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Original Article

Experimental study of stage I *in situ* bone lengthening technology for gunshot bone defects

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Abstract: Objective: The aim of this study was to evaluate Stage I *in situ* bone lengthening technology as a treatment for gunshot bone defects, examining its biological foundation. Methods: Dogs with gunshot bone defects were treated with Stage I *in situ* bone lengthening and conventional late bone grafting. Local microcirculation blood flow changes were compared. Results: No significant differences in local blood flow changes were observed between the two groups of animals with fractures. Bone length increased in the Stage I *in situ* bone lengthening group (2.82±0.94 cm). Relative length increased by 19.3% (15.8-21.4%). Bone healing times were obviously shorter and injured limb function was better in the Stage I *in situ* bone lengthening group than the late bone grafting group. Endochondral bone formation mainly occurred during bone lengthening. Healing time of the wound tract was 21.9±5.3 days in the L group and 23.3±7.8 days in the G group ($P > 0.05$ by t-test). Bone healing time was 8.8±3.2 weeks in the L group and 11.7±5.4 weeks in the G group (6 weeks before bone grafting was not included) ($P < 0.01$ by t-test). After treatment, injured limbs were longer (0.21±0.08 cm) and shorter (0.56±0.18 cm) than healthy limbs in L and G groups, respectively ($P < 0.05$ by t-test). Conclusion: Stage I *in situ* bone lengthening technology for gunshot bone defects demonstrated obvious superiority. It shortens the treatment course and simplifies the operation, showing no obvious adverse effects on the bone healing process and local blood supply.

Keywords: Gunshot injury, bone defect, Stage I *in situ* bone lengthening

Introduction

Bone grafting is contraindicated for contaminated gunshot wounds. Gunshot bone defects often require multiple surgeries and long sessions [1]. Complications, including infections, bone nonunion, and limb dysfunction, however, still occur. Therefore, treatment of gunshot bone defects has been regarded as an puzzle in orthopedic traumatology [2, 3]. Current routine treatment includes bone defect exclusion at the initial stage and bone grafting at the later stage after soft tissue recovers. This treatment method has a long-term course. Repeated surgeries increase the risk of complications, reduce success rates of bone healing, and long-term inactivity of the limbs increases dysfunction. Researchers have performed delayed transplantation of osseous myocutaneous flaps and completed simultaneous reconstruction of bone and soft tissues, shortening the treatment course. However, this has resulted in greater damage to the donor site, while the complex microsurgical reconstruction increased operation times [4]. It has been reported that the use of intramedullary nails will maintain the length of limbs. However, bone defects with a larger distance between fracture ends increases incidence of bone nonunion [5]. Bone transport [6-8] and compression distraction [9, 10] using external fixators, have been used as treatment for bone defects. Both require incisions and cutting of the bone at the distal metaphysis, which increases patient trauma. Furthermore, no application to gunshot bone defects has been reported. Stage I *in situ* bone lengthening technology (Minimally invasive osteotomy; Delayed pulling; Stable and adjustable way of fixing; Slowly divided and extended, extending 1-1.5 mm in 4-6 times per day) with external fixator has been designed as a primary treatment. This technology has been successful in clinical application with bone lengthening of approximately 6 cm and good recovery of limb function [11].

The conventional bone lengthening method is applied if the periosteum is intact. Bone defects due to gunshot injuries are often accompanied by severe periosteum defects [12]. Hence, the question remains whether Stage I *in situ* bone lengthening technology has universal significance as a treatment for gunshot bone defects. What is the pathological basis of bone lengthening in the presence of a periosteum defects? To answer these questions, the current study was conducted.

Materials and methods

Animals and injury

A total of 32 adult hybrid dogs of either gender (15±5 kg in weight), from the Experimental Animal Department of the First Affiliated Hospital of PLA General Hospital, were injected with thiopentone (8 mg/kg) for anesthesia and randomly divided into the Stage I *in situ* bone lengthening group (L group) and late bone grafting group (G group). There were 16 dogs per group. This study was approved by the Ethics Committee of First Affiliated Hospital of PLA General Hospital.

A 5.56 mm ballistic rifle was used to shoot the dogs, with an initial speed of 950±10 m/s and a projectile mass of 4.05±0.1 g. The warhead speed was measured, before and after firing, while the dogs were injured. The right hind limbs in all dogs were suspended. Firing was aimed at the middle diaphysis (the great vessels and nerve were avoided), 6 m away from the bore exit. The projectile hit the femur and caused fracturing.

Treatment method

For the L group, the operation was performed under aseptic conditions using 0.1 L normal saline to wash the wound tract and remove visible foreign bodies, contaminated necrotic tissues, and free bone fragments [4, 5]. Unilateral adjustable external fixator was used. Two fastening nails were screwed into the femur at the proximal and distal fracture ends and external fixed support was installed at the nail end. Fracture reconstruction at the site of the bone defect was shortened to achieve good alignment. After surgery was completed, 0.1 L normal saline was used to wash the wound tract again. The wound was drained using sterile binding buffer. Penicillin and gentamicin sulfate (0.1 g [100,000 IU]) were administered through intramuscular injections twice per day. The antibiotic was changed daily and delayed or two-stage suturing was performed 7 days after surgery, as appropriate. Based on the condition of the fracture, the bone was lengthened about 14 days after the injury, with a lengthening speed of about 1 mm/day. This was perfromed until its length was equivalent to that of the femur of the uninjured side. After lengthening was stopped, the fractured part continued to be fixed until healing.

For the G group, wound management and removal of free bone fragments were performed as above. The injured limb was fixed using gypsum under traction of the femur condyle. At 6 weeks after the injury, a self-iliac bone was grafted at the femur defect and then fixed until healing.

Local microcirculation blood flow measurement

Microcirculation blood flow at the fracture end in both groups was measured immediately and at 1 day, 3 days, 7 days, 3 weeks, and 6 weeks after the injury. Laser Doppler Flowmetry (Periflux 4001 Master, Perimed AB, Stockholm, Sweden) was used. The probe was placed on the bone surface at the fracture end. Results were processed using a microcomputer and special software by Perisoft to display and record the waveform. Blood perfusion was then calculated.

Pathological observation

A dog in each group was randomly killed immediately and at 1 day, 3 days, 7 days, 3 weeks, and 6 weeks after surgery. Bone tissue samples were obtained from the fracture end. They were then fixed in 10% formalin, with routine decalcification, paraffin embedding, and hematoxylin and eosin staining. Afterward, they were was observed under a light microscope.

Statistical analysis

Experimental data are presented as mean standard deviation (x±s). SPSS 13.0 software (SPSS Inc., USA) was used to calculate Student's *t*-test. χ^2 test was used to determine sig-

Table 1. Blood perfusion (pu) at the fracture end in both animal groups after gunshot injuries

	Immediate moment	1 d	3 d	7 d	3 weeks	6 weeks	Normal
L Group	27.21±7.88	19.95±4.67	18.39±4.01	33.07±8.50	50.51±9.55	78.74±12.96	79.06±16.69
G Group	23.93±5.76	22.48±5.39	17.53±3.60	32.33±6.94	45.28±10.75	77.90±13.52	81.12±19.05

No significant difference between groups at various time points was observed ($P > 0.05$ by *t*-test).

nificant differences between groups. *P*-values less than 0.05 indicate statistical significance.

Results

Gunshot injuries in both animal groups

For target velocity measurement, animal tissues in the experiment absorbed 387.1±40.5 J of energy. One dog in the G group died of respiratory arrest due to excessive anesthesia. Two dogs in the L group and one dog in the G group died of hemorrhagic shock due to femoral artery injury. One dog in the G group died at 3 days after injury due to poor constitution. The remaining 27 dogs had a perforating wound with an inlet area of 0.3±0.13 cm^2 and exit area of 6.31±2.1 cm^2. A highly comminuted fracture of the femur was noted after the injury and obvious bone defects were observed after removing the severely contaminated free bone fragments in the conventional bone lengthening method. The bone defect measured 2.61±0.73 cm in 13 dogs in the L group and 2.58±0.65 cm in 12 dogs in the G group ($P > 0.05$ by *t*-test).

Treatment outcomes

No complications, such as septicemia and osteofascial compartment syndrome, were observed in either group. Infection rates for the surviving animals in L and G groups were 15.4% and 16.7%, respectively ($P > 0.05$ by χ^2 test). In the G group, one dog was infected after bone grafting, with an infection rate of 25.0%. This was significantly higher than that in the L group ($P < 0.05$ by χ^2 test). One dog each in the L and G groups had bone nonunion, with an incidence rate of 7.7% and 8.3%, respectively ($P > 0.05$ by χ^2 test).

Bone healing was evaluated based on bone healing criteria and indications of the United States Food and Drug Administration [13-15]. Healing time of the wound tract was 21.9±5.3 days in the L group and 23.3±7.8 days in the G group ($P > 0.05$ by *t*-test). Bone healing time was 8.8±3.2 weeks in the L group and 11.7±5.4 weeks in the G group (6 weeks before bone grafting was not included) ($P < 0.01$ by *t*-test). After treatment, injured limbs were longer (0.21±0.08 cm) and shorter (0.56±0.18 cm) than healthy limbs in the L and G groups, respectively ($P < 0.05$ by *t*-test).

Limb function was indicated by knee-joint passive flexion. Injured limbs had a knee-joint passive flexion of < 25.1±10.6° and < 32.7±16.3° and a knee-joint passive extension of < 17.8 ±9.3° and < 25.4±11.2, compared to healthy limbs in L and G groups, respectively ($P < 0.05$ by *t*-test).

Local microcirculation blood flow measurement

Blood perfusion was significantly reduced at the fracture end immediately after the gunshot injury, further declining at 1 day and 3 days after the injury. Subsequently, it started to improve at 7 days after the injury and become close to the normal value at 6 weeks after the injury ($P > 0.05$ by *t*-test). No statistical differences between groups were observed at various time points (**Table 1**).

Radiographic findings

Immediately after the injury, many bone fragments were observed at the wound tract exit and fracture end (**Figure 1A**). Fracture fixation was shortened after debridement in the L group, with good alignment (**Figure 1B**). The fractures were fixed using plaster, with poor alignment. At 2 weeks, the wound tract healed and a fusiform external callus was occasionally observed at the fracture end in both groups (**Figure 1C**). At 6 weeks, bone grafting was performed in the G group. The bone was continuously lengthened in the L group (**Figure 1D**). At 8 weeks, a callus formed at the recipient area in the G group. The femur in the injured limbs in the L group was basically similar in length to that in the opposite limbs after lengthening (**Figure 1E**). At 12 weeks, the bone in the L group healed and the external fixator was

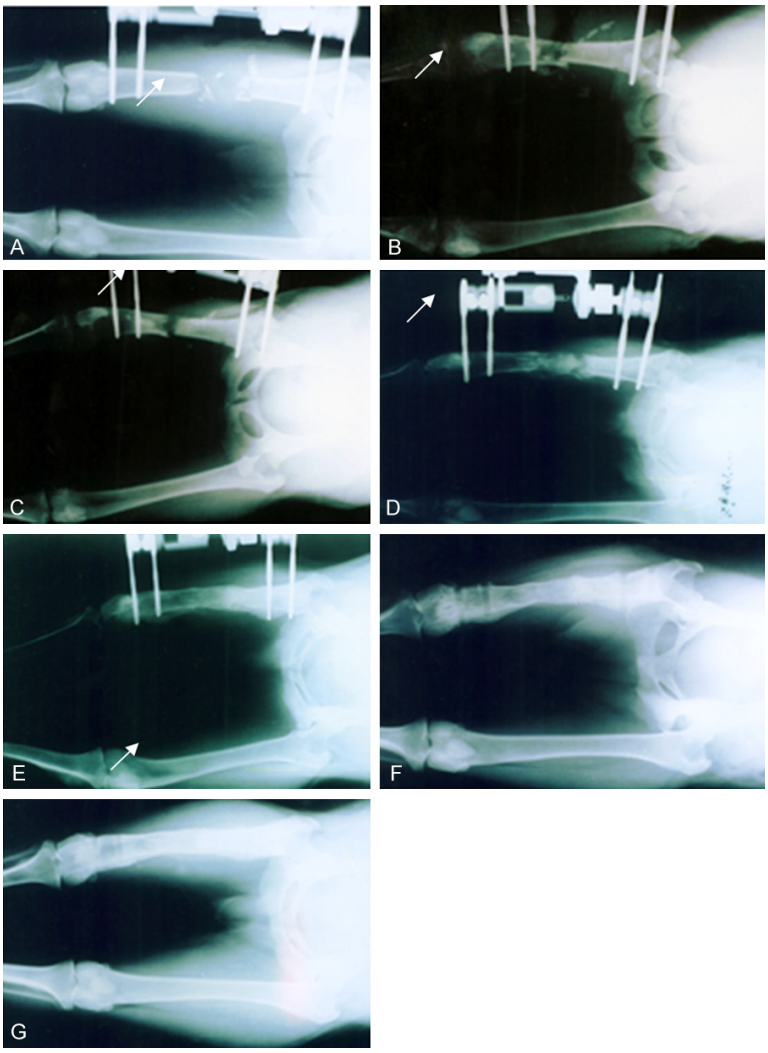

Figure 1. Radiographic findings during stage I *in situ* bone lengthening. A. Femur with defect and healthy femur immediately after gunshot injury. B. When the soft tissues were disposed, external fixation was shortened to achieve good alignment. C. At 2 weeks after surgery, the bone was lengthened after the fracture ends were connected. D. Bone lengthening process. E. At 8 weeks after surgery, bone lengthening was completed, and the external fixator continued to be used. F. After bone healing, the external fixator was removed, and the length of the injured femur was similar to that of the healthy femur. G. Bone modeling was completed in the L group based on bone healing.

removed (**Figure 1F**). At 16 weeks, density at the bone graft area in the G group increased, the bone graft area fused preliminarily with the recipient area, and the bone substitution process was basically completed. Bone modeling in the L group was basically completed based on bone healing (**Figure 1G**).

Radiographic findings for the hind limbs at both sides were estimated to be the same on the same plane. The length of the lengthened bone in the L group was 2.82±0.94 cm. Compared with the limb length before the injury, relative length increased by 19.3% (15.8-21.4%). The length of the filling bone defect in the G group was 1.95±0.76 cm. Relative length increased by 12.2% (9.7-15.1%) ($P < 0.01$ by *t*-test). Magnification rates for the radiograph averaged 11.6%.

Pathological examination

At 2 weeks, hyperplasia in the external periosteum at the fracture end newly formed bone trabecula was observed in the subperiosteum and hyperplasia in the thin-walled capillaries was detected between the bone trabeculae (**Figure 2**). Cells at the recipient area in the G group actively proliferated and a newly formed bone matrix was seen around the grafted bone.

At 3 weeks, many chondrocytes differentiated (**Figure 3**), a few new bone trabeculae formed, and osteoclasts and lymphocytes were observed in the L group. Many osteoblasts and newly formed bone tissues encircled the bone graft and grew in the bone graft area in the G group.

Discussion

Stage I *in situ* bone lengthening technology for gunshot bone defects can be applied with debridement at the initial stage of gunshot injury [16, 17]. Gradual bone lengthening to recover limb length is easier with stage I *in situ* bone lengthening than with bone grafting. Furthermore, with stage I *in situ* bone lengthening, insufficient bone source and failure are not a concern and the long bone substitution process is avoided [18]. Function of the injured limbs is also not limited after surgery and can be maintained at their maximum level. The use of a fixation apparatus for the wound tract is

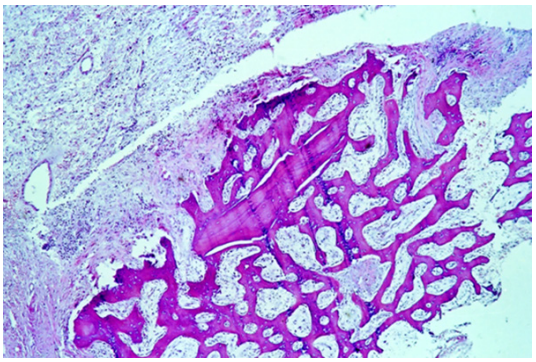

Figure 2. Formation of local new capillaries. At 2 weeks after injury, some intramembranous bone was noted at the fracture site in the periosteum, and local new capillaries formed (HE × 100).

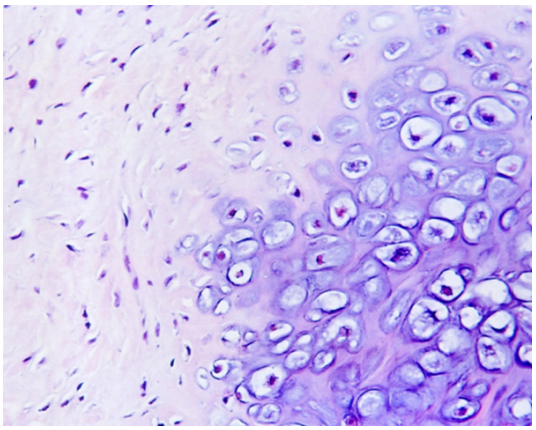

Figure 3. Formation of endochondral bone. At 3 weeks after injury, bone healing during stage I *in situ* bone lengthening was based on endochondral bone formation (HE × 100).

not increased, making stage I *in situ* bone lengthening an obviously superior treatment for bone defects with contaminated or infected gunshot wounds. Based on accumulated experience, this method can be applied to individuals with 15-20% bone defects.

Other studies have shown that blood perfusion significantly decreased at the fracture end after gunshot injuries and started to increase at 7 days after the injury [19, 20]. After the bone in the L group was lengthened, blood perfusion at the fracture end was in accord with that in the G group and continued to increase. At 6 weeks after the injury, blood perfusion in both groups recovered to normal levels. Changes in blood supply were similar to that preoperatively at the fractured part [15]. Currently, there is no unified understanding regarding the effects of bone lengthening on the local blood supply and whether it could lead to delayed bone healing. The bone was lengthened by 1 mm per day in the present experiment. Results showed no significant differences in the local blood supply between the groups. Results suggest that appropriate bone lengthening speed had no adverse effects on the local blood supply to the fracture site. It has been reported that mechanical distraction at 0.35 mm/8 h had a slight effect on the blood supply to the bone tissues [21], in accord with present results.

One study showed that fractures were healed by intramembranous bone formation, which occurred at 14 days after injury, and endochondral bone formation, which occurred at about 21 days, with the endochondral bone mainly forming during osteogenesis. It also showed that intramembranous and endochondral bone formation in mice occurred at 3 and 8 days after the injury, respectively [22]. Postacchini et al. reported that intramembranous and endochondral bone formation in humans occurred at 7 and 12 days after injury, respectively, pointing out that callus formation in the intramembranous bone during bone healing, in general, was less and predominantly occurred in the endochondral bone [23]. The experiment showed that callus formation during bone lengthening mainly occurred in the endochondral bone. This may be due to effects on bone healing of local mechanical tension and micromotion at the fracture end caused by severe damage on the periosteum at the fracture end. Moreover, the local blood supply to the site of gunshot injury recovered slowly, resulting in low oxygen levels at the fracture end. This may be in favor of chondrogenesis.

Osteoblasts during the bone healing process arise from the osteoprogenitor cells in the periosteum endothecium. However, for gunshot injuries, although a few surviving periosteum tissues were observed to be capable of bone formation, endochondral bone formation mainly occurred and osteoblasts were derived from mesenchymal cells. Most authors believe that mesenchymal cells originate from vascular endothelial cells [24]. The experiment also showed more mesenchymal cells around the blood capillaries and venules. It seems that local fracture revascularization not only provides blood sup-

ply for bone healing but also may provide a source for osteoblast progenitors.

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Disclosure of conflict of interest

None.

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