J Orthop Sci (2003) 8:16–19

Journal of
Orthopaedic Science

Histological and radiological analysis of autoclaved bone 2 years after extirpation

Norio Yamamoto[1], Hiroyuki Tsuchiya[1], Takayuki Nojima[2], Hisashi Sumiya[3], and Katsuro Tomita[1]

[1] Department of Orthopaedic Surgery, Graduate School of Medicine, Kanazawa University, 13-1 Takaramachi, Kanazawa 920-8641, Japan
[2] Department of Pathology, Kanazawa Medical University Hospital, Kahoku-gun, Ishikawa, Japan
[3] Department of Nuclear Medicine, Graduate School of Medicine, Kanazawa University, Kanazawa, Japan

Abstract We examined grafted distal femoral autoclaved bone radiologically and histologically 24 months after surgery. The patient was a 16-year-old boy with osteoblastic-type osteosarcoma in the distal part of the left femur. The patient received pre- and postoperative chemotherapy and underwent limb reconstruction surgery using an autoclaved autograft. He was forced to undergo hip disarticulation because of local recurrence in the soft tissue. Radiologically and histologically, we were able to detect newly formed bone at the site of the distal junction and surrounding the autoclaved autograft, although most of the autoclaved bone remained without substitution even 24 months after implantation. The layer of newly formed bone surrounding the autoclaved autograft was so thin that it seemed to be ineffective for weight-bearing. Drilling into the autoclaved autograft appeared to promote little bone regeneration inside the autoclaved autograft. A bone scintigram showed newly formed bone around the autoclaved autograft, but the scan tended to exaggerate such bone formation beyond that actually confirmed by histological examination. We should be careful when applying autoclaved bone for weight-bearing parts.

Key words Autograft · Autoclaved bone · Histology · Radiology · Remodeling

Introduction

In Asian countries the autoclaved autograft is still one of the options for reconstructing defects resulting from surgery for bone tumor. However, few articles refer to the comparatively early stages of bone formation in the autoclaved autograft in humans based on radiological and histological findings. We report here our experience with one patient whose autoclaved autograft was examined both radiologically and histologically 2 years after surgery.

Offprint requests to: N. Yamamoto
Received: June 26, 2001 / Accepted: August 26, 2002

Case report

A 16-year-old boy suffered from osteosarcoma (osteoblastic type) in the distal left femur (Fig. 1a). We used five courses of intraarterial neoadjuvant chemotherapy combined with cisplatin (120 mg/m^2), adriamycin (30 mg/m^2 × 2), and caffeine (1500 mg/m^2 × 3).[20] The patient underwent wide excision (Fig. 2) and reconstruction with an autoclaved autograft (length 240 mm; diaphyseal width 26 mm; condylar width 85 mm) combined with an intramedullary nail, plates, and screws during the first operation (Fig. 1b). The bone graft was returned to its original location after autoclaving at 135°C for 10 min.[2] Intravenous adjuvant chemotherapy was given using the same protocol for five courses. Twenty-four months later the patient had to undergo left hip disarticulation because of tumor recurrence in the soft tissue.

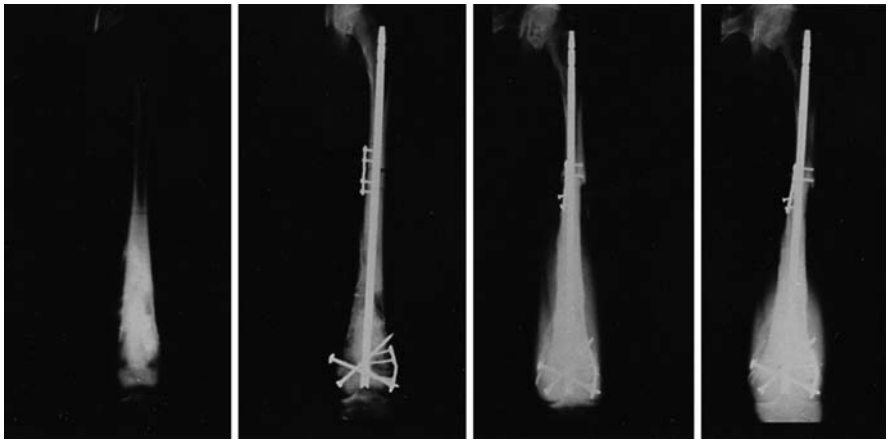
Results

Radiological findings

Radiographs showed bone fusion at the distal junction, but the proximal junction was in a condition of nonunion (Fig. 1c,d). The bone scintigram obtained 2 months after the operation revealed uptake around the autoclaved bone circumference and successive improvement, although uptake was not observed at the inside of the autoclaved bone even 24 months after surgery (Fig. 3).

Histological findings

We evaluated whole sections of the extirpated grafted bone in this case (Fig. 4a). There seemed to be sufficient osteogenesis at the junction of the grafted bone and the distal femoral fragment (Fig. 4b,c). Although nonunion



a,b **c,d**

Fig. 1. Anteroposterior radiographic features of the left femur before and after surgery. **a** Before surgery. **b** Immediately after initial surgery. **c** Two months after surgery. **d** Two years after surgery. Wide excision and reconstruction with an autoclaved autograft was performed during the initial surgery. At the distal site satisfactory bone formation and fusion was observed on the radiograph, whereas at the proximal site bone fusion was poor and nonunion finally developed

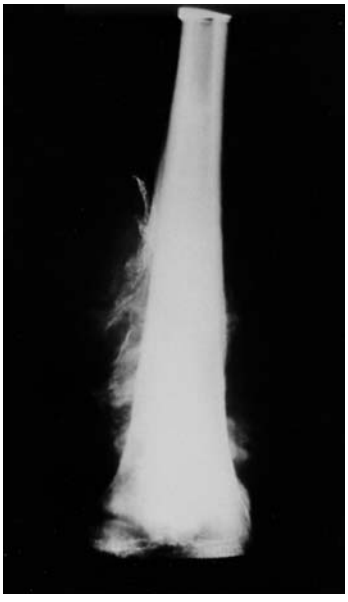


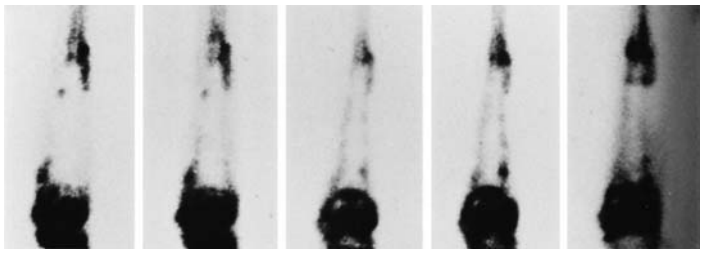
Fig. 2. Anteroposterior radiographic features of the resected specimen. Wide excision was performed after neoadjuvant chemotherapy. The autoclave process was carried out at 135°C for 10 min

was observed at the proximal junction, a bone bridge from the proximal femoral bone had been formed, and synovium-like tissue was observed at the site of nonunion (Fig. 4d). In the remaining portions, only a thin layer (about 1–2 mm thickness) of newly formed bone was observed around the external surface (Fig. 4e,f). The walls of the small holes created by a Kirshner wire were minimally covered by a thin layer of new bone only around their orifices (Fig. 4g). Most of the autoclaved bone had not been substituted.

Discussion

Because of recent improvements in multimodal therapies for malignant bone and soft tissue tumors, limb salvage surgery is performed in many cases. Although various methods, such as tumor prostheses,[12] allografts,[4] and autografts, are used for limb reconstructions, none leads to permanent reconstruction. Even for tumor prostheses, Kawai et al.[9] reported a 10-year survival ratio of about 50%. Allografts are often used for reconstruction in Western countries, but in Japan it is difficult to obtain patient consent for allografts. To overcome this problem, many methods such as autoclaving,[1,3,5,7,8,11,14,17–19,22] irradiation,[24] and pasteurization[6,16] have been applied to the resected bones so they can be used for reconstruction. Clinical application of autoclaved bone was initially reported by Thompson and Steggal[19] in 1956. Since then, clinical findings of various cases have been reported,[1,3,5,7,8,11,14,17,18,22] but reports of the histological examination of the autoclaved bone in clinical material have been sparse.[5,8,11,22] In cases of autoclaved bone, the bone induction potency is lost owing to denaturation of the protein by heat treatment.[21] Only bone conduction potency and structural support functions remain.[23] Furthermore, the dynamic strength of the bone is weakened after autoclaving.[10] Despite such disadvantages, autoclave treatment is still one of the options for limb reconstruction because of its easy management.

In the present case, we were able to evaluate the whole of the autoclaved bone histologically and radiologically because recurrence was confined to soft tissue; it was not seen in the grafted bone. Based on radiographic results (Fig. 1) and histological findings (Fig. 4b,c), we concluded that comparatively rich osteogenesis and bone fusion occurred at the distal junction of the transplanted bone and the distal femoral fragment. In contrast, the proximal junction was in a condition of nonunion. Histologically, synovium-like tissue was observed as a result of nonunion (Fig. 4d). This is because initially diaphysis has disadvantages for callus formation; the fixation technique might also be insufficient. Ortiz-Cruz et al.[13] reported that even in the case of allografts there was a high incidence of nonunion. We determined that nonunion is not a problem with the



a,b,c d,e

Fig. 3. Bone scintigram of the left femur after surgery. **a** Two months after initial surgery. **b** Three months after surgery. **c** Six months after surgery. **d** One year after surgery. **e** Two years after surgery. Radioactivity accumulation was observed at the proximal and distal sites of the junction on the initial scan. At 6 months after operation, accumulation was observed surrounding the autoclaved bone. As time passed, accumulation gradually subsided

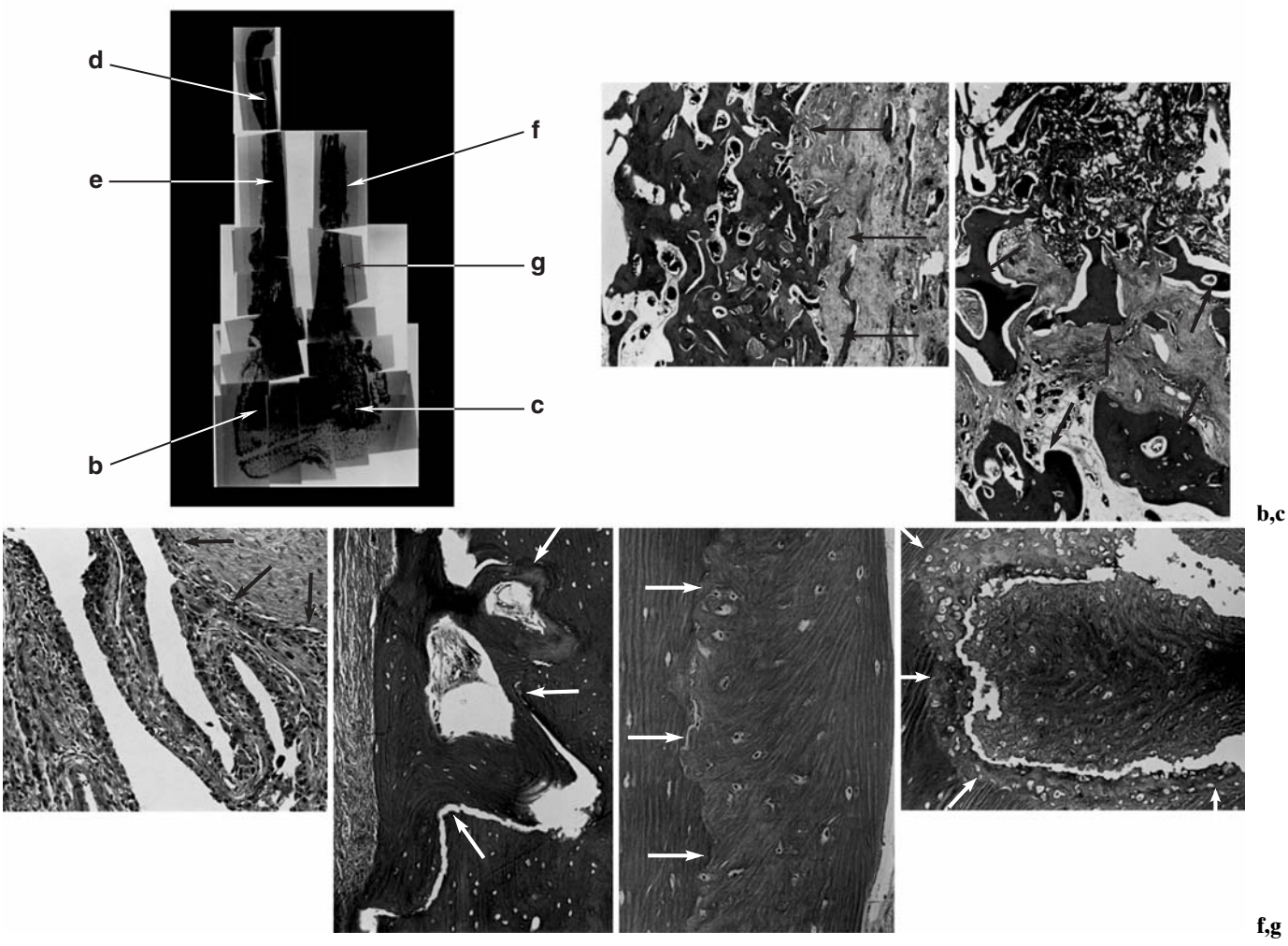


Fig. 4. Histological findings of autoclaved bone. **a** Whole sections of the autoclaved bone. **b,c** At the site of the distal junction with the remaining bone, the volume of newly formed bone entering the autoclaved bone was greater than that of the newly formed bone (*arrows*) surrounding the autoclaved autograft. **d** At the site of the proximal junction where nonunion could be seen on radiographs, synovium-like tissue (*arrows*) had developed, and no bone fusion was achieved. **e,f** A layer of newly formed bone (*arrows*) was observed surrounding the autoclaved autograft, but it was extremely thin. **g** At points where soft tissue had entered from a small hole made with a 2-mm Kirshner wire, bone regeneration occurred even inside the autoclaved autograft (*arrows* indicate newly formed bone). However, inner bone of the autoclaved autograft at the other sites remained without replacement. **a–g** H&E; **b,f,g** ×400; **c,d** ×200; **e** ×100

autoclaved bone but a problem with any transplanted dead bone.

The outer surface of the autoclaved bone was surrounded by only a thin layer of newly formed bone (1–2 mm thick) (Fig. 4e,f). This newly formed layer was obviously thinner than that of the distal junction, which may reflect differences in remodeling processes of the grafted bulky bone that is adjacent to the host bone or to soft tissue. However, a tiny, newly formed bone layer was observed inside the small holes made for the purpose of introducing progenitor cells inside the grafted bone (Fig. 4g). These small holes seemed to be less effective for advancing bone remodeling inside the grafted bone. Most of the autoclaved bone has re-

mained without replacement 24 months after implantation. We determined that this newly formed bone was not useful for improving dynamic strength because of the lack of recovery from the grafted autoclaved bone. In the case of allografts, San-Julian and Canadell[15] noted that drilling holes for fixation increased the possibility of fractures in the transplanted bone. Therefore, we must be careful not to damage the autoclaved bone and not to decrease its strength by ill-advised drilling. This is because the strength of the reconstructed bone is almost entirely due to the residual strength of the autoclaved bone and not to newly formed bone.

Bone scintigraphy 2 months after the primary operation showed that there was already uptake around the autoclaved bone and that the uptake improved. However, even 24 months after surgery uptake was observed only around the circumference of the autoclaved bone, and there was no uptake inside the bone (Fig. 3). In the case of allografts, Bar-sever et al.[2] reported that uptake was observed only around the circumference of the transplanted bones in most cases (mean follow-up period 1.9 years). This seems to indicate that allograft bone remains without replacement, as is the case for autoclaved bone. Furthermore, bone scintigraphy showed newly formed bone, but the scan tended to exaggerate bone formation — beyond what was confirmed by histological examination. We should therefore take care when evaluating bone scintigrams of autografts.

Of course we investigated only one case in this report, and we cannot ignore the influence of the size of the graft, the chemotherapy, and the condition of the soft tissue coverage of this case. However, most patients who undergo reconstruction with an allograft or autograft are treated with chemotherapy and soft tissue resection at the time of surgery. From this point of view, it seems that substitution of the grafted bone was minimal, and the bone remained without replacement, even several years after the operation (as in our case). We must take into consideration the location of autoclaved bone application, especially when it is a weight-bearing part.

We do not have a permanent reconstruction method; and even using a tumor prosthesis, reoperation is required. If autoclaved bone can be regarded as performing the role of temporary structural support, the autoclave technique is one option for reconstruction because of its advantages of easy management and reducing medical expenses.

References

1. Asada N, Tsuchiya H, Kitaoka, et al. Massive autoclaved allografts and autografts for limb salvage surgery. Acta Orthop Scand 1997;68:392–5.
2. Bar-sever Z, Connolly LP, Gebhardt MC, et al. Scintigraphy of lower extremity cadaveric bone allografts in osteosarcoma patients. Clin Nucl Med 1997;22:532–5.
3. Bohm P, Springfeld R, Springer H. Re-implantation of autoclaved bone segments in musculoskeletal tumor surgery: clinical experience in 9 patients followed for 1.1–8.4 years and review of the literature. Arch Orthop Trauma Surg 1998;118:57–65.
4. Choong PF. The role of allografts in tumour surgery. Acta Orthop Scand Suppl 1997;273:89–94.
5. Duparc J, Massin P, Bocquet L, et al. Autoclaved tumoral autografts: apropos of 12 cases, 6 of which highly malignant. Rev Chir Orthop Reparatrice Appar Mot 1993;79:261–71.
6. Ehara S, Nishida J, Shiraishi H, et al. Pasteurized intercalary autogenous bone graft: radiographic and scintigraphic features. Skeletal Radiol 2000;29:335–9.
7. Harrington KD, Johnston JO, Kaufer HN, et al. Limb salvage and prosthetic joint reconstruction for low-grade selected high-grade sarcomas of bone after wide resection and replacement by autoclaved autogeneic grafts. Clin Orthop 1986;211:180–214.
8. Johnston JO, Harries TM, Alexander CE, et al. Limb salvage procedure for neoplasms about the knee by spherocentric total knee arthroplasty and autogenous autoclaved bone grafting. Clin Orthop 1983;181:137–45.
9. Kawai A, Muschler GF, Lane JM, et al. Prosthetic knee replacement after resection of a malignant tumor of the distal part of the femur: medium to long-term results. J Bone Joint Surg Am 1998;80:636–47.
10. Kohler P, Kreicbergs A, Stromberg L. Physical properties of autoclaved bone: torsion test of rabbit diaphyseal bone. Acta Orthop Scand 1986;57:141–5.
11. Lauritzen C, Alberius P, Santanelli F, et al. Repositioning of craniofacial tumours bone after autoclaving. Scand J Plast Reconstr Surg Hand Surg 1991;25:161–5.
12. Mittermayer F, Krepler P, Dominkus M, et al. Long-term followup of uncemented tumor endoprostheses for the lower extremity. Clin Orthop 2001;388:167–77.
13. Ortiz-Cruz E, Gebhardt MC, Jennings LC, et al. The results of transplantation of intercalary allografts after resection of tumour: a long-term follow-up study. J Bone Joint Surg Am 1997;79:97–106.
14. Sanjay BK, Moreau PG, Younge DA. Reimplantation of autoclaved tumour bone in limb salvage surgery. Int Orthop 1997;21:291–7.
15. San-Julian M, Canadell J. Fractures of allografts used in limb preserving operations. Int Orthop 1998;22:32–6.
16. Seki M, Kawamura M, Horinouchi H, et al. Chest wall reconstruction with pasteurized autogenous bone grafts after resection of desmoid tumor: a case report. Nippon Kyobu Geka Gakkai Zasshi 1997;45:1143–6.
17. Smith WS, Simon MA. Segmental resection for chondrosarcoma. J Bone Joint Surg Am 1975;57:1097–103.
18. Smith WS, Struhl S. Replantation of an autoclaved autogenous segment of bone for treatment of chondrosarcoma. J Bone Joint Surg Am 1988;70:70–5.
19. Thompson VP, Steggal CT. Chondrosarcoma of the proximal portion of the femur treated by resection and bone replacement. J Bone Joint Surg Am 1956;38:357–67.
20. Tsuchiya H, Tomita K, Mori Y, et al. Caffeine-assisted chemotherapy and minimized tumor excision for nonmetastatic osteosarcoma. Anticancer Res 1998;18:657–66.
21. Urist MR, Dawson E. Intertransverse process fusion with the aid of chemosterilized autolyzed antigen-extracted allogenic (AAA) bone. Clin Orthop 1981;154:97–113.
22. Wagner M, Pesch HJ. Autoclaved bone grafts in prosthesis replacement of the hip. Orthopade 1989;18:463–7.
23. Wangerin K, Ewers R, Kestel M. Interaction of bone resorption and bone synthesis using an autoclaved bone graft. Prog Clin Biol Res 1985;187:343–51.
24. Yamamuro T, Kotoura Y. Intraoperative radiation therapy for osteosarcoma. Cancer Treat Res 1993;62:177–83.