Clinical Evaluation of Uniaxially Oriented Poly-L-Lactide Rod for Fixation of Experimental Femoral Diaphyseal Fracture in Immature Cats

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ABSTRACT. Transverse diaphyseal fractures of the femur were experimentally made in immature cats, and were fixed by an intramedullary pinning technique using an uniaxially oriented poly-L-lactide (PLLA) rod, a biodegradable polymer. The healing process was evaluated radiographically and histologically. Formation of bony callus was completed in 8 weeks, and cortical bony union followed. The remodeling process was then observed form 12 to 16 weeks. The healing process was almost the same as when a metallic implant was used. Abundant periosteal callus formation may be attributable to the lower elasticity of the PLLA rod compared with metallic implants. Since no other abnormalities such as growth deformities were detected, it was concluded that the combined use of a uniaxially oriented PLLA rod and an external splint is clinically useful for the repair of diaphyseal fractures in immature cats.—KEY WORDS: biodegradable polymer, feline, femur, osteotomy, poly-L-lactide (PLLA).

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The treatment of fractures with biodegradable material was first described by Cutright and Hunsuck, who experimentally applied a polylactate suture to fix a fractured mandible in the monkey in 1971 [5]. This has encouraged a number of studies on bone fixation with polyglycolate and polylactate materials. Axelon *et al.* reported the use of polyglycolate pins in the treatment of bone fracture in dogs and cats. They reported that this material lost its bending strength *in vivo* in 4 weeks [1]. In most of the earlier studies, biodegradable materials have been used in attempts to treat fractures in areas not subjected to great external force such as cancellous bones and mandible, since relatively low strength polymer has been used. The application of biodegradable materials to compact bones like the diaphysis of a long bone has been attempted in only a few cases [1, 5, 9]. Uniaxially oriented poly-L-lactide (PLLA) rods have mechanical characteristics with an initial bending strength of 220 MPa, and more than 50% of its initial bending strength is maintained *in vivo* for 12 weeks [11]. In this paper, the healing process was radiographically and histologically evaluated in the case of experimental diaphyseal fracture treated with PLLA rods in immature cats.

MATERIALS AND METHODS

Uniaxially oriented poly-L-lactide (PLLA) rod: PLLA rods were prepared as described by Matsusue *et al.* [10, 11]. This material has a molecular weight of $2.0–2.5 \times 10^5$ Dalton, initial bending strength of 220 MPa, initial tensile strength of 110 MPa and a bending modulus of 13 GPa. It is cylindrical in shape with a diameter of 3.5 mm and a length of 50 mm (Fig. 1).

Experimental animals: Fifteen (9 male and 6 female) clinically normal cats aged 5 to 6 months maintained in our laboratory were used. Their body weight ranged from 0.8 to 1.3 kg (mean: 1.1 kg).

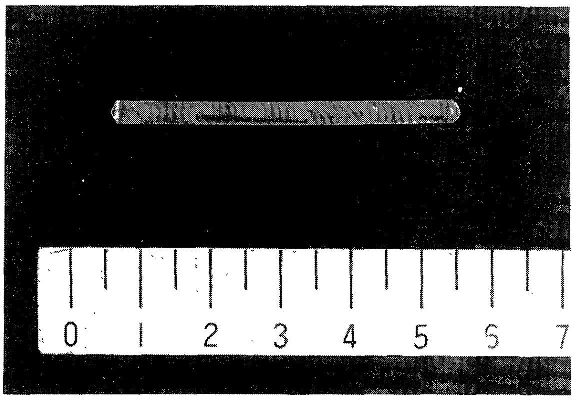

Fig. 1. Uniaxially oriented PLLA rod used in this study.

Surgical procedures: Under general anesthesia with halotane, a transverse osteotomy was made at the middle portion of the right femur with a bone saw. The fracture was then fixed with a PLLA rod by a basic intramedullary pinning technique.

Postoperative management: Ampicillin (Meiji, Tokyo) was administered at a dose of 30 mg/kg for 10 days after surgery and the cats were then kept in a cage for 45 days. A Thomas splint was applied to the right hindleg for fixation during the same period to prevent rotational movement of the femur [16]. These procedures were conducted according to the Guidelines for Care and Use of Laboratory Animals of Nippon Veterinary and Animal Science University.

Experimental groups and observation: The cats were divided into 5 groups (Groups A to E) of 3 cats each for the duration of follow-up observation (2, 4, 8, 12 and 16 weeks after surgery).

Radiographic examination: Anteroposterior (AP) and lateral radiographs were taken for all groups before and immediately after surgery, and at 2-week intervals thereafter. The formation of callus, condition of the osteotomy line and displacement of the fractured fragment were observed on these radiographs. At the same time, the total femoral length and minimum diaphyseal width were measured on the lateral radiographs for both the osteotomized and the contralateral intact femur. At 2, 4, 8, 12, and 16 weeks after surgery, X-ray computed tomography (CT) was conducted with an X-ray CT scanner (IMAGE-MAX II, Yokogawa Medical, Tokyo). Under general anesthesia, transversal scanning was carried out with a slice thickness of 2 mm at the femoral diaphysis including the osteotomy line. Based on these images, the maximum diameter of the femur including the external callus, the diameter of the femur excluding the external callus and the diameter of the marrow cavity were measured. The CT number of the external callus was measured to estimate the ossification process of newly formed cortex [13]. In addition, that of the PLLA rod was also measured to estimate the absorptive process.

Histological examination: In experimental animals euthanatized by overdosing with pentobarbital each time, the treated and the intact femurs were collected, fixed in 70% ethanol, and then decalcified with formic acid. The longitudinal tissue sections of the femoral shaft were stained with hematoxylin and eosin and the time-course changes in osteotomy line and the histological change around the PLLA rod were investigated.

Statistical analysis: The data were statistically analyzed by Student's *t*-test, and $p<0.05$ was defined as statistically significant.

RESULTS

Radiographic examination: The anchoring callus extending toward the fractured ends in contact with the bone cortex was found at 2 weeks in all the animals. The amount of external callus formation further increased, and at 6 to 8 weeks the callus from both fractured ends completely united with disappearance of the fracture line. At 10 weeks, the distinction between the superficial layer and the inner layer in the external callus became clear, and the density of the old cortex at the region of the callus decreased. At 12 to 16 weeks, the width of the osseous callus decreased and the density at the margin of the osseous callus increased with time. After 4 weeks, the density around the PLLA rod increased with time (Fig. 2).

Through the radiographic findings, there was almost no difference in total femoral length in both the fractured and contralateral femurs on the lateral radiograms. However, minimum diaphyseal width increased rapidly on the treated side at 4 weeks.

Changes in CT findings are shown in Fig. 3. Anchoring callus appeared around the bone cortex at 2 weeks after surgery, and its amount increased up to 8 weeks (Fig. 3-A, B, C). At 12 weeks, the width of the osseous callus in the superficial layer was increased, and the margin of the old cortex became irregular because of partial absorption (Fig. 3-D). At 16 weeks, the osseous callus further increased in thickness, compared to that at 12 weeks, and grew larger than the old cortex. The density and the width of the old cortex further decreased (Fig. 3-E).

The CT number of the PLLA rod during repairing period was constantly 151.7–163.2 Hounsfield unit (H. U.). However, that of the external callus increased continuously until 16 weeks after surgery (Fig. 4).

The maximum diameter of the femur including the external callus continued to increase until 8 weeks, and then subsequently decreased until 16 weeks. In contrast, the diameter of the femur excluding the external callus tended to decrease with time. The diameter of the marrow cavity stayed almost constant throughout the period of the

Fig. 2. Radiographic changes in the treated femur in the lateral projection.

Fig. 3. Change in the transversal CT-images at the osteotomy line. Each image was photographed at 2 (A), 4 (B), 8 (C), 12 (D), and 16 months (E) after surgery, respectively.

experiment (Fig. 5).

Histological examination: At 2 weeks, proliferation of mesenchymal cells and the formation of woven bone were observed in the area of periosteum near the fracture ends. Intervention of granulation, cartilage, and a little necrotic tissue were noticed between the fracture ends. At 4 weeks, the volume of periosteal callus had greatly increased. Enlargement of the Harvesian system was noticed in the cortex near the fracture ends. At 8 weeks, completion of the external bony callus was confirmed. The border of the osteotomy line end was not distinguishable because the dead portions of the bone ends were displaced by newly formed woven bone (Fig. 6). Moreover, formation of bone marrow was noticed in the endosteal area. At 12 weeks, formation of compact bone was confirmed in the superficial region of the external callus, and had progressively increased in width at 16 weeks. Absorption of woven bone was noticed gradually from 12 to 16 weeks. On the other hand, formation of fibrous membrane and continuous new bone were observed circumferently around the PLLA rod from 4 to 16 weeks. No inflammatory or foreign body reaction to the PLLA rod was found in any stage.

DISCUSSION

In the field of veterinary orthopedic surgery, surgical fixation of fractures has commonly been achieved with

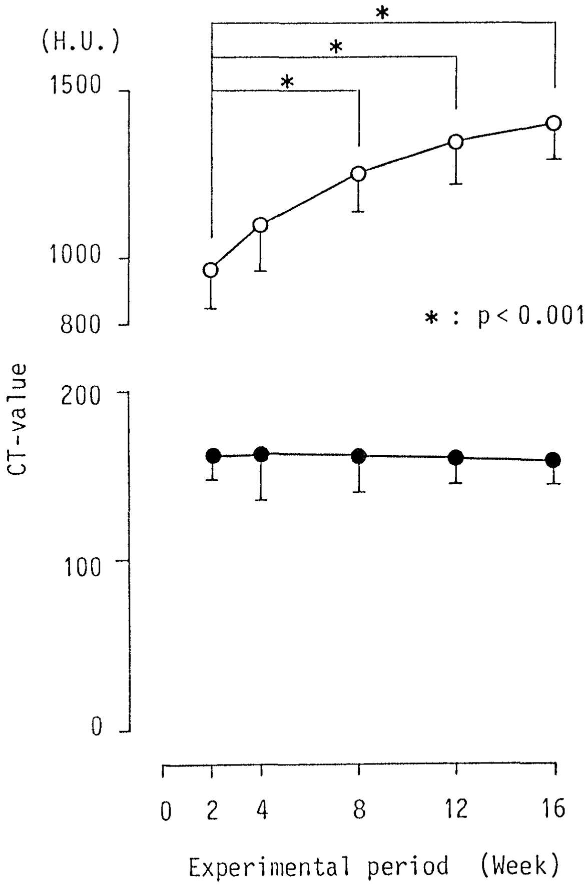

Fig. 4. Change in CT-value for the external callus (○) and PLLA rod (●) with time. Each point represents the mean ± S.D. The CT numbers for the external callus were significantly increased at 8, 12 and 16 weeks, compared to that at 2 weeks (p<0.001).

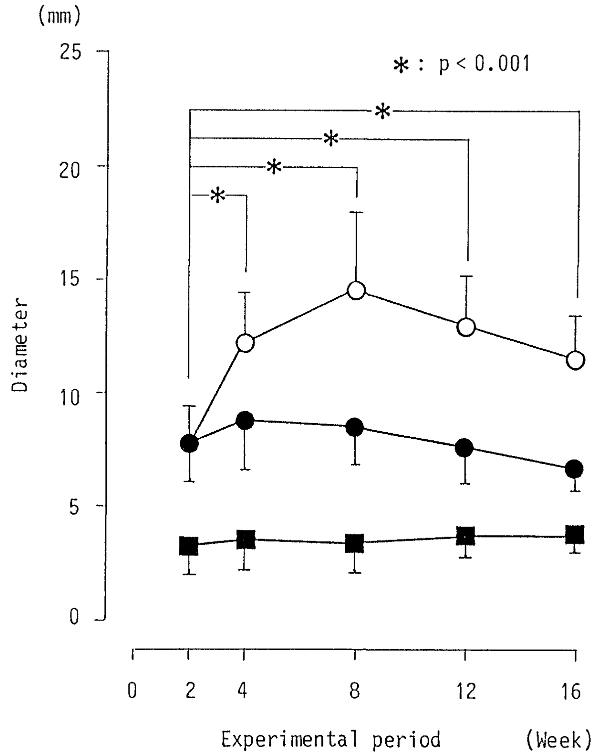

Fig. 5. Change in the diameter of the external callus (○), original femoral shaft (●), and medulla cavity (■) with time. Each point represents the mean ± S.D. Significant differences were found for the maximum diameter of the femur including the external callus at each observational time compared to the value at 2 weeks after surgery (p<0.001).

metallic materials. These have gained general acceptance as highly reliable materials for bone union [3, 8, 15], but some problems are involved in the use of such metallic materials, including the necessity removal of the implant, occurrence of osteoporosis in fixed cortical bone, development of inflammatory responses due to corrosion of the pins in the marrow or loosening of the implant, and induction, though rare, of a malignancy [4, 7, 18]. The properties demanded as indispensable in such materials include the ability to retain adequate dynamic strength until healing is completed, and to immobilize bone fragments that were reduced and to be degraded and absorbed in the body without a toxic effect after the healing so as not to need reoperation [5, 10].

Radiographic examination confirmed the completion of external callus formation and the disappearance of the fracture line at 8 weeks after surgery. It was also histologically confirmed. This is interpreted as a sign of secondary bone healing. In X-ray CT examination, the maximum diameter of the femur including the external callus increased to the maximum at 8 weeks after surgery and subsequently decreased. The abundant callus forma-

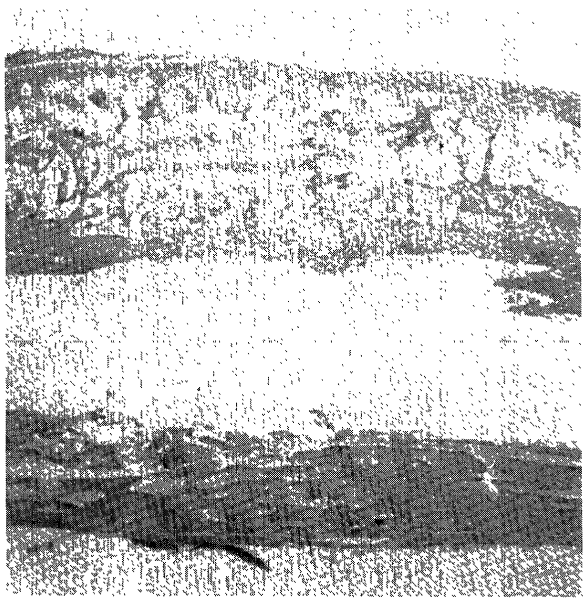

Fig. 6. Photomicrograph of a section in the area of the osteotomy line at 8 weeks after surgery.

tion noted in this study was considered to be a characteristic finding when compared to the healing process of diaphyseal fractures for which metallic intramedurally pins were used [12]. The amount of callus formation has been reported to increase in proportion to the instability of the bone fragments at the fracture site [12]. When this information is taken into consideration, the decrease in the amount of callus at 8 weeks after surgery seems to suggest that the fractured bone fragments gained stability at 8 weeks after surgery. The rotational movement of the tested femur was prevented by immobilization with an external splint and the femur itself was fixed with the intramedullary rod. Nevertheless, a lot of callus was formed. This may be attributable to the lower elasticity of the PLLA rod compared to metallic materials. The modulus of elasticity of metallic material used for bone union (e.g., SUS316) is 200 GPa, while those of a PLLA rod and bone cortex are 13 GPa and 10–17 GPa, respectively [6, 11, 17]. Due to the low elasticity of the PLLA rod, micromotion was loaded onto the region of the fractured ends, and this might accelerate callus formation until 8 weeks after surgery.

In this study, bone union was almost completed in 8 weeks after surgery and the reconstruction process was in progress thereafter. There has been no report on the bending strength of the cortical bone of the feline femur, but that of the human femoral bone cortex is known to be in the 120–200 MPa range [17]. Matsusue *et al.* reported that a PLLA rod buried in the marrow cavity retains the same level of bending strength as the cortical bone for a period of 8–12 weeks [11]. The healing period in the present study was found to correspond to the duration for which the PLLA rod maintained the same level of bending strength *in vivo* as the bone cortex. Bohler described how differences in age and fixation technique influenced the time required for fracture healing [2]. In animals below 12 months of age treated by an intramedullary pinning method, the period required for bone union is estimated to be 8 weeks or less, which is similar to the results of this study.

Daniel *et al.* said that scrupulous care was required for the choice of material shape and method of fixation, and deciding when the repair of fractured bone should be done with biodegradable material as an implant [6]. In the present study, an experimental model of osteotomies was made in the femoral diaphysis of animals of immature and normal status. Clinical application of the PLLA rod must therefore demand special knowledge for patients in which bone union is affected or delayed by other complications.

In conclusion, the uniaxially oriented PLLA rod is a useful and safe biodegradable material for diaphysial fracture fixation with the use of an external splint in immature cats.

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