The Reduction of Humeral Fracture in Pigeons with Intramedullary Poly (Methyl Methacrylate) and Neutralization Plate Fixation

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ABSTRACT. The reduction of experimental humeral fracture in pigeons was performed with intramedullary bone cement (poly (methyl methacrylate); PMMA) and neutralization plate fixation to investigate the effect on bone fracture healing and the recovery of flying ability. As a result, neither plate nor bone cement fixation held for more than 2 weeks, but no re-fracture was observed in any cases with both plate and bone cement. In the latter group, it was confirmed by flight tests that almost normal flying ability was recovered in 6 weeks. In pathologic findings, blood supply to the fractured ends recovered within 2 weeks and no cortical bone necrosis was observed at the time except for a disturbance of endosteal callus formation. The process of fracture healing on the plate side took place slightly later than that on the non-plate side, and when much PMMA remained between the fractured ends of the cortical bone the bone formation became spongyoid. In view of the quick recovery of flying ability, however, plate and bone cement fixation is considered appropriate to prevent the displacement of a humeral fracture in the pigeon.—KEY WORDS: bone cement, humeral fracture, microangiogram, pigeon, plating.


In birds, reduction of a fracture is difficult, especially with the humerus, which is pneumatic bone and easily shattered by excessive stress [3, 9]. External fixation is not stable and continuous motion readily causes excessive callus at the fracture site [3, 4, 6, 7, 9, 11]. In internal fixation, the intramedullary pinning procedure is not strong enough to bear the rotational force exerted on the wing [4, 7, 9, 11], and plate fixation is stable itself [10]. However, in birds, unlike mammals, it is rarely applied for the characteristic bone structure [6, 11]. It has been well documented that bone cement in the medullary space is useful in securing the fractured humerus [1, 2, 9]. Kuzma and Hunter also used AO/ASIF plate at the same time, attaining faster healing in function, as confirmed both clinically and radiographically [6]. In view of this result, bone necrosis and the disturbance of endosteal blood supply by heat of polymerization are not supposed to be serious problems [6].

In the present study, internal fixation of humeral fracture in a pigeon was done by a modification of the method of Kuzma to investigate the process of healing by radiographic, microangiographic and histopathologic comparison.

MATERIALS AND METHODS

Animals: This study was done in accordance with a permission of Gifu municipal authorities to exterminate the pigeons within Gifu University. Twenty-four adult pigeons kept over one week on a commercial diet for pigeons and water ad libitum were used in this experiment.

Groups: Pigeons were divided into three groups of 4, 4, and 16, and fixed in the following ways: with an individual plate only, bone cement (poly(methyl methacrylate), PMMA), or both plate and bone cement for the humeral fracture.

Anesthesia: Ketamine at 50 mg/kg of body weight was administered intramuscularly, following ampicillin at 400 mg/kg of body weight just before the operation.

Surgical procedure: Under anesthesia, the surgical procedure was carried out by a modification of the method described by Kuzma and Hunter [6]. In short, after disinfecting with 0.1% benzalkonium chloride, the humeral bone was approached from lateral side to expose its diaphysis, then cut with an electric cutter (BLF electric dental microengine, Osaka Electric Co., Tokyo) right across the long axis of the bone to avoid the branch of the median nerve. Plate fixation (group A) was performed by a standard technique with 2.8 cm × 5 mm AO/ASIF plate (mini straight plate OR234–15, Mathys Ltd., Switzerland) with 5 holes and 8 mm × 1.5 mm of bone screws (mini screws for cortical bone OR210–08). The plate was fitted along the bone axis and four holes of 1.2 mm in diameter were drilled, two in the proximal and two in the distal humerus. After the fractured bone was secured, muscles and subcutaneous tissue were closed with 4–0 polyglycolic acid suture (Dexon Plus), and 4–0 monofilament nylon suture was used for skin suture. Bone cement fixation (group B) was performed with PMMA by mixing two components (2 g of powder polymer and 1 ml of liquid monomer) for a few minutes. A disposable syringe to which a 3-cm-long pre tube was attached was filled with about 3 ml of PMMA before polymerization and injected into the medulla above each fragment of the humeral bone. At the time a pre tube was inserted into the medulla near the epiphysis to inject PMMA while withdrawing gradually. About 0.5 g PMMA was injected so as to form a good seal between the ends. Any excess protruding PMMA was discarded. After fixing by polymerization for
about 8 min to be reduced en bloc, muscles, subcutaneous tissue and skin were closed in the same manner as above. The fixation of group C was performed by plate fixation, as group A after bone cement fixation, as group B.

Postoperative treatment and flight test: The pigeons, without external fixation postoperatively, were kept alone in their 45 × 30 × 35 cm cage for one week, then transferred into individual 42 × 59 × 50 cm cages in which a few were kept together. Four pigeons in group C were given test flights to determine the degree of wing recovery. Radiography: Apart from detecting possible refracture, radiographical examination of the rest of the pigeons was performed every 2 weeks postoperatively.

Microangiography: The pigeons were euthanized with an overdose of pentobarbital sodium (100 mg/kg) and heparin sodium (500 IU/kg) injected into the left ventricle. The aorta was cannulated to perfuse with about 100 ml of lactate solution and ultrasonicated for 4 hr before injecting 20 ml/kg of 90% barium sulfate. The right humerus and peripheral tissue were then immersed in 10% formalin for one week. After fixation, decalcification was performed with a modification of the method of Plank and Rychlo [8]. In short, bone tips with peripheral tissue removed were immersed for one day in 50 to 100 ml decalcification solution for each bone tip containing 7.0 g of aluminium chloride, 8.5 ml of hydrochloric acid and 5.0 ml of formic acid, adjusted to 100 ml with distilled water, and then steeped for one day in 5% sodium sulfate, followed by rinsing in running water. The tissue sections obtained were sliced into 1 mm thickness after the bone tips were cut along the long axis of the bone to remove PMMA. Radiography was performed at 32 cm FFD, 15 kvp, 8 mA and 1 to 4 min exposure, to observe the microvascular structure of the humerus.

Histopathological examination: After that, the diaphysis of the bone was dissected out of the epiphyses and embedded in tissue embedding medium (paraplast, Sherwood Medical Co., U.S.) in the conventional manner. 6 μm thick serial sections were cut and stained with HE and Azan solutions for observation.

RESULTS

In groups A and B refractures were found on radiographic examination within 2 weeks, but group C showed no refractures during the experiment. Moreover, flying ability in group C did not fully recover within 4 weeks, even though the form was virtually normal. Both vertical and horizontal ability eventually recovered, however, in 6 weeks except for No. 2 (Table 1). Five meters was the measured maximum height, and a pigeon which had flown to five meters flew beyond that height to fly away the next day.

Radiographic, microangiographic and pathological findings in group C: At the second week postoperatively, the fracture line was clear and no external callus was observed on the radiograph. In pathological findings, there was fibrous external callus at the fractured ends on the plated sides (Fig. 1), though there were both a cartilaginous callus near the proximal and an osseous callus near the distal fractured end on the non-plated side (Fig. 2). Tissue necrosis was seen at the fractured ends on the plated side, but no cortical bone necrosis due to polymerizing heat was observed. PMMA in the medulla and at the fractured ends was covered with fibrous connective tissue.

On microangiography, capillary vessels were seen around the fractured end of the non-plated side and in the cancellous bone of the distal epiphyses (Fig. 3). Furthermore, compared with the pathological findings, on the non-plated side, the capillary vessels were seen in the fibrous tissue inside the cortical bone and external callus, and the vessels in the cartilage were sparse, whereas the vessels observed in the bony trabeculae were dense. The lesser capillary vessels were only pathological around the fractured ends of the plated side.

At the 4th postoperative week, the fracture line was still clear, but an external callus bridged the fractured ends on the non-plated side on the radiograph (Fig. 4).

Among the pathological findings, the external callus composed of cancellous bony trabeculae involving Harversian lamellae bridged the fractured ends, forming a

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<th>Table 1. Degree of recovery of flying ability in horizontal and vertical flight tests for group C</th>
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Fig. 1. Histology of the plated side at 2nd postoperative week. Fractured ends are separated, and the gap, in which PMMA intermediates, is seen. Fibrous connective tissue is seen outside the cortical bone. P: Proximal, D: Distal, and BM: Bone marrow. HE. × 93.

Fig. 2. Histology of the non-plated side at 2nd postoperative week. a) Fractured ends are separated, and the gap, in which PMMA intermediates, is seen. Fibrous connective tissue involving hematoma is seen. P: Proximal, D: Distal, and BM: Bone marrow. HE. × 109. b) External callus composed of bony trabeculae and thick cartilage is seen. HE. × 104. c) Spongioid external callus composed of immature bony trabeculae is seen. HE. × 111.

Fig. 3. Microangiograph around fractured ends at 2nd postoperative week. Capillary vessels are seen in the external callus and inside the cortical bone on the non-plated side (below). P: Proximal, D: Distal, and BM: Bone marrow. × 8.5.
bony union, whereas those on the plated side evidenced cartilaginous bony union (Figs. 5–6). PMMA in the medulla was covered with fibrous connective tissue in which a few giant polymuclear cells were seen.

On microangiography, many capillary vessels were seen around the fractured ends on the non-plate side, in the external callus on the plated side and in the proximal cancellous bone of epiphysis (Fig. 7). Compared with the pathological findings, many reticulated vessels were seen among the bony trabeculae, in the fibrous connective tissue of the Harversian canals and inside the cortical bone on the non-plated side.

At the 6th postoperative week, the fracture line became fairly unclear. There was a case in which ossification was found on the non-plated side (Fig. 8). Among the

Fig. 4. Radiograph at 4th postoperative week.

Fig. 5. Histology of the non-plated side at 4th postoperative week. Bony union involving bony trabeculae is seen in the external callus and at the fractured ends. P: Proximal, D: Distal, and BM: Bone marrow. HE. × 90.

Fig. 6. Histology of the plated side at 4th postoperative week. Cartilaginous bony union is seen in the external callus and bony trabeculae seen between fractured ends. P: Proximal, D: Distal, and BM: Bone marrow. HE. × 100.

Fig. 7. Microangiograph of around fractured ends at 4th postoperative week. Reticulated capillary vessels are seen in the external callus on the non-plated side (below). P: Proximal, D: Distal, and BM: Bone marrow. × 7.8.
pathological findings, new bone formation involved more mature Harversian layers than those in the 4th week over the fractured ends without excessive callus or cortical necrosis (Figs. 9–10). On microangiography, capillary vessels were seen around the fractured ends on the non-plated side and in the cancellous bone of the epiphyses. Compared with the pathological findings, reticulated microvessels were seen in Harversian canals and in the fibrous connective tissue at the fractured ends on the non-plated side. On the plated side, capillary vessels were only noted on the outside of the cortical bone as well as in the cortical bone itself at the fractured ends.

At the 8th postoperative week, the fracture line became very indistinct, and the ossification which bridged over the non-plated side by means of the external callus was clearer than at the 6th week. Pathologically speaking, the new bone formation involving many Harversian layers was seen to be a bony union at the fractured ends on the non-plated side but not on the plated side. Microangiography revealed capillary vessels in the cortical bone, outside the cortical bone and the epiphyses on the plated side (Fig. 11). Compared with the pathological findings, reticulated vessels were seen in the cortical bone and in the fibrous tissue outside the cortical bone on the plated side, but hardly in the cortical bone on the non-plated side.

From the pathological findings at the 12th postoperative week, bony union was seen to be reforming on the plated side. On the non-plated side, due to the intervention of PMMA, the cortical structure became spongoid around the proximal fractured end, but the rest formed compact bone. Giant cells were no longer observed in the fibrous connective tissue.

**DISCUSSION**

The pattern for fracture healing in birds is similar to that in mammals [4], and endosteal callus appears in fractured ends at first, then periosteal callus to support the bone [4, 11]. The healing period depends on fixing stability, blood supply and infection [11]. For instance, if fixed rigidly, it takes about three weeks for the radial fracture to heal and begin to remodel [3, 7]. But much longer is required for recovery of a pneumatic bone such as the humerus [3, 6]. Putney et al. [9] reported that when the wing was bandaged to the body for a humeral fracture by osteotomy, it suffered from displacement in a few days, and 10 weeks later the fractured ends were covered with much fibrous connective tissue, indicating no prospect of recovery or that it would take a long time for the unstable fragments of the humerus to repair by means of external fixation. In the preliminary study, external fixation caused extreme stress in the pigeons, eventually leading to refracture due to excessive movement in all the cases tried. For that reason, the present study was carried out by either plate or bone cement fixation without external fixation. But continuous stable fixation was not attained by plate fixation alone because of the cracking or the crushing of the bone at the time of the plating procedure. On the other hand, Putney et al. [9] reported that when bone cement was poured into the intramedullary space to reduce the fractured humerus under external fixation, no displacement was seen at the fractured ends. The bone cement fixation of the humerus in the present study

Fig. 8. Radiograph at 6th postoperative week.

Fig. 9. Histology of the non-plated side at 6th postoperative week. Stable bony union is seen. P: Proximal, D: Distal, and BM: Bone marrow. HE. × 92.

Fig. 10. Histology of the plated side at 6th postoperative week. Bony union (arrow heads) and a gap (arrow), in which PMMA intermediates are seen at the fractured ends. P: Proximal, D: Distal, and BM: Bone marrow. HE. × 92.
resulted in re fracture in all cases, indicating the need for some internal fixation. Furthermore, Kuzma and Hunter [6] reported that plate fixation was used 10 min after polymerization of PMMA in each bone fragment. In the preliminary study, their procedure was carried out but failed in re fracture on all the cases tried. In the present study, accordingly, each fragment was reduced to seal between both ends with PMMA which was to be a single mass in the medulla, then plate fixation was done to attain a good result. It is therefore important for PMMA to be continuous between the fractured ends, as Kuzma and Hunter admitted a fault in their method [6].

With regard to the blood supply at the fractured ends, Chaffee [5] reported that humeral fracture in osprey was reduced with intramedullary pinning and external fixation but re fractured at the 7th postoperative week, even though the pin was removed 2 weeks before. At necropsy, the distal epiphysis of the humerus was found to show signs of bone necrosis due to the pin, by which the blood supply was disturbed. Intramedullary pinning of that kind occasionally causes injury to the epiphysial and endosteal nutritive vessels resulting in bone necrosis. Similarly, PMMA in the medullary canal is feared to disturb the endosteal blood supply and callus formation; but at the 2nd postoperative week in group C in which plate fixation was used after injecting bone cement, capillary vessels were seen in the periosteal callus composed of fibrous connective tissue, and in the cancellous bone of the epiphyses. Furthermore, PMMA in the medullary space and soft tissue was covered with fibrous connective tissue, and necrosis of the cortical bone due to polymerization heat was no longer seen. These findings indicate that the function of periosteal repair was proceeded and that the injection of PMMA was scarcely damaged for the nutritive vessels.

The periosteal callus containing spongioid new bone was seen on both sides at the 4th week, and cortical bony union at the 6th week. Bush [3] reported that, in the process of natural healing with no fixation in humeral fracture, the callus was still fibrous connective tissue at 9 days and composed mainly of fibrocartilage at 16 days. Then, at 21 days, the callus bridged the fractured ends, and at 12 weeks, decreased to indicate remodeling of the bone. According to Putney, the fractured humerus reduced with bone cement was covered with fibrous callus at 10 days postoperation, bridged with thick bony callus at 30 days and eventually healed at 75 days [9].

In the present study, however, the period of bone healing was not particularly delayed, but the process of fracture healing was slightly behind that on the non-plated side. On the other hand, in some cases, the residue and/or intervention of PMMA between the fractured ends caused the cortical bone to become even spongioid. It is important not to use PMMA between them, as Kuzma and Hunter [6] indicated, because re fracture tends to occur at the site where both cortical bone fragments are not continuous. Putney et al. [9] reported that giant cells reacting to PMMA appear at the 4th week postoperation, then gradually disappear. In the present study, these were also observed from 4 to 10 weeks. PMMA was covered with fibrous connective tissue and no toxic symptoms were observed, and bone cement in the intramedullary space was considered not to have a toxic effect, as Putney et al. [9] indicated. Besides, no toxic symptoms have been seen in two years in a pigeon with PMMA.

In any case, given the almost normal flying ability
evidenced by the flight test at the 6th postoperative week, bone function recovered, and, despite injury induced by PMMA injection into the endosteal callus, there was maintenance of nutritive vessels at the fractured ends, and good support for the fracture site with both plate and PMMA. Furthermore, histological cortical bony union and the radiographical ossification were observed even on the plate side at the 6th postoperative week, suggesting the possibility of the plate removal at the time in a case with no PMMA between the fractured ends.

REFERENCES