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Rod-Through-Plate Fixator for Long Bone Fractures: A Morphological Study on Rabbits

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Summary

We study the effect of a new fixator for treatment of diaphyseal bone fractures of small animal. The experiments were performed on 12 New Zealand White rabbits. Transverse osteotomy was made in the central third of the diaphysis of the rabbit's femur and a rod-through-plate fixator was used to treat the bone fracture. The regeneration of the bone tissue was studied by means of radiography and histomorphology at postoperative weeks 2, 4 and 8. The rod-through-plate fixator gives stable fixation and early mobilisation of the limb. Radiographic images showed that the fractures had repaired well. Morphologically, all the parts of the callus had a typical structure. An immunohistochemical examination showed elevated levels of extracellular matrix proteins collagen type II, osteocalcin and osteopontin in the callus area. The new fixation method gives strong fixation of bone fragments with minimal traumatization of soft tissue during the operation. The construction of the rod-through-plate fixator allows one to reduce pressure in the area of the fracture and the dynamic fixation given by intramedullary rods allows micromotions stimulating callus formation and avoiding implant-induced osteoporosis. The fixator has a simple construction and its use expands the treatment possibilities for diaphyseal fractures of long bones. The rod-through-plate fixator use does not require special training for the surgeon.

Introduction

In orthopaedic surgery of small animals both conservative and operative methods are used for the treatment of fractures of long bones. This is due to the diversity of bone fractures, whereas none of the methods can have maximal efficiency for all types of fractures of long bones (*Brinker et al., 1991*). The main factors that ensure speedier recovery from a bone fracture are precise reposition, stable fixation of the fracture and as early mobilisation of the limb as possible (*Perren, 1979*). Nevertheless, the most important factor that affects recovery from a bone fracture is considered to be the strong and stable fixation of bone fragments and for that different methods have been developed including plate fixators and intramedullary nailing (*Brinker et al., 1991*). In treatment of long-bone diaphyseal fractures of small animals, combination of these two approaches gives additional stability, especially considering resistance to the rotational forces. The combined fixator was first introduced by A. Seppo in 1979 in humans (*Seppo et al., 1979*), where extramedullary and intramedullary osteosynthesis was employed. It has to be noted that post-operative treatment in humans and animals is based on completely different factors. In animals, it is extremely important that the construction used for fixing the broken fragments has maximal strength and is compact and stable, because with animals one cannot expect them to stay immobile during the process of recovery. Therefore, the rod-through-plate fixator for small animals has to enable the use of the dam-

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aged limb immediately after the operation.
The aim of this study was to use a new rod-through-plate fixator (Fig 1) to treat experimental femoral fractures of rabbits and to assess fracture healing radiologically and by histomorphology. Also, we aimed to detect extracellular matrix proteins collagen type II, osteocalcin and osteopontin to trace cartilage formation, osteoblastic activity and bone turnover.

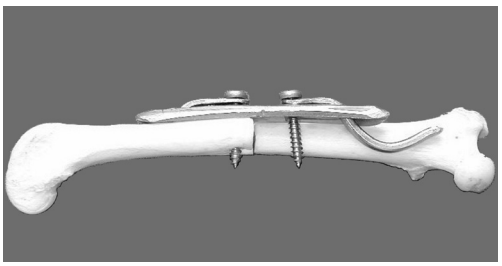

Figure 1. Rod-through-plate fixator`s construction.

Material and Methods

Animals

The experiments were performed on 12 male New Zealand White rabbits. The animals were kept in the animal house at 20±2°C under a 12-h/12-h light/dark cycle (lights on at 0700 hours). Tap water and food pellets were available ad libitum. All animal procedures were approved by the Estonian Agricultural University Animal Care Committee in accordance with the European Communities Directive of 24 November 1986 (86/609/EEC). The animals were in the experiments for 2, 4 or 8 weeks and each group contained 4 animals.

Fixator

The rod-through-plate fixator is made of stainless steel and consists of a support plate, two curved rods and two cortical bone screws (Fig 2). The support plate is 2 mm thick and has a curved cross-section profile. The length of the plate is approximately 2/5 of the length of the rabbit's femur and the width 2/3 of the diameter of the bone. At 10 mm from the ends of the plate there are milled openings

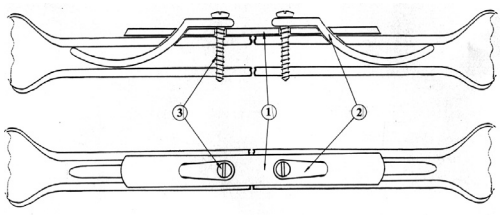

Figure 2. Rod-through-plate fixator`s connection principle: 1 – support plate, 2 – curved rods, 3 – bone screws.

with an inclination of 45º and with a diameter 3.5 mm in the direction outwards from the centre. The diameter of the inclination must correspond to the diameter of the curved rod. The curved part of the rod (length 25-27 mm, width 3.4 mm, thickness 2 mm) that enters the bone marrow cavity has an oval cross-section and is minimally conical (0.5-0.8 mm) towards the periphery. Such a design makes it easier to remove the rod after the bone fracture has healed. The other end of the rod (tail of the rod), measuring 15 x 6 x 1.5 mm is located on the support plate, it is flat and has the same curve as the support plate. At the end of the tail of the rod there is a hole with a diameter of 3 mm, which during the operation is matched with the opening of the same diameter in the support plate. Such a construction makes it possible to attach simultaneously both the rod and the support plate to the bone with screws. Cortical screws with a length of 18 mm and width of 2.8 mm are used, which have to penetrate both cortexes of the bone. The size of the described fixator is intended for an average-size rabbit (4-5 kg). If the animal is of a different size, the dimensions of the rod-through-plate fixator have to be adjusted proportionally based on the radiographic image.
The original construction of the rod-through-plate fixator is currently in the process of patenting.

Operating technique

The animal was placed on the surgery table with warming pad and during the operation all requirements were strictly followed (*Huber et al., 2006*). Before the operation, the support plate and curved

rods of the required size were made based on the radiographic image, so that they would extend to the maximal distance from the fracture area. The bend of the curved rods is correct if they attach to the bone cortex at three points: at the top of the rod, in the middle point of the curve and in the area of the milled opening of the support plate. For anaesthesia, Domitor (400 µg/kg; Orion Pharmas, Finland) and ketamine (20 mg/kg; Ketanest®, WDT, Germany) in one syringe simultaneously under the skin were used. A permanent cannula was inserted into the auricular vein through which 0.9% NaCl solution was administered during the operation with the interval of one drop per second. Lateral access to the femur was used and the fixator was placed on the femur. The diaphysis of the femur was osteotomized transversely with a thin pad saw. The ends of the fragments were repositioned and the support plate was placed on the bone. Through the support plate the rods were inserted and attached with the screws. The so-called "pincers effect" appears between the support plate and the rods and thus the rod-through-plate fixator attaches to the bone. Such an attachment ensures the maximal stability of the construction, avoids rotation and the occurrence of new fractures. After the operation, radiographic images were taken in the mediolateral projection to assess the reposition of the fragments and the position of the rod-through-plate fixator. The animals received an analgesic (carprofen, Rimadyl®, Pfizer, Germany) for three days postoperatively.

Histology

Sections of the bone were fixed for histopathological evaluation with 4% buffered formalin solution, decalcified with EDTA during three weeks and embedded in paraffin wax according to classical methods. Seven-µm thick paraffin sections were dewaxed and brought to water through graded ethanols. Sections were stained with hematoxylin and eosin or van Gieson, then dehydrated through graded ethanols, cleared in xylene and mounted with DPX (Fluka, Switzerland). Sections were examined using the Olympus BX-50 microscope.

Immunohistochemistry

Four-µm thick paraffin sections were mounted on poly-L-lysine coated SuperFrost slides (Menzel, Germany), deparaffinized and rehydrated. Peroxidase activity was removed by 0.6% hydrogen peroxide (Fluka, France) in methanol (Fluka, Germany), then sections were washed in PBS (pH=7.4). The sections were treated with normal 1.5% goat serum for 30 min at room temperature and then incubated with the primary antibody to collagen II Ab-2 (2B1.5, NeoMarkers, USA) diluted 1:100, to osteocalcin (Abcam, UK) diluted 1:500 overnight at 4°C. The next day, the sections were washed in PBS and incubated with diluted biotinylated secondary antibody (VECTASTAIN, ImmunoVision Technologies, Co, USA) for 30 min at room temperature. The sections were washed with PBS and incubated for 30 min with VECTASTAIN ABC Reagent and then incubated in DAB solution (VECTOR Laboratories, USA) for 10 min at room temperature. Cell nuclei were counterstained with hematoxylin or toluidine blue. The sections were dehydrated through graded ethanols, cleared in xylene and mounted with Eukitt (Fluka, Switzerland). The amount of osteopontin in the tissue was determined with polyclonal antybody to osteopontin (ab14715, Abcam, UK) with a dilution 1:1000. For determination, the shorter version of the same protocol was used, where the sections were incubated with a primary antibody during 45 min at room temperature. The collagen II, osteocalcin and osteopontin were expressed by a subjective scale ranging from 0 to 3 (0 – no staining, 1 – weak staining, 2 – moderate staining, 3 – strong staining). Two independent observers in a blinded fashion performed the evaluation, their scores were averaged and data were tested nonparametrically using the Mann-Whitney U test. The level of significance was set at $p<0.05$.

Results

Operated animals were lodged in an animal house, where after cessation of anaesthesia they immediately started to load their weight on to the operated

limb. Throughout the first postoperative week the limb function was estimated clinically every day. After the operation the appetite and general activity of the animals declined, but recovered during the first week. No functional disturbances or muscle atrophies were clinically noticed in the rabbits.

Radiographical monitoring of fracture healing

Radiographic images taken directly after surgery, and then after 2, 4 and 8 weeks after the operation showed that the fractures had healed well in 11 animals, where the intensive growth of the periosteal callus at the proximal edge of the support plate was clearly visible. All the elements of the rod-through-plate fixator were at the required positions (Fig 3). In one case the healing of the fracture was slower and had not yet reached the final stage as the callus was not evenly dense. Nevertheless, radiographic image showed that the position of the rod-through-plate fixator and the screws had not changed.

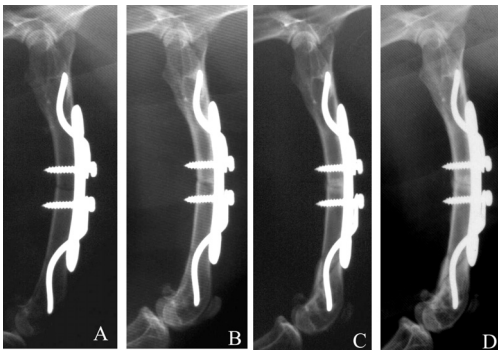

Figure 3. Radiographs taken during the experiment. a – directly after surgery; b – 2 wk after surgery; c – 4 wk after surgery; d – 8 wk after surgery.

Histopathology

Experiments with duration of two weeks

In the bone fragments the bone canals had insignificantly expanded and were often empty. In a few cases, loose connective tissue and blood vessels were present in the canals. The cambial layer of the periosteum contained 1-2 rows of osteoblasts, the fibrous layer was relatively thicker and dense. The periosteal and intermediate callus consisted of thin trabeculae, which had mostly developed from the connective tissue. In the central callus, endochondral ossification was prevalent, whereas the transfer of the cartilage to the bone tissue was smooth. Around the endochondrally formed bone trabeculae there was a regular chain of osteoblasts, which showed ongoing formation of the bone tissue. On the side of the periosteum, the callus was surrounded by young bone tissue which was penetrated by loose connective tissue in the form of strands. In the deeper layer there was also an insignificant amount of hyaline cartilage tissue. The trabeculae of the bone tissue were of different volume and demonstrated little mineralization and contained young and large osteocytes. The newly formed nature of the bone was also confirmed by the overwhelming prevalence of the cells in the cell/matrix ratio.

Experiments with duration of four weeks

In the bone fragments bone canals had significantly widened. In one experiment, a slot of the fixation screw was clearly visible, surrounded by a thin layer of connective tissue and then up to three times thicker layer of new bone tissue. This finding allows us to claim that the material used for the fixator does not cause extensive acute tissue reactions. No signs of inflammation were found during the experiment. Compared to the experiment with shorter duration, the periosteum was thicker, in particular in the fibrous layer, surrounded by coarse collagenous fibres in the outside. Fibrous callus was massive and with abundant cells, which indicated the active nature of the tissue. The cartilaginous callus, which was relatively rare, was dominated by hyaline cartilage tissue. Trabeculae of the bony callus were quite massive and ossification was both intramembranous and endochondral. In the first case, there was either a regular chain of osteoblasts around the bone trabeculae or the cells of the periost participated in osteogenesis. Endochondral ossification in the experiment took place in two ways. In the first case, there was no

clear line between the hyaline cartilage tissue and the bone tissue and there were islands of cell-rich cartilage tissue in the bone trabeculae. In the second case, condrocytes swelled followed by destruction and the bone tissue began to develop around the preserved matrix. As compared to the experiments with duration of two weeks, a significant difference was the development of the canal system in the newly formed bone, containing connective tissue and blood vessels.

Experiments with duration of eight weeks

In bone fragments normal osteocytes were seen, lacunae were less widened as compared to the experiments with shorter duration. Bone canals were wider than normal. As a rule the periosteal callus was voluminous and contained abundant young cell-rich connective tissue. In the fibrous periosteal callus there were smaller bone trabeculae surrounded by a regular chain of osteoblasts. In the cartilaginous periosteal callus there was both hyaline and fibrous cartilage tissue. In the trabeculae of the bone tissue, canals containing connective tissue and blood vessels had formed. In the trabeculae there were osteocytes in different stages of development. In the intermediate callus and endosteal callus there was abundant connective tissue, the trabeculae were clearly expressed, but osteoblasts around them were located irregularly.

In one case the bone fragments were not properly arranged because of the comminuated fracture. Normal osteocytes were seen in the compact bone of the fragments, lacunae were differently widened, but less than in experiments with shorter duration. As the fragments are intermingled it enables ingrowth of periosteal connective tissue with low ostegenic activity.

Immunohistochemistry

The bone fracture areas were studied for the presence of collagen II, osteocalcin and osteopontin (Fig. 4). Weak osteocalcin and osteopontin expression was seen 2 weeks after the beginning of the experiment; later the level of both proteins increased (Fig 5). Collagen II expression was high already through the first weeks, but still increased at week 8 (Fig 5).

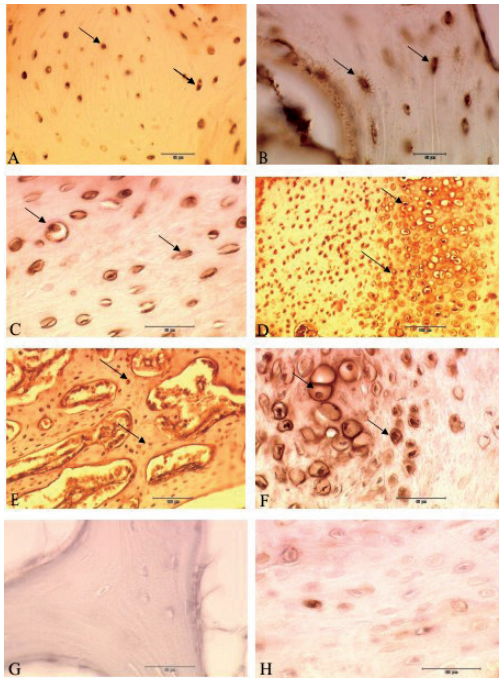

Figure 4. Immunohistochemistry. A– weak osteopontin staining in the 2 wk group; B – intense osteopontin staining in the 4 wk group; C – intense collagen II reaction in the 2 wk group; D – intense collagen II reaction in the 8 wk group; E - intense osteocalcin staining in the 8 wk group; E – weak osteocalcin staining in the 4 wk group; G – collagen II control (primary antibody omitted); H – osteocalcin control (primary antibody omitted).

Discussion

Healing of bone fractures is affected by several factors, but the stable fixation of bone fragments is of ultimate importance (*Perren, 1979; Uhthoff & Dubuc 1971*). In this study we introduce an original rod-through-plate fixator for maximal stabilisation of the diaphyseal fractures of small animals. With this rod-through-plate fixator the support plate

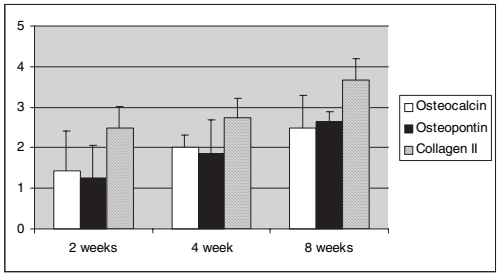

Figure 5. Estimation of immunostaining for osteocalcin, osteopontin and collagen type II in the callus area. Staining graded as: 0- absence; 1 - weak; 2 – moderate; 3 – strong; 4 - intensive. Four rabbits per each time point, values are expressed as mean±SD. Statistically significant ($p<0.05$) differences in staining intensity: osteocalcin 2 weeks vs 8 weeks, osteopontin 2 weeks vs 8 weeks and collagen II 2 weeks vs 8 weeks.

placed on the bone is relatively short, while intramedullar rods lengthen the shoulder of the fixator proximally and distally almost up to the metaphyses. As compared to the conventional plate fixator, the installation of this rod-through-plate fixator does not require intensive surgical access, thus reducing the length of the operation and traumatisation of tissue ensuring optimal conditions for the healing of the fracture. Curved intramedullar rods give dynamic fixation as they are attached to endosteum at three points and so give extensive and stable contact with bone (*Wolff, 1975*). Traditional intramedullary nails do not give adequately strong contact with bone and therefore are less resistant to rotational forces. This especially applies for transverse fractures, which often require additional fixation (*Vasseur et al., 1984*). The specific feature of our method can be considered the "pincers effect", which is produced by the special attachment principal to the bone cortex – the effect is generated between support plate and intramedullar rods when the screws are fixed. This kind of attachment, where the force vectors are directed opposite to one another, is believed to be mechanically the most secure and stable. At the same time the fixation is not absolutely rigid and because of amortisation of rods during limb loading, micro-movements are produced in the fracture area (*Uhthoff & Dubuc, 1971*). By that, more intensive callus formation and faster bone consolidation is provided (*Goodship & Kenwright, 1985*). When using extremely rigid fixators formation of osteoporosis induced by implants has been described and, in connection with that new, fractures occur after removal of implants (*Field, 1997*).

All the experimental animals loaded their weight on the operated limb already during the day following the operation. Both radiography and histomorphology showed that the rod-through-plate fixator had no adverse effect on bone structures and the fracture healing was normal. Callus formation and maturation as followed at weeks 2, 4 and 8 showed typical transformations. The early soft callus containing mostly fibrous tissue underwent successive degeneration, whilst the hard callus expanded constantly, where bone tissue was formed both by intramembraneus and endochondral ossification. Immunohistochemical staining showed gradual increase in the level of all three matrix proteins studied, e.g. weak reaction to osteocalcin, osteopontin and collagen II found at week 2 was changed to intense staining at week 8 (Fig 4). Still, collagen II staining was relatively intensive already at week 2 and 4, but clear increase was noted at week 8 (Fig 5). Increased expression of osteocalcin was expected as being the major component of the non-collagenous bone matrix binds to hydroxy-apatite during matrix mineralization (*Hoang et al., 2003*) and evidence of high osteoblastic activity. Osteopontin, also one of the major non-collagenous proteins of bone extracellular matrix, is a glycoprotein that promotes the attachment of osteoblasts to the extracellular matrix and modulates formation of new bone through multiple mechanisms (*Alford & Hankenson, 2006*). Increased expression of osteopontin in our study is in concordance with the study by Yamazaki and co-authors (*Yamazaki et al., 1999*) reporting expression of osteopontin in mineralization and the remodeling phase of the healing frac-

ture. Furthermore, osteopontin mRNA was detected in cells of the fracture callus associated with tissue turnover - in osteocytes, osteoclasts and osteoprogenitor cells, but not in osteoblatsts, which expressed osteocalcin (*Yamazaki et al., 1999*). Increased expression of type II collagen, being predominantly synthesized by cartilage cells (*Thorwarth et al., 2005*), found at week 8 in our study is related to the cartilaginous periosteal callus with both hyaline and fibrous cartilage tissue. Thus, strong expression of these three extracellular matrix proteins at week 8 evidence ongoing chondrogenesis, osteoblastic activity and bone turnover.

The modest amount of cartilage tissue seen in the callus is a good sign indicating the normal ossification process of the tubular bone (*Hsu et al., 2003*) as the large volume of poorly vascularized cartilage tissue can become a precondition for pseudoarthrosis (*Harrison et al., 2003*). A good result is the absence of inflammatory processes and the foreign body reaction as well as destruction of the newly formed tissue, which shows that the combined method is suitable for attaching the fixator to the bone. The latter is important, as the bone-metal compatibility is a serious problem in orthopaedics (*Uhthoff et al., 2006*).

Our study also emphasized the necessity to consider the unique anatomy of rabbits as the cortical layer of their tubular bones is relatively thin and has a fragile structure (*Crigel & Balligand, 2002*). Therefore, it is important to avoid physical overstress during the operation or mistakes in the choice of the diameter of the screw. One case of comminuated fracture seen in our study confirms that necessity.

Conclusion

In conclusion, the new relatively inexpensive combined bone fixation method for diaphyseal fractures of long bones allows one to lengthen the shoulder of the rod-through-plate fixator and by that to reduce pressure in the area of the fracture, to ensure stability and normal fracture healing. The traumatisation of soft tissue during the operation is minimal and the operated limbs are fully weight bearing. Nevertheless, as the results are based on a relatively small number of rabbits, additional experiments are required including studies on larger animals, e.g. sheep, to confirm the usefulness of the rod-through-plate fixator.

Acknowledgements

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