Original article

Experimental study of progressive tibial lengthening in dogs using the Ilizarov technique. Comparison with and without associated intramedullary K-wires

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A R T I C L E   I N F O

Article history:
Accepted 24 June 2014

Keywords:
Bone lengthening
Ilizarov
Intramedullary nailing
Experimental surgery
Dog

A B S T R A C T

A delay in the union of bone regenerate in surgical lengthening procedures and the healing index (HI) are major factors in the quality of the results in progressive bone lengthening. Early removal of the external fixator (EF) is associated with a low rate of postoperative complications, including pin track infection, and with better muscle and joint function recovery. Addition of intramedullary wires (IMWs) to the EF led to a 9–46% decrease in the HI depending on the clinical series. We hypothesized that IMWs may accelerate the ossification process of bone regenerate and tested it in this experimental study.

Methods: Progressive tibial lengthening of 28 mm was obtained in 12 dogs operated with the classical Ilizarov technique (group I) and in 12 dogs operated with the same technique and addition of two IMWs 1.5 mm in diameter (group II). The following criteria were assessed: HI, X-ray measurements, and histological aspect of the bone regenerate and postoperative complications.

Results: The mean HI was 32.3% lower in group II than in group I. The radiological bone union criteria were observed on day 15 of the fixation period in group II versus day 30 in group I. Histology showed that maturation occurred earlier and bone cortices were thicker in group II than group I. Intramedullary ossification was present along the IMW in group II, whereas it was absent in group I. No clinical complications were observed in either group.

Discussion: The presence of the IMWs clearly contributes to stimulation of the ossification processes of the bone regenerate and to acceleration of bone union. IMWs allowed an earlier removal of the external fixator for a 32% time reduction compared to cases without IMWs. In addition, new intramedullary bone formation and presence of IMWs are expected to increase the mechanical resistance of the bone regenerate.

Conclusion: Improvement of quantitative and qualitative criteria of bone regenerate in progressive bone lengthening with an EF combined with IMWs was demonstrated in this experimental study.

Significance: Favorable results encourage the authors to continue using IMWs in addition to the EF in patients treated with long-bone progressive lengthening.

Level of evidence: II.

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1. Introduction

Significant unequal limb length is most often treated with progressive bone lengthening procedures. Among the surgical techniques available, bone lengthening is often obtained using different types of external fixator (EF) [1–3]. With the objective of reducing the complication rate related to EFs, alternatives including intramedullary nailing began to be used in 1983 [4]. The number

of complications associated with the use of EFs in bone lengthening is reduced by lowering the healing index (HI). The HI corresponds to the ratio of the number of days an EF is in place compared to the amount of lengthening measured in centimeters [5]. This is reduced by very regular lengthening of 1 mm per day distributed over 60 days using automatic distraction osteosynthesis [6–8], by multifocal and/or multisegmental lengthening [9], or by adding intramedullary implants such as nails [10–17] or elastic stable intramedullary nailing (ESIN) [18–21]. This association of intramedullary implants with EFs has made it possible to lower the rate of deformities and fractures after removal of the EF. Cases of osteitis have been reported in the association with intramedullary nails [17], although not when associated with flexible intramedullary nailing (FIN) when combined with flexible intramedullary wires (IMWs) [18,19].

In children and adolescents, the radiological criteria allowing removal of the EF material at the end of the lengthening phase are the return to continuity of at least three of four cortices in the lengthening zone and the disappearance of the growth zone (GZ). This zone, described by Ilizarov [1], corresponds to a fibrous layer located in the middle of the regenerate and populated by osteoblasts. The lack of mineralization makes it radiologically transparent [6].

To better understand the reasons for the acceleration of the maturation of bone regenerate (BR) during the EF–IMW association, this experimental study in dogs compared a group operated with only EF with a group operated associating EF and IMW. Earlier experimental studies had been conducted in dogs [1,21–23], sheep [24,25], goats [26], and rabbits [27,28]. For the present study, the choice of adult dogs was dictated by earlier experiments conducted in our institution [6].

We sought to experimentally validate that BR was formed more rapidly in presence of IMWs than in their absence. The objective of the study was not only to quantify the radiographic progression of BR, but also to qualify the differences observed between the two methods through histological analyses.

### 2. Material and methods

An experimental study of progressive 28-mm lengthening of the tibia was conducted in 24 adult mixed-breed dogs divided into two groups of 12 dogs each: in group I the lengthened tibias were fixed using an Ilizarov EF adapted to dogs; in group II the tibias were operated identically with an EF also fixed by two steel IMWs using the ESIN method [29].

The weight and age as well as the initial length of the tibia were recorded for all dogs.

#### 2.1. Protocol

The study protocol included the following phases:

- surgery on D0;
- lengthening period (D5–D33): after five days, progressive lengthening was undertaken at a rate of 1 mm per day distributed into four times 0.25 mm daily over 28 days for a total of 28 mm. AP and lateral X-rays were taken every week during the lengthening period (D12, D19, F26, and D33) to assess the progression of regenerate growth;
- fixation period (beginning on D34): this period ended with the removal of the EF when continuity of the BR GZ and three bone cortices was visible on the X-rays. The radiographic follow-up was pursued every week until EF ablation: D64–66 and D90–97 (for group I corresponding to the 30th–32nd and 64th–66th days of the fixation period itself), and D48 and D64 for group II (14th and 30th days of the fixation period). This difference was explained by the results of a preliminary experimental study [29] and a clinical study [20], which demonstrated an acceleration of BR healing when IMW was associated compared to EFs alone;
- finally, the third period corresponded to the duration separating fixation removal and killing the animals. In each group, six animals were killed when the EF was removed and six others 30 days later.

#### 2.2. Surgical technique

All the surgical interventions were performed under general anesthesia with a sodium thipental 5% solution (10–15 mg/kg) with atropine as needed. The same surgeon performed all surgeries.

In both groups, the fixators comprised two distal circular rings and two proximal rings in three quarters connected by three threaded stems. A percutaneous osteotomy of the fibula and an osteoclasis of the tibia were performed in the middle third of the leg. In group II, the ESIN included two Kirschner steel wires 1.5 mm in diameter, approximately 30% of the dogs’ tibial medullary canal. These two wires, curved along the entire length and supported at their ends, were introduced after the osteotomy. The surgical technique was thus comparable to that performed in children [29].

At the end of the intervention, the two wires presented their curvature slightly below the osteoclasis site. Their free end was cut several millimeters outside the bone and bent to approximately 60° so as to prevent intraosseous migration of the IMWs.

All the dogs received the same postoperative care and the same feeding. They were allowed to walk with no limitations. They were killed by an intravenous injection of sodium thipental 5% (45 mg/kg). In group II, the IMWs were removed. The operated tibias were decalcified in a solution of formic acid 5% for the histological study. The specimens preserved in paraffin were cut longitudinally in 6- to 7-μm slices using a microtome. The slices obtained were stained using a Van Gieson picrofuchsin solution.

#### 2.3. Data studied

The healing index (HI) was calculated for both groups by comparing the duration of EF wear to the 28 mm of lengthening.

During the lengthening period, the objective BR criteria were measured on the weekly AP X-rays (Fig. 1):

- the diameter of the BR versus the diameter of the tibia (dBRVt): the diameter of the regenerate in its middle part was compared to the diameter of the tibia near the osteotomy. This diameter could be less than, equal to, or greater than the tibia. A 1-mm difference corresponded to an approximately 10.5% gap;
- the length of the periosteal reaction;
- the thickness of the periosteal reaction;
- the height of the growth zone.

During the fixation period, the following radiological criteria were studied:

- the number of bone cortices on the AP and lateral X-rays;
- complete ossification of the growth zone;
- visualization of the medullary canal within the regenerate.

The histological study of the regenerate was descriptive:

- aspect of the bone regenerate: woven primary bone or cancellous bone;

The complications studied concerned the cutaneous lesions caused by the IMWs in group II as well as fractures and deviations of the regenerates after removal of the EF for both groups.

2.4. Statistical analysis

The healing index was compared between the two groups using the Student t-test.

2.5. Ethics

In agreement with current legislation, the research protocol was approved by the scientific committee of the Ilizarov Academic Institute, Kurgan, Russia.

3. Results

The two groups of dogs were similar: the mean age was 2.3 ± 0.9 years and the mean weight 18.8 ± 1.06 kg. The mean length of their tibia was 18.7 ± 0.18 cm for both groups. The 28 mm obtained corresponded to a mean 15% lengthening.

3.1. Lengthening period

In group I, the lengthening rate was maintained as designated by the protocol at a speed of 1 mm per day for all dogs. However, in group II, the X-rays of certain regenerates showed beginnings of early bone union. In these cases, it was necessary to accelerate the lengthening speed as needed (Table 1).

Table 1

<table>
<thead>
<tr>
<th>Study protocol, duration of the different periods, and healing index.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Operation D0</strong></td>
</tr>
<tr>
<td>Group I</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>4</td>
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<tr>
<td>5</td>
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<tr>
<td>6</td>
</tr>
<tr>
<td>7</td>
</tr>
<tr>
<td>8</td>
</tr>
<tr>
<td>9a</td>
</tr>
<tr>
<td>10</td>
</tr>
<tr>
<td>11</td>
</tr>
<tr>
<td>12</td>
</tr>
<tr>
<td>Mean</td>
</tr>
<tr>
<td>Standard deviation</td>
</tr>
</tbody>
</table>

Group II

| 13               | 1                                           | 1                               | 15            | 17.1                                         | D48                           |
| 14a             | 1                                           | 1                               | 15            | 17.1                                         | D48                           |
| 15a             | 1 – 1.25                                     | 1                               | 30            | 22.5                                         | D93                           |
| 16a             | 1 – 1.50                                     | 1                               | 15            | 17.1                                         | D78                           |
| 17               | 1                                           | 1                               | 15            | 17.1                                         | D78                           |
| 18               | 1                                           | 1                               | 15            | 17.1                                         | D78                           |
| 19a             | 1 – 1.50                                     | 1                               | 15            | 17.1                                         | D48                           |
| 20a             | 1 – 1.50                                     | 1                               | 30            | 22.5                                         | D93                           |
| 21a             | 1 – 1.25                                     | 1                               | 15            | 17.1                                         | D78                           |
| 22a             | 1 – 1.25                                     | 1                               | 30            | 22.5                                         | D63                           |
| 23a             | 1 – 1.50                                     | 1                               | 15            | 17.1                                         | D78                           |
| 24               | 1                                           | 1                               | 15            | 17.1                                         | D48                           |
| Mean             | 1                                           | 1                               | 15            | 17.1                                         | D48                           |
| Standard deviation | 1                                           | 1                               | 15            | 17.1                                         | D48                           |

LP: lengthening phase; FP: fixation phase; HI: healing index; EF: external fixator. Modification of lengthening speeds.

* 1.25 mm/d during the 2nd week.
  1. 1.05 mm/d during the 2nd week.
  2. 1.25 mm/d during the 3rd week.
  3. 1.50 mm/d during the 3rd week.
  4. Dogs illustrated in Figs. 2 and 3.

Table 2
Radiological aspect of the bone regenerate during the lengthening period. Number of cases per category (n).

<table>
<thead>
<tr>
<th></th>
<th>dBRvT</th>
<th>L. periosteal reaction (mm)</th>
<th>Periosteum thickness (mm)</th>
<th>GZH (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D12 Group I</td>
<td>NA</td>
<td>Absent (6)</td>
<td>Absent (6) 0.5–1.5 mm (6)</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10–20 mm (6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D19 Group I</td>
<td>NA</td>
<td>10–20 mm</td>
<td>1–3 mm</td>
<td>NA</td>
</tr>
<tr>
<td>Group II</td>
<td></td>
<td>2–4 mm (10)</td>
<td>1–6 mm</td>
<td>NA (6) 3–5 mm (6)</td>
</tr>
<tr>
<td>D26 Group I</td>
<td>Equal (4) &gt;1–3 mm (8)</td>
<td>3–30 mm</td>
<td>1–3 mm</td>
<td>3–9 mm (12)</td>
</tr>
<tr>
<td>Group II</td>
<td>&gt;2–8 mm (12)</td>
<td>10–67 mm</td>
<td>1–6 mm</td>
<td>2–6 mm (12)</td>
</tr>
<tr>
<td>D33 Group I</td>
<td>&lt;2–3 mm (3) equal (4) &gt;1–3 mm (5)</td>
<td>10–30 mm</td>
<td>1–6 mm</td>
<td>2–9 mm (12)</td>
</tr>
<tr>
<td>Group II</td>
<td>Equal (2) &gt;2–7 mm (10)</td>
<td>10–75 mm</td>
<td>2–6 mm</td>
<td>1–4 mm (12)</td>
</tr>
</tbody>
</table>

dBRvT: diameter of bone regenerate versus tibia; L: length; GZH: growth zone height; NA: not applicable

Table 3
Radiographic aspect during the fixation period (FP). Number of dogs responding to the criteria over the number of dogs alive.

<table>
<thead>
<tr>
<th></th>
<th>3–4 corticals visible</th>
<th>Complete ossification of the GZ</th>
<th>IMC visible</th>
</tr>
</thead>
<tbody>
<tr>
<td>Since the intervention</td>
<td>D40</td>
<td>D47</td>
<td>D61</td>
</tr>
<tr>
<td>From the FP</td>
<td>1 wk</td>
<td>2 wk</td>
<td>4 wk</td>
</tr>
<tr>
<td>Group I</td>
<td>0/12</td>
<td>0/12</td>
<td>6/12</td>
</tr>
<tr>
<td>Group II</td>
<td>2/12</td>
<td>8/12</td>
<td>8/8</td>
</tr>
</tbody>
</table>

GZ: growth zone; IMC: intramedullary canal; wk: week(s).

3.2. Healing index

The HI was significantly lower (−32.3%) in group II than in group I (Table 1).

3.3. Radiological aspect

The dBRvT values, the length of the periosteal reaction, the thickness of the periosteum, and the height of the GZ measured weekly during the lengthening period are reported in Table 2. Comparing group II to group I, the first three values were higher, and the height of the GZ was narrower in group II than in group I (Table 2).

During the fixation period, the appearance of cortices and the disappearance of the GZ occurred earlier in group II than in group I. The group II X-rays showed the presence of a medullary canal beginning in the 4th week of this fixation period, which was not visible at this stage in group I (Table 3).

3.4. Histology

Group I: At D63 the regenerate was mainly represented by cancellous bone tissue, the GZ was partially bridged by beginning trabeculae and included several islets of fibrous tissue. In the peripheral zone, the cortex surrounded the regenerate by relatively compact immature bone tissue. Toward D93, the regenerate was formed by an association of mature cancellous and trabecular tissue with, however, an aspect of this bony tissue less compact than the adjacent bone tissue. The cortices were composed of compact cancellous tissue and the medullary canal of bony trabeculae and fatty marrow.

Group II: as early as D48, the BR presented a fine network of primary bone with trabeculae that were clearly anastomosed; the GZ showed no fibrous tissue and cortices were being formed. At D63, the periphery of the regenerate was composed of mixed cortical and trabecular compact bone tissue. Intramedullary osteogenesis was observed, particularly along the trajectory of the wires that had been removed.

Comparing the two groups, the fibrous tissue was replaced with bony tissue much earlier in group II than in group I. Moreover, the primary bone tissue and the cortices appeared beginning at D48 in group II and D63 in group I (Figs. 2 and 3).

3.5. Complications

No material breakage or sepsis around the wires was observed in either group. In terms of the BR, no cases of deformity or fracture after EF material removal were observed. In group II, no cutaneous lesions or IMW migration were observed.
4. Discussion

The group I experimental model is the model described by Ilizarov and since then revised within the institution by Shevtsov [2]. This validated model has been used by many authors, who, in the last century, also described the progression of BR and emphasized the favorable role played by bone and periosteal vascularization [22–28,30]. Although it is desirable to protect intramedullary vascularization during corticotomy, it has been shown in rabbits that even if this is destroyed, it repairs itself in a few days [28]. According to these authors, revascularization required a delay between surgery and the beginning of progressive lengthening. For this reason, lengthening only begins five days after surgery. However, BR can appear even when intramedullary vascularization is destroyed, either during the osteotomy itself or with the presence of intramedullary nails. In the mini-goat, Lin et al. observed comparable results of BR ossification after lengthening with intramedullary nailing, with and without reaming [26].

In rabbits [27], BR ossification includes an endochondral ossification phase, mainly membranous in dogs [22,23,30]. De Pablos and Canadell studied BR in 6- to 12-month-old dogs and 6- to 12-month-old sheep. They noted no significant difference in BR maturation between these groups [25].

In the present study investigating dogs, groups I and II also presented intramembranous-type BR. This type of ossification has also been reported in children [31]. The organization of bony trabeculae seems, however, affected by the type of EF: the Ilizarov EF provides more regular and more parallel bony trabecular organization than the unilateral Wagner fixator [22]. Regular alignment of the bony trabeculae was also observed in both groups in the present study.

Hamdy et al. insisted that BR formed both in the intramedullary cavity and the periosteum [23]. The histological studies of our two groups found this type of ossification in two areas. It should be noted that the presence of IMWs induced complementary ossification at their contact points in group II.

The possibility of early removal of IMWs in group II was related to the appearance of better-quality BR obtained more rapidly than in group I. Already during the lengthening phase, it was necessary to accelerate the lengthening speed to 1.25 and even 1.5 mm/day during the 2nd or 3rd week because of excessively early ossification of the regenerate (six dogs: no. 15, 16, 19–23). The occasional need to accelerate the daily lengthening rate to prevent excessively premature fusion of the GZ has been described in children [31].

During the lengthening phase, the X-rays showed parameters indicating an acceleration of the healing processes in group II: dBRVT, GZ thinness, as well as thickness and length of the periosteal reaction.

During the fixation period, the X-rays showed more rapid development of the bone cortices and ossification of the GZ in the EF–IMW association. The appearance of the medullary canal within the BR, visible in group II beginning on D61, was not noted in group I. The histological analyses showed that the organization of bone tissue was earlier in group II than in group I in terms of bone maturity. In addition, mature bone trabeculae were observed along the IMWs, which incontestably reinforces the mechanical resistance of BR. This advance in maturation of the ossification in group II allowed us to remove the EF at D48 for eight dogs and at D63 for the four others. In group I, the EFs could not be removed until D63–65 for six dogs and D89–96 for the six others.

The comparative experimental studies published earlier did not consider the question of the effect of IMWs during progressive bone lengthening using the circular EF. Yet clinical experience has demonstrated the value of this method in children and adolescents: the association EF + ESIN compared to EF alone reduced the HI between 8.9 and 48.7% depending on the etiology and whether the length inequality was congenital or acquired, and depending on the bone segments lengthened [20,21].

This study found that the HI was at times shortened: it was 18.9 days/cm in group II and 27.9 days/cm in group I, a significant 32% reduction (p = 0.0001). Of the experimental studies in the literature, Aronson reported a HI of 26.9 days/cm in dogs [25].

One of the limitations of our study is the absence of a mechanical analysis of the bone regenerate. The solidity of the BR is what determines removal of the EF at a given date without risk of fracture or deviation of the BR. It is certain that the presence of intrasosseous IMWs plays an additional mechanical role that needs to be evaluated. Another limitation involves the analysis of the BRs that was solely radiographic before removal of the EF, possible when the GZ was completely ossified and when three of the four cortices were visible. Bone density studies would contribute additional information and could possibly shorten the HI even more and therefore the time the EF is in place based on more precise criteria than those used herein. Finally, we preferred the study of quantifiable radiological measurements to observational criteria such as those proposed by Li et al. [32], validated by an interobserver study [33].

An attempt should be made to understand the reasons for the more rapid appearance of BR maturation observed in group II compared to group I. Do the IMWs play a role in BR formation by increasing the overall rigidity of the system, by stimulating angiogenesis, or by another unknown mechanism? We are convinced that the intramedullary ossification observed in group II and the presence of IMWs left in place after EF removal contribute to mechanically reinforcing the BR. Complementary studies are required to examine bone vascularization and to measure the biomechanical aspect of BR with and without IMWs.

In clinical practice with children and adolescents, one of the objectives of progressive bone lengthening is to reduce the HI, i.e., shorten as much as possible the length of time EF must be worn without risking the onset of complications such as BR deformities or fractures. Currently, it is acknowledged that the EF can only be removed when the GZ continuity is replaced with bone tissue and there are at least three of four cortices visible on the AP and lateral X-rays. However, the presence of IMWs and associated intramedullary ossification along these IMWs may allow even earlier removal of the EF.

5. Conclusion

The comparative study of progressive bone lengthening using circular EF according to the Ilizarov technique in dogs showed that adding intramedullary wires significantly reduced the healing index by 32.3%. The EF can therefore be removed earlier when using IMWs than when not using them. The reasons the HI was reduced in the EF–IMW group compared to the EF-only group are more rapid ossification of the BR with an advanced disappearance of the EZ, the earlier appearance of the cortices surrounding the regenerate, and the appearance of a medullary canal. The histological study clearly showed earlier bone maturation of the BR in group II compared to group I.

This experimental study confirms the clinical data and warrants the pursuit of the EF–IMW association in progressive surgical bone lengthening in children. Complementary biomechanical studies and the study of bone vascularization would make it possible to envision even earlier removal of the EF with the objective of reducing the complications related to its presence.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

References
