in the waxed bones in the inner 1/3 of cortex. In reamed bones large amounts of endosteal bone formation and limited subperiosteal bone formation were seen, whereas the wax-filled bones revealed large amounts of subperiosteal bone formation (fig. 3). The remodelling activity (table III) was predominant after 4 weeks in the outer 1/3 of cortex on the side filled with wax

($p = 0.03$), whereas the predominant activity was located in the inner 1/3 of the cortex on the reamed side ($p = 0.03$). In the middle zone no larger differences were found.

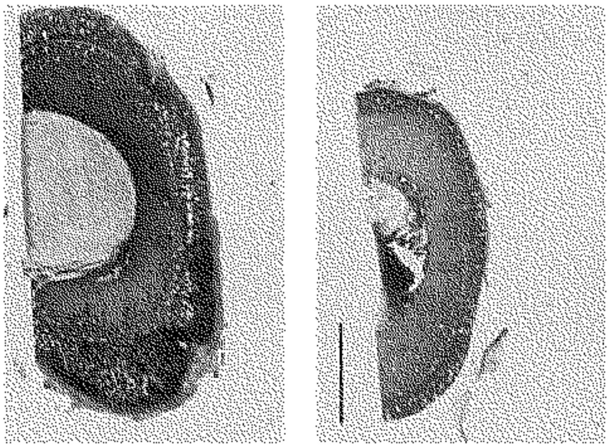

Fig. 3a *Fig. 3b*

Fig. 3. — Histological sections (65) stained with Stevenels blue (bar = 5 mm). Left bone wax-filled, right bone reamed. The wax-filled bone demonstrates large subperiosteal apposition while the reamed bone demonstrates some endosteal apposition. In the wax-filled bone heavy remodelling with many resorption lacunae in the outer part of cortex can be seen.

DISCUSSION

Reaming of the medullary cavity resulted in 5 to 10 times greater blood flow and remodelling activity in cortical bone at 4 weeks observation as compared to untouched bone. However the blood perfusion in reamed bones was nearly normalized after 12 weeks.

Increasing operative trauma, i.e. reaming, and reaming with filling of the medullary cavity produced an increasing response from the diaphyseal bone with increased blood perfusion and remodelling activity. This is in accordance with Danckwardt-Lilienström (2), who found the largest vascular response and new bone formation in areas where most bone had been removed from the endosteal surface by reaming.

If the medullary cavity is left empty and not obturated, endosteal revascularization is made possible, and endosteal apposition is a predominant reaction. In addition, remodelling activity is increased throughout the entire cortex as assessed by ^{99m}Tc measurements, autoradiograms and histologically. If the medullary cavity is obturated with an inert material like bone wax the endosteal revascularization is blocked, and the repair response is a large subperiosteal apposition with

Table III. — Histological evaluation of cortical bone remodelling, counted as grid points (max 500), in inner, middle and outer 1/3 of cortex

Identification	Cortical bone remodelling							
	Reaming				Wax			
54	95	26	13	134	30	49	55	134
55	127	47	54	228	75	98	59	232
62	52	48	68	162	41	62	132	235
64	52	73	59	184	10	57	75	142
65	92	63	51	206	22	36	93	151
72	98	51	46	195	45	51	87	183
Median	94	50	53	190	36	54	81	167
	Reaming				No operation			
4 weeks								
29	89	38	72	199	12	6	6	24
30	57	50	59	166	17	13	12	42
12 weeks								
20	120	102	67	289	13	12	15	40
21	97	62	48	207	18	15	12	45

higher bones remodelling activity than in the endosteal apposition of reamed bones. Histologically the predominant remodelling activity is located in the outer 1/3 of the cortex, as apposed to the inner 1/3 of reamed bone. Thus the higher median ^{99m}Tc activity, and probably the increased bone blood perfusion in bones filled with wax, can at least partly be explained by the large subperiosteal apposition. However, cortical remodelling activity, as assessed from the autoradiograms, was also higher after filling with bone wax in 5/6 cases. However, remodeling activity in the histological sections was only larger on the wax-filled side in 2/6 cases. Although barely consistent this difference in results can be explained by differences in the methods. The autoradiograms mirror the activity in bone remodelling activity at the time of killing, whereas the histological sections demonstrate the remodelling activity over a period of weeks prior to killing of the dogs. Thus bone remodelling in reamed bones might have reached a maximum earlier in the observation period and before the bones filled with wax.

The large subperiosteal apposition found in bone filled with wax indicates that other factors than instability of implants (5) may contribute to this phenomenon.

The differences between reamed and obturated bones can be explained by two different ways of revascularization (6). Old cortex can simply be revascularized, but it can also be totally remodeled through osteoclasts removing dead bone followed by formation of new osteones. The latter process gives rise to more bone remodelling activity, although at a later stage. This is supported by our histological findings, which after reaming showed only few areas of dead bone within old cortical bone even in the endosteal parts of the cortex, whereas larger areas of dead bone endosteally were found in the bone filled with bone wax. This is in accordance with Rhinelander (6) who found total revascularization of diaphyseal bone 6 months after operation in areas where the cement did not completely fill the medullary cavity, while other areas with adequate cement filling remained avascular for more than one year.

Thus obturation of the medullary cavity produces severe and long-lasting necrosis. The reason for this long-lasting avascularity is probably due to problems of revascularization when the medullary cavity is obturated, making restoration of normal centrifugal blood flow impossible, the only remaining source of vascular supply being from the periostium.

We conclude that obturation of the medullary cavity of a reamed bone with an inert material (bone wax), severely changes the process of revascularization and bone remodelling. Consequently experimental models testing the effects of bone cement on bone should employ a control operation which, besides identical surgical preparation, should create the same degree of medullary obturation as created by modern cementing techniques using cement guns and pressurizing devices.

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SAMENVATTING

J. STÜRUP, L. NIMB en J. S. JENSEN. Studie op de cortex van de tibia bij de hond na uitboren en vervolgens opvullen met inerte botwas.

Bij 4 honden werden de gevolgen op de tibiadiafyse nagegaan van intramedullaire boring. Bij 6 honden werd vervolgens de tibia uitgefreesd en opgevuld met inerte botwas. De doorbloeding werd gemeten door de techniek van microsferen en de botremodelage werd gevolgd met de concentratie van ^{99m}Tc-MDP. De botnecrose en de botremodelage werden geëvalueerd op histologische coupes. De biologische respons nam toe na chirurgisch trauma. Na eenvoudig uitboren was de endostale appositie de belangrijkste reaktie. Obturatie van de mergholte lokte een meer uitgesproken subperiostale en corticale reaktie uit. De auteurs konkluderen dat de botremodelage opmerkelijk verschilt na het eenvoudig uitboren van het bot en het opvullen met botwas na uitboring. Wanneer een botcement getest wordt, moet de studie een vergelijking inhouden van het aspect na opvulling van de mergholte met een substantie die botcement simuleert.

RÉSUMÉ

J. STÜRUP, L. NIMB et J. S. JENSEN. Étude de la corticale du tibia chez le chien, après alésage et remplissage par une cire à os inerte.

Chez 4 chiens, le tibia fut alésé. Chez 6 chiens témoins, le tibia fut alésé et comblé de cire à os, inerte. Les auteurs ont comparé l'aspect histologique des corticales prélevées dans les 2 groupes. La vascularisation osseuse fut mesurée par la technique des microsphères et le remodelage osseux par l'importance de la concentration en ^{99m}Tc-MDP. La nécrose osseuse et l'activité de remodelage furent observées sur les coupes histologiques. La réponse biologique augmenta après traumatisme chirurgical. Le simple alésage fut suivi d'une apposition endostéale comme réaction principale. L'obturation subséquente de la cavité médullaire provoqua une réaction sous-périostée corticale plus importante.
Les auteurs en déduisent que le processus de remodelage diffère, de manière significative, entre la corticale simplement alésée et la corticale alésée et ensuite obturée à l'aide de cire à os.
Lors des épreuves d'évaluation d'un ciment à os, l'étude doit inclure une comparison des effets observés après obturation du canal médullaire, simulant le remplissage par un ciment à os.