Effects of polymerization heat and monomers from acrylic cement on canine bone

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We investigated the effects of polymerization heat and toxicity of polymethylmetacrylate bone cement in the canine tibial diaphysis. Heat was studied by filling the tibias with either bone cement or bone wax contained in a monomer tight membrane pouch. Toxicity was studied by filling both tibias with cement, with the control side contained in the membrane pouch. Bone blood perfusion was measured by microsphere technic, and bone remodeling by ^{99m}Tc-methylene diphosphonate uptake and by histologic technique. In bone exposed to the combination of polymerization heat and monomer, both perfusion and remodeling were impaired. We did not find any effects of polymerization heat alone.

We conclude that hot toxic chemicals from bone cement during polymerization may inhibit bone blood perfusion and remodeling, whereas heat alone seems to be of minor importance for the regenerative processes in cemented diaphyseal bone.

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In comparison with an inert material (bone wax), conventional polymethylmetacrylate (PMMA) bone cement may cause increased necrosis with reduced bone blood perfusion and delayed bone remodeling (Stürup et al. 1990, 1992a,b, Jensen et al. 1991a). The harmful effects of bone cement have been attributed to polymerization heat (Mjöberg 1986) and leakage of toxic substances, notably methylmetacrylate (Feith 1975, Pedersen et al. 1983). However, quantitative assessments of these effects have apparently not been investigated utilizing a control operation with reaming and obstruction of the medullary canal, as obtained with bone cement. Our experiment aimed at separating the effects of polymerization heat and toxicity.

Animals and methods

16 adult mongrel dogs, weighing 24 (20–35) kg, were operated on. Both tibiae were reamed, brushed, flushed and sucked dry. The first series of 7 dogs was used to evaluate the effect of polymerization heat alone, and the second series of 9 dogs to evaluate the effect of leaking monomer. A 150-µm membrane consisting of a 50-µm monomer-tight polyvinylalcohol (PVA) membrane on the inside protected by a 100-µm layer of polyurethane on the outside was formed into condom-shaped pouches. The membrane was tested in the laboratory by filling it with MMA and immersing it in a water, methyl alcohol solution. Test samples were collected and analyzed by gas chromatography (Perkin Elmer, UK) and the membrane could withhold MMA completely for 10 minutes, which is equivalent to the polymerization time of conventional PMMA bone cement. Hereafter, a minimal penetration of MMA occurred as the PVA dissolved. However, as leakage of MMA monomer stops within a few minutes after mixing of cement components (Sylvest et al. 1992, Trap et al. 1992), the bone was considered completely protected from MMA. The thermal conductivity of the condom was 0.15 $W \times m^{-1} \times K$ and was not considered to influence heat transmission between bone and cement significantly. In each dog the condom was inserted with a blunt rod into the reamed marrow cavity of the tibia, in series 1 on both sides and in series 2 on either side. In series 1, one tibia was filled with Palacos® bone cement and the contralateral tibia with bone wax containing radiopacifier (15 percent zirconium oxide).

In series 2, one tibia was filled with Palacos® bone cement within the membrane, while the contralateral tibia was filled with cement without a membrane to evaluate the effect of leakage of monomer. Test and control sides were randomly chosen between the left and right bones. Postoperative radiographs confirmed adequate filling of the tibial diaphysis in all cases.

Through a catheter introduced into the inferior vena cava via the external jugular vein, blood samples were collected before, 30 sec and 60 sec after cementation in the pouch to test the tightness of the membrane. The blood samples were collected in glass tubes with teflon lid, frozen immediately and quantitatively analyzed for MMA content by head space gas chromatography (Perkin Elmer®, level of resolution 1 ppm) within 24 h. The choice of sampling times corresponded to maximal values for monomer concentrations in blood (Sylvest et al. 1992). One dog in the first series was excluded due to monomer leakage (19 ppm). Monomer could not be traced in the blood in the other dogs with cemented-in condoms.

After 4 weeks' observation, estimation of avascularity was performed by vital staining with 30 mL, 7.5% disulphine blue injected intravenously 30 min before the dogs were killed by an overdose of Mebumal sodium. Tibiae were collected, stripped from soft tissue and divided longitudinally in the sagittal plane. Grading of avascular areas was based on a visual impression by one author (JS).

Before the dogs were killed they received propionylpromazine 0.1 mg/kg as premedication, and anesthesia was induced with thiomebumal sodium 12.5 mg/kg. The dogs were intubated and ventilated with 20 percent O_2/80 percent N_2O, using a Servo respirator. Anesthesia was maintained with fentanyl (Haldid) 0.2–0.4 mg i.v. Muscle relaxation was obtained with intermittent doses of pancuronium bromide (Pavulon) 0.2 mg/dose.

Blood perfusion measurements with 1.2×10^7 Sc-46 labeled 16.5 μm microspheres (Nen-Track®), were performed (Tøndevold and Bülow 1983, Stürup et al. 1990) by injecting them into the left ventricle of the heart, simultaneously with collection of reference blood samples from the abdominal aorta with a suction pump; catheters were introduced through the carotid arteries.

Bone remodeling activity was estimated by the uptake of 30 mCi ^{99m}Tc-methylene diphosphonate (MDP) injected intravenously 2 hours before killing the dogs (Christensen 1985).

From alternately the medial and lateral halves of the central diaphysis of the tibia 5, 1 cm long bone specimens were collected and counted for Tc activity. A week later the specimens were counted for Sc-46 activity, together with the reference blood samples.

Blood flow rates (mL/100g/min) were calculated from Sc-46 counts (Tøndevold and Bülow 1983, Stürup et al. 1990) and Tc activity was calculated per gram tissue. Diaphyseal blood flow rates and diaphyseal ^{99m}Tc activity were calculated as mean values of the 5 central specimens from the tibial diaphysis. Results from ^{99m}Tc counts are given as ratios between test and control bones for each animal.

The other half of the tibias was freeze-cut on a hard-tissue cryostat, photographed, and placed on a photographic film for 12 hours to produce an autoradiogram depicting the distribution of ^{99m}Tc-MDP (Figure 1) (Stürup et al. 1992a). A section of the autoradiograms, corresponding to the 5 central centimeters of the diaphysis, was analyzed in a TV-based image analyzer (Leitz TAS-plus, Germany) for areas and degree of blackening in the subperiosteal apposition and the cortex. Blackening was presented as ratios between mean values of the two sides. The area of subperiosteal apposition was calculated as total area (cm^2).

Following the autoradiography, 2 transverse diaphyseal segments were cut from each leg and prepared for histological examination, without decalcification (Jensen et al. 1991b). The sections were evaluated, with transmitted light microscopy, and the amount of cortical remodeling was quantified. A grid-counting method was employed, using a 125 times magnification and counting 100 points in a 100 μm grid. Counting was performed in 5 random locations in each of the 3 inner, middle and outer third cortical zones, and the results presented as the median number of grid points intercepting osteons with remodeling activity. The Wilcoxon two-sided paired rank sum test was employed using a significance level of 0.05.

Results

The disulphine blue-stained sections revealed no obvious differences in unstained areas between the different groups. Blood perfusion (Table 1) in bones exposed to polymerization heat was lower in 5 of 6 cases, as compared to bone wax (NS). Blood perfusion in bones exposed to hot monomer, compared to heat alone, was lower in 8 of 9 cases (P 0.02).

^{99m}Tc counting did not disclose any difference in uptake in bones exposed to polymerization heat, as compared to the controls, median ratio between test and control sides being, respectively, 1.03 (0.78–1.45) and 0.76 (0.51–1.13). However, in bones exposed to hot monomer, the ^{99m}Tc-MDP uptake was lower in 7/9 cases (P 0.03).

The area of subperiosteal apposition as depicted on the autoradiograms (Figure 1) was not affected by either polymerization heat or hot monomer. The area of original cortex did not differ significantly between the legs of a pair, indicating that the sections studied were anatomically comparable. There was no difference between the 2 sides in blackening in the autoradiograms located on the subperiosteal apposition and

Table 1. Blood perfusion (mL/100g/min) in canine tibial diaphysis in bones exposed to polymerization heat from bone cement (A) or bone wax (B), both isolated in pouch, or to combined local toxicity of leaking chemicals and polymerization heat of cement (C) or cement in pouch (D)

Dogs	Tibial location[a]	A	B	C	D	Ratio
Series 1						
31	L	14.5	15.6			0.93
35	M	19.7	22.9			0.86
53	L	10.7	6.5			1.65
60	L	7.4	9.4			0.79
61	M	23.4	26.3			0.89
63	L	30.2	30.8			0.98
Median		17.1	19.3			0.91
Series 2						
66	L			3.4	3.7	0.92
69	L			5.0	9.0	0.56
70	M			2.6	1.5	1.73
71	M			8.0	15.3	0.52
73	M			3.4	3.7	0.92
74	M			11.7	25.1	0.47
74x	L			8.8	13.8	0.64
75	L			11.4	16.9	0.67
76	M			13.7	14.8	0.93
Median				8.0	13.8	0.67

[a] M medial, L lateral tibia used for perfusion measurements.

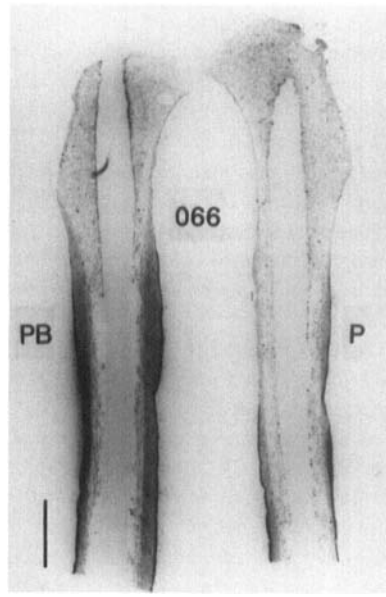

Figure 1. Autoradiograms of canine tibial diaphysis (bar 2 cm), exposed to bone cement polymerization heat (left), or to hot cement monomer (right). Note the inhibited bone remodeling activity, including subperiosteal apposition after exposure to the hot monomer.

Table 2. Cortical remodeling in canine tibial diaphysis, quantified on histological sections by grid counting, in 3 cortical zones (inner, middle and outer), revealed no influence of polymerization heat (A), compared to a control (B), but inhibited remodeling in bones exposed to toxicity of bone cement (C), compared to a control (D). See text and Table 1 for experimental design. Median *range*

	A		B		C		D	
Inner	79	*35–115*	88	*23–112*	65	*11–140*	96	*10–195*
Middle	80	*44–89*	86	*27–95*	69	*28–106*	94	*58–198*
Outer	63	*38–80*	64	*33–92*	84	*58–98*	83	*58–137*
Total	232	*149–238*	231	*110–276*	231	*125–316*	259	*158–438*

original cortex in either of the 2 series.

Cortical remodeling (Table 2) did not reveal any effect of polymerization heat in either of the 3 cortical zones or in the cortex as a whole. Hot monomer, however, inhibited remodeling in the cortex as a whole (P 0.04) and in the middle zone (P 0.03), with a similar trend in the inner zone (P 0.06).

Discussion

Quantification of remodeling activity is based on the assumption that ^{99m}Tc-MDP is bound to bone with remodeling activity (Christensen 1985). However, blood perfusion can affect tracer uptake (Sagar et al. 1978) as it has been shown that a 4 times higher blood perfusion resulted in a 70 percent increase in tracer uptake. However, as the median ratios in the series with the highest percentual blood perfusion difference, cement with and without the membrane, for blood perfusion and ^{99m}Tc-MDP uptake were nearly identical, 0.67 and 0.76, respectively, only a minor part of the increased uptake on the membrane-protected side can be explained by increased blood perfusion.

Reports suggest that damage arising from polymerization heat is limited to a narrow rim located on the endosteal surface (Toksvig-Larsen et al. 1991), probably because the isotherm penetration depth is limited to 1.3–1.6 mm for the 50 degree isotherm (Huiskes 1980). In this area, however, blood perfusion has already been destroyed by intramedullary reaming. These considerations are in accordance with our

results, as we could not find any additional effects of polymerization heat on cortical remodeling, bone perfusion, or bone remodeling activity, in comparison with bone reamed and obturated with bone wax in a similar fashion. However, polymerization heat may add to the toxicity of bone cement (Huiskes 1980).

The largest amount of monomer leaks from the cement within the first minutes after admixing of the components (Homsy et al. 1972, Lee et al. 1973, Linder et al. 1976, Schoenfeld 1979, Sylvest et al. 1992) and in vitro studies have shown that as much as 14 percent of the MMA may escape into the surroundings (Lee et al. 1973). Delayed leakage after cementation is negligible (Trap et al. 1992). The amount of MMA leakage seems primarily to depend on surface area rather than thickness of the cement (Homsy et al. 1972, Linder et al. 1976). In the immediate vicinity of the cement, much higher concentrations of monomer are probably reached than are measured in the central venous blood. In a study on mice calvaria, Pedersen et al. (1983) found toxic effects of MMA at concentrations of only 1 μg/mL, increasing with higher concentrations, indicating a high sensitivity of bone tissue. Our results are consistent with these observations, as we found depressed bone blood perfusion and remodeling activity in cortical bone exposed to hot leaking MMA. The remodeling activity was primarily depressed in the inner 2/3 of the cortex. This can be explained by the MMA concentration declining with increasing distance to the cement interface which is due to dilution and removal by the still existing periosteal blood circulating in the outer part of cortex. We concluded that leakage of hot monomer from PMMA bone cement, implanted in the intramedullary area, impairs bone blood perfusion and remodeling in canine tibial diaphysis. We have not in this fairly limited series been able to find any effect of polymerization heat alone.

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References

Christensen S B. Osteoarthrosis. Changes of bone, cartilage and synovial membrane in relation to bone scintigraphy. Acta Orthop Scand (Suppl 214) 1985.

Feith R. Side-effects of acrylic cement implanted into bone. A histological, (micro)angiographic, fluorescence-microscopic and autoradiographic study in the rabbit femur. Acta Orthop Scand (Suppl 161) 1975.

Homsy C A, Tullos H S, Anderson M S, Diferrante N M, King J W. Some physiological aspects of prosthesis stabilization with acrylic polymer. Clin Orthop 1972; 83: 317–28.

Huiskes R. Some fundamental aspects of human joint replacement. Analyses of stresses and heat conduction in bone-prosthesis structures. Acta Orthop Scand (Suppl 185) 1980.

Jensen L N, Stürup J, Kramhøft M, Jensen J S. Histological evaluation of cortical bone reaction to PMMA cement. Acta Orthop Belg 1991a; 57 (3): 254–9.

Jensen L N, Jensen J S, Gotfredsen K. A method for histological preparation of undecalcified bone sections containing acrylic bone cement. Biotech Histochem 1991b; 1 (2): 82–6.

Lee A J, Ling R S, Wrighton J D. Some properties of polymethylmethacrylate with reference to its use in orthopedic surgery. Clin Orthop 1973; 95: 281–7.

Linder L G, Harthon L, Kullberg L. Monomer leakage from polymerizing acrylic bone cement. An in vitro study on the influence of speed and duration of mixing, cement volume and surface area. Clin Orthop 1976; 119: 242–9.

Mjöberg B. *Loosening of the cemented hip prosthesis.* The importance of heat injury. Acta Orthop Scand (Suppl 221) 1986.

Pedersen J G, Lund B, Reimann I. Depressive effects of acrylic cement components on bone metabolism. Isotope release and phosphatase production studied in vitro. Acta Orthop Scand 1983; 54 (6): 796–801.

Sagar V V, Piccone J M, Charkes N D, Makler P T Jr. Skeletal tracer and bone blood flow in dogs. J Nucl Med 1978; 19: 705–6.

Schoenfeld C M, Conard G J, Lautenschlager E P. Monomer release from methacrylate bone cements during simulated in vivo polymerization. J Biomed Mater Res 1979; 13 (1): 135–47.

Stürup J, Madsen J, Tøndevold E, Jensen J S. Decreased blood perfusion in canine tibial diaphysis after filling with acrylic bone cement compared with inert bone wax. Acta Orthop Scand 1990; 61 (2): 143–7.

Stürup J, Bødtker S, Jensen J S. Bone remodelling in tibial diaphysis following cementation with bone cement and bone wax. Eur J Exp Musculoskeletal Res 1992a; 1: 171–4.

Stürup J, Nimb L, Jensen J S. The effect on cortical bone of reaming and filling of canine tibial diaphysis with inert bone wax. Acta Orthop Belg 1992b; 58 (4): 388–94.

Sylvest A, Kramhøft M, Jensen J S, Thomsen L D, Jensen J C. Monomer in central venous blood during polymerization of bone cements, and toxicity of a new monomer. Eur J Exp Musculoskeletal Res 1992; 1: 47–50.

Toksvig-Larsen S, Franzén H, Ryd L. Cement interface temperature in hip arthroplasty. Acta Orthop Scand 1991; 62 (2): 102–5.

Trap B, Wolff P, Jensen J S. Acrylic bone cements: Residuals and extractability of methacrylate monomers and aromatic amines. J Appl Biomat 1992; 3: 51–7.

Tøndevold E, Bülow J. Bone blood flow in conscious dogs at rest and during exercise. Acta Orthop Scand 1983; 54: 53–7.