Cengiz Sen · Taner Gunes · Mehmet Erdem ·
Resid Dogan Koseoglu · Nurper Onuk Filiz

Effects of calcitonin and alendronate on distraction osteogenesis

Received: 14 November 2005 / Accepted: 15 November 2005 / Published online: 8 March 2006
© Springer-Verlag 2006

Abstract In this experimental animal study the effects of calcitonin and alendronate on distraction osteogenesis are investigated. Forty-five mature female New Zealand type rabbits were used. Rabbits were randomized into three groups, each consisting of 15 animals. Under optimal operating conditions a pre-reconstructed circular external fixator was applied to the right tibia of rabbits, and osteotomy was performed with a Gigli saw just below the tibial tuberosity. During the ten days of distraction, a placebo was administered for group I (control group), group II received 10 U of calcitonin, and group III was treated with alendronate (0.5 mg/kg/day) gavages. Rabbits were examined histologically (at the third and eighth weeks, according to Huddleston et al.), radiologically (at the third, sixth, and eighth weeks, according to Lane and Sandhu), and mechanically (at the eighth week, as torsional loading). During the first three weeks, the groups did not differ much with regard to radiological parameters. However, in the calcitonin and alendronate groups improved histological scores were detected compared with the control group. Radiological images obtained at the end of six weeks demonstrated improved consolidation in the calcitonin and alendronate groups compared with the control group. At the end of eight weeks postoperatively, radiological and histological parameters did not differ among the groups, while torsional failure load was found to be much improved in the calcitonin group ($p=0.006$). During the distraction osteogenesis period, calcitonin and alendronate affected the quality of regenerate favourably. This effect is sustained until the end of the consolidation period. However, this finding needs to be supported by experimental and human studies.

Résumé Etude expérimentale des effets de la calcitonine et de l'alendronate sur l'ostéogénèse en distraction. Quarante-cinq lapins adultes femelles de Nouvelle Zélande ont été le support de l'expérimentation. Les lapins ont été randomisés en trois groupes de 15 animaux dans des conditions opératoires satisfaisantes. Il a été mis en place un fixateur externe sur les tibias droits de chaque lapin. L'ostéotomie a été réalisée avec la scie de Gigli juste en dessous de la tubérosité tibiale. Pendant les dix premiers jours d'allongement, un placebo a été administré au premier groupe (groupe contrôle), le groupe 2 a reçu 10 unités de calcitonine, le groupe 3 a été traité par l'alendronate (0,5 mg/kg/jour). Les lapins ont été sacrifiés et examinés histologiquement à la troisième et la huitième semaines (Ref. Huddleston et col.), radiologiquement à la troisième, sixième et huitième semaines (Ref. Lane et Sandhu) et mécaniquement à la huitième semaine avec tests de torsion. Dans les trois premières semaines, on ne retrouve pas de différences radiologiques entre les trois groupes, cependant l'on constate une amélioration histologique sur les groupes calcitonine et alendronate comparés au groupe contrôle. Les images radiologiques obtenues à la fin de la sixième semaine nous montrent une meilleure consolidation pour les groupes calcitonine et alendronate. A la fin de la huitième semaine, post-opératoire il n'y avait pas de différences radiologiques et histologiques dans les différents groupes, les tests de torsion sont simplement améliorés dans le groupe calcitonine ($p=0,006$). En conclusion, durant la période d'ostéogénèse en distraction, la calcitonine et l'alendronate ont des effets positifs sur la qualité du régénérat, ces effets se maintiennent jusqu'à la période de consolidation. Cependant, ces effets positifs doivent être confirmés par des études chez l'homme.

C. Sen · T. Gunes (✉) · M. Erdem
Orthopaedics and Traumatology, Gaziosmanpasa University,
Tokat, Turkey
e-mail: drtgunes@gmail.com

R. D. Koseoglu · N. O. Filiz
Pathology, Gaziosmanpasa University,
Tokat, Turkey

Introduction

The technique of “distraction osteogenesis” (DO), which is unique in the regeneration of bone, is frequently used in the treatment of bony loss, pseudoarthrosis, chronic osteomy-

elitis, leg length discrepancy, and deformity. According to Ilizarov [10], who introduced this technique, and transferred his experience into the world of orthopedics, certain principles must be obeyed. Important principles to be considered are preservation of blood circulation of the bone segment to be lengthened, stable external fixation, optimal latency period before distraction, determination of the rhythm of lengthening as 1 mm a day divided into four different sessions, and allowing time for the consolidation of the regenerate segment.

Since the introduction of this method into the western world, it has been increasingly used. Factors affecting the success of distraction osteogenesis have been investigated in various reports [2, 6, 13, 15–17, 18, 23–25]. The most important expectation in this study was to improve the quality of the regenerated bone over a shorter period.

It has been reported that calcitonin improves the mechanical features of fractured and intact bone [11] and has positive effects on bone matrix synthesis [7]. It has been reported that bisphosphonates improve callus formation and mineralization during fracture healing [17].

A comparative study on calcitonin and alendronate, which are used widely for the treatment of osteoporosis, has not yet been encountered to our knowledge. In this study, the effects of calcitonin and alendronate on distraction osteogenesis were investigated with regard to radiological, histological, and biomechanical parameters.

Materials and methods

In this study, 45 mature New Zealand-type rabbits (mean weight 3.5 kg; range 2.5–4.5 kg) were used. Before initiation of the study, approval from the Ethics Committee of Gaziosmanpasa University, School of Medicine was obtained. The study was carried out in the “Center for Experimental Animals” at the same institution. Rabbits were randomised into three groups, each consisting of 15 animals. As a prophylaxis cefazoline sodium (20 mg/kg/day i.m.) was injected preoperatively, and eight h postoperatively. Interventions were performed under ketamine (10 mg/kg i.m.) and xylazine (8 mg/kg i.m.) anesthesia. Skin subdermis, and periosteum were exposed with an anteromedial longitudinal incision on the right tibia. Then a pre-reconstructed two-ring circular fixator was applied. Next, a Gigli saw was used for the osteotomy (Fig. 1). Subsequently the periosteum and skin were closed properly. After a seven day waiting period, distraction was applied to each tibia on a schedule of 2×0.5 mm/day. Furthermore, for group I (control group) a placebo was administered over the distraction period, while for ten days group II received 10 MRC-U (Medical Research Community-Unit) of salmon calcitonin [13] and group III was treated with alendronate (0.5 mg/kg/day in distilled water) [8]. At the end of ten days, distraction was terminated. Twenty-one days postoperatively, five rabbits in each group were sacrificed with high doses of sodium pentothal, and evaluated with regard to radiological and histological

Fig. 1 Application of circular fixator

parameters. The remaining rabbits in each group were sacrificed similarly, and underwent radiological, histological, and biomechanical investigations at the end of eight weeks.

After sacrifice, all tibias were harvested, stripped from their soft tissue, and sent to a pathologist who was unaware of the allocation of rabbits into groups. For histological evaluation, extremities dissected from their soft tissues were fixed in 10% buffered formaldehyde solution for 48 h, and then treated with a rapid decalcification solution for 12 h. After the decalcification procedure, tibias were sampled longitudinally from distracted segments. For each tibial segment, four cuts were taken from the distraction area and routine histological preparation was carried out. After routine histological preparation, samples were embedded in paraffin. From paraffin blocks sections of 4 μ m in width were taken and stained with hematoxylin-eosin. Sections stained with hematoxylin-eosin were examined under light microscope. For each subject, four cuts from distraction area were taken for analysis. For radiological evaluations, the scoring system developed by Lane and Sandhu [14] and for histopathological assessments the grading system of Huddleston et al. [9] were used. Biomechanical tests were performed using the Materials Testing Machine (Lloyd Instruments, Fareham, UK) using torsional loading forces with a rhythm of 5°/second.

Kruskal–Wallis variance analysis and the chi-squared test were used for statistical evaluations between all groups and for intergroup assessments the Mann–Whitney *U* test (Bonferroni correction) was performed. Statistical significance ($p < 0.05$) was determined in consideration of 95% confidence limits.

Table 1 Mean radiological and histological scores of the groups at 21 days

Groups	Radiological scoring	Histological scoring
Group I ($n=5$)	3	3
Group II ($n=5$)	3	4
Group III ($n=5$)	3	4.2

Fig. 2 a–c Radiological appearance of the three groups at the end of 6 weeks **a** Control group **b** Calcitonin group **c** Alendronate group

Results

The study was completed with 36 rabbits due to the death of four rabbits in group I, three in group II, and two in group III due to unexpected death, infection, and diarrhea. Radiological evaluations at the end of three, six, and eight weeks were performed separately by the investigators in charge (CS, TG, ME) regardless of the assignment of study groups or allocation of numbers, and an average of all scores was taken into consideration. According to these results 21 days postoperatively, a statistically significant difference was not detected during intergroup evaluations in terms of radiological and histological results ($p>0.05$). Histopathologically, although a statistical difference was not demonstrated, callus formation was of a higher quality in groups II and III (group III > group II; Table 1). In radiographs taken at the end of six weeks, consolidation in group II and III was better than that found in group I (Fig. 2, Table 2). Radiological and histological results obtained at the end of eight weeks were not statistically significant, but they demonstrated better regeneration in groups II and III (Table 3). During histopathological examinations, apparent osteoblastic activity in the distraction area, neovascularization, cartilage tissue islets, and trabecular bone formation were observed. Trabecular bone tissue was the predominant component in callus tissue compared with cartilaginous areas in the control group (Fig. 3a). Although marked osteoblastic activity and markedly improved callus formation were observed in the calcitonin and alendronate groups compared with the control group, trabecular bone formation was more prominent in the alendronate group, and especially in the calcitonin group compared with the control group. In these three groups, trabecular bone tissue was the dominant component in callus formation (Fig. 3b,c). When the results were evaluated statistically, a significant difference could not be found among the groups ($p>0.05$).

Table 2 Mean radiological scores at the end of 6 weeks

Groups	Mean scores
Group I (n=6)	6.8
Group II (n=7)	7
Group III (n=8)	7.3

Maximal torsional failure loads are shown in Table 3. When the results of biomechanical tests were evaluated, a significant difference was found among all groups ($\chi^2=13.278$, $p=0.001$). When groups were compared with each other, there was a significant difference between group II and group I ($U=0.00$, $p=0.001$) and also between group III and group I ($U=8.00$, $p=0.043$). When compared with group III, the maximal torsional failure load was found to be higher in group II ($U=5.00$, $p=0.006$).

Discussion

The distraction osteogenesis technique, which was introduced by Ilizarov, has been used extensively in the Western World since the late 1980s [10]. This technique has gained in popularity as it can both correct various bone problems and lead to fewer complications [22].

The histological findings of distraction osteogenesis were reported by Aronson [1]. This distraction process was recognized as complete “intramembraneous ossification” as young mesenchymal cells transform into highly vascularized fibroblasts, and then type I collagen fibers are formed that are aligned parallel to the lengthening vector and longitudinal axis of the bone. During this period, chondroblasts differentiate from young mesenchymal cells, cartilage tissue ossifies, and the periosteum of the bone also participates in this process. During the stable consolidation period osteoblastic activity takes over fibroblastic and chondroblastic activity. When the distraction process is terminated, trabeculi are formed in the matrix localized

Table 3 Radiological, histological, and biomechanical test results at the end of 8 weeks

Groups	Mean radiological score	Mean histological score	Maximal torsional failure load (N)
Group I (n=6)	8.6	8.5	167.6
Group II (n=7)	9.4	9.4	234.4
Group III (n=8)	9.2	9.1	186.2

Fig. 3 Histopathological examinations. **a** Callus formation consisting of roughly equal amounts of trabecular bone and chondroid tissue in the control group sacrificed by the 8th week (HE, $\times 20$). Osteoblasts were rarely observed at the rim of the trabeculae (HE, $\times 60$). **b** Callus formation consisted of predominantly well-developed

trabecular bone in the calcitonin group sacrificed by 8th week (HE, $\times 20$). The rows of osteoblasts at the rim of well-developed trabeculae were noted (HE, $\times 60$). **c** Callus was formed by mainly well-developed trabecular bone and a limited chondroid tissue area in the alendronate group sacrificed by the 8th week (HE, $\times 20$)

between the bone ends, osteon formation and osteoblastic activity increase immensely, and mineralization starts to take place. These newly formed trabeculi align parallel to the axis of lengthening, join each other through bone bridges, and the bone rapidly remodels.

The principles of the accomplishment of high quality regeneration with distraction osteogenesis were set out in full by Ilizarov. These are: protection of the periosteum during corticotomy, low-energy corticotomy, stable osteosynthesis with a circular fixator, a 7–10-day latency period and waiting period for consolidation [10].

In our study, distraction procedures were applied in all groups in accordance with these principles except two. We lengthened tibias with a rhythm of 2×0.5 mm/day instead of 4×0.25 mm/day. Sen et al. concluded that with a rhythm of 1 mm/day, regeneration of higher quality can be obtained than those achieved with 0.5 mm/day [21]. On the other hand, Kojimoto et al. demonstrated that preservation of the periosteum rather than the endosteum is more important for callus formation [12]. Gigli saw osteotomy recommended by Paley and Tetsworth reinforces this argument [20]. Therefore, in our study we believe that lengthening on a schedule of 2×0.5 mm/day and Gigli saw osteotomy do not affect callus formation adversely. Compliance of radiological and histological findings of rabbits in all three groups with those provided by Aronson demonstrates the validity of our method.

Many investigators have been searching for ways of obtaining higher quality regenerates [6, 23, 24]. Yamane et al. found that a vitamin D derivative, ED-71, increased callus volume after termination of the lengthening procedure resulting in thicker callus formation [25]. Mizumoto et al. demonstrated that recombinant human bone morphogenetic protein-7 increased bone mineral density during distraction osteogenesis [18]. Bail et al. observed that growth hormone increased the rigidity of callus formation during distraction osteogenesis [2]. Li et al. found that recombinant human bone morphogenetic protein -2 (rhBMP-2) reinforced the consolidation phase of distraction osteogenesis in rabbit models and also enhanced volumetric density [15]. Li et al. also revealed that thrombin-related peptide (TP 508) enhanced bone formation and the consolidation phase of distraction osteogenesis [16].

In this study, for the first ten days of lengthening, group II received calcitonin and group III was treated with a

biphosphonate derivative, alendronate. Calcitonin and biphosphonate derivatives, which are specific inhibitors of bone resorption, are frequently used in the treatment of osteoporosis. It is known that the effects of calcitonin are mainly the inhibition of bone resorption by osteoclasts. It is recognized that through this mechanism of action calcitonin increases the bone mineralization rate [3, 5].

Biphosphonates are known to prevent bone resorption through their direct effects on osteoclastic function. In addition, higher doses of biphosphonates demonstrated suppression of bone formation and osteoblastic activity [3–5, 8, 17]. In some studies long-term use of pamidronate pre- and postoperatively increased callus mineralization, but decreased remodeling [17]. These studies demonstrated that during long-term use of biphosphonates relatively higher quality callus had formed; however, a delay in mature bone formation had occurred.

The effects of these two drugs on the outcome of distraction osteogenesis have not been sufficiently investigated. Kokoroghiannis et al. lengthened right tibias of 26 New Zealand rabbits on a schedule of 2×0.375 mm/day for ten days, while 14 of them received 10 IU/day of salmon calcitonin, and the remaining 12 rabbits were treated only with placebo (control group). The degree of bone mineralization was monitored at various intervals during experiments with DEXA (dual energy X-ray absorptiometry). Increases in bone density occurred over time, while no significant difference was found between the calcitonin and the control groups [13]. However, in their study a lack of histological and biomechanical evaluation constitutes the weak point of this investigation.

We did not encounter any studies on the effects of alendronate on the outcome of distraction osteogenesis. However, in a study by Little et al., the right tibia of 17 rabbits underwent distraction for 15 days on a schedule of 2×0.375 mm/day after osteotomy; nine rabbits received only placebo (control group) and eight rabbits were treated with pamidronate (3 ml/kg). As for evaluations carried out using DEXA, and histological examinations, in the pamidronate group bone formation was significantly enhanced, but re-modelling was delayed. In addition, the total lengths of both regeneration and bone segments were found to be shorter than those detected in the control group. This phenomenon was attributed to the bone formation-accelerating effects of pamidronate resulting in premature consolidation [17].

In our study groups, both calcitonin and alendronate were given at previously recommended doses only during the distraction period. Since our aim was to investigate the effects of the drugs used on the outcome of distraction osteogenesis, we believe that ten days of treatment with these drugs would suffice. In addition, as is widely accepted, after entering into the consolidation phase, which is called the stable phase of new bone formation, drugs do not have any effect on bone formation.

At 21 days we did not find any significant difference among the groups of sacrificed rabbits when assessed radiologically. We attributed this finding to our method of osteotomy. In a study conducted by Kojimoto et al. only after 14 postoperative days was endosteal re-vascularization established because of medullary injury [12]. In our Gigli saw osteotomies, 21 days postoperatively a significant difference was not found among the groups on radiological examination. However, with regard to the results of the histological evaluation, we detected better callus formation in the calcitonin and alendronate groups compared with the control group. New bone formation in group III fared better in the scoring system, despite the lack of a statistically significant difference. This finding complies with the results of investigations indicating the favourable effects of alendronate on new bone formation in the early stages. According to the results of radiological findings, we discovered at the end of six weeks that the total scores in groups II and III were better, despite the absence of a statistically significant difference.

At the end of eight weeks, according to our radiological findings relating to sacrificed rabbits, intergroup differences were not apparent. We attribute this result to strict observance of the principles set forth by Ilizarov for distraction osteogenesis. When the histological results are reviewed, although the differences are not statistically significant, our mean scores in group II were better than those in groups I and III. Since various investigations revealed that biphosphonates delay bone re-modeling, we thought that this result was in accordance with those found in the literature. Furthermore if we take a look at the biomechanical findings, a significant difference in favour of the groups treated with alendronate and calcitonin was observed with regard to the torsional failure load compared with control group. In addition, in group II failure of the torsional loading was significantly better than in group III. We believe that the relatively higher forces against torsional loading in group II in comparison with the other groups is an important conclusion of this study.

From the 1990s onward distraction osteogenesis has been used successfully for the resolution of many bone disorders. However, in addition to the success of this technique, the relatively high rates of complications are important issues. Delay in callus formation and fracture of the regenerated bone after removal of the frames are among the most frequently seen complications. For the management of these complications surgical interventions are usually recommended [19]. As for the biomechanical test results obtained in our study, since higher tolerability rates to torsional strain were detected in the calcitonin group, we

believe that calcitonin might be useful for the prevention of complications, such as delay in consolidation and refractures. However these findings must be verified by further experimental and human studies.

Acknowledgement For statistical analyses we thank Ass. Prof. Dr. Ilker Etikan in the Department of Statistics, School of Medicine, Gaziosmanpaşa University.

The authors did not receive any grants or outside funding in support of their research or preparation of this manuscript.

References

1. Aronson J (1997) Current concepts review. Limb lengthening, skeletal reconstruction, bone transport with the Ilizarov method. *J Bone Joint Surg [Am]* 79:1243–1253
2. Bail HJ, Rachke MJ, Kolbeck S, Krummrey G, Windhagen HJ, Weiler A, Raun K, Mosekilde L, Haas NP (2002) Recombinant species-specific growth hormone increases hard callus formation in distraction osteogenesis. *Bone* 30:117–124
3. Baron R (1993) Anatomy and ultrastructure of bone. In: Favus MJ (ed) *Primer on the metabolic bone disease and disorders of mineral metabolism*, 2nd edn. Lippincott-Raven, Philadelphia, pp 3–10
4. Day JS, Ding M, Bednarz P, Linden JC, Mashiba T, Hirano T, Johnston CC, Burr DB, Hvid I, Sumner DR, Weinans H (2004) Bisphosphonate treatment affects trabecular bone apparent modulus through micro-architecture rather than matrix properties. *J Orthop Res* 22:465–471
5. Deftos LY (1993) Calcitonin. In: Favus MJ (ed) *Primer on the metabolic bone disease and disorders of mineral metabolism*, 2nd edn. Lippincott-Raven, Philadelphia, pp 70–76
6. El-Mowafi H, Mohsen M (2005) The effect of low-intensity pulsed ultrasound on callus maturation in tibial distraction osteogenesis. *Int Orthop* 29:121–124
7. Farley JR, Tarbaux NM, Hall SL, Linkhart TA, Baylink DJ (1988) The anti-bone-resorptive agent calcitonin also acts in vitro to directly increase bone formation and bone cell proliferation. *Endocrinology* 123:159–167
8. Hu JH, Ding M, Soballe K, Bechtold JE, Danielsen CC, Day JS, Hvid I (2002) Effects of short-term alendronate treatment on the three-dimensional microstructural, physical and mechanical properties of dog trabecular bone. *Bone* 31:591–597
9. Huddleston PM, Steckelberg JM, Hanssen AD, Rouse BS, Bolander ME, Patel R (2000) Ciprofloxacin inhibition of experimental fracture healing. *J Bone Joint Surg [Am]* 82:161–173
10. Ilizarov GA (1990) Clinical application of the tension-stress effect for limb lengthening. *Clin Orthop* 250:8–26
11. Karachalios T, Lyritis GP, Giannarakos DG, Papanicolaou G, Sotopoulos K (1992) Calcitonin effects on rabbit bone. Bending tests on ulnar osteotomies. *Acta Orthop Scand* 63: 615–618
12. Kojimoto H, Yasui N, Goto T, Matsuda S, Shimomura Y (1988) Bone lengthening in rabbits by callus distraction. *J Bone Joint Surg [Br]* 70:543–549
13. Kokoroghiannis C, Papaioannou N, Lyritis G, Katsiri M, Kalogera P (2003) Calcitonin administration in a rabbit distraction osteogenesis model. *Clin Orthop Relat Res* 415: 286–292
14. Lane JM, Sandhu HS (1987) Current approaches to experimental bone grafting. *Orthop Clin North Am* 18:213–225
15. Li G, Bouxsenin ML, Luppen C, Li XJ, Wood M, Seeherman HJ, Wozney JM, Simpson H (2002) Bone consolidation is enhanced by rhBMP-2 in a rabbit model of distraction osteogenesis. *J Orthop Res* 20:779–788

16. Li G, Ryaby JT, Carney DH, Wang H (2005) Bone formation is enhanced by thrombin-related peptide TP508 during distraction osteogenesis. *J Orthop Res* 23:196–202
17. Little DG, Cornell MS, Brody J, Cowell CT, Arbuckle S, Cooke-Yarborough CM (2001) Intravenous pamidronate reduces osteoporosis and improves formation of the regenerate during distraction osteogenesis. A study in immature rabbits. *J Bone Joint Surg [Am]* 83:1069–1074
18. Mizumoto Y, Moseley T, Drews M, Cooper VN, Reddi H (2003) Acceleration of regenerate ossification during distraction osteogenesis with recombinant human bone morphogenetic protein-7. *J Bone Joint Surg [Am]* 85 [Suppl]:124–130
19. Paley D (1990) Problems, obstacles and complications of limb lengthening by the Ilizarov technique. *Clin Orthop* 250:81–104
20. Paley D, Tetsworth K (1991) Percutaneous osteotomies: osteotome and Gigli saw techniques. *Orthop Clin North Am* 22:613–624
21. Sen B, Çakmak M, Arıtamur A, Göğüş A, Olgaç V (1991) Tavşanlarda kallus distraksiyonu ile uzatma. *Acta Orthop Traum Turc* 25:170–178
22. Sen C, Kocaoglu M, Eralp L, Gulsen M, Cinar M (2004) Bifocal compression-distraction in the acute treatment of grade III open tibia fractures with bone and soft-tissue loss. *J Orthop Trauma* 18:150–157
23. Shimazaki A, Inui K, Azuma Y, Nishimura N, Yamano Y (2000) Low intensity pulsed ultrasound accelerates bone maturation in distraction osteogenesis in rabbits. *J Bone Joint Surg [Br]* 82:1077–1082
24. Subasi M, Kapukaya A, Kesemenci C, Balci TA, Büyükbayram H, Ozates M (2001) Effect of chemotherapeutic agents on distraction osteogenesis. An experimental investigation in rabbits. *Arch Orthop Trauma Surg* 121:417–421
25. Yamane K, Okana T, Kishimoto H, Hagino H (1999) Effect of ED-71 on modeling of bone in distraction osteogenesis. *Bone* 24:187–193