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The comparison of the negative effect of autoclaving and pasteurization on bone healing

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Objectives: The aim of this study was to compare the effects of autoclaving and pasteurization on bone healing.

Methods: Twenty-five full-grown male rabbits were included in the study; all 25 had bone blocks resected and reimplanted. In group 1, bone blocks were autoclaved; in group 2, bone blocks were pasteurized; and in group 3 (controls), resected bone blocks were reimplanted without sterilization.

Results: Heiple scores of the proximal parts of the fusion surfaces in group 1, group 2, and group 3 were 12.8±0.4, 6.8±1.2, and 10.2±1.9, respectively. Heiple scores of the distal parts of the fusion surfaces in group 1, group 2, and group 3 were 10.8±0.8, 6.0±1.1, and 9.8±1.5, respectively. Differences in radiologic scores were not statistically significant between the groups for proximal or distal fusion surfaces at 3 and 6 weeks.

Conclusion: In conclusion, pasteurization has a less negative effect on bone healing than autoclaving, and can be considered for bone sterilization in certain circumstances.

Key words: Autoclaving; bone healing; bone sterilization; pasteurization; rabbits.

Limb-saving techniques have become popular for reconstruction after resection of malignant tumors in orthopedics. As there is limited access to autologous bone grafts, which are the gold standard for bone transplantation, several methods have been proposed for using the same bone part which is affected by the tumor. These techniques include, but are not limited to, pasteurization, autoclaving, and irradiation.[1-3]

The procedures using resected bone have the advantages of adaptation of the graft completely to the host in size and shape, as well as having no risk of disease transmission.[3,4] However, it is well defined in the literature that treating the bone with high heat such as autoclaving or irradiation has a negative effect on bone morphogenic protein and other growth factors responsible for osteogenic properties.[2,5] Pasteurization, which is defined as low heat treatment at 60° to 65°, is believed to have the effect of killing malignant tumor cells while preserving the properties of the bone's morphogenic proteins.[6,7]

The aim of this study was to compare the effects of autoclaving and pasteurization on the bone's ability to heal.

Materials and methods

The animal experiment was carried out after having the permission of institutional review board in accordance with the Guidelines for the Care and Use of Laboratory Animals. All measures were taken to minimize animal suffering. Twenty-five full-grown

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male rabbits were included in the study. Mean weight of the rabbits was 2400 g (range 2150-2750 g). Animals were housed in standard cages with access to water and food. The animals were divided into 2 testing groups and 1 control group. Ten animals were included in each testing group (group 1 and group 2), and the remaining 5 animals were included in the study as control group (group 3).

Surgical procedure

Rabbits were anesthetized with intramuscular injection of ketamine hydrochloride (30-40 mg/kg) and xylazine hydrochloride (3-5 mg/kg).[8] Each rabbit received infection prophylaxis (20 mg/kg cefazolin sodium). After appropriate cleaning and draping, the middle part of the right ulna was exposed and the periosteum removed. Before creating a defect by removing bone, a 2-mm K-wire was inserted to the proximal part of the ulna. Then, a 20-mm bone block was removed by a bone cutter manually in order to avoid potential thermal damage. All surgical procedures were carried out by one author (RV) to achieve uniform technique.

In group 1, resected bone blocks were autoclaved (at 120 °C for 20 min, at room temperature for 15 min, and in sterile saline 15 min); bone blocks were pasteurized in group 2 (at 60 °C in sterile saline, at room temperature for 30 min, and in sterile saline for 15 min).[9,10] Following these sterilization procedures, bone blocks were re-implanted to their original place and fixed with the K-wire inserted previously. In group 3, resected bone blocks were re-implanted and fixed with K-wire without being treated by any type of sterilization. All procedures were completed without complications. No external fixation was used after the surgery.

Postoperative pain control was maintained by adding acetaminophen (1-2 mg/mL) to the water supply of the animals for 3 days.[8] Conventional postoperative radiographs were obtained at 3 and 6 weeks under sedation by ketamine hydrochloride (10-15 mg/kg). These were done in order to check the position of the grafts and to assess callus formation. However, final evaluation of fusion was done histologically. Rabbits were sacrificed by an overdose of thiopental sodium in the 6th postoperative week. After sacrifice, ulnar bones were resected en bloc.

Histological evaluation

Resected bones were fixed in 10% formaldehyde for 24 hours and decalcified in 10% formic acid for 15 days. Serial slices (4 µm thick) including proximal and distal osteotomy cuts parallel to the long axis of the ulna were taken by a microtome. Tissue slices were placed on silanated slides and dried at 37 °C. Slices were then stained with hematoxylin–eosin (H-E).

Tissue morphology and healing process of the distal and proximal fusion surfaces between the autografts and host bone were evaluated separately

Table 1
Numerical histological evaluation of Heiple[11]

Score	0	1	2	3	4	5
Appearance of allograft	No cellular activity on the either surface	New cellular activity on each surface	Fully incorporated	-	-	-
Continuity between bone edges	No	Yes				
Callus	No	Fibrous callus	Chondral callus	Bone callus	-	-
Bone edges	Necrotic	Resorbed	New bone apposition	-	-	-
Quality of new bone	-	Initial apposition	Single nodule of woven bone	Trabeculae of woven bone	Bridging trabeculae	Trabeculae of lamellar bone

Table 2

Radiologic scoring of the radiographs at postoperative 6th week

0	No change from immediate postoperative appearance
1	Slight increase in radiodensity
2	Bridging of one cortex, recognizable increase in radiodensity
3	Bridging of at least one cortex, early incorporation of the graft
4	Defect bridged on both medial and lateral sides, graft and new bone not easy to differentiate
5	At least one of four cortices obscured by new bone
6	Defect bridged by uniform new bone, graft no longer visible, cut ends of cortex no longer distinguishable

according to the system of Heiple et al.[11] (Table 1). Fusion and bone healing quality were evaluated in 5 different subcategories with a maximum total score of 13. In addition to histology, radiographic evaluation was done with the radiographs obtained at postoperative 3 and 6 weeks using the grading scale proposed by Lane (Table 2).[12]

Statistical analyses were performed using the Mann-Whitney U test (SPSS 16.0 for Windows). The level of statistical significance was defined as $p<0.05$.

Results

Histological evaluation

Heiple scores of the proximal fusion surfaces in group 1, group 2, and group 3 were 12.8 (median 13, range 12-13), 6.8 (median 10, range 8-13), and 10.2 (median 6.5, range 5-9), respectively ($p<0.05$ between group 1 and 2, between group 1 and 3, and between group 2 and 3). Heiple scores of the distal fusion surfaces in group 1, group 2, and group 3 were 10.8 (median 11, range 10-12), 6.0 (median 9.5, range 8-13), and 9.8 (median 6, range 4-8), respectively ($p<0.05$ between groups 1 and 2, between groups 1 and 3, and between groups 2 and 3) (Table 3).

Radiographic evaluation scores for proximal fusion surfaces 3 weeks postoperatively in group 1, group 2, and group 3 were 4 (median 3, range 2-4), 3 (median 4, range 1-4), and 3.2 (median 4, range 2-6), respectively. Radiographic evaluation scores for distal fusion surfaces 3 weeks postoperatively in group 1, group 2, and group 3 were 3.6 (median 3, range 2-4), 3 (median 3.5, range 1-6), and 3.2 (median 4, range 2-4), respectively. Radiographic evaluation scores for proximal fusion surfaces 6 weeks postoperatively in group 1, group 2, and group 3 were 5.4 (median 5, range 4-6), 5 (median 6, range 4-6), and

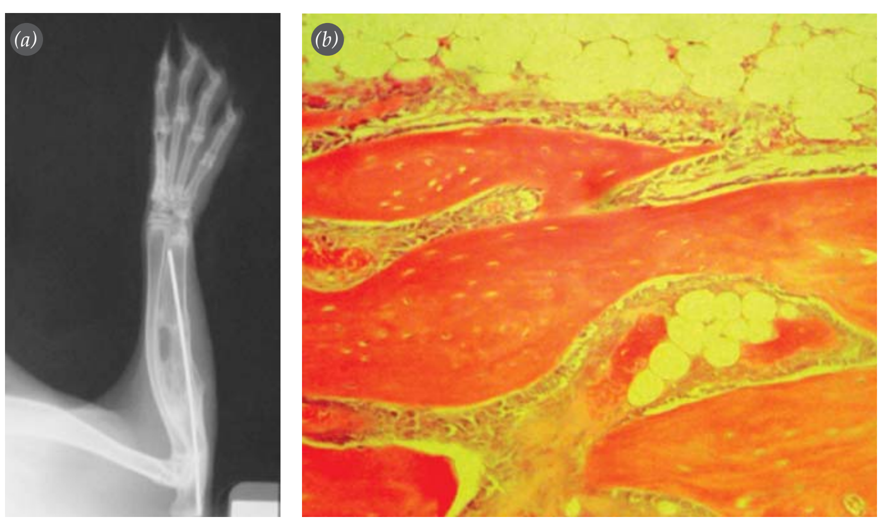

Fig. 1. (a) Radiograph of a rabbit's forearm in control group at postoperative 6th week. **(b)** Histological appearance of the distal part of the fusion surface of ulnar bone of rabbit revealing bone trabeculae surrounded by osteoblastic rim (H-E x200).

Table 3
Histological and radiological scores of study groups

	Histological (proximal)	Histological (distal)	Radiological (proximal)		Radiological (distal)	
			3 weeks	6 weeks	3 weeks	6 weeks
Control group (Group 1)						
1	13	10	2	6	4	4
2	13	11	4	5	4	6
3	13	10	6	6	4	6
4	13	12	4	6	4	4
5	13	11	4	4	2	6
Mean score	12.8	10.8	4	5.4	3.6	5.2
Autoclaving group (Group 2)						
1	6	6	2	4	2	4
2	7	5	4	6	3	5
3	6	6	2	4	3	5
4	9	6	4	6	3	4
5	6	6	2	5	3	4
6	8	5	2	4	4	5
7	6	8	4	4	3	4
8	5	4	2	5	4	6
9	7	7	4	6	2	4
10	8	7	4	6	3	4
Mean score	6.8	6.0	3.0	5.0	3.0	4.5
Pasteurization group (Group 3)						
1	8	8	2	5	2	5
2	12	10	4	6	6	6
3	9	8	2	4	1	3
4	12	9	4	6	4	6
5	11	11	4	6	3	6
6	9	9	1	6	4	4
7	13	13	3	6	4	6
8	12	10	4	6	4	5
9	8	11	4	4	3	6
10	8	9	4	5	2	4
Mean score	10.2	9.8	3.2	5.4	3.2	5.1

5.4 (median 6, range 4-6), respectively. Radiographic evaluation scores for distal fusion surfaces 6 weeks postoperatively in group 1, group 2, and group 3 were 5.2 (median 4, range 4-6), 4.5 (median 5.5, range 3-6), and 5.1 (median 6, range 4-6), respectively. Differences in radiological scores were not statistically significant between the groups ($p>0.05$) (Fig. 1-3).

Discussion

Different techniques have been described to reconstruct bone defects after tumor resection in orthopedic oncology. Advanced techniques in arthroplasty and prosthetic implants are the first choice for replacement of bone defects, and their biomechanical advantages are well described. However, loosen-

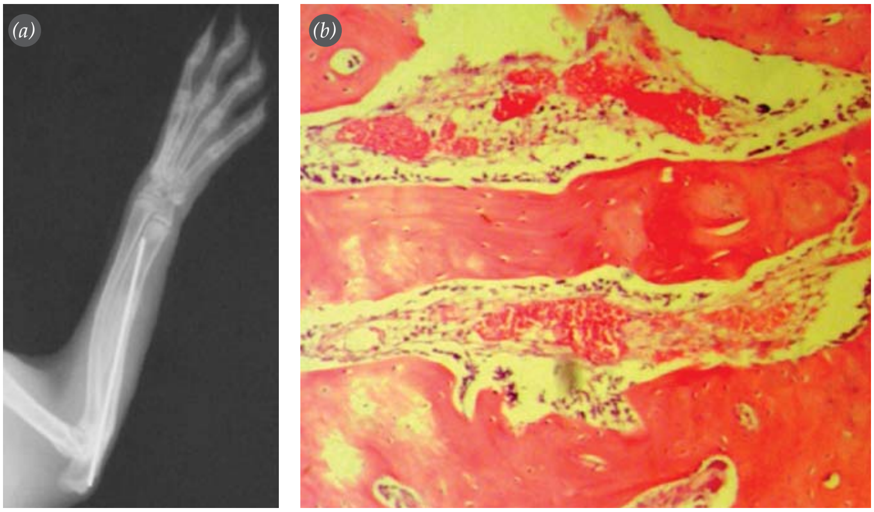

Fig. 2. (a) Radiograph of a rabbit's forearm in group 1 (autoclaved) at postoperative 6th weeks. **(b)** Histological appearance of the distal part of the fusion surface of ulnar bone of rabbit revealing bone trabecular anastomosis (H-E x200).

ing, implant failure, and wear still remain the main concerns in prosthetic replacement. These potential disadvantages have led researchers and physicians to develop biologic reconstruction techniques. Also, because of the high morbidity and mortality associated with prosthesis surgery, techniques requiring less invasive surgery have gained importance.[13-16]

Replacement of the resected bone by allograft is a common technique. However, immunologic reactions, disease transmission, risk of nonunion, and requirement of bone bank are significant drawbacks of this technique.

The idea of using the same bone affected by the tumor for reconstruction after killing the tumor cells has gained popularity. Curative treatment is possible as well as minimizing the potential risks of prostheses or other implants. The most important issue in this technique clearing tumor cells from the affected bone piece before using it as an autograft. Pasteurization and autoclaving are the main methods used to kill the tumor cells. Also, these two techniques have been used widely to sterilize allografts.[1,2,15]

The main concern about these techniques is the decrease in biologic and mechanical properties of the bone. In a biomechanical study, Kohler et al.[17] reported that autoclaving had a negative effect on rabbit bones in terms of strength and bone mass. They hypothesized that the reason was collagen de-naturation and transformation of the collagen into gelatin solution in temperatures over 70 °C. Pasteurization

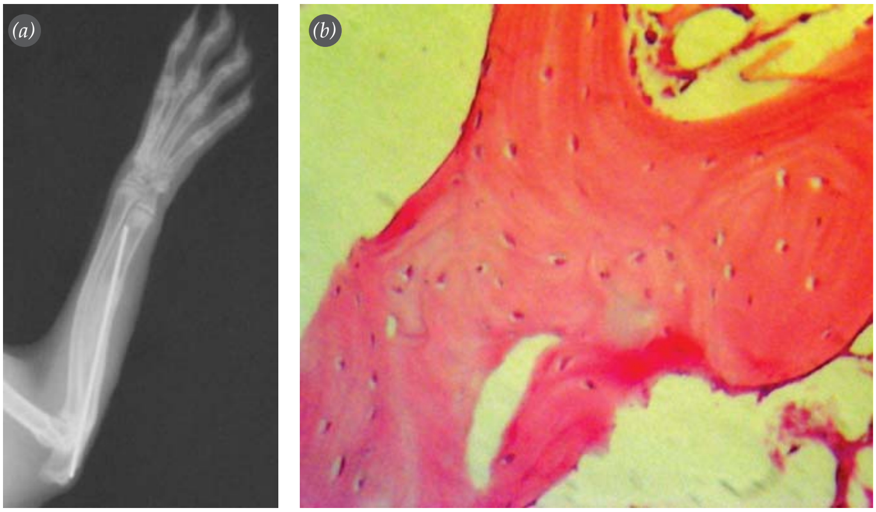

Fig. 3. (a) Radiograph of a rabbit's forearm in group 2 (pasteurized) at postoperative 6th week. **(b)** Histological appearance of the distal part of the fusion surface of ulnar bone of rabbit revealing mature bone (H-E x200).

when compared to autoclaving has been proposed to spare the growth factors responsible for osteoinduction.[2] Ehara et al.[5] used pasteurized autografts and reported 5 complete radiologic unions in 8 patients. Two patients had pseudoarthrosis, and 1 had infection.

Zoricic et al.[2] compared biologic qualities of pasteurized, autoclavized, and frozen allografts in rabbits. They claimed in their study that the temperature applied was quite sufficient to disinfect the bone allograft of HIV without interfering with the osteogenic properties of a pasteurized bone graft. However, they did not use the same resected bone after sterilization; instead, resected bones were used as allografts, in contrast to the present study. In our study, resected bone parts were re-implanted to their original place, as occurs in a real surgical scenario.

In an animal study, Manabe[6] compared pasteurized, autoclaved, boiled, and fresh bone by testing the biomechanical strength and osteoconductivity. It was concluded that autoclaving bones resulted in degeneration of the bone material, and grafts were absorbed with little new bone formation and union. In contrast, the structures of pasteurized bones were preserved, and the grafts were gradually replaced with viable bone in a manner similar to that of fresh bone graft.

In the present study, two non-unions and graft resorption occurred at the distal fusion surface in the autoclaved autografts. Unfortunately, we cannot explain why this occurred at the distal end. Although histological and radiographic results were similar at the end of the 6th week, histological union rate was higher in pasteurization group.

In conclusion, pasteurization has a less negative effect on bone healing than autoclaving, and can be considered for bone sterilization in certain circumstances.

References

1. Araki N, Myoui A, Kuratsu S, Hashimoto N, Inoue T, Kudawara I, et al. Intraoperative extracorporeal autogenous irradiated bone grafts in tumor surgery. Clin Orthop Relat Res 1999;(368):196-206.
2. Zoricic S, Bobinac D, Lah B, Maric I, Cvijanovic O, Bajek S, et al. Study of the healing process after transplantation of pasteurized bone grafts in rabbits. Acta Med Okayama 2002;56:121-8.
3. Kim HS, Kim KJ, Han I, Oh JH, Lee SH. The use of pasteurized autologous grafts for periacetabular reconstruction. Clin Orthop Relat Res 2007;464:217-23.
4. Uyttendaele D, De Schryver A, Claessens H, Roels H, Berkvens P, Mondelaers W. Limb conservation in primary bone tumours by resection, extracorporeal irradiation and re-implantation. J Bone Joint Surg Br 1988;70:348-53.
5. Ehara S, Nishida J, Shiraishi H, Tamakawa Y. Pasteurized intercalary autogenous bone graft: radiographic and scintigraphic features. Skeletal Radiol 2000;29:335-9.
6. Manabe J. Experimental studies on pasteurized autogenous bone graft. [Article in Japanese] Nippon Seikeigeka Gakkai Zasshi 1993;67:255-66.
7. Izawa H, Hachiya Y, Kawai T, Muramatsu K, Narita Y, Ban N, et al. The effect of heat-treated human bone morphogenetic protein on clinical implantation. Clin Orthop Relat Res 2001;(390):252-8.
8. Hrapkiewicz K, Medina L, Holmes DD. Clinical medicine of small mammals and primates: an introduction. 2nd ed. London: Manson Publishing; 1998. p. 135-72.
9. Köhler P, Kreicbergs A. Incorporation of autoclaved autogenic bone supplemented with allogenic demineralized bone matrix. An experimental study in the rabbit. Clin Orthop Relat Res 1987;(218):247-58.
10. Ahmed AR, Manabe J, Kawaguchi N, Matsumoto S, Matsushita Y. Radiographic analysis of pasteurized autologous bone graft. Skeletal Radiol 2003;32:454-61.
11. Heiple KG, Chase SW, Herndon CH. A comparative study of the healing process following different types of bone transplantation. J Bone Joint Surg Am 1963;45:1593-616.
12. Lane JM, Sandhu HS. Current approaches to experimental bone grafting. Orthop Clin North Am 1987;18:213-25.
13. Harris IE, Leff AR, Gitelis S, Simon MA. Function after amputation, arthrodesis, or arthroplasty for tumors about the knee. J Bone Joint Surg Am 1990;72:1477-85.
14. Gitelis S, Piasecki P. Allograft prosthetic composite arthroplasty for osteosarcoma and other aggressive bone tumors. Clin Orthop Relat Res 1991;(270):197-201.
15. Cannon SR. Massive prostheses for malignant bone tumours of the limbs. J Bone Joint Surg Br 1997;79:497-506.
16. Böhm P, Fritz J, Thiede S, Budach W. Reimplantation of extracorporeal irradiated bone segments in musculoskeletal tumor surgery: clinical experience in eight patients and review of the literature. Langenbecks Arch Surg 2003;387:355-65.
17. Köhler P, Kreickbergs A, Strömberg L. Physical properties of autoclaved bone. Torsion test of rabbit diaphyseal bone. Acta Orthop Scand 1986;57:141-5.