Efficacy of frozen autograft treated with liquid nitrogen in limb-sparing surgery in feline scapular osteosarcoma: A case report

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ABSTRACT. In recent years, a novel technique of limb preservation has been used in human medicine that involves frozen autograft treated with liquid nitrogen. In this case, frozen autograft treatment along with shoulder joint reconstruction was performed in an 11-year-old cat with osteosarcoma of the distal scapula. Surgical site infection, shoulder dislocation, local recurrence, and pulmonary metastases were not reported for 24 months after surgery. Moreover, the patient was able to bear weight on the operated limb after 2 months of surgery, and excellent limb function without lameness was demonstrated after 15 months. The frozen autograft technique is advantageous because it is inexpensive, simple, and retains its initial strength after treatment, and could be a novel treatment in feline osteosarcoma.

KEY WORDS: frozen autograft treatment, limb-sparing surgery, osteosarcoma, scapulectomy

Osteosarcoma is the most common primary bone tumor in cats, frequently affecting the femur, tibia, and humerus, but rarely involving the scapula. Typically, procedures of scapulectomy or amputation are performed for osteosarcoma of the scapula, but scapulectomy does not preserve complete limb function [6]. To improve quality of life, limb-sparing surgery is becoming a more popular alternative. Several surgical techniques have been reported for limb preservation, including the use of viable bone materials (autografts and allografts), non-viable bone materials (bone prostheses), and reusing the resected neoplastic bone. Several methods have been developed to enable the reuse of resected neoplastic bone. Autoclave and pasteurization methods can thermally sterilize the bone. Autoclaving uses high temperatures to devitalize tumor cells via heating of the resected neoplastic bone segments [4], while pasteurizing uses low temperatures [1]. Furthermore, irradiation of the resected neoplastic bone induces necrosis of the tumor cells [11]. The resected bones treated by the autoclave method tend to weaken and lose inductivity. Furthermore, autoclaving impairs bone healing, whereas the pasteurization and irradiation methods help maintain the initial bone strength. Nonetheless, pasteurization requires a strict heating procedure and irradiation requires special equipment.

The frozen autograft method using liquid nitrogen has been developed for use in humans to address these issues [9]. Benefits of the liquid nitrogen technique include its lack of requirement for special equipment, as well as the maintenance of protein and cell components and initial bone strength within the frozen autograft [10]. This technique has shown good surgical outcomes with longer survival times in human patients [3]. Therefore, it is being used more commonly in such surgical cases. However, only few reports have demonstrated limb preservation using treated autografts in dogs. Additionally, the incidence of complications, such as postoperative infection and construct failure, is high [5, 7]. To the best of our knowledge, there have been only two reports of frozen autograft reconstruction in dogs [2, 8], but none in cats.

An 11-year-old, 5 kg, spayed female Japanese domestic short hair cat visited our medical center with a history of a mass on her right shoulder without pain or lameness. Physical examination revealed a firm and sessile mass on the right scapula. Radiography showed bone augmentation outside of the scapula (Fig. 1A and 1B). Computed tomography (CT) revealed that the mass had developed from the acromion process and bone sclerosis proximal to the body of the scapula without metastasis (Fig. 1C). A biopsy was performed and histopathological analysis revealed that the mass was an osteosarcoma.

Informed consent was obtained from the owner, and limb-sparing surgery was performed. The patient was anesthetized, the right shoulder was shaved, and general antiseptic was applied. Antibiotic agents were administered intravenously (cefazolin...
sodium 20 mg/kg Nichi-Iko Pharmaceutical Co., Ltd., Toyama, Japan) 40 min before surgery and every 2 hr during the perioperative period. Ketamine (1 mg/kg/hr, Daiichi Sankyo Propharma Co., Ltd., Tokyo, Japan) and fentanyl (10 µg/kg/hr, Janssen Pharmaceutical K.K., Tokyo, Japan) were administered using a constant-rate infusion during the perioperative period.

The patient was placed in the left lateral recumbent position, and the scapula was exposed using a lateral approach. The omotransversarius and trapezius muscles were dissected at a sufficient distance from the mass border. The deltoideus muscle was separated in the center, and the supraspinatus and infraspinatus muscles were separated from the body of the scapula and preserved (Fig. 2A). The biceps tendon, lateral and medial glenohumeral ligaments, and subscapularis tendon were dissected, and the glenohumeral joint capsule was incised and disarticulated. The brachial plexus and subscapularis nerve were preserved. Next, the subscapularis muscle was transected at the level of the planned osteotomy (Fig. 2A), and the glenohumeral joint capsule was incised and disarticulated. The osteotomy was performed 12 mm from the proximal end of the scapula with an oscillating saw (Fig. 2B), and the distal scapular bone particle containing the osteosarcoma was removed (Fig. 3A). After resection, the operative field was irrigated using Ringer’s lactate solution. To prevent dissemination, the mass with the spine and acromion process was resected from the distal scapular bone particle on a separate operating table (Fig. 3B). Frozen autograft processing was performed as described previously [9]. Briefly, the excised bone was frozen in liquid nitrogen for 20 min (Fig. 3C and 3D), then thawed at room temperature for 15 min, and finally thawed in distilled water for 10 min for devitalization. A 1.5 mm diameter bone tunnel at the humeral head and 2 bone tunnels at the scapular neck were created using a power drill. The bone tunnels were used to anchor 60-lb nylon monofilament sutures (Varivas shock leader, Morris Co., Ltd., Saitama, Japan) that were sterilized using hydrogen peroxide gas plasma in a V-shaped manner. Four bone tunnels were created in the remaining bone particle for the anchoring sutures (Fig. 3E). The graft was attached to the trunk of the body using pre-drilled holes in the scapular body with 2–0 absorbable monofilament suture (Monodiox, Alfresa Pharma, Osaka, Japan) (Fig. 3F). Following this, the biceps tendons were sutured together, and the dissected muscles were attached to the synergetic muscles (Fig. 3G). The lymph nodes related to the site were not removed. An active drain was placed in the subcutaneous space, and subcutaneous tissue and skin were closed in a routine manner.

The patient was administered with constant rate infusion of fentanyl (3–5 µg/kg/hr) for the first 24 hr after surgery, while cefalexin (20 mg/kg, orally, every 12 hr, VMDP, Saitama, Japan), enrofloxacin (5 mg/kg, PO, every 24 hr, Bayer, Osaka, Japan), and robenacoxib (1 mg/kg, orally, every 24 hr, ElancoJapan, Tokyo, Japan) were administered for 10 days postoperatively. Since there was minimal serous exudation, the active drain was removed 2 days postoperatively. The patient was discharged to the care of the owner 5 days after surgery, and spica splinting was applied to the patient for 4 weeks.

There was no indication of any postoperative infection in the. Furthermore, the patient was able to bear weight on the operated limb after 2 months of surgery (Supplementary movie 1), and muscular atrophy of the affected limb and restricted amplitude of the shoulder joint gradually improved after 3 months of surgery (Supplementary movie 2). We observed the cat walking on a leveled floor, and the lameness had disappeared almost completely in 6 months (Supplementary movie 3). Additionally, limb function was evaluated as excellent after 15 months. Although osteoarthritus of the shoulder joint of the affected limb worsened slightly, the shoulder dislocation and bone fracture were not visible on radiographs during follow-up (Fig. 4). Postoperative radiographs at 6 months showed that the bone tunnels, where the sutures passed for reconstruction of the ligamental strength of the joints, had enlarged (Fig. 4A–C). This could be attributed to friction of the sutures. At 24 months postoperatively, the hole became smaller in size (Fig. 4F), indicating that the suture had weakened or chafed, and that the treated bone had regained its ability to ossify. CT

Fig. 1. Lateral (A) and craniocaudal (B) radiographs of the right scapula with osteosarcoma at the initial visit; (C) Computed tomography reveals bone sclerosis (white arrow) proximal to the body of the scapula, and possible presence of tumor cells observed near the shoulder joint and proximal to the body of the scapula.
scans did not reveal any evidence of recurrence or metastases at 6 months postoperatively. Bony union between the scapula and frozen autograft appeared to have been established, and the subchondral bone surface of the shoulder joint appeared slightly rough on CT examination (Fig. 5). Local recurrence and pulmonary metastases were not observed on radiographs up to 24 months postsurgery.

Fig. 2. The red dotted line in a coronal section image (A) describes the area of resection (Blue area: osteosarcoma, s: supraspinatus, i: infraspinatus, ss: subscapularis muscle). Three-dimensional computed tomography image of the right scapula (B) describes the position of osteotomy (white arrow).

Fig. 3. The scapula with osteosarcoma is resected and returned to the body after the devitalization of the tumor cells with liquid nitrogen. Intraoperative findings (A) after the muscles are separated or dissected and osteotomy is performed; (B) Following resection of the mass, including the spine and acromion process from the distal scapular bone particle; (C) Prior to dipping the excised bone in the liquid nitrogen; (D) Showing excised bone after treatment with liquid nitrogen; (E) During preparation for anchoring the bone graft to the shoulder joint, the remaining scapula, and body trunk; (F) During return of the bone graft to the body; and (G) Following attachment of the dissected muscles to the synergetic muscles.
In the present case, scapulectomy or amputation could have been performed. However, the patient had no lameness or pain at the initial visit, and the owner was reluctant to provide consent for amputation or scapulectomy due to the risk of reducing patient activity or developing lameness. Additionally, partial scapulectomy could not be performed because the tumor was close to the shoulder joint. Devitalization of the tumor cells was required to rescue the scapula, because bone sclerosis indicated the possible

**Fig. 4.** Postoperative radiographs of the shoulder joint and scapula at (A) 1 month; (B) 3 months; (C) 6 months; (D) 12 months; (E) 24 months; (F) The shoulder dislocation and bone fracture are not visible on radiograph at any follow-up time points.

**Fig. 5.** (A) No local recurrence is observed on the short axis view of the scapula on computed tomography; (B) Bony union of the scapula is observed on the long axis view; (C) The articular surface of the shoulder joint before surgery; (D) The articular surface of the shoulder joint after surgery. Bony union between initial scapula and frozen autograft is established without local recurrence, and the postoperative articular surface of the shoulder joint appears slightly rougher.
presence of tumor cells proximal to the scapular body. We decided to use the liquid nitrogen technique to devitalize the tumor cells. The owner was informed of all possible complications arising from the limb-sparing technique using liquid nitrogen. They were also informed that the technique was still being developed for use in animal medicine. This report demonstrates the successful use of the liquid nitrogen technique to kill tumor cells, and avoiding limb loss, local recurrence, and metastases for up to 24 months post-surgery.

Shoulder arthrodesis was a possible option in this case; however, the prognosis following shoulder arthrodesis in cats is poorly understood. In this case, we were able to retain the shoulder joint by anchoring the scapula and humerus, and the range of motion in the shoulder joint was normal on palpation. Further studies involving magnetic resonance imaging may be needed to reveal whether preservation of the cartilage contributes to the functional recovery of the shoulder joint.

Bony union was visible on CT at 6 months post-surgery. In human surgery, bony union of the treated and host bone is defined as complete cortical bridging, and bony union has been reported at a mean of 6.7 months postoperatively [9].

The liquid nitrogen technique was a simple and inexpensive method that enabled the devitalization of tumor cells of the neoplastic bone. The scapula was preserved as a point of attachment of muscles, which enabled full functional recovery in this case. This technique has the potential to become a novel surgical treatment for feline osteosarcoma of the scapula.

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