성장기 가토에서 골 결손 유발 후 자연골 형성에 관한 분석

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INTRODUCTION

Several studies have described spontaneous regeneration of long bones in children and preadolescents who have a large bone defect after trauma or after resection of sequestrum in osteomyelitis1,3,5,7,10,11,14). Intramembranous ossification caused by the intact periosteal sleeve is known to be the main mechanism of spontaneous regeneration. Previous studies have described the repair process in segmental bone defects that were treated by a biodegradable implant or with osteoinductive factor substitutes2,8,9,12,13,15). However, there has been no study that investigated the repair process in a large bone defect when the periosteal sleeve was intact. If the repair process of spontaneous bone regeneration in the intact periosteum is well understood, perhaps spontaneous bone regeneration can replace the use of an internal bone transport or bone grafting in the treatment of large bone defects in the long bones of children.

The present study was designed to examine the histopathologic structure of the regenerate bone in surgically induced bone defects of the femur. We compared the healing time period between groups of young rabbits that had different amounts of bone defects.

MATERIALS AND METHODS

This study consisted of 50 New Zealand White rabbits (6 weeks old) that weighed an average weight of 1.0 kg (range, 0.9-1.1 kg). A bone defect was made at the mid-shaft of the femur and fixed with an external fixator. The periosteum was resected in 10 rabbits (defect size: 15%, 25%) and was untouched in 40 rabbits. The 40 rabbits were divided into four groups of 10 rabbits each. Each group was divided into two subgroups of 5 rabbits each. The first subgroup was fixed with only an external fixator. The second subgroup was fixed with an external fix-
ator and an adjunctive intramedullary Kirschner wire (K-wire) fixation. A total of 8 rabbits (2 rabbits from each main group) underwent histopathologic examination during the follow-up period. Groups were classified according to the percentage of bone defects: group 1 (n=8), was 15% of the original femoral length; group 2 (n=8), was 20%; group 3 (n=8), was 25%; and group 4 (n=8), was 30%. Anteroposterior radiographs were taken weekly, and the progress of the bone regeneration was documented. Bone union was defined when corticomedullary differentiation was seen in the anteroposterior radiographs and the bone union time (weeks) was measured between the time of osteotomy and the bony union. The healing index was calculated to be the union time (weeks) per amount (cm) of bone defect. The healing index between the groups of bone defect was compared using a one-way analysis of variance (ANOVA) test and multiple comparison tests (Scheffe and Dankun). Data with $P$ values $<0.05$ were considered significantly different.

**Operative Technique**

After the rabbits had received general anesthesia (25 mg/kg ketamine HCl, intravenously), and lateral longitudinal skin incision was made over the right femur under aseptic conditions. The fascia was cut longitudinally and the periosteum was exposed between the quadriceps and the biceps. The periosteum was incised longitudinally 2 to 4 cm at the resection area.

Fig. 1. Radiographs after the operation, at 5 weeks after the operation, and at 8 weeks after operation according to the femoral bone defect size. AI-III. 15% defect, BI-III. 20% defect, CI-III. 25% defect.
of the midshaft of the femur and carefully retracted. Four half-pins (2.3 mm) were inserted into the femoral shaft after drilling. A double-transverse osteotomy was performed using an air saw between the second and third pins at the mid-shaft of the femur. A segment of bone (ranging from 15% to 30% of the total femoral length) was excised from beneath the periosteum. In the group that had adjunctive K-wire fixation, the K-wire (1.8 mm) was inserted before the application of the external fixator. The periosteum was left intact in 40 rabbits. In 10 rabbits, the periosteum with bony segment was resected. The pins were then clamped to the external fixation device and the bone gap was maintained. The fascia surrounding the muscles and skin were repaired. The rabbits were killed and specimens from each group were obtained for histopathologic examination of the regenerated bone. The femora and surrounding muscles were removed and frozen at -60°C for 24 hours. The frozen specimens were then bisected longitudinally along the coronal plane and fixed in 10% neutral formalin for 24 hours. They were decalcified in 5% nitric acid solution overnight and embedded in paraffin blocks after tissue processing. The tissue specimens were cut (5 μm thick) and then stained with hematoxylin-eosin and toluidine blue.

RESULTS

In the bone defect group that underwent the periosteum resection, there was no callus formation at the bone defect and there was no bone union. Bone union in the femora was obtained in all the bone defect groups in which the periosteum was preserved (Fig. 1). The mean bone union time was 7.2 weeks in the first group (16 femurs) that were fixed with only an external fixator and 7.3 weeks in the second group (16 femurs) that were fixed with an external fixator and intramedullary K-wire fixation. There was no significant difference in the bone union time between the two groups (P>0.05). In the first group, 38% had union with an angular deformity ranging from 10° to 20°, whereas 15% of the second group had union with an angular deformity ranging from 5° to 10°. According to these results, the adjunctive K-wire fixation did not affect the healing period and also provided stability while preventing angular deformity.

Radiographs showed that callus formation started on the medial side of the bone gap longitudinally along the periosteum at 1 week after surgery and progressed to the lateral side by 2 weeks after surgery. The early callus formation on the
medial side of bone defect may have resulted from an intact periosteum. Late callus formation on the lateral side is caused by the injured lateral periosteum after the longitudinal incision. The bone defect was filled with callus formation within 5 weeks after surgery and recanalization or corticalization occurred between 7 weeks and 9 weeks after surgery (Fig. 2).

There was no significant difference of bone union time between the bone defect groups ($P > 0.05$). However, the healing index was significantly different ($P < 0.05$) between the 15% bone defect group and the other bone defect groups (Table 1). These results mean that the union time is not changed regardless of the amount of the bone defect.

Histopathologic examinations showed intramembranous and atypical endochondral ossifications along the periosteum and a typical endochondral ossification at the center of the bone defect (Fig. 3). In atypical endochondral ossifications, there was no capillary invasion. Chondrocyte-like cells (which are metachromatic after a toluidine blue stain) are formed from the periosteum and are directly transformed to osteocytes without capillary invasion (Fig. 4).

**Table 1. Union time and healing index of the bone defects groups**

<table>
<thead>
<tr>
<th>Bone defect</th>
<th>Union time (weeks)</th>
<th>Healing index (weeks/cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 (n=8)</td>
<td>7.29±0.75</td>
<td>6.67±0.79</td>
</tr>
<tr>
<td>Group 2 (n=8)</td>
<td>7.14±0.89</td>
<td>4.70±0.55</td>
</tr>
<tr>
<td>Group 3 (n=8)</td>
<td>7.43±0.7</td>
<td>3.92±0.88</td>
</tr>
<tr>
<td>Group 4 (n=8)</td>
<td>7.00±0.8</td>
<td>3.08±0.64</td>
</tr>
<tr>
<td>Mean</td>
<td>7.19±0.78</td>
<td>4.59±1.51</td>
</tr>
</tbody>
</table>

**DISCUSSION**

There have been no experimental studies as to the pathogenesis of spontaneous bone formation in bone defects of rabbit where the periosteum is preserved, although some studies have described the pathogenesis of spontaneous bone formation during limb lengthening\(^6,6\). Previous studies report using implants with or without using osteoinductive factors, such as bone morphogenetic protein (BMP) and autologous mesenchymal stem cells, in segmental bone defects\(^8,12,13,15\). Nielsen \(\textit{et al.}\)^8 and Nyman \(\textit{et al.}\)^9 reported membrane-guided bone regeneration in rabbits having segmental radius defects.

**Fig. 3.** Along the periosteum, intramembranous and atypical endochondral ossifications are noted. The endochondral ossification is also noted at the center of the bone defect (H-E stain, \(\times 40\)).

**Fig. 4.** A: Typical endochondral ossification at the center of the bone defect shows capillary invasion of the cartilage, cartilage resorption, and new bone deposition along the calcified cartilage in sequence. Along the periosteum, atypical endochondral ossification is also noted (H-E stain, \(\times 100\)). B: Toluidine blue staining (\(\times 100\)).
regenerating periosteum from each end of the bone gap contributed to new bone formation. Implants using osteoinductive factors have been reported to be useful in the repair of segmental bone defects\textsuperscript{2,12,13,15}. There was an intramembranous ossification within the interstices of the implant. Our study demonstrated that the intact periosteum at the bone defect could lead to spontaneous bone formation without using osteoinductive factors when an external fixator is used, this providing enough stability. The intramembranous ossification from the periosteum and the typical endochondral ossification at the center of bone defect contributed to new bone formation.

Spontaneous regeneration of bone after traumatic bone loss or tumor resection in children and adolescents has been reported in previous studies\textsuperscript{1,3,5,7,10,11,14}. These studies reported that, when the peristeal sleeve was untouched, the bone defects (range, 4-20 cm) were spontaneously filled with a callus in children or adolescents (age range, 3-18 years). The mean healing index was 2 wk/cm (range, 0.8-5 wk/cm), regardless of the cause of the bone loss in these studies. The amount of bone loss did not affect the healing time period. For example, a 4-cm femoral bone loss in a 17-year-old patient and a 17-cm humeral bone loss in a 16-year-old patient healed by 5 months after the initial injury. These clinical results were compatible with results from our study using young rabbits. These clinical and experimental studies support the theory that new bone formation at the bone defect is possible in children and young animals when the periosteum is preserved and the healing period is not correlated with the amount of bone loss.

In children who have a large bone loss, it is difficult to fill the bone defect with the autograft because of limitation in the donor site. Using an allograft has disadvantages, which include a high incidence of nonunion and necrosis of the allograft. Internal bone transport is one method to fill the bone defect, but the consolidation takes a long time and additional surgery is often necessary to solve associated major or minor complications.

**CONCLUSION**

According to the results from our present study, spontaneous bone regeneration from an intact periosteum can be used to fill a bone defect. This method can replace the internal bone transport method because the consolidation time period of callus formation at the bone defect is shorter or the same as compared with the consolidation time period at the distraction site. This method could be useful when the bone defect is located at the midshaft of a long bone and the remaining bone fragment on each side is large enough to use an external fixation for stability.

**REFERENCES**


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Abstract

Spontaneous Bone Regeneration in Surgically Induced Bone Defects in Young Rabbits

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Purpose: To evaluate and compare the spontaneous regeneration repair process in femoral bone defects in 6-week-old rabbits and to compare the healing time periods between different rabbit groups.

Materials and Methods: Bone defects were created at the femur mid-shaft and an external fixator was applied in 50 rabbits. The periosteum was resected in 10 rabbits (defect size: 15%, 25%) and left untouched in the remaining rabbits. Forty rabbits were divided into four groups according to the percentage of bone defects (15%, 20%, 25%, 30%). Radiographs were taken weekly to evaluate the bone regeneration and union. The bone union time was measured between the osteotomy and the cortico-medullary differentiation by examining radiographs. The healing index was defined as the union time (week) per amount (cm) of bone defect. Eight rabbits, 2 from each groups with the bone defects, were investigated by histopathologic examination.

Results: The mean union time was approximately 7.0 to 7.3 weeks. The healing index in groups that had a large percentage of bone defects was less than in groups that had a small percentage of bone defects. The periosteum-resected group did not show bone regeneration. Histopathologic examinations showed intramembranous and atypical endochondral ossifications along the periosteum and typical endochondral ossification at the center of the bone defects.

Conclusion: Spontaneous bone regeneration may be used in children to fill the bone defect instead of performing an internal bone transport. Spontaneous bone regeneration is useful in cases of mid-shaft bone defects or when the remaining bone fragments are large enough for an external fixation application.

Key Words: Bone defect, Femur, Spontaneous bone regeneration, Young rabbit

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