Eesti Maaülikool
Estonian University of Life Sciences

ROD-THROUGH-PLATE FIXATOR IN THE TREATMENT OF LONG BONE FRACTURES OF SMALL ANIMALS

KOMBINEERITUD METALLOOSTEOSÜNTEES PIKKADE TORULUUDE MURDUDE RAVIKS VÄIKELOOMADEL

GARRI TRALMAN

A Thesis
applying for the degree of Doctor of Philosophy in Veterinary Medicine and Food Science
(veterinary surgery)

Väitekiri
filosoofiadoktori kraadi taotlemiseks veterinaarmeditsiini ja toiduteaduse
(veterinaarkirurgia)
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LIST OF ORIGINAL PUBLICATIONS

This thesis is based on studies reported in the following papers, referred to in the text by their respective Roman numerals:

I Andrianov V., Tralman G., Hõim R., Haviko T., Lenzner A., Pääsuke M., Arend A., Aunapuu M. Rod-through-plate fixation of canine diaphyseal fractures. *Vet Comp Orthop Traumatol* 2007, 20: 308-311.

II Tralman G., Nõupuu K., Uksov D., Kibur R.T., Männik P., Talve N., Andrianov V., Roosaar P., Arend A., Aunapuu M. Using internal fixation for the treatment of long tubular bones in small animals: Evaluation of the plate fixator for tibial fractures in sheep. *Papers on Anthropology* 2008, XVII: 311-319.

III Tralman G., Andrianov V., Talvi N., Arend A., Aunapuu M. Standard surgical technique for applying rod through plate (RTP) internal fixator in small animals. *Scand J Lab An Sci* 2010, 37:141-146.

IV Tralman G., Andrianov V., Arend A., Männik P., Kibur R.T., Nõupuu K., Uksov D., Aunapuu M. A novel combined method of osteosynthesis in treatment of tibial fractures: A comparative study on sheep with application of Rod-through-plate fixator and bone plating. *Anato Histol Embryol* 2013, 42:80-89.

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I The author designed the study 30%, participated in data collection 80%, data analysis 30%, and prepared all the parts of the manuscript 90%.

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III The author designed the study 80%, participated in data collection 80%, data analysis 90%, and wrote the manuscript 90%.

IV The author designed the study 50%, participated in data collection 60%, data analysis 60%, and prepared all the parts of the manuscript 80%.

ABBREVIATIONS

ALP	alkaline phosphatase
AO	Arbeitsgemeinschaft für Osteosynthesefragen
a – p	anterio-posterior
ASIF	Association for the Study of Internal Fixation
b. i. d.	twice a day
bw	body weight
CRIF	clamp rod internal fixator
DCP	dynamic compression plate
EDTA	ethylenediaminetetraacetic acid
EU	European Union
H&E	haematoxylin-eosin
ILN	interlocking nail
IM	intramedullary
i/m	intramuscular
i/v	intravenous
IU	international units
kN	kilonewton
LC-DCP	limited contact dynamic compression plate
mA	milliamper
N/mm	pressure/millimetre
PC-Fix	point-contact fixator
RTP	rod-through-plate
s/c	subcutaneous
s. i. d.	once a day

1. INTRODUCTION

Bone fracture treatment in humans goes back many centuries (Clark, 1937). Development of orthopaedic surgery started in the middle of the 18th century and fracture treatment was mainly by means of external support. Since the second part of the 19th century after the developement of anaesthesiology, antiseptic techniques and the discovery of x-rays the developement of orthopaedic surgery has been rapid. Dogs have been used as experimental animals to develop methods for fracture treatment in humans, thus these methods are often appicable for fracture treatment in dogs but this does not mean that these methods are always the best choice for fracture treatment in dogs. The principles of intramedullary nailing in humans were formulated by Küntscher in 1940 (Küntscher, 1958).

At the present time the most common method for fracture treatment is internal fixation by bone plating. This system was first described by A. Lane and A. Lambotte (Broos and Sermon, 2004). The idea of interfragmentary compression came into use after works by Danis in 1949 (Danis, 1979).

The main breakthrough in the internal fixation of fractures was made by the AO/ASIF group in Switzerland in 1958 when the principles of successful osteosynthesis were established (Rosen, 1984; Allgöwer and Spiegel, 1979). The veterinary surgeons then became familiar with biomechanical principles of internal fixation, bone physiology, fracture classification and the entire system of implants and instruments for osteosynthesis. Progress was also made with the developement of stainless steel implants of suitable rigidity, ductility and tissue neutrality. Different biomaterials have been developed to decrease the risk of complications but the stainless steel is still the most widely used material for implants in veterinary surgery. The principle of combined bone fixator was introduced by Seppo et al. in humans (Seppo et al., 1979). A reponator-fixator constructed by them (patent nr US3680553), consisting of curved rods, was used, which formed a so-called „anchor system" away from the fracture site. Seppo first described the formation of the massive callus at the fracture site and quick consolidation of fractures. He explaned it by electrochemical reactions caused by the fixator because of its special construction (Seppo, 1978). Seppo reported the successful use of this model for fracture fixation of long tubular bones. A modification of this system was developed by Andrianov for the use of fracture repair in small animals (Andrianov et al., 2003). The purpose was to achieve stable internal fixation for long tubular bone fractures and minimize soft tissue trauma during operation.

In contemporary small animal orthopaedic surgery, conservative and operative methods are used for the treatment of fractures of long tubular bones. In small animal surgery fixation of fractures should be simple to use and strong enough to allow immediate ambulation of the affected limb with no restrictions.

2. REVIEW OF THE LITERATURE

2.1. Bone fracture and bone repair

Bone fractures can be caused by direct or indirect forces on the bone. There are several classifications of the fractures. Generally bone fractures are classified as follows:

1) open fractures (fracture site communicates with the outer environment);
2) closed fractures (fracture site does not communicates with outer environment).

In complicated cases main blood vessels and nerve trunks can be also damaged.

The direction of the fracture line can be: transverse, oblique, spiral, comminuted and segmental. Transverse and oblique fractures are caused by direct forces acting on the bone. In oblique fractures the fracture line is at 30° or less to the long axis of the bone. A spiral fracture is caused by indirect forces, and the fracture line is at 30° or more to the long axis of the bone.

There are three objective clinical signs for the diagnosis of the fracture: deformity, crepitus and abnormal mobility. Also subjective clinical signs such as pain, local swelling and loss of function are present. Radiographic examination is essential for the diagnosis of the fracture. Radiographs have to be made in at least two views, at right angles to each other, to determine the localisation of the fracture and to describe its nature.

One of the main functions of bone tissue is load bearing. Bone tissue is the strongest tissue in the body and has the highest demand for regeneration, and because of this there needs to be a different approach to bone injury treatment than to injury of other organ systems. The first response to fracture is similar to the response to any injury that leads to tissue destruction and haemorrhage. After the fracture bone tissue can regenerate so that the pre-fractured properties are mostly restored. Bone regeneration is a very complex process depending on several local and systemic factors (Tsiridis et al., 2007). To enhance fracture healing four main factors have been identified, including growth factors (Einhorn, 2003; Tsiridis et al., 2007), osteoconductive scaffolds (Vaccaro, 2002; Babis and Soucacos, 2005; Stylios et al., 2007; Franch, 2008), osteogenic cells (Brighton and Hunt, 1991; Connolly, 1998; Bielby et al., 2003) and also the mechanical environment and stability (Augat et al., 1998; Lanyon, 1992). Mechanical relative stability and a soft tissue envelope with adequate vascularity are essential factors for bone healing (Giannoudis et al., 2007).

There are two basic histological types of fracture healing: direct bone healing, or contact healing, and indirect healing, or healing with callus formation (Hulse and Hyman, 2003). Direct bone healing is rare and can take place only in the case of absolute stability of bone fragments and the elimination of interfragmentary strains (Giannoudis et al., 2007).

Indirect bone healing with callus formation bone heals by different stages: inflammation, soft callus, hard callus and remodelling (Hulse and Hyman, 2003). The healing process goes through formation of different types of tissues which transform from one to another by increasing strength and stiffness of the fractured area. The bone regenerative process begins with haematoma formation, where the leucocytes (neutrophils and macrophages) „clean up" the traumatized area. During the first days haematoma is replaced by granulation tissue - loose tissue, with little stiffness but which can tolerate elongation. Granulation tissue undergoes maturation and becomes more dense connective tissue with collagen fibres. With maturation connective tissue stiffness increases and tolerance of elongation decreases. Fibroblasts and periosteal cells are involved at this stage of the healing process.

Connective tissue and fibrocartilage at the fracture site form the callus. The callus helps to stabilize the fractured bone, as the external callus formation increases the diameter of the bone and therefore increases the ability of the lever arm to resist fracture forces.

Mineralization of the fibrous tissue and fibrocartilage starting from the fragment ends toward the fracture gap. This process will lead to increased stiffness of the newly formed tissue. At the same time osteoprogenitor cells of the periosteum differentiate into osteoblasts and start to form new bone. Local resorption of the mineralized cartilage is replaced by new blood vessels and lamellar bone, which gradually replaces fibrous and cartilaginous callus and forms new bone trabeculae. The same process also takes place in the marrow cavity where bone growth proceeds from both ends of the fracture towards the fracture gap. This united bone is spongy bone and will be gradually replaced by compact bone during remodelling to its original shape. Fixation of the bone usually speed healing and result in better structural and functional restoration (Rahn, 2002; Ross et al., 1995).

Conservative and operative methods are used for fracture management in veterinary surgery. It is extremely important for a surgeon to make the right decision about which type of fixation to use after proper examination of the patient. The method of fracture repair is based on the location type of the fracture, the size and the age of the animal as well as the number of bones involved and any concurrent soft tissue injury (Denny and Butterworth, 2000).

The methods for fracture treatment can be classified as follows:

1. limb splintage with casts, coaptation splints, Thomas splints
2. bone splintage with external skeletal fixators, intramedullary pins, bone plates, bone screws, tension band wires and cerclage wires

The second method requires operative treatment and is preferred for most fractures of long tubular bones.

2.2. Conservative treatment

The conservative management of fractures is performed with bandages, splints and casts. Coaptation splints and casts are made of different materials. They are useful for the treatment of stable (nondisplaced) fractures. The basic objective is immobilisation of the joint above the fracture and all joints below the fracture. Temporary splintage is most useful if there is a reason for the delay until operative treatment can be done to reduce additional trauma to soft tissues by fractured bone ends (Brinker et al., 1990a). It can be used also as extra support following internal or external fixation (DeYoung and Brobst, 1993).

2.3. External skeletal fixation

External skeletal fixation is a means to stabilise fractures using percutaneous fixation pins that penetrate both corticles internally and are connected together externally to form an external frame. The device consist of pins and connecting elements to complete the frame. The first use of external skeletal fixation in man goes back to the end of 19th century. In the 1930s Anderson, Hoffmann and Stader developed the basic design of external skeletal fixation (Egger, 1993). This type of fixation is especially useful in open, highly comminuted and infected fractures. It provides stable fixation of bone fragments with minimal additional damage of blood supply to the traumatized bone and soft tissues (Egger, 1991). Half pins that penetrate one side of the limb and both corticles and full pins that penetrate both corticles and both sides of the limb are used according to need and anatomical location. This provides a stable fragment fixation without the need for a foreign body at the fracture site. A circular external skeletal fixation system where transosseus wires are connected to the rings under tension was developed by Ilizarov for human orthopaedics. Using smooth wires under tension the system configuration gives more stability between the fracture fragments, and protects the soft tissues and intraosseus and extraosseus blood supply (Ilizarov, 1989). Ilizarovs method was also introduced for use in veterinary surgery (Ferretti, 1991).

2.4. Internal fixation

Internal fixation methods apply plates and screws or intramedullary nails. The simplest way to compress fracture fragments is by using lag screws. Screws are the most important part of any stable internal fixators (Schacker and Houlton, 2002). At the present the most common method for fracture stabilisation is using bone plates. The dynamic compression plate (DCP) developed by the AO group came into use in 1969. In the DCP plate design the screw holes are spherical and, as the screw is tightened, the spherical screw head glides towards the centre of the plate until the deepest portion of the hole is reached. The result is that the bone fragment into which the screw is being driven is displaced at the same time, and in the same direction (toward the centre), and the fracture line is compressed. The aim is to cause interfragmentary compression and rigid internal fixation to allow the fragments to unite without callus formation. These AO/ASIF basic principles are well described by Brinker, Flo and Piermattei in „Manual of Internal Fixation in Small Animals” (Brinker et al., 1984).

Over time „biological osteosynthesis” has gained acceptance (Broos et al., 2004). There have been changes in plate design. This technique focus on the maintenance of soft tissue attachments and blood supply to the fractured bone and absolute anatomical reduction is not so critical, with the exception of intraarticular fractures. Limited contact dynamic compression plate (LC-DCP) and point contact fixator (PC-Fix) came into use to minimize vascular impairment between plate and bone contact (Field and Törnkvist, 2001; Frigg, 2001; Uhthoff et al., 2006; Vannini, 2008a; Hudson et al., 2009). The locking plate system works like internal fixators and there are different designs available for veterinary use (Tepic, 2008; Petazzoni, 2008; Ness, 2008). Also, good results have been described by using the clamp rod internal fixator (CRIF)system for veterinary use (Zahn and Matis, 2004).

Since 1930 intramedullary (IM) nails have gained wide acceptance after works of Küntscher (Küntscher, 1958). Stainless steel pins are used for fracture stabilisation by intramedullary placement. Intramedullary pins with different cross-sectional geometry are available to reduce interfragmentary rotational movement (Tarr and Wiss, 1986; Hach, 2000). Generally, the main principle is to insert the nail as tight to medullary canal as possible. The principle of dynamic fixation was developed by Rush, who used flexible pin fixation for the treatment of long tubular bone fractures in human surgery. Since then different IM dynamic fixation nails and methods have been described in the literature for fracture treatment in human and veterinary orthopaedics (Rush, 1968; Hasenhuttl, 1981; Shelbourne and Brueckmann, 1982; Velazco et al., 1983; Zickel et al., 1986; Pankovich et al., 1981; Pankovich, 1981; Wolff, 1975). In veterinary surgery the Rush pinning technique is restricted to fractures in the methaphyseal regions of bone (Brinker et al., 1990b; Jackson, 1998). The most commonly used nails in veterinary surgery are with round cross sectional diameter Steinmann pins or Kirchner wires, for their easy application and probably their low cost. In 1985 to overcome problems with nails, interlocking nail (ILN) came into use (Kempf et al., 1985; Diaz-Bertrana et al., 2005).The intramedullary nail is locked by screws proximally and/or distally. This method eliminates rotational forces on the facture site and can be used in transverse and oblique fractures. The ILN was also used successfully in veterinary surgery (Durall and Diaz, 1996; Dueland et al., 1996). In small animals, in many instances, a combination of different types of fixation are required to achieve stable fixation of the fragments, allowing free movement, until consolidation of the fracture can occur.

2.5. Problems with fracture fixation

Every fracture repair requires careful planning to choose right method for fracture management. It is very difficult to make the right decision and very often the use of different methods at the same time is the most appropriate option to achieve interfragmentary stability. The main reason for the failure of fracture treatment is selection of the wrong method of fixation or improper use of the fixator.

Non-operative treatment with various casts and splints requires a lot of experience from surgeon to ensure good results. Such treatment is limited to nondisplaced fractures and preferrably distal to the elbow or stifle joint. The problem is to ensure adequate interfragmentary stability and to maintain blood circulation in the limb and to the injured bone. The results are often skin ulcers, swellings, nonunions, malunions or infections. As the limb is immobilised muscle atrophy is usually severe, and as the adjacent joint is involved in fixation, joint contractures can develop. Therefore operative methods are preferred to conservative management, especially for long tubular bones.

External skeletal fixation, if correctly assembled, is simple to apply, well tolerated by animals, preserves blood supply to the bone and gives good interfragmentary stability; however it has several problems. Most of them are associated with transcortical pins. The most common problems are drainage, infection and skin irritation at the pin-skin interface. These can be minimized by avoiding pin penetration through a large muscle mass and controlled by disinfection of the irritation site. A more serious problem is pin loosening, this can be caused by thermal necrosis or by infection (Matthews et al., 1984). This causes instability of the fragments and can lead to nonunion or malunion if not corrected. If the pins penetrate large muscles, the range of motion of the adjacent joints can be limited and the functioning of the leg can be impared, especially if applyed to the canine femur (Egger, 1991).

Internal fixation with plates and intramedullary pins are the most frequently used methods for fracture repair currently. For intramedullary osteosynthesis, round Steinmann pins are used most commonly in veterinary surgery. As Steinmann pins are round in cross section, for transverse and short oblique fractures a combination of other fixation points is always required (Vasseur et al., 1984). In the ILN system locking screws can resist most of the fracture forces, but in correct placement tight fitting to the medullary canal is required (Suber et al., 2002). Because of natural curvature and variations in the cross sectional diametre of the bone, pins rarely fill the medullary canal. Filling the medullary canal at the fracture site is even undesirable, because the pin interferes with medullary blood supply (Chapman, 1986). The cranial bowing of the canine femur makes it difficult to insert the pin to its full extent. Bone pinning is adequate for smaller dogs and cats but for larger breeds other methods of fixation are advised. It requires the great skill to insert the pin in the desired direction, and cortical shift is common if the pin enters the second fragment. Possible complications also include articular damages, seroma formations and nerve entrapment in femoral fractures (Palmer et al., 1998; Schrader, 1991; Langley-Hobbs et al., 1996). Bone plating for long tubular bone fractures is more time consuming, but more stable fixation can be achieved and no additional fixation is neccessary. It is recommended that at least six corticles should be engaged on both fragments with bone screws. For the bone plating great exposure to the injured bone is necessary and thus more damaging to the blood supply to the fractured bone and soft tissues. Weakening of the bone resulting from osteoporosis and demineralisation of the repaired bone, so called „stress protection", can be caused by too rigid fixation (Uhthoff and Dubuc, 1971; Braden et al., 1973; Akeson et al., 1976; Tonino et al., 1976; Moyen et al., 1978; Paavolainen et al., 1978; Bradley et al., 1979; Chao et al., 1989; Claes et al., 1997; Field, 1997). This problem can lead to refractures after fixation removal (Hidaka and Gustillo, 1984; Solheim, 1972; Dencker, 1964; Frankel and Burstein, 1968; Chrisman and Snook, 1968).

The need for an internal fixation system of reduced rigidity has been clearly identified. The primary focus to reduce rigidity was addressed and new, more flexible and less rigid materials were developed (Woo, 1985; Woo et al., 1985; McKibbin, 1985). There have been several investigations into flexible implants and interfragmentary movement which have lead to nonunions and delayed unions if too flexible implants were used or interfragmentary movement was too great (Church and Schrader, 1990; Claes et al., 1997). In recent years new designs of fixators have also been the subject of attention. The main goal is to reduce the rigidity of fixation and to preserve perfusion to the injured bone. Fixation of the fragments is necessary for early limb ambulation to maintain leg function and to keep the fractured bone in alignment during the healing period. However, it does not neccessarily mean that bone union does occur. Preservation of intact biology of the bone is an important factor for fracture healing with minimal complications, therefore careful handling of the soft tissues of the injured limb is necessary to preserve any soft tissue attachement to the bone if possible during surgical intervention. Surgery itself causes disruption of the blood supply and also the implants lead to disturbed perfusion of the injured bone (Perren, 2001). Several investigations have shown implant-induced vascular problems (Anderson et al., 1962; Wilson, 2002; Kessler et al., 1986; Rand et al., 1981; Tarr and Wiss, 1986). Less invasive surgery and different implants have also been investigated in veterinary surgery (Schatzker, 1996; Vannini, 2008b; Schmökel et al., 2003; Savoldelli and Montavon, 1996).

2.6. Combined fixators

In 1970 Seppo patented his reponator-fixator for the fracture treatment of long tubular bones in human surgery. The idea of this type of fixator was strong stabilisation of the fracture to allow early ambulation of the limb without using any external support. The fixator was made of two different metals and worked like a galvanic element to stimulate callus formation, as described by Seppo and his co-workers (Seppo et al., 1979). Because of its special design the fixator was not in contact with fracture ends, and therefore did not interfere with the circulation outside or inside the bone.

The fixator comprises two pairs of curved rods which are crosswise interconnected, and the ends of the rods are positioned in opposite sides. These rods create a three point support in the medullary cavity on both sides of the fracture site without crossing the fracture site.

Figure 1. Original Seppos fixator for diaphyseal fractures (Estonian Health Care Museum,Tallinn).

Inspired by the work of Seppo, a fixator for small animals was introduced by Andrianov; the rod-through-plate (RTP) fixator (European Union patent No 1682008, Estonian Republic patent No 04899). The main principle, combined fixation, was derived from the fixator-reponator of Seppo. The fixator was simplified by the use of one pair of intramedullary rods and bone plate extramedullary. No different metals were used to ensure more tissue neutrality.

The fixator is handmade from medical stainless steel and comprises one supporting plate, two curved rods, two 4.5 mm cortical screws and two 3 mm fixation screws. The supporting plate is 2 mm thick and convex in cross section. The length of the plate has to be 3/5 of the length of the fractured bone and have a width of 1/3 of the bone diameter. At 30 mm from each end of the plate there are milled channels, 5 mm in diameter, at a 45 degree angle towards the end of the plate. The diameter of the channels must be equal to the diameter of the curved rods. Two 4.5 mm and 3 mm diameter holes are located 20 mm from the centre of the plate and are perpendicular to it. The curved rods are 5 mm broad, 4 mm thick, 40-50 mm long, oval in cross section and conical towards the perphery, from about 1.5 mm of the edge. Such a structure facilitates rod removal after the fracture has healed. The other end of the rod (the tail-end) that lies on the plate is flat and equally convex as the plate. The tail of the rod has two holes in it, a 4.5 mm hole for a cortical screw and a 3 mm hole for the fixation screw, which correspond to the holes in the plate. The size of the holes and the interval between the holes and channels in the plate and rods are standard in order to allow configuration of the different sized plates and rods. Standard 3.5-4.5 mm cortical screws are inserted through these holes and the plate and the rods are simutaneously fixed to the bone, ensuring that both corticles are completely engaged. The fixation screw is 3 mm in diameter and 5 mm in length. These screws are fixed to the plate and they do not reach to the surface of the bone (Fig. 2, 3).

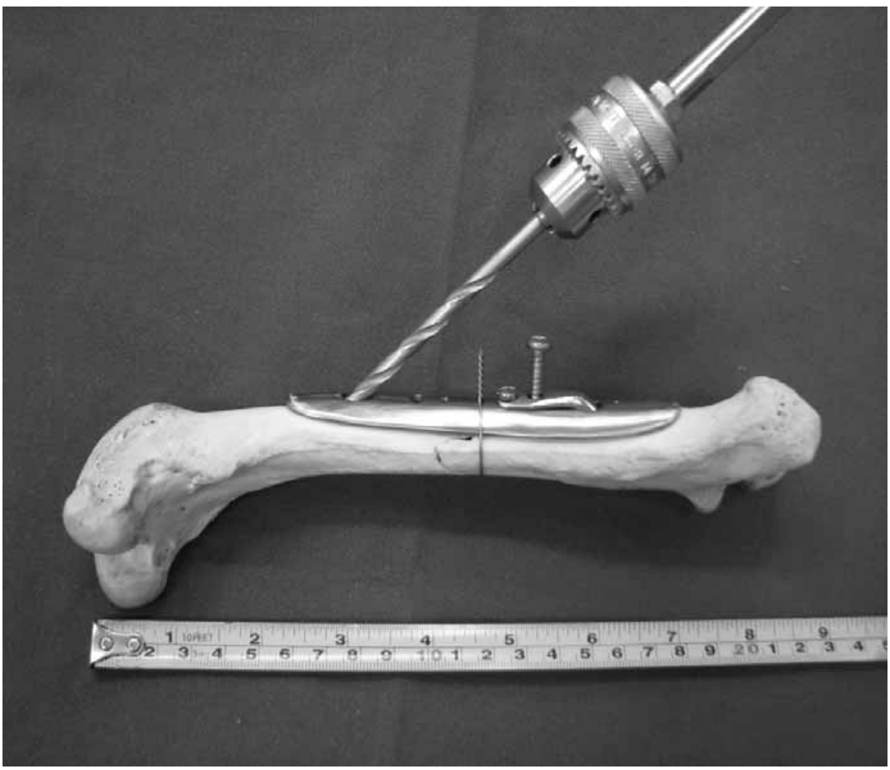


Figure 2. RTP fixator on a cadaver bone.

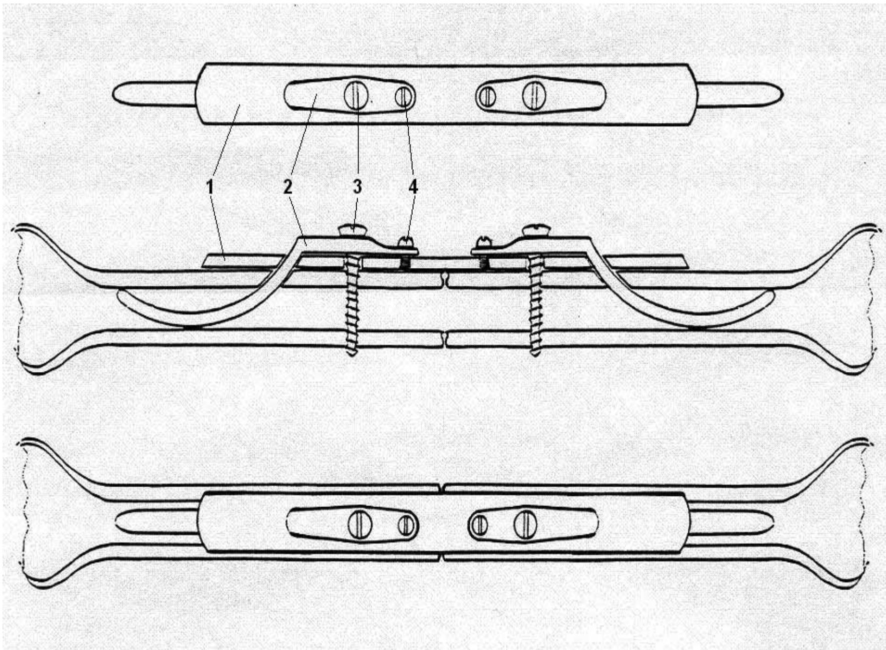


Figure 3. Rod-through-plate fixator. 1 – supporting plate; 2 – curved rod; 3 - cortical screw; 4 - 3 mm locking screw.

3. AIM OF THE STUDY

The purposes of the study were:

1. To compare the effectivness of plate and RTP fixation in experimental tibial osteotomies in sheep (Papers I, III, IV).
2. To test RTP fixator useability in clinical cases for long bone fracture repair in dogs (Paper II).
3. To develop a standardized technique for RTP-fixator placement (Paper III).

4. MATERIALS AND METHODS

4.1 Experimental study

4.1.1. Experimental animals

The experiments were performed on seven male Estonian Blackhead sheep. All sheep were from Karula farm (Estonia) and were six months old. The mean body weight of the sheep was 35 (±5.0) kg. The animals were kept in the university animal housing facility at 20 ± 2°C under a 12-h/12-h light/dark cycle (lights on at 07.00 hours). The animals had free access to food and water. The duration of the experiment was ten weeks. At the end of the experiment the animals were euthanazed. Euthanasia was carried out after sedation with 20 mg/ml Xylazin hydrochloride 0.15 mg/kg according to body weight by intravenous (i/v) (Xsylapan®) and by i/v administration of T61® (Embutramide 200 mg + Mebezonium iodide 50 mg + Tetracaine hydrochloride 5 mg) 5 ml/50 kg. Permission (No. 75, 16 March 2007) for the study was given by the Estonian National Board of Animal Experiments in accordance with the European Communities Directive (86/609/EEC).

The seven sheep were divided into two groups: in four sheep the rod-through-plate (RTP) fixator was applied (the RTP group), and in three sheep the bone plating was used (the Plate group).

4.1.2. Anaesthesia procedure

Operations were performed on the sheep under general anaesthesia. After premedication with atropine 0.1 mg/kg intramusculary (i/m), induction was made with medetomidine hydrochloride 0.01 mg/kg i/v. All animals were intubated and anesthesia was maintained with a mixture of isoflurane 2% and pure oxygen with a flow rate of 11 ml/kg/min (Riebold et al., 1995). For gas anaesthesia a Komesaroff inhalation anaesthesia device was used. Lactated Ringers solution 11 ml/kg/h was administrated intravenously during the anaesthesia. At the end of the operation, for antibiotic prophylaxis, procainpenicillin 50 mg/kg i/m was adminisetred after surgery and on the first and second postoperative day. An analgetic, carprofen, was administered at 2 mg/kg according to body weight subcutaneously (s/c) once a day (s.i.d) after the procedure and continued for two postoperative days.

4.1.3. Surgical technique

4.1.3.1. Surgical technique for RTP fixator

Radiographs of the left tibia were made in mediolateral and craniocaudal projections from a distance of one meter by *Medlink URS Veterinary Portable X-ray SP-VET-4.0*, parameters 50 kVp and 10.0 mAs were used. A radiographic screen *AGFA CR MD 4.0 General Plate* was used and images were loaded onto a computer by an *AGFA ADC Solo Digitizer*. The size of the plate and rods were chosen according to the bone size on the craniocaudal radiograph. The plate was accurately contoured to the shape of the bone. Curved rods correspond to the diameter of the medullary canal and extended in maximal distance from the osteotomy site proximally and distally, according to the radiograph. Intramedullary rods were curved and were in contact with the cortex at three points: at the end of the rod, at the centre of the rod arc and at the point of rod and plate union.

Before surgery on experimental animals hair was clipped from the region of the jugular vein, the *cephalica antebrachii* vein and also from the left hind leg from the centre of the femur to the centre of the metatarsals. An intravenous cannula was placed into the cephalic vein.

The animal was placed in lateral recumbency, such that the leg to be operated on was on the top. The operation field was disinfected with 75% ethanol, followed by covering with towels to achieve an aseptic working area.

A skin incision, about 12 cm in length, was made in the middle third of the tibia on the lateral side. Fasciae were identified and cut in line with the skin incision (Fig. 4).

The intermuscular septum between the deep digital flexor muscle and the lateral digital extensor muscle was identified and separated by blunt dissection. The peroneal nerve was identified and protected (Dyce et al., 1987).

Self-retaining retractors were used to maintain the exposure of the tibia. Muscle attachements were separated from bone from about one third around the bone throughout the length of the plate using a periosteal elevator (Fig. 5).

The plate was then applied to the lateral side of the tibia, and held in position by bone clamps. The plate was conformed to the bone contour, if necessary, using plate benders.

The forceps holding the plate and the bone were placed in the centre of the plate. In this position they did not hinder the following manipulations.

Starting proximally, a 4 mm hole was drilled through the lateral cortex of the bone at approximately 45° to its longitudinal axis. The channel for the rod in the plate served as a guide ensuring the correct angle and position of the 4 mm hole (Fig. 6).

The intramedullary rod was inserted through the hole into the medullary cavity, held from the tail of the rod with forceps. If necessary, the arc of the rod was changed according to the need not to apply too much pressure to the cortex of the bone. After insertion of the rod the distance between the tail of the rod and the plate should be about 2-3 mm. At the same time, the holes for cortical screws in the plate and in the rods tail should be aligned. If necessary, rod must also be bent in a craniocaudal direction. For correct alignment of the holes, 2 mm Kirschner wire was used through both holes and the wire was moved in the required direction until both holes were perfectly aligned (Fig. 7).

Figure 4. Skin incision.

Figure 5. Approach to the bone.

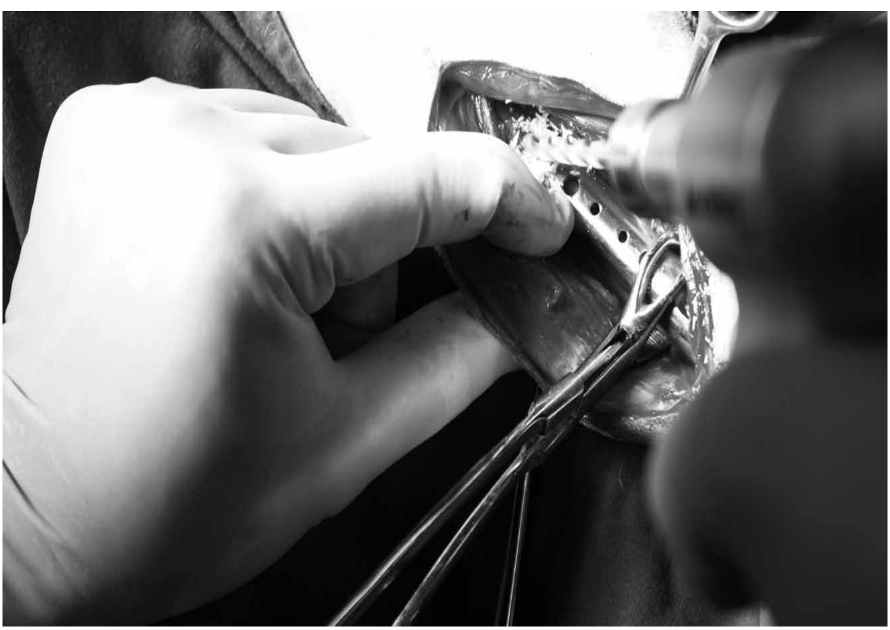


Figure 6. Channel for intramedullary rod.

Figure 7. Supporting plate and proximal intramedullary rod in place.

A cortical screw was inserted through the rod and plate holes to the bone. Screws were inserted according to the AO standard technique (Brinker et al., 1990a). Using a 2.5 mm drill bit holes through both corticles were drilled. The length of the hole was measured, both corticles tapped and appropriately sized 3.5 mm screws were inserted. Tensioning the screw pushed the rods tail to the plate and gave tension to the bone corticles from the medullary canal. The same procedure was performed distally.

For osteotomy the fixator was removed and osteotomy was performed perpendiculary to the long axis of the bone. Gigli wire was used for osteotomy. Osteotomy was centred to the midway point between the screw holes.

The fixator was returned to the osteotomised bone using the same locations. In the modification of the RTP fixator there are two additional screws for fixation of the rods to the plate.

Figure 8. RTP fixator on bone.

The wound was closed in layers, starting with deep fascia with continuous suture and subcutaneous tissue and skin with interrupted manner with absorbable material. After closure chlortetracycline spray 20 mg/ml to the wound was applied.

No bandages on the operated leg were used. Radiographs of the operated leg were made after surgery in a craniocaudal direction to identify the location of the fixator on the bone. Free movement of the animals was allowed immediately after the operation. To ensure similar handling of all operated animals, all surgical procedures were performed by the same surgeon with a single assistant. Average operation time was 1 hour and 40 minutes. After eight weeks from the beginning of the experiment the fixator was removed. Removal of the implant took about 20 minutes. Monitoring time of the animals after fixator removal was 2 weeks to ensure the absence of any possible complications.

4.1.3.2. Surgical technique for plate fixation

The plate was selected by length and contoured to fit the surface of the bone using preoperative radiographs. The anaesthesia procedure and preparation for surgery were performed as described for RTP fixation. The bone plate was also placed on the lateral side of the sheep tibia using the same approach as described for the RTP fixator. The approach to the tibia was made along the length of the plate. A six hole plate was fixed to the bone temporarily by bone-holding forceps. Contouring of the plate was done if necessary. After predrilling a 2.5 mm hole, measuring the depth with a depth gauge and pre tapping both corticles, three 3.5 cortical screws distally and three 3.5 cortical screws proximally were inserted. The screws were released and tibial osteotomy was carried out using a gigly saw, using the centre of the plate as the mark for the cut. After osteotomy the screws were again tightened. No compression between fragments was made. The wound was closed in layers with a synthetic resorbable suture material. The closed wound was covered with an oxytetracyclin spray, at 36 mg/g. Antibiotics were given intramusculary, 5 ml Amoxycillin 150 mg/ml. Carprofen, at 2 mg/kg s.c., was given after surgery and for three postoperative days.

4.1.4. Follow up

Experimental animals of both group were evaluated for use of the operated leg on first two weeks.

4.1.4.1. Roentgenological evaluation

The operated leg was radiographed following surgery and then after every two weeks up to the end of the experiment. Radiographs were made on anterio-posterior (a-p) projections. Callus formation and implant positioning were evaluated from the radiographs at 2, 4, 6, 8 and, 10 weeks following surgery in two groups (RTP and Plate). The height of the fracture callus on radiographs was measured on the medial side of the tibia using software CELL ^ 2.5 (Olympus Soft Imaging Solutions GmbH). The nonparametric Mann–Whitney U-test was applied for statistical analysis (GraphPad Software, Inc, San Diego, CA, USA).

4.1.4.2. Biochemical analysis of blood

Blood samples were taken from the jugular vein of sheep. The samples were taken before the operation and subsequently in week 2, 4, 6 and 8 after surgery. Blood biochemical data such as levels of serum alkaline phosphatase (ALP), osteocalcine, total protein and C-reactive protein were studied.

4.1.4.3. Histology

In one animal from the RTP group, biopsy under anaesthesia was taken 2 weeks and, in another animal, 4 weeks after the operation. At the end of the experiment, the animals were euthanized and bone biopsy was taken from all animals of both groups from the medial side of the tibia. Two cortical screws served as landmarks for the correct location of the osteotomy site. A full thickness section of bone was removed by oscillating saw at the osteotomy site.

Sections of the bone were fixed for histopathological evaluation with 10% buffered formalin solution, decalcified in the Sakura TDE™ 30 Decalcifier System with Decalcifier Solution (Code 1428; Sakura, Alphen aan den Rijn, the Netherlands) during 3 days, and embedded in paraffin with vacuum infiltration processor (Tissue-Tek® VIP™ 5 Jr; Sakura, San Francisco, CA, USA). Specimens were cut with microtome Ergostar HM 200 (Microm, Walldorf, Germany) at 7 µm thickness and stained using the haematoxylin-eosin (H&E) and van Gieson methods for general orientation to sections. Sections were examined using the Zeiss Axiophot-2 microscope (Zeiss, Munich, Germany).

4.1.4.4. Immunohistochemistry

Collagen I, collagen V, osteocalcin and osteopontin were evaluated in bone tissue immunohistochemically. 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 the 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 I (diluted 1:100, Abcam, UK), collagen V (diluted 1:100, Abcam, UK), osteopontin (diluted 1:1000, Abcam, UK) or osteocalcin (diluted 1:500, Abcam, UK) overnight at 4°C. On the next day, 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. Then the sections were incubated in DAB solution (VECTOR Laboratories, USA) for 4 min at room temperature, rinsed in the PBS and cells nuclei were counterstained with hematoxylin. Sections were dehydrated through graded ethanols, cleared in xylene and mounted with Eukitt (Fluka, Switzerland). For osteopontin detection in the tissues, polyclonal antibody to osteopontin (ab14715, Abcam UK), diluted 1:1000, was used. A shorter version of the above-described protocol was applied, which includes incubation of sections with antibody to osteopontin during 45 min at room temperature. Staining intensity of collagen I, collagen V, osteonectin, osteocalcin and osteopontin was expressed by a subjective scale ranging from 0 to 3 (0 – no staining, 1 – weak staining, 2 – moderate staining, 3 – strong staining). Immunohistochemistry negative controls were performed by omitting the primary antibody. Two independent observers in a blinded fashion performed the evaluation and their scores were averaged.

4.2. Mechanical testing of the fixator (unpublished data)

In order to verify the stability of the construction of the RTP fixator mechanical tests were performed. The mechanical load bearing tests of the RTP fixator were performed at the Laboratory of Metrology at the Tallinn University of Technology. Deep frozen tibiae of one-year-old sheep were used. The bones were thawed to room temperature before the tests. The average length of the bones was 200 to 210 mm, and the mid-diaphyseal cross-sectional measurements were 14 x 18 mm, respectively. The cortical thickness of the diaphysis was 3.0 mm. Bending and rotation tests were performed in the course of the trials.

The first bending test was performed using a whole tibia, without applying the fixation device. Tibial osteotomy was used with the rest of the tests in which the fixation device was applied. The fixator was placed on the medial side of the bone. Osteotomy was performed in the middle of the diaphysis. Two osteotomy profiles were used. In the second test, osteotomy was performed crosswise to the anatomical axis of tibia. In the third test wedge-shaped osteotomy was performed, whereas the wedge-shaped opening in the bone was on the lateral side. 50 x 50 mm metal blocks were placed on both ends of the tibia, and fixed to the bone by using methyl methacrylate. The axis of the bone was in the centre of the block. The sides of the block were fixed parallel to each other.

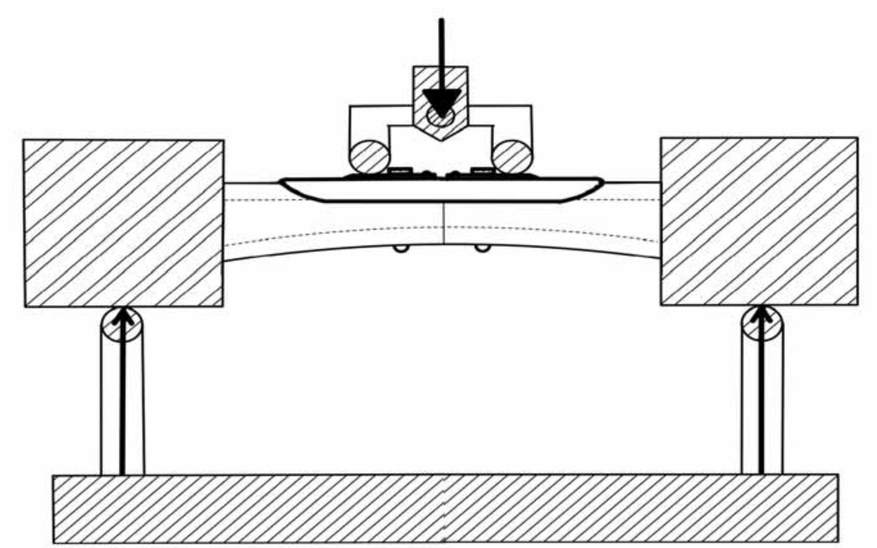
The bending test was performed by using the INSTRON 8802 Servohydraulic Fatigue Testing System. The four-point test was used. The load points were selected so that the distal points were located in the centre of the blocks while the proximal points were placed randomly, considering that the load in both points was on intramedullary lamella tails. The distal load points were placed 90 mm from the centre of osteotomy and the proximal load points were located 20 mm from the centre of osteotomy. In all bending tests the location of load points was similar. Uniformly distributed maximum load on the device was applied by gradually increasing the loads. Strength test of the unbroken bone was performed at 6 mm/min, and that with the fixation device at 3 mm/min. The load was increased until the total failure or destruction of the device.

The testing system registered the changes in the device that had occurred due to the load. Charts and tables were electronically created to reflect the changes. Based on the chart the yield point and the point of deformation or complete failure were determined. Also, displacement at the yield point and complete failure were determined. According to the obtained parameters stiffness of the device was calculated. The first test was conducted using an intact (unbroken) tibia of sheep without osteotomy.

In the course of the second test the direction of force vector was towards the fixator side of the bone. The fixator was pressed against the bone. The proximal load points were seated on the tails of the rods of the fixator adjacent to the cortical screws (Fig. 9).

The third tests was carried out under the conditions, where using the tails of the rods, the fixator was pulled farther from the bone. (Fig. 10).

Rotation tests were performed using a mechanical testing device KM-50-1, N81, 1967. The principle of fixing the bone in the blocks was the same, whereas extra metal rods were founded in both ends of the blocks into the longitudinal axis of the bone. These rods were used to fix the device in a vertical position in the testing system. The upper end was fixed stiffly while the lower end was moveable, which made it possible to turn the device along the longitudinal axis of the bone (Fig. 11).



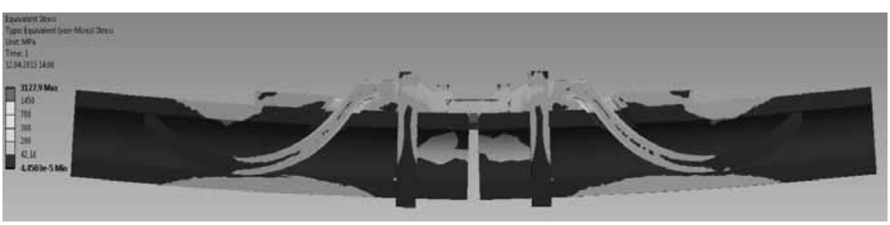


Figure 9. Points of application of force (above) and distribution of load to the construction (below).

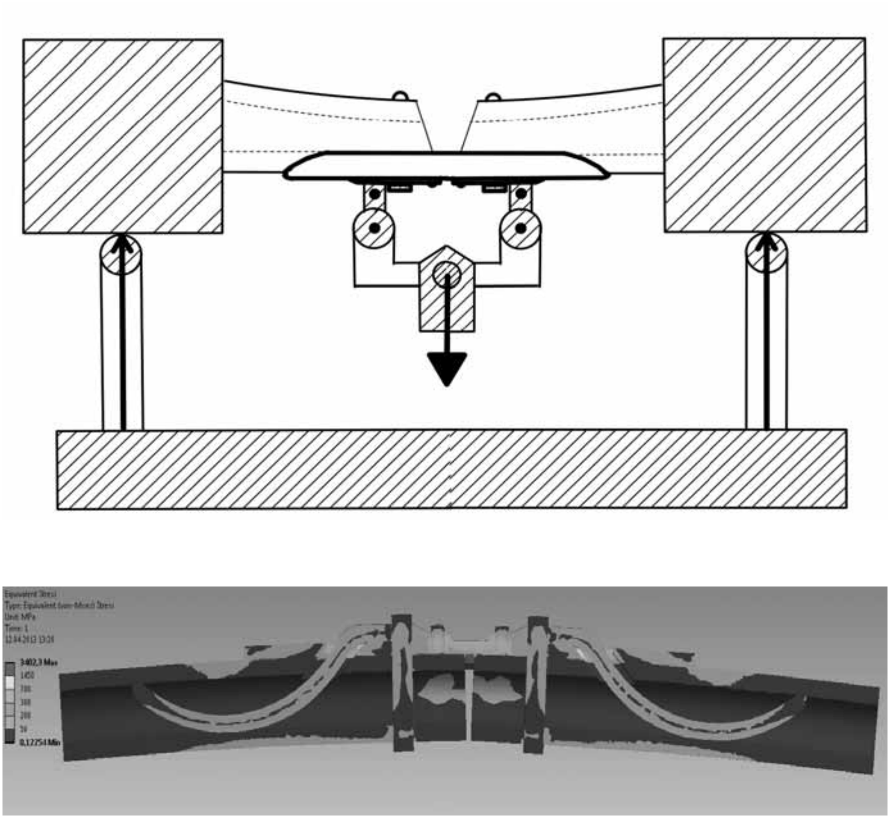


Figure 10. Points of application of force (above) and distribution of load to the construction (below).

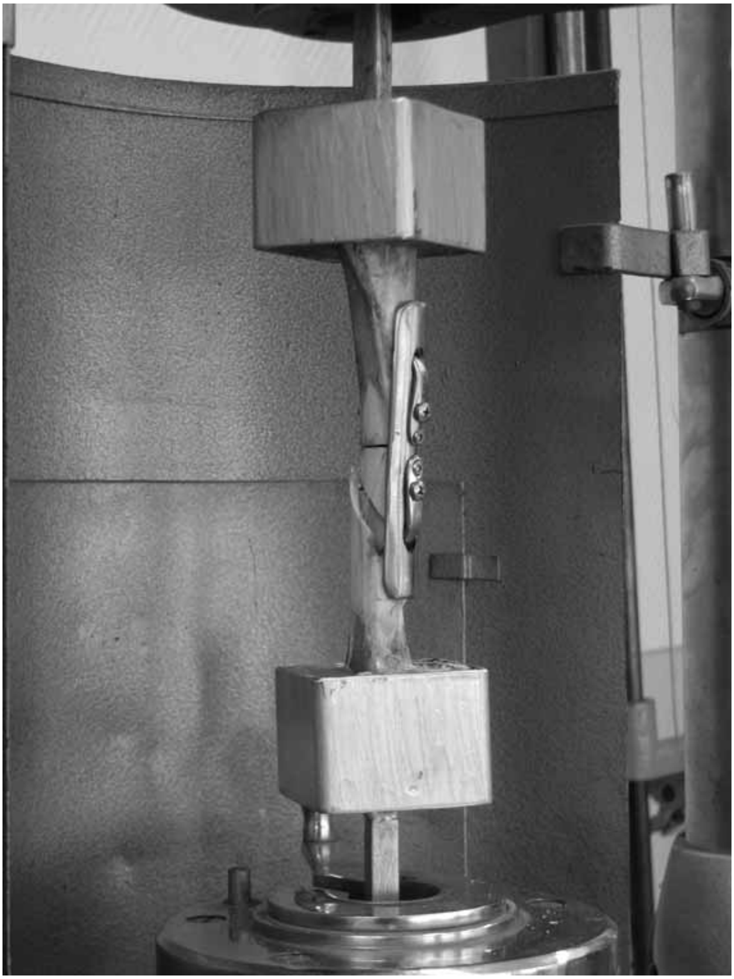


Figure 11. Rotation test.

4.3. Clinical study

4.3.1. Clinical patients

A total of six dogs, with transverse or short oblique diaphyseal fractures of the femur or tibia, were treated between the years 2000-2002 in the Small Animal Clinic of the Estonian University of Life Sciences, using the combined osteosynthesis technique. The median age of the dogs was 4.5 years. Two dogs were female and four male with body weight ranging between 24 and 58 kg. All bone fractures were caused by trauma: motor vehicle accidents in five cases and falling from a height in one case. Fractures occurred in the middle third of the femoral (two patients) or tibial (four patients) diaphyses. In one case some small fragments were seen, which were left untouched. In one case two supplemental full cerclage wires were used because of small fissure lines in the distal fragment.

4.3.2. Operating technique and follow-up

Before surgery the size of the plate and rods were chosen; the choice was determined by the size of the bone on the radiographs and the plate was accurately contoured to the shape of the bone. The rods were also selected according to the radiographs; they were to correspond to the diameter of the medullary canal and extended to their maximal distance proximally and distally according to the radiographs. The operative procedure required anaesthesia. This was induced by medetomidine hydrochloride (Domitor 1 mg/ml) 0.04 mg/kg/i.m. and Ketamine (Ketanest® 100 mg/ml) 4 mg/kg/i.m. Approaches to the bone were performed as described by Piermattei D. L. (Piermattei, 1992). Adequate exposure of the injured bone was performed, preserving all the surrounding muscle and tendon insertions. The fracture was reduced and the plate was applied and held in position by two bone holding forceps. The plate was conformed to the bone contour if necessary, using plate benders. The forceps holding the bone were placed on the plate and the bone near the holes, in this position they did not hinder the following manipulations. A hole was drilled through the near cortex of one bone fragment with the drill bit entering the bone at a 45° angle to its longitudinal axis. The channel in the plate served as a guide, ensuring the correct angle and position of the hole. The end of the rod was held with forceps and the rod was gently hammered through the plate and cortex into the medullary cavity until the tail of the rod met the plate. The second rod was inserted in a similar way into the other fragment. If necessary, each rod was adjusted with plate benders so that it could pass freely through the hole and a slight tension was created in the rod in the medullary cavity. The tail of the rod was placed on the plate so that the holes were aligned. Through them the screw hole was drilled with a 3.2 mm drill bit. The hole penetrated both cortices. The length of the hole was measured with a depth gauge and the appropriately sized self-tapping type screw was inserted using a screwdriver. As the screws were tightened, the intramedullary rod began to act like a spring, providing pressure onto the plate. This created the "forceps-effect" between the plate and rod, fixing the device to the bone (Fig. 12). Routine closure of the wound was performed. Postoperatively a protective bandage was applied for the first three to five postoperative days to prevent swelling of the surgical wound. No supplementary external coaptation splints or bandages were used. The dog was allowed to put weight on the affected limb on the following day; restricted activity on a leash was recommended for the following two weeks. Amoxicillin 15 mg/kg b.i.d. was administered orally for five days after the operation. Carprofen (Rimadyl 50 mg/ml) 2 mg/kg s.i.d. was injected subcutaneously for three days postoperatively as an analgesic. The reduction, alignment, the axis of the bone and fixator positioning was confirmed radiographically immediately after surgery. The projections were mediolateral and anteroposterior. Case 1 was examined radiographically 11 days after operation, because of new trauma and worsening of the limb function. All animals were returned 10 days postoperatively for suture removal and clinical examination. Clinical re-examination was performed again at seven and 12 weeks after the operation. The functional status of the limb was recorded. Clinical and radiographical evaluations were performed between week 7 to 18 months after surgery before implant removal.

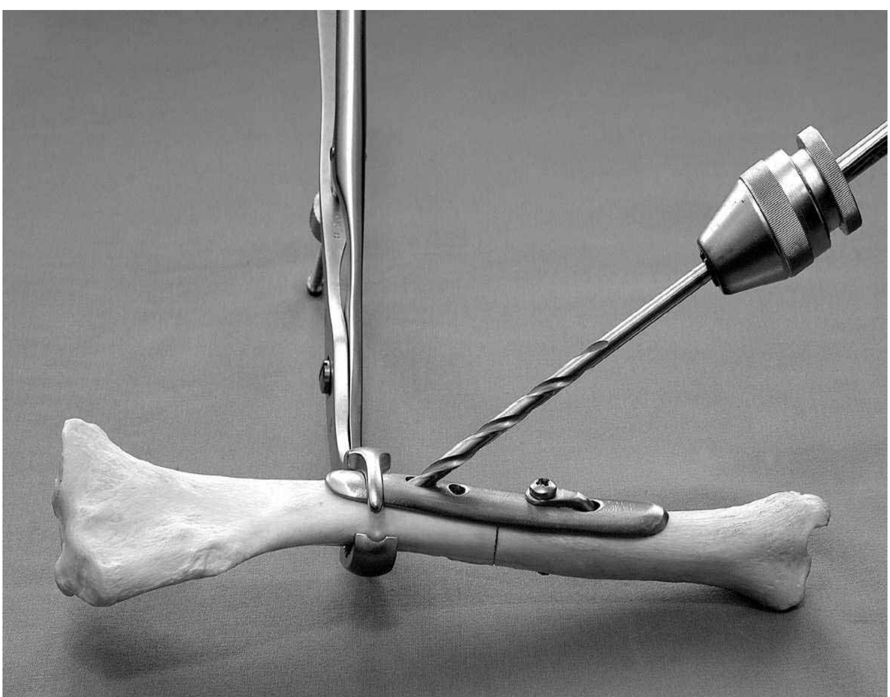


Figure 12. Fixator used on clinical cases.

4.4. Statistical analysis

In paper IV staining intensity of collagen I, collagen V, osteonectin, osteocalcin and osteopontin was 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 and their scores were averaged. and data were tested nonparametrically using the Mann-Whitney *U*-test (GraphPad Software, Inc, San Diego, CA, USA).

The Mann-Whitney test (STATA 10.1 Statacorp, Texas, USA, 2009) was used to determine whether differences existed between the two experimental groups in the median amount of visible callus, in the change of visible callus amount during follow up period (2 week result minus 10 week result) and in the amount of callus between investigation weeks.

5. RESULTS

5.1. Experimental study

In an experimental survey of seven animals it was found that, with the operative technique described, it is possible to attain good postoperative results. No complications after the surgery on the experimental animals were detected. Postoperative swelling of the operated leg was minimal and no clinical problems were noted. In the RTP group, animals started to load body weight on the operated limb the next day after the surgery and in the plate group, animals started to use the operated limb on the seventh day. After two weeks post operation minimal lameness was noted on both groups of animals.

5.1.1. Roentgenological evaluation

Examination of postoperative radiographs showed clearly visible osteotomy lines. In the RTP group two weeks after the operation periosteal callus formation was seen on radiographs, and after four weeks massive callus formation was noted. After six weeks the osteotomy line was still visible, but disappeared on the eight-week radiographs. No implant loosening was noted on radiographs during the experiment. In the plate group, callus formation was minimal and was seen on radiographs starting from week 4. A gap between the fragments was visible even after 10 weeks, at the time of implant removal (Fig. 13, 14).

Extensive callus formation in the RTP group and modest formation in the plate group were also confirmed by measurements of the height of callus on radiographs (Table 1).

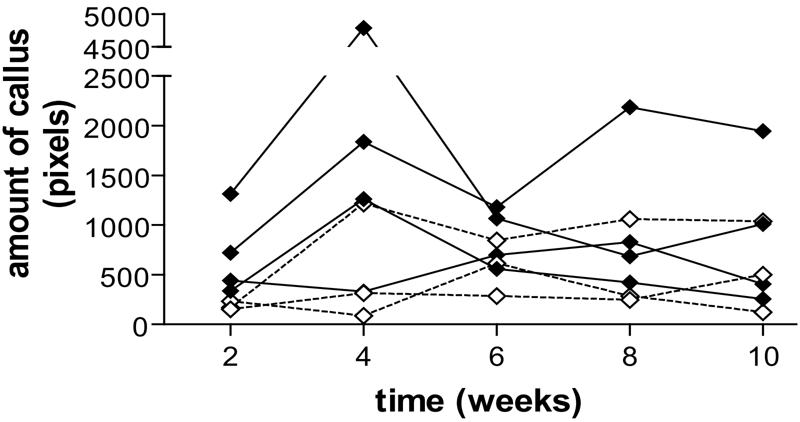


Table 1. Dynamics of callus formation in both groups. RTP experimental group (*n*=4; ♦) and plate group (*n*=3; ◊).

Figure 13. Bone healing in experimental animals with RTP fixator.

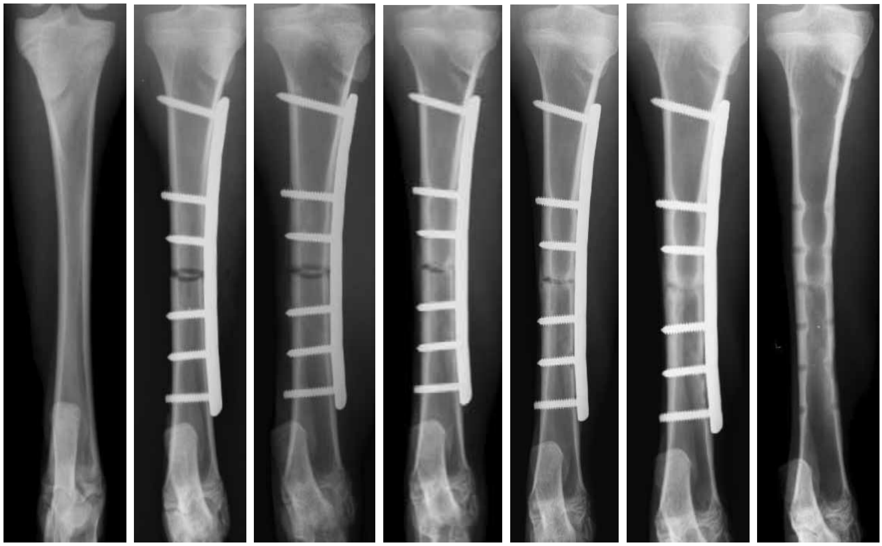


Figure 14. Bone healing in experimental animals with plate fixation.

5.1.2. Biochemical analysis of blood

Serum total protein level was within normal limits and statistically identical in the animals of both groups during the experiment. The C-reactive protein level also remained within normal limits and constant in both groups of animals throughout the whole experiment. However, difference was found in serum ALP levels between the test groups. Serum ALP levels of the animals of the RTP group had increased by week 2 after surgery, and thereafter ALP started to decline gradually, reaching the preoperative level by week 8. Serum ALP levels of the animals of the plate group, however, had decreased by week 2 after surgery and remained at a low level during the observation period (Fig. 15).

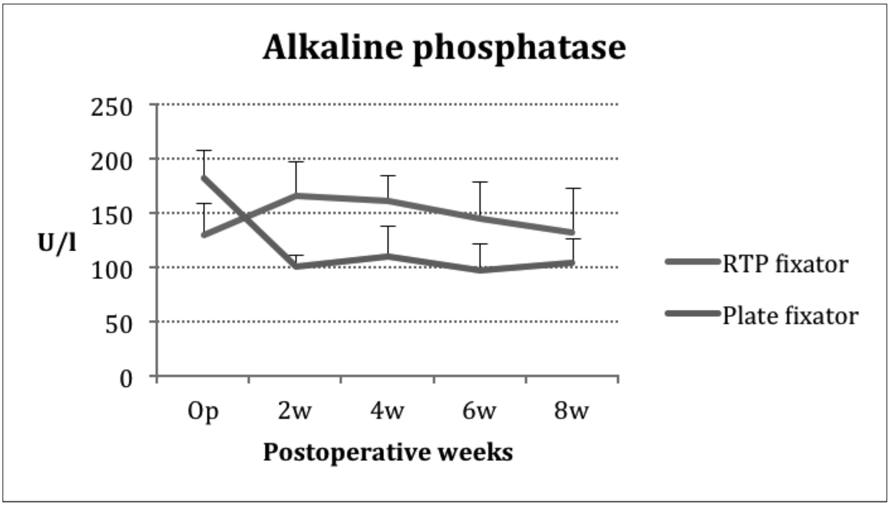


Figure 15. Levels of serum alkaline phosphatase in experimental groups (RTP and plate groups); Op – values on operation day, on day 14 (2w), day 28 (4w), day 42 (6w) and day 56 (8w). $P < 0.05$ 2w RTP group versus plate group.

On the other hand, osteocalcin levels remained constant in both groups of animals throughout the whole experiment (Fig. 16).

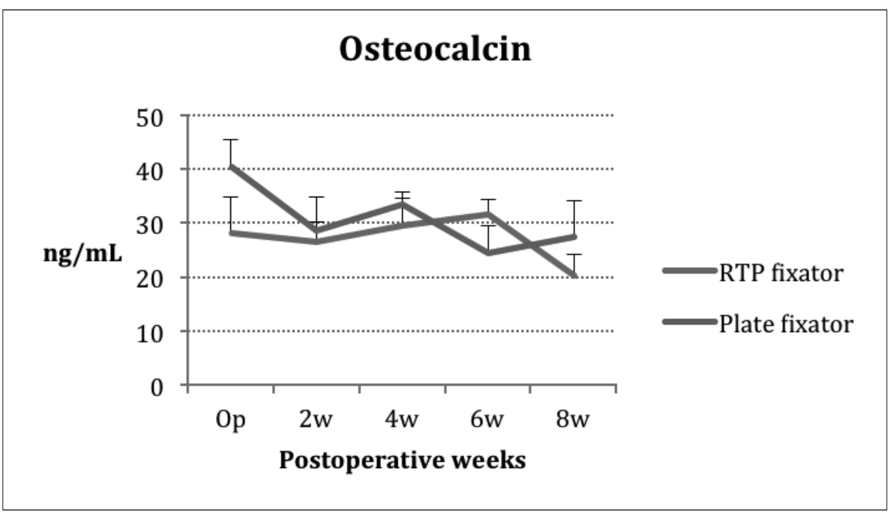


Figure 16. Levels of osteocalcin in experimental groups (RTP group and plate group); Op –values on operation day, on day 14 (2w), day 28 (4w), day 42 (6w) and day 56 (8w). Differences between groups are statistically not significant.

5.1.3. Histology

On week 2, bone biopsy was taken from one experimental animal in the RTP group (Fig. 17A). In this sample, there was no remarkable widening of bone channels. In the cambial layer of the periosteum, one or two rows of osteoblasts were seen; the fibrous layer was thickened and dense. Periosteal callus and intermediate callus were formed by fine bone trabeculae that had mostly developed from the connective tissue. However, in the intermediate callus, endochondral ossification was obvious with a smooth transition from the cartilage tissue to the bone. From the side of the periosteum, callus was lined by young bone tissue into which strands of loose connective tissue invaded. In deeper layers, a small amount of hyaline cartilage was found. Bone trabeculae were differently sized and scantly mineralized (contained young and large-sized osteocytes). The clear predominance of cells in the cells to matrix ratio revealed that the bone is newly formed.

On week 4, bone biopsy was taken from another RTP group animal (Fig. 17B). In this sample, bone channels were markedly widened. No changes indicating inflammation were found. Periosteum, especially its fibrous layer, was markedly thickened as compared to the week 2 biopsy specimen. Coarse collagen fibres were externally attached to the fibrous layer. Callus was massive and rich in cells indicative of active tissue. Trabeculae of the bony callus were massive, and formation of the bone occurred both by intramembranous and endochondral ossification. In the first case, bone trabeculae were surrounded by a regular chain of osteoblasts, or periosteal cells were involved in osteogenesis. Endochondral ossification in the current experiment occurred in two ways. The first way of ossification was characterized by the lack of a clear border between the hyaline cartilage and the bone tissue. During the bone formation, islets of cell-rich cartilage tissue were entrapped. In the second way, chondrocytes swelled, perished and bone tissue started to form around the areas of preserved ground substance. The main difference in the newly formed bone, as compared to the week 2 biopsy specimen, was the development of the system of canals, which contained connective tissue and blood vessels, that is, the process of regeneration was more advanced, and the set-up of the system of canals was in progress.

On week 10, all animals were sacrificed and osteotomy areas were studied histologically. In the RTP group, normal osteocytes were seen in the bone fragments. Comparing to samples taken on week 2 and 4, the lacunae were less widened (Fig. 17C). Bone canals were enlarged, but widening of canals near the ends of fragments is a normal reaction to the trauma. As a rule, periosteal callus was very voluminous and contained a large amount of young cell-rich connective tissue. Bony callus contained differently sized bone trabeculae, the amount of which seemed to increase. This is indicated by the presence of osteoblasts around the trabeculae. Remodelling appeared to be limited, as no osteoclasts, resorption lacunae or macrophages were found.

Much less periostal callus formation and less connective tissue were seen in the plate group. In the connective tissue of the periostal callus, small bone trabeculae surrounded by chains of osteoblasts were seen (Fig. 17D). Cartilagineous periosteal callus contained hyaline and fibrous cartilage tissue, while in the bony callus, woven bone tissue dominated. Bone trabeculae were very different in size; osteocytes were normal and lacunae more narrow as compared to the RTP group. On the bone trabeculae, chains of osteoblasts were seen. In bony callus, connective tissue was located between the trabeculae; osteoclasts were not present. Connective tissue and canals containing blood vessels had formed into bone trabeculae. In bone trabeculae, osteocytes in different developmental stages were localized. Intermediate and endostal callus contained a large amount of connective tissue; bone trabeculae were clearly expressed, but the few osteoblasts around them were placed irregularly.

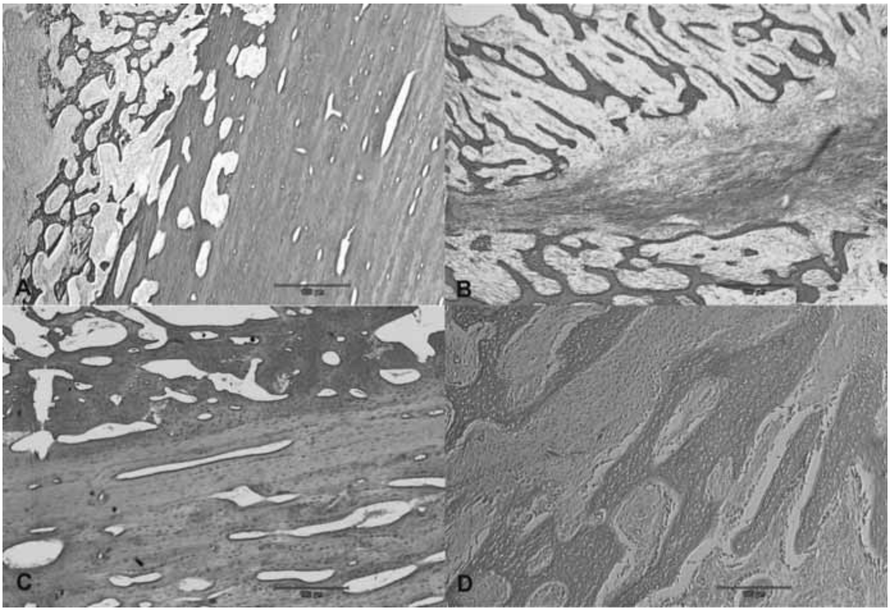


Figure 17. Formation of bone in sheep (RTP fixator: A-wk 2; B-wk 4; C-wk 10; Plate fixator: D-wk10). H&E staining (A, B, D), van Gieson staining (C).

5.1.4. Immunohistochemistry

Content of collagen I, collagen V, osteopontin and osteocalcin was detected in bone biopsies in the RTP group on the second, the fourth and on the tenth postoperative week, and on the tenth post-operative week in plate fixator group (Fig. 20). In the RTP group, the level of the collagen I increased during the follow-up weeks: if on the second post-operative week collagen I content was low (Fig. 18A), then on the fourth week it was elevated (Fig. 18B) and reached its maximum on week 10 (Fig. 18C). In the plate group, collagen I content was statistically lower on week 10 ($P < 0.05$) compared with the RTP group (Fig. 18D). The level of collagen V was low in the RTP group on week 2, but was markedly elevated on week 4 and especially on week 10 (Fig. 18E). In the plate group, collagen V level was statistically lower ($P < 0.05$) on week 10 as compared to the RTP group (Fig. 18F). Osteopontin level was low in the RTP group on weeks 2 and 4 (Fig. 19A, 19B), but on week 10 its level was equally high in both groups of animals (Fig. 19C, 19D). There were no clear differences in osteocalcin level between both groups, though on week 10 osteocalcin level was slightly higher in the RTP group than in the plate group (Fig. 19E, 19F).

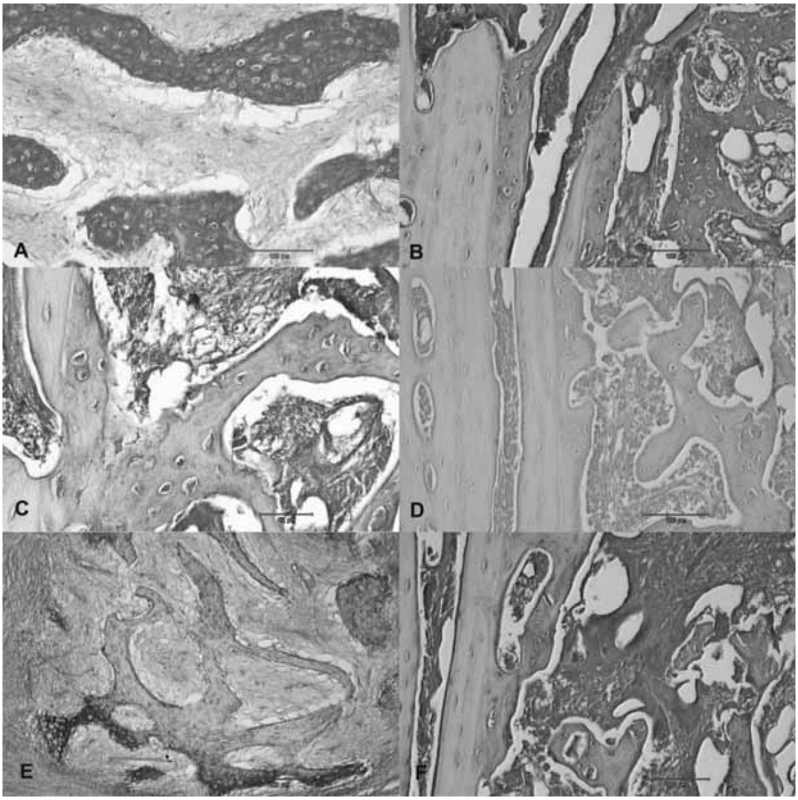


Figure 18. Immunohistochemical detection of collagen I in samples of RTP fixator group: moderaate immunostaining on week 2 (A), string staining of weeks 4 (B) and 10 (C), and moderaate staining in bone plating group on week 10 (D). Strong immunostaining for collagen V in samples of RTP group (E) and plating group (F) on week 10. Staining DAB+haematoxylin.

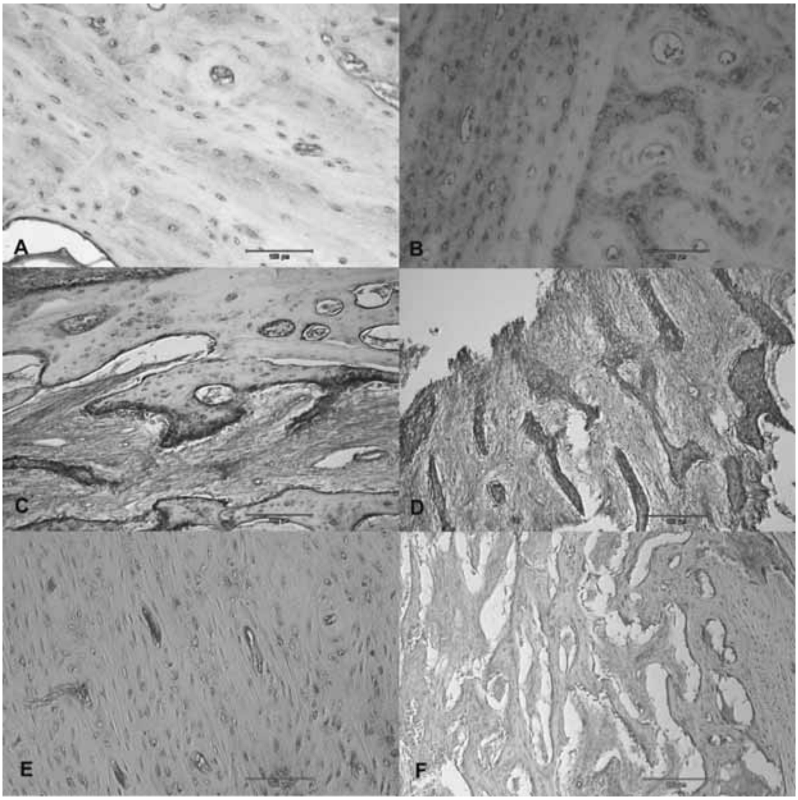


Figure 19. Immunohistochemical detection of osteopontin in samples of RTP fixator group: moderate immunostaining on week 2 (A), weak staining on week 4 (B), stronger staining on week 10 (C), and strong staining in bone plating group on week 10 (D). Strong immunostaining for osteocalcin in samples of RTP group (E) and moderate staining in bone plating group (F) on week 10. Staining DAB+haematoxylin.

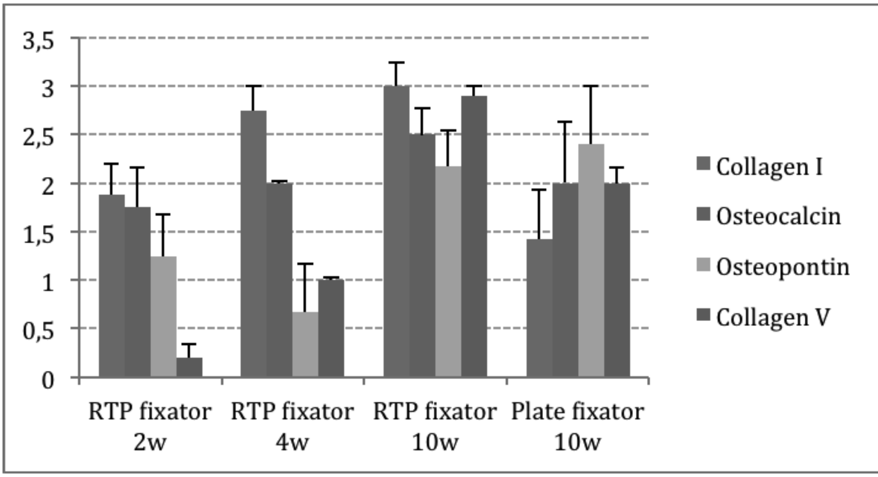


Figure 20. Collagen I, collagen V, osteocalcin and osteopontin immunostaining in samples of fracture area of RTP group animals (RTP fixator) on week 2 (w2), week 4 (w4) and week 10 (w10) or plate group animals (plate fixator) on week 10 (w10). Staining intensity was expressed by a subjective scale ranging from 0 to 3 (0 – no staining, 1 – weak staining, 2 – moderate staining, 3 – strong staining). Statistically significant differences ($P < 0.05$; the Mann–Whitney U-test): collagen I – RTP fixator 10w versus plate fixator 10w; collagen V – RTP fixator 2w versus RTP fixator 4w and 10w; RTP fixator versus plate fixator 10w; osteopontin – RTP fixator 4w versus RTP fixator 10w.

5.2. Results of mechanical testing of the fixator (unpublished data)

The failure load of unbroken tibia was 2.5 kilonewton (kN), and displacement at this load was 9 mm. Fracture of the bone occurred at 3 kN maximum load. Displacement of the bone from its axis was 10.16 mm. The calculated bending stiffness of the bone was 277.78 pressure millimetre (N/mm).

In the second test the failure load was 0.58 kN. Displacement at this load was 3.8 mm. Visible/detectable deformation of the device occurred due to the supporting plate. At the end of the test the load was 1.33 kN that caused the complete failure of the device. At the end of the test the bone broke at the entry point of the cortical screw. The displacement at this moment was up to 28.09 mm. All other elements of the fixing device remained unbroken. The stiffness of the device used in the second test was calculated to be 128.89 N/mm.

In the third test the measured failure load was 0.3 kN. Displacement was 1.75 mm. At the end of the test the bone broke at the entry point of the cortical screw. Visible deformation occurred due to intramedullary bone lamellae. Displacement of the device at the moment of deformation was 10.202 mm. The stiffness of the device was calculated to be 171.42 N/mm.

In the rotation test the bone broke at 245.166 kN/m^2, whereas at this moment the angle of rotation was 47°. The bone broke at the entry point of the intramedullary rod. The components of the fixation device remained without visible deformation until the fracture of the bone.

5.3. Clinical study

The clinical study was performed on six dogs, with transverse or short oblique diaphyseal fractures of the femur or tibia. The fractures healed by secondary bone union with callus formation. In all cases the functional recovery of the operated limb was considered to be good by ten days after surgery. In five cases no complications associated with the surgical technique were encountered, the dogs put weight on the affected limb and no significant muscular atrophy had developed. The fixator did not show any adverse effects on the bone structure on follow-up radiological evaluations, and remained in the same position in which it had been applied. In one case proximal screw migration occurred due to a new trauma to the limb. The curved rod changed its positioning a little in the medullary canal, but maintained its function as a fixative agent. The device was not removed, and no additional immobilization was used. The limb showed good function, fracture displacement did not occur, the alignment remained unchanged, and the callus was more massive (Fig. 20).

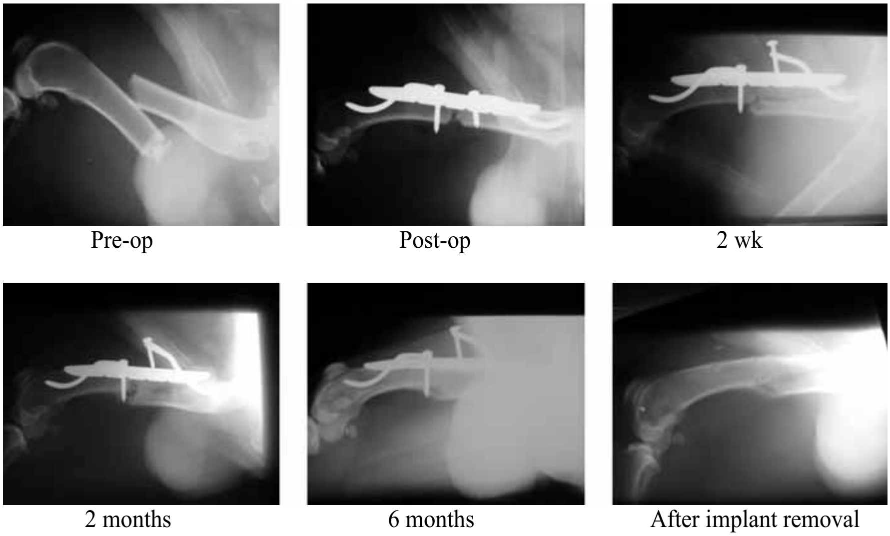


Figure 21. RTP fixator on dogs femur. Proximal screw loosening and fissure in fragment.

The fixator was removed from four to 18 months after surgery. No complications were noted after removal. Clinical and radiographic control and time of the removal of the fixator was very much dependent on the patient owners (Fig. 21).

Figure 22. Bone healing with RTP fixator.

6. DISCUSSION

Healing of bone fractures is affected by several factors (Tsiridis et al., 2007), but the stable fixation of bone fragments and the preservation of blood supply to the bone are the most important factors (Hulth, 1989).

In this study we tested an original rod-through-plate (RTP) fixator for stabilisation of the fractures of long bones both in experimental conditions and clinical cases. In the experimental part tibial osteotomies were performed on sheep. During the following treatment for comparison either the RTP fixator or conventional bone plating was applied (Papers I, III, IV). In the clinical part the RTP fixator was used to treat transverse or short oblique diaphyseal fractures of tibia or femur of dogs (Paper II).

The idea of the design of the RTP fixator is to reduce surgical exposure to the bone when compared to the conventional plates, and at the same time to increase the lever arm of the fixator proximally and distally. Furthermore, the design of the RTP fixator gives stable fixation with adequate rigidity and maintains enough axial micromovement between the fragments to stimulate callus formation. In the RTP fixator the support plate, which is placed on the bone is relatively short, while intramedullar rods lengthen the shoulder of the fixator proximally and distally up to the metaphyses. When compared to the conventional bone plating, the installation of this RTP fixator does not require extensive surgical access, thus reducing the time of the operation and also reducing traumatization of tissue, which ensures optimal conditions for the healing of the fracture. The technique enables stable and dynamic fixation of the fragments. In construction, the intramedullary parts of the fixator are curved and exert dynamic pressure on the bone cortex at three points: at the end of the rod, at the centre of the rod arc and at the point of rod and plate union. The fixator exerts a continuous dynamic force and transfers the load effectively in the axial direction, which is similar to Rush pin fixation. The rods are not too tight in the medullary cavity and do not pass through the fracture site intramedullary. This reduces disruption of the medullary blood supply which is considered as a critical factor in fracture fixation (Anderson et al., 1962; Wilson, 2002). The curved rods are oval in cross-section, which provides resistance to rotation. Thus no additional fixation device is necessary. The resistance of the RTP fixator to rotational force became aslo evident in our metodological mechanical testings. The curved rods extend from the fixation points proximally and distally in the medullary cavity, thus minimizing mechanical load at the fracture site, and improving the conditions for bone tissue regeneration. The same principle was used by Seppo and his coworkers in their fixator (Seppo et al., 1979).

The extramedullary part of the fixator is applied to the bone in the same manner as conventional bone plates. The difference is that the connecting plate is relatively short and only two cortical screws are needed, one for each bone fragment. The screws are inserted according to standard AO principles (Brinker et al., 1984). Fewer screws enable preservation of bone tissue from excessive pressure to the periosteum caused by screw tightening and minimize the rigidity of the fixation device. As the screws are tightened, the intramedullary rod begins to act like a spring, providing pressure on the plate. This creates the "forceps-effect" between the plate and rod, fixing the device to the bone. At the same time, it is not totally rigid and the elasticity of the rods allows micromovement at the fracture site when the limb is being used. It is widely accepted that small axial micromovement will stimulate callus formation, which gives more external support at the fracture site (Akeson et al., 1980; Claes, 1989; Goodship and Kenwright, 1985; Terjesen and Johnson, 1986; Wu et al., 1984). Callus formation is not the purpose of the procedure by itself, but it clearly shows the presence of instability of fragments and the formation of bridging external callus helps to increase the stability of fragments, by increasing cross section of the fracture site (Perren, 1979). With callus formation, more axial loading is transferred from the fixator alone to the centre of the bone and helps to resist fracture forces. The phenomena of stress protection, osteopenia and refracture following plate removal as the long-term consequences of too rigid stabilization techniques are ruled out.

The new system of fixation introduced here improves the methodologies of fixation and allows the use of a different principle of fixation which has its positive qualities. Furthermore, mechanical testing of the RTP fixator where bending and rotational forces were applied demonstrated strength and stability of the construct. The use of the RTP fixator allows an increase in the shoulder of the device and avoids extensive damage to the surrounding soft tissue. At the same time, thanks to three support points (intramedullar canal), which form a triangle, the possibility of rotation is excluded. The structure of the fixation device, which forms an anchor system and in which the fixating powers are aimed at compression, not tearing, ensures stronger fixation. The fixator is limited to use on long tubular bones and in the case of transverse or short oblique fractures in the middle third of the bone. The fixator has been clinically tested on animals with femoral and tibial fractures (Paper II), and experimentally on tibial osteotomies on sheep (Papers III and IV) and rabbits (Andrianov et al, 2007), as it is easiest to apply the device to these bones. The standardized operation technique, step-by-step application of the RTP fixator has been described in Paper III.

In a clinical study on dogs (Paper II) no additional fixation devices were used, and no restrictions to the activity of dogs were made. Small fragments between fracture line or small fissure lines in the fragments were not limitations for fixation with RTP fixator. The follow-up radiographs were made before implant removal surgery. Complete union and callus formation was seen at the fracture site by the time of the implant removal. It was impossible to make follow-up clinical and radiographical examination on clinical cases because of the dog owners very poor cooperation. Actually, the only clinical examinations were at the time of suture removal and when the dogs were brought in for implant removal surgery. The exception was one dog with sudden lameness two weeks after surgery, caused by proximal screw loosening. This case showed to us a possible weak point of the construct design and therefore a small modification was made (modification includes addition of locking screws, what is discussed below).

In an experimental study the speed of regeneration of the long tubular bone osteotomies was evaluated using the new RTP fixator and this was compared with bone plating without compression at the osteotomy site. A comparative histological and immunohistochemical study of the newly formed bone was carried out (Paper IV). Some blood serum markers reflecting bone regeneration were determined (Cox et al., 2010; Oni et al., 1989; Nyman et al., 1991). All surgeries were performed by one surgeon to guarantee similar conditions of the experiment. Also, implants were removed and bone specimen were taken by single surgeon. The new RTP method of fixation reduced the interfragmentary strain by intra- and extramedullary fixation and gave enough stability for fracture healing. In this experimental part, the RTP fixator had a modification with the addition of one locking screw on each rod to fix the intramedullary rods to the plate. Cortical screws can become loose and this causes loosening of the rod, and instability at the fracture site, as discussed above in one case in the clinical study on dogs and as described previously in experiments on rabbits (Andrianov et al., 2003). Locking screws were inserted through holes in rods and fixed to the plate, which has threaded holes in it. Locking screws are short enough not to penetrate the surface of plates contacting the bone. This type of design should maintain stability at the fracture site even in case of cortical screw loosening. Thus, the purpose of the RTP fixation is to reduce the traumatization of the soft tissues during the operation, guarantee the stability of fractured fragments and to allow unlimited weight bearing by the animal immediately after surgery. On clinical examination of experimental animals, no differences were noted between the groups - with plate vs. RTP fixator, except that in the RTP group animals started to load body weight to the operated limb much earlier as compared to the Plate group. As seen on radiographs, more massive callus formation was found in the RTP group. The bridging callus formation was visible much earlier on the radiographs, which indicated more rapid bone healing and provided the opportunity for earlier implant removal. Through for statistical analysis the sample size was small, our results indicated some differences in callus formation between the two groups. In the RTP group, as compared to the Plate group, callus formation was more intensive throughout the whole follow-up period. In particular, on radiographs after two weeks from the operation strong callus formation in the RTP group was seen, while in the Plate group it was undetectable (Table 1). Increase callus formation in the RTP group is also confirmed by histological studies. By the end of the second week, histological findings of the bone biopsy of the RTP group samples matched the normal bone healing process (Murray et al., 1996). At that time, two stages (destruction and inflammation) of the bone healing were completed, and intense tissue regeneration and callus formation were seen on histological examination. Formation of fibrous connective tissue together with a smaller amount of cartilage tissue and young bone tissue was seen. After 4 weeks, the regeneration process was in progress, and in the newly formed bone, the characteristic formation of the canal system was noted together with the increasing number of cells (McKibbin, 1985). Lack of processes typical of inflammation can be considered as a positive result, which is confirmed by low C-reactive protein levels. After 10 weeks, a small amount of cartilage tissue was seen in the RTP group, which indicates a normal ossification process (Ekholm, 2001). A large amount of avascular cartilage tissue can lead to the formation of the fracture non-union (Harrison et al., 2003). As seen on radiographs and also histologically, a large amount of periosteal callus was found on week 10. At the same time, in the Plate group, the amount of callus was modest. The large amount of callus is obviously produced by the construction principles of the RTP fixator, where the elasticity of the rods ensures micromovement between the fragments, which stimulates callus formation and thus bone healing (Goodship and Kenwright, 1985; Jagodzinski and Krettek, 2007). If in the early stages of bone healing micromovements between bone fragments stimulate callus formation, then in the later stages in calcifying callus movements should be minimal, but required stability is achieved naturally by increasing ossification of the callus (Jagodzinski and Krettek, 2007). Although the levels of blood serum markers reflecting the process of bone regeneration fluctuated and the sample size was small, the dynamics of these parameters generally correlated with the morphological findings. While no changes were found in the serum total protein level in the Plate group during the post-operative weeks, then in the RTP group, the levels were elevated on week 2, followed by a gradual decline during the next weeks. As deviations were high, the results were not statistically significant (Fig. 15). Clear dynamics could be seen in differences of the alkaline phosphatase (ALP) content. In the Plate group, the levels of serum ALP decreased on week 2 and remained at the same level during the rest of the experiment. On the contrary, in the RTP group, ALP levels were elevated on week 2 and decreased during the following weeks, reaching pre-operative levels on week 8. On week 2, serum ALP differences between the groups were statistically significant (Fig. 15). As the ALP is considered a parameter of intensity of bone formation, then this marker also refers to more intensive ossification in the RTP group compared to the Plate group. A similar increase in the level of serum ALP in sheep after experimental osteotomy during post-operative weeks 2–4 with a decline in the following weeks has been described by Klein et al. (2004). The same dynamics of serum ALP level has also been reported in humans after femoral fractures– an increase during the second and the third week and a decrease during the next weeks (Ikegami et al., 2009). On the other hand, no changes between experimental groups were found in serum levels of osteocalcin (Fig. 16), another bone formation marker (Cox et al., 2010), although a declining tendency as compared to pre-operative levels was seen in both groups on week 8. A serious deviation of parameters reflecting bone formation in sheep has also been reported by other investigators (Klein et al., 2004). The results of immunohistochemical investigation of bone biopsy samples indicated differences in the intensity of bone formation between the two experimental groups. The levels of collagen I, the main component of the extracellular bone matrix, were relatively high in the RTP group on week 2 already, but still increased on week 4 and reached their maximum level on week 10. In the Plate group, the content of collagen I was significantly lower on week 10 as compared to the RTP group (Fig. 18). The results of collagen V immunostaining were relatively similar. Collagen V is a component of the bone matrix, which is found in a significantly smaller amount than collagen I. Collagen V is considered to regulate the formation and orientation of type I collagen fibres (Birk, 2001). In the RTP group, the level of collagen V was very low on week 2, but on week 4 the levels elevated and on week 10 the collagen V content was almost equal with collagen I. In the Plate group, the content of collagen V remained low, thus followed the levels of collagen I (Fig. 18). Similarly to the osteocalcin levels in blood serum, no differences were found in immunohistochemical staining of osteocalcin in biopsy samples from the RTP group. On week 10, osteocalcin immunostaining intensity was not different between the two experimental groups either (Fig. 19). The tissue content of osteopontin, on the other hand, showed some differences. Osteopontin is one of the main non-collagen proteins of the extracellular matrix of the bone. The elevation of its level has been described during bone remodelling and mineralization (Yamazaki et al., 1999). In our study, an increase in osteopontin level on week 10 as compared to week 4 was noticed in the RTP group. At the same time, no considerable differences in the osteopontin level between the RTP group and the Plate group were seen on week 10. (Fig. 19). A similar increase in the content of osteopontin, but also of osteocalcin, has been described in rabbits 8 weeks after femoral osteotomy (Andrianov et al., 2007). From our experimental study it can be concluded that application of RTP fixation is more efficient in early mobilization and faster bone regeneration than conventional bone plating in the treatment of experimental osteotomies of long bones in sheep: in the RTP group, consolidation of fractures was faster, as demonstrated by radiographical, histological, and immunohistochemical investigations and in part by blood serum markers for bone formation.

To reduce perioperative problems and operation time during the application of the RTP fixator, preoperative planning is a very important step as emphasized in Paper III, where step by step fixator placement has been described. From preoperative radiographs plate length should be selected and also should be prebent to fit the countour of the bone. Also, it is necessary to choose the curved rods length such that they do not penetrate the bone ends and articular surface either proximally or distally. Rods should be also prebent as close as possible to the curve such that it allows the centre of the rods arch to be in contact with the inner surface of the bone on one side and the tip of the rod to the opposite cortex. Minor corrections of plate and of rods can be made during the operation. The most difficult part of the fixator placement is the insertion of the intramedullary rods. It is possible to bend the rods during the operation in either direction or cut them shorter as necessary. Removal of the fixator does not require as much access to the bone as traditional plates, thus reducing operation time and preserving tissues. Because of the conical form of the intramedullary rods the removal of the rods is very easy.

Thus, the design of the RTP fixator is simple and its use in veterinary medicine expands the possibilities for the fixation of long bone fractures in small animal surgery. As the use of RTP fixator is simple no special training is required for the surgeon but some experience is of benefit, especially for rod insertion. Basic osteosynthesis instruments were used for the experimental and clinical surgical procedures, and therefore in our experience special instruments for the fixator placement are not needed.

7. CONCLUSIONS

In our experimental study on sheep we compared an RTP fixator with plate fixation in tibial osteotomies for bone healing radiographically, and limb function clinically. In the clinical section we tested the suitability of the RTP fixator for traumatic long tubular bone fracures in dogs.

1. On clinical examination function of the leg was good in all experimental animals and was similar with both types of fixation. However, animals with the RTP fixator started to load body weight to the operated limb much earlier as compared to animals, where osteotomies where treated with conventional bone plating. With radiographs callus formation was evaluated and measured on all experimental animals and compared with both types of fixation (RTP and Plate). From radiographs, more intense callus growth was seen in the RTP fixator group after two weeks leading to more rapid fragment stabilisation. Histological studies also demonstrated bone mass increase due to new tissue formation (Papers I, III and IV).
2. In clinical cases no surgical or other complications were seen. The exception was one dog with femoral fracture, who had proximal screw stripping resulting in intramedullary rod dislocation. Improvement of the RTP fixator design was required. Limb function was good in all cases. In all operated bones strong osseus union was achieved between fragments. There were no abnormalities noted after implant removal (Paper II).
3. Using standard instrumentation for osteosynthesis we developed a standardized technique which is described for fixator placement for midshaft bone fractures in long tubular bones (Paper III).

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9. SUMMARY IN ESTONIAN

Kombineeritud metalloosteosüntees pikkade toruluude murdude raviks väikeloomadel

Sissejuhatus

Luumurde on inimestel ravitud juba sajandeid. Peamiseks ravimeetodiks oli jäseme väline immobiliseerimine erinevast materjalist lahasega. Alates 18. saj keskpaigast on aseptika ja anestesioloogia arengu ning röntgenikiirte avastamise tõttu toimunud kiire areng ka ortopeedias ja traumatoloogias.

20. sajandi neljakümnendatel aastatel formuleeris Küntscher esmased intramedullaarse e. üdiõõnesisese naelastamise põhitõed (Küntscher, 1958). Tänapäeval kasutatakse luumurdude fikseerimiseks kõige enam luuplaate. Sellist plaatfiksaatorite e. metallist luuplaatide süsteemi kirjeldasid esimestena A. Lane ja A. Lambotte (Broos ja Sermon, 2004). Surve tekitamist murdunud fragmentide vahel luumurru ravis kirjeldas esmakordselt Danis (Danis, 1979). Viiekümnendatel aastatel sõnastas Sisemise Fiksatsiooni Uurimise Assotsiatsioon n.n AO/ASIF-i grupp Šveitsis sisemise fikseerimise põhitõed, osutades uusimatele saavutustele luude füsioloogias, metallograafias ja biomehhaanikas (Rosen, 1984; Allgöwer ja Spiegel, 1979). Veterinaarkirurgias loodi luumurdude fikseerimiseks implantaatide süsteem. Kõige olulisemaks peeti murdunud luu otste head anatoomilist paigaldamist ning võimalikult jäika ja tugevat kinnitamist. Viimastel aastatel on hakatud rohkem tähelepanu pöörama nn bioloogilisele osteosünteesile. Tänapäeval pannakse rohkem rõhku traumeeritud luu verevarustuse säilitamisele, fragmentidevaheline ideaalne anatoomiline repositsioon ei ole enam nii primaarne. Liigespindade anatoomiline repositsioon on oluline vaid liigesesiseste murdude fikseerimisel. Luumurdude ravimise metoodika valik sõltub konkreetse murru iseloomust ja murru asukohast.

1979. aastal kirjeldas meditsiinidoktor Arnold Seppo omaloodud reponaator-fiksaatorit, mis koosnes luu peal paiknevast tugielemendist ja kõveratest varrastest üdiõõnes, mis moodustasid nn ankursüsteemi. Oma fiksaatori kasutamisel täheldas dr Seppo massiivset kalluse moodustumist murru kohal ja murru kiiret konsolideerumist. Seda süsteemi täiustas ja lihtsustas veterinaariakandidaat Vladimir Andrianov, kes esitles 2003. aastal oma nn RTP (*Rod-through-plate*) fiksaatorit väikeloomade toruluude murdude raviks (Eesti Vabariigi patent nr 04899, EL patent nr 1682008). Dr Vladimir Andrianovi konstrueeritud fiksaator on mõeldud pikkade toruluude risti- ja põikimurdude raviks luu keskmises kolmandikus. Fikseerimise eesmärgiks oli saavutada maksimaalselt tugeva ja suhteliselt lihtsa konstruktsiooni abil piisav fragmentidevaheline fikseerimine kuni murru täieliku paranemiseni ning tagada samal ajal jäseme täielik ja vaba funktsioon luumurru ravi ajal.

Käesoleva töö raames uuritakse nii eksperimentaalselt kui ka kliiniliselt luumurdude regeneratsiooniprotsessi RTP-fiksaatori kasutamisel.

Fiksaator

Fiksaator on valmistatud roostevabast meditsiinilisest terasest. See koosneb tugiplaadist, kahest kaarja kujuga vardast, kahest toruluu kortikaal- ehk kompaktainesse keeratavast kruvist ja kahest lukustuskruvist. Tugiplaat on 2,5 mm paksune ja kaarja läbilõikeprofiiliga. Plaadi pikkus on 3/5 traumeeritud luu pikkusest ja laius 1/3 luu läbimõõdust. Plaadi otstest 30 mm kaugusel on freesitud 5,5 mm diameetriga kaldkanalid 45° nurga all plaadi otste suunas. Kaarja kujuga vardad on pikkusega 40–50 mm, laiusega 5 mm ja paksusega 4 mm. Vardad on ristlõikes ovaalse kujuga ja minimaalse koonilisusega 1,5–2 mm nende otste suunas. Selline ehitus kergendab varraste eemaldamist peale luumurru paranemist. Varraste tugiplaadipoolsed otsad on lapiku kujuga ja sama kumerusega nagu tugiplaat, mõõtudega 25 x 8 x 2 mm. Lapikutes otstes on kaks avaust. Üks on mõeldud 4,5 mm diameetriga korteksikruvile teine 3 mm diameetriga lukustuskruvile. Mõlemad avaused on vastavuses tugiplaadi peal paiknevate avaustega. Nende eesmärk on üheaegselt kinnitada vardad ja tugiplaat kruvide abil luu külge ja lukustada vardad tugiplaadi külge. Kasutatakse korteksikruve läbimõõduga 4,5 mm, mis peavad ulatuma mõlemast luu korteksist läbi. Lukustuskruvi on tavalise M3 metallivintlõikega, 5 mm pikk ja ei tohi ulatuda luukorteksini. Ülalnimetatud fiksaatori mõõdud on arvestatud keskmise suurusega loomale (mass 20–30 kg). Fiksaatori mõõte on võimalik proportsionaalselt korrigeerida eri suuruse ja massiga loomade jaoks. Tugiplaadi peal paiknevate avauste vahe on standardne, see võimaldab asendada erineva pikkusega vardaid omavahel. Töö fiksaatoriga on tunduvalt kergem ja mugavam, kui komplektis on mitu eri mõõtudega tugiplaati ja kaarjaid vardaid.

Töö eesmärgid

1. Võrrelda katseliselt RTP-fiksaatori ja luuplaadiga fikseeritud sääreluude osteotoomiate paranemist lammastel.
2. Testida RTP-fiksaatorit kliiniliselt.
3. Kirjeldada RTP-fiksaatori paigaldamise standardtehnikat.

Materjal ja metoodika

Eksperimentaalne uuring

Loomad

Katsed tehti EMÜ loomakliinikus. Katseloomadeks oli 7 eesti tumedapealist lammast vanuses 4 kuud. Loomad olid pärit Karulast. Eksperimentide korraldamisel arvestati loomkatse läbiviimise eeskirjadega ning eksperimendi tegemiseks oli olemas katseloomaluba (No.75, märts 2007). Lammaste keskmine kaal oli 35 kg ning lambaid hoiti sisetingimustes, kus neile oli tagatud püsiv temperatuur ja 12-tunnine valgustsükkel. Loomadel oli vaba juurdepääs toidule ja veele. Eksperiment kestis kümme nädalat. Katse lõpus loomad eutaneeriti.

Anesteesia

Operatsiooniks kasutati üldtuimastust. Premedikatsiooniks kasutati atropiini 0,1 mg/ kg i/m. Üldtuimastus juhatati sisse medetomidiinhüdrokloriidi 0,01 mg/kg i/v manustamisega. Seejärel loomad intubeeriti ja anesteesia säilitati isofluraani ja hapniku seguga. Tilkinfusiooniks kasutati ringerlaktaadi lahust 11 ml/kg/h.

Operatsioonitehnika RTP-fiksaatoriga

Enne operatsiooni valiti röntgenipildi alusel vajalike mõõtmetega fiksaatorikomplekt. Jälgiti, et tugiplaadi profiil oleks maksimaalselt kooskõlas katselooma luu profiiliga. Varraste pikkus ja paine valiti arvestusega, et varda kaared vastaksid üdiõõne diameetrile ja ulatuksid murrutsoonist maksimaalselt kaugele. Vardakaare paine on õige, kui varras toetub luukorteksile kolmes kohas: varda tipus, kaare keskpunktis ja tugiplaadi freesitud avause piirkonnas, moodustades üdikanalis kolmnurkse profiili.

Fiksaator paigaldati katselooma sääreluu lateraalsele pinnale. Juurdepääs tehti lateraalse külje poolt, arvestades lammaste anatoomilisi iseärasusi. Peale operatiivse juurdepääsu tegemist ja luurevisiooni korrigeeriti tugiplaadi painet vastavalt vajadusele. Luutangidega asetati tugiplaat luule nii, et ümbritsevad pehmed koed saaksid minimaalselt viga. Tugiplaati hoiti luutangidega nii, et tugiplaadi otstes asuvad kaldkanalid oleksid vabad. Seejärel puuriti läbi tugiplaadi kaldkanali ning luu ülemise kortikaalse kihi ühes kaldavauses umbes 45° all kanal. Läbi tugiplaadi puurides toimib kaldkanal ise juhina. Puuri läbimõõt peab olema vastavuses tugiplaadi kaldavauste läbimõõtudega.

Seejärel viidi üks kaarjas varras tugiplaadi ja korteksisse puuritud kaldkanali kaudu üdiõõnde nii, et varda sabaosa ulatus tugiplaadini. Varda painet korrigeeriti vajaduse korral selliselt, et varda kaarjas osa liiguks kanalis suhteliselt vabalt (piisab keskmise tugevusega näpuvajutusest) ja üdiõõnde asetatud vardas tekiks väike pinge. Varda sabaosas ja tugiplaadi peal asuvad augud peavad olema kohakuti. Esimesest august puuriti avaus läbi mõlema korteksi. Seejärel mõõdeti luu diameeter koos tugiplaadi ja varda sabaosaga, et määrata vajalik kruvi pikkus. Vindilõikajaga lõigati mõlemasse korteksisse keere ja kogu konstruktsioon kinnitati luu külge kruviga. Analoogiliselt paigaldati ka teine varras. Seejärel fiksaator eemaldati ja diafüüsi keskpaigas tehti Gigli traatsaega luulõikus, mille järel paigaldati fiksaator uuesti luule. Seejärel kinnitati lukustuskruvid ja haav suleti.

Postoperatiivselt süstiti loomadele prokaiinpenitsilliini 50 mg/kg i/m ja selle süstimist jätkati edaspidi üks kord päevas kahe päeva jooksul. Valuvaigistina kasutati karprofeeni 2 mg/kg s/c kohe pärast operatsiooni ja kahel järgneval päeval üks kord päevas. Eksperimendis ei kasutatud lisaks ühtegi immobiliseerivat konstruktsiooni ja loomadel lubati vabalt liikuda kohe pärast ärkamist. Eksperimendi lõpus peale eutanaasiat võeti opereeritud piirkonnast luukoe histoloogiliseks uuringuks biopsia.

Operatsioonitehnika luuplaadiga

Operatsiooniks ettevalmistus ja anesteesia tehti samal viisil, nagu on kirjeldatud RTP-fiksaatori paigaldamist. Plaadi pikkus valiti preoperatiivsete röntgenipiltide alusel ja paine korrigeeriti luu kuju järgi. Juurdepääs sääreluule viidi läbi luu lateraalse külje pealt nagu ka RTP-fiksaatori puhul. Lõike ulatus vastas luuplaadi pikkusele. Luuplaat kinnitati luule kolme kortikaalkruviga distaalselt ja kolme kortikaalkruviga proksimaalselt. Seejärel kruvid lõdvendati ja tehti luulõikus luuplaadi keskkohas. Pärast lõiget kruvid pingutati taas.

Fragmentide vahele survet ei tekitatud. Samuti ei kasutatud ühtegi täiendavat immobiliseerivat konstruktsiooni ja loomadel lubati vabalt liikuda kohe pärast ärkamist.

Hindamine

Katseloomadel hinnati röntgenoloogiliselt kalluse moodustumist. RTP grupis oli neli ja plaatfiksaatori grupis kolm looma. See toimus kohe pärast operatsiooni ja järgnevalt iga kahe nädala tagant kuni katse lõpuni. Hinnati kalluse teket ja fiksaatori positsiooni. Uuriti luu regeneratsiooni histoloogiliselt ja immunohistokeemiliselt. Määrati ka mõned vere biokeemilised markerid, mis viitavad luu regeneratsiooni protsessile.

Statistiline analüüs

Kalluse moodustumise intensiivsust analüüsiti röntgenipiltidel 2., 4., 6., 8., ja 10. nädalal. Kallus mõõdeti mõlema grupi loomadel (RTP-fiksaatori grupp ja plaatfiksaatori grupp) sääre mediaalselt küljelt pikslites, kasutades programmi CELL ^ 2.5 (*Olympus Soft Imaging SolutionsGmbH*). RTP-fiksaatori grupis oli neli ja plaatfiksaatori grupis oli kolm looma. Analüüsiks kasutati mitteparameetrilist Mann-Whitney testi.

Histoloogia

Luu biopsia võeti sääre mediaalselt küljelt, kasutades kortikaalkruvide otsi õige asukoha määramiseks. Biopsia võeti kogu läbilõikes ja selleks kasutati ostsilleeruvat saagi. Võetud tükid fikseeriti 4% formaliinilahuses, hiljem need dekaltsineeriti ja sisestati parafiini. Preparaadid värviti hematoksüliin-eosiin, van Giesoni järgi.

Vere biokeemiline uuring

Vere biokeemilistest näitajatest määrati osteokaltsiini, üldvalgu, C-reaktiivse valgu ja aluselise fosfataasi tasemed vereseerumis.

Immunohistokeemia

Immunohistokeemiliselt uuriti kollageen I ja V, osteokaltsiini ja osteopontiini sisaldust luukoes.

Kliiniline uuring

Kliinilistest juhtudest on RTP fiksaator paigaldatud kuuele koerale; neli sääreluu ja kaks reieluu diafüsaarsete murdudega keskmises kolmandikus. Kõik operatsioonid viidi läbi Eesti Maaülikooli loomakliinikus. Koerte keskmine vanus oli 4,5 aastat ja kehakaal 24 kuni 58 kg. Ühel juhul kasutati lisaks RTP-fiksaatorile ka distaalses fragmendis luulõhede esinemise tõttu traatligatuuri.

Fiksaatori valimiskriteeriumid, ettevalmistus ja operatsioonitehnika olid samad, nagu eelnevalt kirjeldatud. Kõik loomad opereeriti üldanesteesias. Juurdepääs luudele sooritati vastavalt kirjanduses antud kirjeldustele (Piermattei, 1992). Murru fragmendid reponeeriti, luule asetati tugiplaat, mida hoiti paigas luutangidega. Reieluule paigaldati fiksaator lateraalselt või kraniolateraalselt ja sääreluule mediaalselt. Postoperatiivselt mingeid immobiliseerivaid vahendeid ei kasutatud. Röntgeniülesvõtted tehti kohe pärast operatsiooni, et hinnata murru repositsiooni ja fiksaatori asendit. Loomad lasti koju samal päeval pärast operatsiooni. Loomaomanikele soovitati loomade liikumist kahe nädala jooksul pärast operatsiooni piirata.

Tulemused

Eksperimentaalne uuring

Kaks nädalat pärast operatsiooni ei erinenud jäseme kasutusvõime mõlema grupi loomadel. Katseperioodi jooksul ei täheldatud opereeritud jäsemetel ühtegi komplikatsiooni. Röntgenoloogilised uuringud viidi läbi kohe pärast operatsiooni ja edaspidi iga kahe nädala tagant kuni fiksaatori eemaldamiseni kümnendal nädalal. Juba kahe nädala pärast peale operatsiooni oli kalluse teke röntgenoloogiliselt nähtav ja neljandaks nädalaks oli kallus juba tunduvalt suurenenud.

Statistilise analüüsi tulemuste põhjal ilmnes RTP-fiksaatori grupi loomadel ulatuslikum kalluse moodustumine 2. nädalast kuni 10. nädalani ($P < 0,01$ võrreldes loomadega, kellel oli rakendatud luuplaate). Eksperimendi jooksul ei täheldatud kummalgi grupil fikseerimiselementide nihkumist algsest asendist ega ka fragmentidevahelist dislokatsiooni.

Kalluse sidekude oli kohev ja sisaldas suurel hulgal fibroblaste ning kalluses olid nähtavad peened veresooned. Tihedamas luuosas olid sidekude sisaldavad õõnsused kitsad, kuid vastu luukudet esines korrapärane osteoblastide ahel, mis viitas normaalsele osteogeneesile. Samuti vastas normile luupõrkade struktuur.

Immunohistokeemiliste analüüside tulemusel ilmnes, et 10. nädalaks oli RTP-rühma loomade kalluses tunduvalt rohkem kollageen I ja V kui plaatfiksaatori loomade kalluses (P < 0,05 võrreldes katseloomadega, kus rakendati plaatfiksaatorit). Osteokaltsiini ja osteopontiini osas statistiliselt tõepäraseid muutusi katserühmade vahel ei ilmnenud.

Võrreldes mõlema grupi loomade vere biokeemilisi analüüse, olulisi statistilisi erinevusi ei esinenud, ainult aluselise fosfataasi tase oli teisel nädalal RTP-grupis kõrgem kui grupis, kus kasutati luuplaate (P < 0,05).

Kliiniline uuring
10.–14. päeval pärast operatsiooni hinnati jäseme kasutust heaks. Ühtegi komplikatsiooni seoses operatsiooniga ei täheldatud, välja arvatud üks patsient, kellel opereeritud jäseme funktsioon kaks nädalat pärast operatsiooni halvenes. Röntgenoloogiliselt diagnoositi proksimaalse kruvi ja vastava varda dislokatsioon ja fragmentidevaheline minimaalne nihe. Mingisuguseid lisamenetlusi ei tehtud ja murd paranes ilma lisaprobleemideta.

Fiksaatori eemaldati vahemikus 7 nädalat kuni 18 kuud pärast operatsiooni. Röntgenoloogiliselt olid kõik murrud paranenud kalluse moodustumisega fragmentide vahel.

Lähtuvalt kliinilistest juhtudest ja eelnevatest katsetest küülikutega on fiksaatori konstruktsiooni täiustatud kahe lukustuskruviga nõnda, et varraste kinnitamine tugiplaadi külge tagatakse sõltumatult kortikaalkruvidest. Katsed lammastega tehti täiustatud fiksaatoriga.

Kokkuvõte
Katsete tulemused näitasid, et RTP-fiksaator tagab piisavalt kindla stabiilsuse fragmentide vahel ja võimaldab loomadel jäset kasutada kohe pärast operatsiooni. Puudub vajadus postoperatiivseks jäseme lisaimmobilisatsiooniks. Röntgenoloogilise uurimise tulemustest võib järeldada, et luumurd paranes kalluse intensiivse moodustumisega. See viitab mikroliikumise olemasolule fragmentide vahel. Kalluse intensiivsemal moodustumisel stabiliseerub ka luumurd kiiremini.

Kuna fiksaatori tugiplaat on traditsioonilistest luuplaatidest tunduvalt lühem, siis ei tekita fiksaatori paigaldus ümbritsevatele pehmetele kudedele ulatuslikke vigastusi. Samal ajal stabiliseeritakse fiksaator varrastega, mis pikendavad üldkonstruktsiooni õlga üdiõõne- siseselt.

Fiksaatori tugiplaat ja kaarjas varras kinnitatakse luule üheaegselt standardse AO tehnika abil, kasutades mõlemas fragmendis ainult ühte kruvi. Kortikaalkruvide pingutamisega stabiliseeritakse kogu konstruktsioon. Tugiplaat ja üdiõõnes olevad vardad loovad ka näpitsatele sarnase haarde proksimaalselt ja distaalselt murru otstes. Selline kombinatsioon tagab siiski fragmentidevahelise mikroliikumise. Samal ajal tagab fiksaatori konstruktsioon vajaliku tugevuse ja väldib fragmentidevahelist rotatsiooni. Kalluse intensiivse moodustumisega saavutatakse kiirem fragmentidevaheline stabiliseerumine ja jõud murru piirkonnas kantakse fiksaatorilt rohkem luu tsentrisse. Samuti on välistatud luu demineraliseerumine ja sellest tingitud patoloogilised murrud pärast fiksaatori eemaldamist, mida on täheldatud liiga rigiidsete fiksatsioonide korral. Kliinilistest juhtudest lähtuvalt ei ole luu väiksemad lõhed ega fragmentide vahel olevad väiksemad luukillud vastunäidustuseks fiksaatori kasutamisel. Fiksaatori paigaldamine ei nõua spetsiaalsete instrumentide olemasolu. Preoperatiivne tugiplaadi ja varraste valik ning ettevalmistamine on oluline, see toimub röntgenipiltide alusel ja on aeganõudev protseduur.

Nagu eelpool mainiti, on fiksaatori konstruktsiooni täiustatud kahe lukustuskruviga. Selline tehniline täiustus osutus vajalikuks lähtuvalt kliiniliste patsientide ravi tulemustest ja eelnevatest katsetest küülikutega. Lukustuskruvidega kinnitatakse üdiõõnesiseselt paiknevad vardad jäigalt tugiplaadi külge, mille tulemusena neutraliseeritakse kortikaalkruvidele mõjuv kangikoormus täielikult. Selline kinnitamise viis hoiab ära varraste asendi dislokatsiooni ja sellest tuleneva fragmentidevahelise nihkumise. Fiksaatori kasutamine ei nõua ka ühegi lisafiksaatori kasutamist.

Katsetulemuste põhjal võib järeldada, et RTP-fiksaator on nii eksperimentaalloomade kui ka kliiniliste juhtude puhul osutunud efektiivseks vahendiks pikkade toruluude risti- ja põikimurdude ravil luude keskmises kolmandikus.

Katsete käigus on välja töötatud ja kirjeldatud standardne RTP-fiksaatori paigaldamise metoodika.

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I

PUBLICATIONS

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Rod-through-plate fixation of canine diaphyseal fractures.
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Rod-through-plate fixation of canine diaphyseal fractures

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Summary
Efficacy of the rod-through-plate fixation for fracture repair was evaluated in six clinical cases of canine long bone fractures. This fixation incorporates principles of intramedullary and extramedullary osteosynthesis for transverse and short oblique fractures of long tubular bones. The plate-through-rod device is comprised of one pair of curved rods, a connecting plate and two bone screws. The connecting plate has both a hole and a channel on each end. Curved rods have a long curved part and a straight part with a hole in it for screw fixation. All components are made up of medical stainless steel. Clinical and radiographical examinations were performed from week seven to18 months after the operation. Functional abnormalities were not observed at this period in five patients and all fractures were healed. In one patient screw stripping in the proximal fragment of the femur was detected radiologically and minimal implant dislocation was noted at seven weeks after the initial repair. The rod-through-plate fixation method gives strong fixation of bone fragments with minimal traumatization of soft tissue during the operation. The design of the rod-through-plate is intended to reduce pressure of the plate on the cortex in the area of the fracture, whereas the dynamic fixation provided by the intramedullary rods may allow micromotion, thus stimulating callus formation and avoiding implant-induced osteoporosis. Clinical relevance: The rod-through-plate fixator has a simple construction and its use expands the treatment possibilities for diaphyseal fractures of long bones.

Keywords
Bone, dog, rod-through-plate fixation, internal fixation

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Introduction

Both conservative and operative methods are used for the treatment of long bone fractures in small animals (1–3). However, due to the diversity of bone fractures, no single method can have maximal efficiency for all types of fractures of long bones (3). The main factors that ensure a speedier recovery from a bone fracture are precise reposition, stable fixation of the fracture and as early limb function as possible (4), preservation of soft tissues and blood supply to the bone. 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 (5–8). When treating diaphyseal fractures of small animals, a combination of these two approaches gives additional stability, especially considering the resistance to the rotational forces (9). The principles of combined fixator were first introduced by A. Seppo in humans (10). In 1978–1979, he and coworkers reported the successful use of the first model of the combined fixator in human patients for the repair of long bone fractures (10, 11). Our modification of this model of the combined fixator works on the principle of intramedullary and extramedullary osteosynthesis. The method for the use of this device was developed by a cadaver study and was examined experimentally for the stabilization of transverse femoral osteotomies in rabbits (12). 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 fixation for small animals has to enable the use of damaged limb immediately after the operation.

The purpose of this study was to evaluate functional recovery and fracture consolidation in dogs with transverse or short oblique diaphyseal fractures of long tubular bones treated with rod-through-plate bone fixator.

Materials and methods

Patients

A total of six dogs with transverse or short oblique diaphyseal fractures of the femur or tibia were treated, between the years 2000–2002 in the Small Animal Clinic, using the combined osteosynthesis technique (Table 1). The median age of the dogs was 4.5 years. Two were female and four were male with the body weight ranging between 24 and 58 kg. All of the bone fractures were caused by trauma: motor vehicle accidents in five cases and falling from a height in one case. The fractures occurred in the middle third of the femoral (two patients) or tibial (four patients) diaphyses. In one case some small fragments were seen, which were left untouched. In one case two supplemental full cerclage wires were used because of small fissure lines in the distal fragment.

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Table 1 The dogs operated, fractures and implant removal time.

Case	Breed	Sex	Age	Weight (kg)	Fracture type and side	Implant removal time	Comments
1	German Shepherd Dog	F	13 years	32	Transverse, Femur, R	7 months	Proximal screw stripping with implant displacement
2	St. Bernard	M	2 years	58	Transverse, Tibia, L	18 months	
3	German Shepherd Dog	M	3 years	30	Transverse, Tibia, L	5 months	
4	Collie	F	5 years	25	Transverse, Tibia, L	6 months	Small fragments between fracture line
5	Mongrel	M	4 years	28	Short oblique, Tibia, L	4 months	
6	German Shepherd Dog	M	7 months	24	Transverse, Femur, R	4 months	Distal fragment with long fissures. Two full cerclage wires were used

Fixator

The fixator is handmade of medical stainless steel and comprises of one supporting plate, two curved rods and two cortical screws (Fig.1). The supporting plate is 2 mm thick and convex in cross-section. The length of the plate has to be 3/5 of the length of the fractured bone and the width 2/3 of the bone diameter. The connecting plate has both a hole and a channel on each end. The two channels are 5.5 mm in diameter and located at 30 mm from each end of the plate. They angle 45° towards the end of the plate. The curved rods are 5 mm broad, 4 mm thick, 45 mm long and are oval in cross-section. Such structure facilitates rod removal after the fracture has healed. The size of the channels in plate and rods is standard in order to allow configuration of different sized plates and rods. Two 5 mm diameter holes are located 20 mm from centre of the plate and are perpendicular to it. The end of the rod that lies on the plate is flat and equally convex with the plate. The tail of the rod has a 5 mm hole in it, which corresponds to the hole in the plate. 4.5 mm cortical screws are inserted through these holes and the plate and the rods are simultaneously fixed to the bone, ensuring that both cortices are completely engaged.

Surgical technique

Before the surgery, the size of the plate and rods were chosen. The choice being determined by the size of the bone on the radiographs and the plate was accurately contoured to the shape of the bone. The rods were also selected according to the radiographs. They had to correspond with the diameter of the medullary canal and extend in maximal distance proximally and distally according to the radiograph. Anaethaesia was induced by Domitor[a] (400 µg/kg) and Ketamine[b] (20 mg/kg) i.m. Adequate exposure of the in-

[a] Orion Pharmas, Espoo, Finland.
[b] Ketanest®, WDT, Garbsen, Germany.

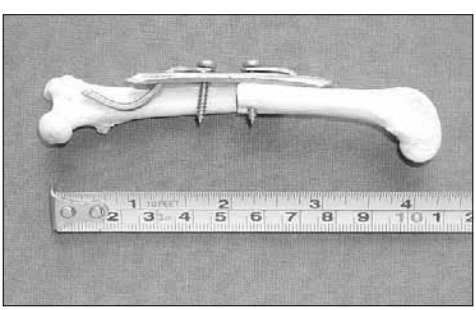

Fig. 1 The fixator comprises one pair of curved rods, connecting plate and two cortical bone screws. Connecting plate has 2 holes and 2 channels. The curved rods lie in the medullary cavity and contact the bone cortex in three points.

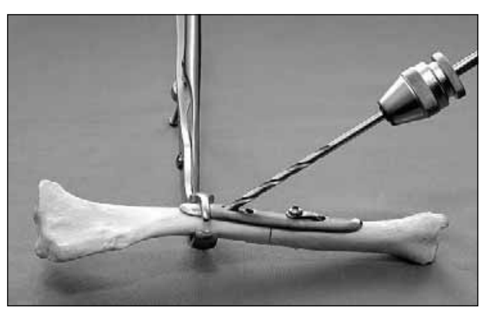

Fig. 2 The connecting plate is positioned on the medial side of a tibia. Bone holding forceps must be placed on the plate and the bone near the plate channels. The channel is drilled through the near cortex of one bone fragment a 45° angle to its longitudinal axis.

Fig. 3 A) Pre-operative lateral radiographic view of the left tibia with a transverse diaphyseal fracture in a two-year old St.-Bernard dog (Patient #2). B) Immediate post-operative cranio-lateral view of the same dog after fracture reposition and fixator installation. C) 18 months after post-operative cranio-lateral view of the same dog after fracture consolidation. Radiographic view showing clinical union with periostal bridging callus. D) Immediate post-operative lateral view of the same dog after fixator removal.

jured bone was performed, thus preserving all of the surrounding muscle and tendon insertions. The fracture was reduced and the plate was applied and held in position by two pairs of bone holding forceps. The plate was conformed to the bone contour, if necessary, using plate benders. The forceps holding the bone were placed on the plate and the bone near the holes (Fig. 2), hence in this position they did not hinder the following manipulations. A hole was drilled through the near cortex of one bone fragment with the drill bit entering the bone at 45° angle to its longitudinal axis. The channel in the plate served as a guide ensuring the correct angle and position of the hole. The end of the rod was held with the forceps and the rod was gently tapped through the plate and cortex into the medullary cavity until the tail of the rod met the plate (Fig. 1). The second rod was inserted in a similar way into the other fragment. Wherever necessary, each rod was adjusted with plate benders in order that it could be passed freely through the hole, and slight tension was created in the rod in the medullary cavity. The tail of the rod was placed on the plate so that the holes were aligned. The screw holes were drilled through them with a 3.2 mm drill bit, penetrating both cortices. The length of the hole was measured with the depth gauge and the appropriately sized self-tapping type screw was inserted using a screwdriver. As the screws were tightened, the intramedullary rod began to act like a spring, providing pressure to the plate. This created the 'forceps-effect' between the plate and rod, fixing the device to the bone.

Routine closure of the wound was performed. In order to prevent swelling of the surgical wound a protective bandage was applied postoperatively for the first three to five days. Supplementary external coaptation splints and bandages were not used. The dogs were allowed to put weight on the affected limb the following day, and restricted activity on the leash was prescribed for two weeks.

Amoxicillin 15 mg/kg b.i.d. was administered orally for five days after the operation. Carprofen[c] (2 mg/kg s.i.d.) was injected subcutaneously for three days as an analgaesic.

The reduction, alignment, the axis of the bone and fixator positioning was confirmed radiographically immediately after surgery. Projections were latero-medial and antero-posterior. Case #1 was examined radiographically 11 days after operation because of new trauma and worsening of the limb function.

All of the animals were returned 10 days postoperatively for suture removal and clinical examination. Clinical re-examination was performed again at seven and 12 weeks after the operation. The functional status of the limb was also recorded. Clinical and radiographical evaluations were performed between week seven and 18 months after surgery before implant removal. Clinical abnormalities were not found in any of the patients.

Results

The fractures healed by secondary bone union with callus formation. In all of the cases the functional recovery of the operated limb was considered good 10 days after the surgery. In five cases, there were not any complications associated with the surgical technique. The dogs put weight on the affected limb and the development of a significant muscular atrophy had not developed. The fixator did not exhibit any adverse effect on the bone structure with follow-up radiological evaluations and remained in the same position that it had been originally applied.

In one case, the proximal screw migration occurred due to a further trauma to the limb (the dog fell from a height). The positioning of the curved rod changed slightly in the medullary canal, but maintained its function as a fixative agent. The device was not removed, and additional immobilization was not used. The limb showed good function, fracture displacement did not occur, the alignment remained unchanged, and the callus was more massive.

The fixator was removed four to 18 months after surgery. Complications were not noted after removal. Clinical and radiographic follow-up and time of the removal of the fixator (Fig. 3) was very much dependent on the patients' owners.

[c] Rimadyl®, Pfizer Animal Health, Denmark.

Discussion

The healing of bone fractures is affected by several factors, but the stable fixation of bone fragments (1, 2), the preservation of soft tissues and blood supply to the bone is of ultimate importance. In this study we introduce an original rod-through-plate fixation for the stabilisation of the diaphyseal fractures of dogs. With this fixation the support plate placed on the bone is relatively short, while intramedullar rods lengthen the shoulder of the fixator proximally and distally almost up to the metaphyses. Compared to the conventional plate fixator, the installation of this rod-through-plate fixation does not require extensive surgical access, thus reducing the length of the operation and the traumatization of tissue, which ensures optimal conditions for the healing of the fracture. The technique enables stable and dynamic fixation of the fragments. In construction, the intramedullary parts of the fixator are curved and exert dynamic pressure on the bone cortex in three points: at the end of the rod, at the centre of the rod arc, and at the point of rod and plate union. The fixator exerts continuous dynamic force and transfers the load effectively in the axial direction, it can be similar to Rush pin fixation (1, 13). The rods are not too tight in the medullary cavity. This reduces a disruption of the medullary blood supply: a factor emphazised in fracture fixation by Anderson et al. (5) and Wilson (15).

The curved rods are oval in cross-section, and provide resistance to rotation. Traditional intramedullary nails and pins have limited implant to-cortex contact and provide little resistance to rotational forces, especially in the case of transverse fractures and additional fixing devices are often necessary (13, 17). The curved rods extend the fixation points proximally and distally in the medullary cavity, thus minimizing mechanical load at the fracture site and improving the conditions for bone tissue regeneration. The same principle was used by Seppo et al. in their fixator (10, 11).

The extramedullary part of the required fixator is applied to the bone in the same manner as conventional bone plates. The difference is that the connecting plate is relatively short and only two cortical screws are needed, one for each bone fragment. The screws are inserted according to AO principles (7). Fewer screws enable the preservation of bone tissue and minimize the rigidity of the fixation device and pressure on the underlying cortex. As the screws are tightened, the intramedullary rod begins to act like a spring, providing pressure to the plate. This creates the 'forceps-effect' between the plate and rod, fixing the device to the bone. At the same time, it is not totally rigid and the elasticity of the rods allows micromovement in the fracture site when the limb is being used. It is well acknowledged that small axial movements can stimulate callus formation, which may enhance bone healing (11).

The fixator has been clinically tested on animals with femoral and tibial fractures, as it is easiest to apply the device to these bones. The new system of fixation which we have introduced improves the methodologies of fixation and uses a different principle of fixation which has its positive qualities. The use of the rod-through-plate fixator enables the shoulder of the device to increase whilst avoiding extensive damage to the surrounding soft tissue. At the same time, due to the three support points (intramedullary canal), which form a triangle, the risk of rotation is eliminated. The structure of the fixation device, which forms an anchor system, and in which the fixating powers, are aimed at compression, and without tearing, ensures stronger fixation.

The phenomena of stress protection, osteopenia and refracture following plate removal present the long-term consequences of too rigid stabilization techniques (6, 16). As the plate is relatively short and fixation points reach more distally and proximally than do other tecniques, less surgical exposure is required in order to repair the fracture, hence reducing surgical time and trauma to the injured area.

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II

Tralman G., Nõupuu K., Uksov D., Kibur R.T., Männik P., Talve N., Andrianov V., Roosaar P., Arend A., Aunapuu M.
Using internal fixation for the treatment of long tubular bones in small animals: Evaluation of the plate fixator for tibial fractures in sheep.
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USING INTERNAL FIXATION FOR THE TREATMENT OF LONG TUBULAR BONES IN SMALL ANIMALS: EVALUATION OF THE PLATE FIXATOR FOR TIBIAL FRACTURES IN SHEEP

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ABSTRACT

This study is part of a larger project for finding optimal methods for fracture fixation of tubular bones in small animals. Strong fracture fixation is important in animals to ensure early weight bearing and quick fracture healing. In this study, plate fixation was used to treat experimental tibial fractures of sheep. Radiographical, histological and immunohistochemical methods were used for evaluation of the healing process. Plate fixation gives good stability for the healing process but traumatisation of soft tissues during the operation is quite extensive. The regeneration process is also slow and, therefore, this method cannot be considered as most appropriate for treatment of fractures of long tubular bones in small animals.

INTRODUCTION

Bone is an essential part of the locomotor system, acting as lever arm during motion and resisting the force of gravity. Bone surgery compared to soft tissue surgery is different, as bone tissue has specific physical and morphological features. Fractures may be classified on many bases: causes, presence of external wounds, extent of damage, direction and location of fracture line – open and closed fractures, complete, green-stick and fissure fractures, transverse, oblique, spiral, comminuted, multiple, avulsion and condylar fractures. Diversity of different fractures requires different approaches for treatment and, therefore, the method of fracture repair is based on the type and location of a fracture. Fixation should be minimally invasive for soft tissues and should preserve blood supply to the healing bone. At the same time, fixation has to be strong enough to resist different forces during the healing period.

Development of orthopaedic surgery started in the middle of the 18th century but was very limited at that time. After development of anaesthesia, antiseptic techniques and discovery of x-rays in the second half of the 19th century the development of orthopaedic surgery was rapid [1]. The first bone surgery by using cerclage wires was performed in 1827 by Rogers [2]. At present, the most common method for fracture treatment is plate fixation. This system was first described by Arbuthnot Lane and Albin Lambotte in 1905 [3]. In 1949 the idea of interfragmentary compression was first described by Danis [4]. The idea was further developed by the AO group in Switzerland where DCP (dynamic compression plate) came into use in 1969. Compression of fractures increases fracture stability through frictional impact loading and narrowing the gap between fragments. It results in optimal conditions for direct bone healing without visible callus formation [5–7]. In DCP plates the design of the screw holes is based on the spherical gliding principle. As the screw is tightened, the spherical screw head glides toward the centre of the plate until the deepest portion of the hole is reached. The result is that the bone fragment into which the screw is being driven is displaced at the same time and in the same direction – toward the centre – and fracture line and the fragments are compressed [5, 8]. As external callus formation increases the diameter and therefore increases the lever arm to resist forces, healing without callus takes much longer and the risk of refracture after implant removal is high. [9, 10]. At the same time, in case of articular and juxtaarticular fractures, stability at the fracture site is of critical importance [11]. Also, disruption of the blood supply and thinning of the cortices may result in a too stiff fixation – known as stress protection [12, 13].

Over time, surgical technique has improved much with a shift to mini-invasive surgery. In 1985 „biological osteosyntheseis" [3] came into use which means less invasive surgery to minimize disruption of blood supply to the bone [3]. Plates are applied mini-invasively (LISS) to preserve soft tissues and, at the same time, maintain blood supply to the bone as much as possible (LC-DCP and Wave plate). In LISS (Less Invasive Stabilization System) plates, monocortical locking screws are used and the plate is applied without excessive surgical intervention. In Wave plates, contact between the plate and the bone is reduced by the presence of grooves on the underside of the plate, thereby improving vascular supply. These grooves also encourage the development of a small bone bridge beneath the plate and circumferential shell of callus around the fracture site. [14]. In 2001 LCP (locking compression plate) came into use. In LCP, screws are locked into plate to eliminate implant loosening.

In 1930 Gerhard Küntscher and brothers Rush developed the method of intramedullary pinning. Pins are driven into the intramedullary canal to stabilise the fracture. In 1985, interlocking nailing became popular after Kempf, Grosse and Beck [15]. The nail is locked by screws proximally and distally from the fracture site. This method eliminates rotational forces in the fracture site and can be used in transverse and oblique fractures [16].

External skeletal fixators are used in comminuted fractures and fractures with excessive soft tissue injury. The fixator consists of connecting bars and trans-skeletal pins which are connected to each other. The principle of external fixation was developed in 1843 by Wutzer [17], but in 1938 after Raoul Hoffmann external fixation came into wide use. Circular external fixation system, where transosseus wires are connected to the rings under tension was developed by G. A. Ilizarov in 1950 [18]. The main problems with external skeletal fixation are pin tract infections and ancyloses of the joints.

The purpose of osteosynthesis is to restore the function of the affected limb as close to normal as possible. Important is stability of fragments on fracture site and proper operation technique. Operation technique and fixation should preserve blood supply to the bone and preserve soft tissues as much as possible.

This study is part of a larger project in finding optimal fixation methods for fracture treatment of long tubular bones. In this experimental study, plate fixation for tibial fractures was evaluated.

MATERIAL AND METHODS

In the experimental study, two sheep were used for fracture stabilisation using round hole neutralization plates and 3.5 mm cortical screws. The animals were kept in the clinic of the Estonian University of Life Sciences. The experiment lasted for 10 weeks. All animal procedures were approved by the University of Tartu Animal Care Committee in accordance with the European Communities Directive of 24 November 1986 (86/609/EEC).

Operation

Hair was clipped for aseptic surgery from the lateral side of the tibia. Anaesthesia was induced with Domitor (*Medetomidinum hydrochloridium* 1 μg/ml, i/v, Pfizer) according to the animal's weight. The animal was fixed to an operation table in lateral recombency, the subsequent intubation and anaesthesia was carried out with isofluran (*isofuranum*). The operation field was prepared for aseptic surgery (Chemisept). Lateral approach was made between muscles (*m. ext. digiti IV pedis proprius; m. digitalis pedis lungus; m. gastrochnemius ja m. flexor hallucis longus*) to bone. A plate was selected by length and contoured to the surface of the bone. The plate was fixed to the bone temporarily. After predrilling 2.5 mm holes and pre-tapping, three 3.5 mm cortical screws were inserted distally and three 3.5 mm cortical screws proximally from the preplanned osteotomy site. Osteotomy was performed with a gigly saw in the centre of the tibia. After osteotomy the screws were tightened. The wound was closed in layers with synthetic resorbable suture material VICRYL® 1, CP-1 or *Safi l ® green 1.* The closed wound was covered by Alamycin spray (Oxytetracyclin 36 mg/g, Norbook Laboratories Ltd (GB), Ireland). The animal was extubated. Antibiotics were given, intramusculary 5 ml Amoxy-kel 15% (150 mg/ml, Hoogstraten, Belgium). Rimadyl

2 mg/kg s.c. (*carprophenum 50 mg/ml*, Pfizer) was used for pain management according to the animal's weight.

Radiology

Radiographic images were taken directly after the surgery and then after every two weeks. Radiographs were taken in two projections: craniocaudally and mediolaterally by *Medlink URS Veterinary Portable X-ray SP-VET-4.0*, parameters 50 kVp and 10.0 mAs were used. Radiographic screen *AGFA CR MD 4.0 General Plate* was used and images were loaded to computer by *AGFA ADC Solo Digitizer*.

Histology

After bone biopsy, the bone was decalcified using „SAKURA TDE™ 30 Decalcifier System". Tissue samples were fixed for histopathological evaluation with 4% buffered formalin solution and embedded in paraffin according to classical methods. Seven-μm thick paraffin sections were dewaxed and brought to water through graded ethanols. Sections were stained with H&E or van Gieson according to classical methods, then dehydrated through graded ethanols, cleared in xylene and mounted with DPX (Fluka, Switzerland). Sections were examined using the Olympus BX-50 light microscope.

Immunohistochemistry

Seven-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), treated with normal 1.5% goat serum for 30 min at room temperature and incubated with the primary antibody collagen I Ab-2 (2B1.5, NeoMarkers, USA) diluted 1:100, and osteocalcin (abcam, UK) diluted 1:500 overnight at 4°C. In the following day, sections were washed in PBS and incubated with diluted biotinylated secondary antibody (VECTASTAIN, ImmunoVision Technologies, Co, USA) for 30 min at room temperature. Sections were washed with PBS and incubated for 30 min with VECTASTAIN

ABC Reagent. Then sections were incubated in DAB solution (VECTOR Laboratories, USA) for 10 min at room temperature. Cells nuclei were counterstained with hematoxylin or toluidine blue. Sections were dehydrated through graded ethanols, cleared in xylene and mounted with Eukitt (Fluka, Switzerland). The collagen I and osteocalcin is expressed by a subjective scale ranging from 0 to +++ (0 – no staining, 1 – weak staining, 2 – moderate staining, 3 – strong staining). Two independent observers in a blinded fashion performed the evaluation.

RESULTS

After the operation no complications were noted. Rimadyl 2 mg/kg (*karprophenum* 50 mg/ml, Pfizer) was used for pain management according to the need. The animals started to use the operated leg two weeks after surgery.

Radiology

In postoperative radiographs, osteotomy line is clearly visible. In both animals callus formation was minimal and was noted on radiographs six weeks after the operation. A gap between the fragments was visible even after ten weeks at the time of implant removal (Fig. 1). It shows that consolidation was slow and not completed. On radiographs no implant failure was found during the monitoring time.

Morphology

The amount of the periosteal callus in animals was minimal and therefore minimal connective tissue was noted. In fibrous callus small bone trabeculae were present, around which a regular chain of osteoblasts was located. In cartilagineous periostal callus both hyaline cartilage and fibrocartilage were present in small amounts. Within bone trabeculae canals with connective tissue and blood vessels were formed. In bone trabeculae osteocytes in different developmental stages were seen. In intermediate and endostal callus a large amount of connective tissue was noted, bone trabeculae were clearly expressed but few osteoblasts surrounded them irregularly.

Collagen I and osteocalcin were studied immunohistochemically. By the tenth week of the experiment the level of both proteins was low (graded as weak by both observers).

DISCUSSION

In this study the effect of plate fixation on bone regeneration in treatment of long tubular bone was examined. Stable fixation of fragments is an important part for fracture healing [19]. For plate application extensive approach to the bone is required which lengthens the anaesthesia time and causes more soft tissue trauma. Therefore, in our opinion this method is not the best for the fixation of long tubular bones. During the radiographic examination the osteotomy gap was visible and all the elements of the fixator were in place during the experiment. At the end of the experiment bone was healed as detected radiographically, but the regeneration had been prolonged.

Morphological examination showed that the process of regeneration was not yet complete, which was proven by the presence of osteoclasts in sample tissue. Good results were shown by absence of inflammatory processes in tissue and degradation of newly formed tissue. As the callus formation was minimal, normal regeneration of tubular bone was noted. [20].

The immunohistochemical study showed that at the end of the experiment the amount of collagen I and osteocalcin was not markedly elevated. Low level of collagen I in bone shows a disturbed biosynthesis of fibrillar collagens after the operation and plate application. Collagen I is the main building component in bone tissue and its low level refers to disturbances in this process.

In conclusion, plate fixation provides stable fixation of fragments and consolidation of the fracture but because of excessive soft tissue trauma and a long healing period it is recommended to use more effective methods for fracture stabilisation. One of the alternative methods is to use a combined rod-through-plate fixator, where both extramedullary and intramedullary osteosynthesis are employed. The advantage of this method is that the traumatization of soft tissues during the operation is minimal and the operated limbs are fully weight bearing. The efficiency of the rod-through-plate fixator in treatment of long bone fractures has already been tested on rabbits [21] and on dogs [22]. The preliminary results also show a positive effect of the rod-through-plate fixator in treatment of tubular bone fractures in sheep (unpublished data).

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Standard surgical technique for applying rod through plate (RTP) internal fixator in small animals.
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Standard Surgical Technique for Applying Rod Through Plate (RTP) Internal Fixator – An Experimental Study in Sheep

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Summary

The standard surgical technique for applying arod through plate (RTP) internal fixator to treat long bone fractures was carried out. The experiments were performed in 4 male Estonian Blackhead sheep. Previously, aRTP fixator for small animal fracture repair has been tested experimentally in rabbits and also on clinical cases in dogs. To reduce complications as such screw loosening and rod displacement, modification to the original fixator was made: one locking screw on each rod was added to fix the rod to the plate. In this study the healing process of tibial osteotomies using the modified RTP fixator was evaluated in sheep. Sheep were used as experimental animals because body weight and bone structure is similar to small animals.

Introduction

To treat fractures of long bones of small animals both conservative and operative methods are used *(Brinker et al, 1991).* Although numerous options are available for fracture treatment surgical, intervention is very often the method of choice, where for the internal fixation, various plates or intramedullary nails are used *(Tarr & Wiss, 1986 ; Uhthoff et al, 2006).* By combining these two methods, i.e. plate fixation and intramedullary nailing, additional stability can be achieved, especially in conjunction with the resistance to rotational forces. In this study a new fixator, where support plates are combined with intramedullary rods, has been tested for treatment of long bone diaphyseal fractures. The combined new rod through plate (RTP) internal fixator has two patents – European Patent No 1682008 (authors Andrianov V, Lenzner A, Haviko T, 30.05.07) and Estonian Republic patent No 04899.

Our RTP fixator is derived from the original method developed by Seppo (*1979)*, but by combining (Fig.1) extramedullary and intramedullary elements of osteosynthesis, is a new fixator, which expands the treatment possibilities for the internal fixation of bone fractures. The main elements of the fixator are extramedullary plates and two curved rods, which are located intramedullary and exert dynamic pressure on the bone cortex in three points proximally and distally from the fracture site. Rods are fixed to the supporting plate with bone screws.

The purpose of this study is to describe the operative technique for applying our modified RTP in experimental animals for use as a standard clinical method in diaphyseal bone fracture treatment in

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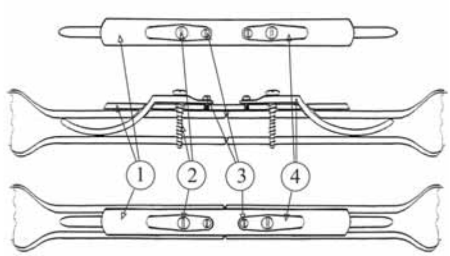

Figure 1. Scheme of the rod through plate fixator.

small animals. This type of fixator has been tested on rabbits for the treatment of femoral osteotomies and also clinically on dogs for the treatment of tibial and femoral fractures *(Andrianov et al, 2007).*

Materials and Methods

The experiments were performed on 4 male Estonian Blackhead sheep. All sheep were from Karula farm (Estonia) and were four months old. Permission (No. 75, 16 March 2007) for the present study was given by the Estonian National Board of Animal Experiments in accordance with the European Communities Directive (86/609/EEC). The mean body weight of the sheep was 35 (± 5.0) kg. 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). The animals had free access to food and tap water.

Medication

Surgery was performed under inhalation anesthesia, which was premedicated by atropine 1 mg/ml (Atropine sulfate, Verofarm, Russia) 3 ml i/m and induced by Medetomidine hydrochloride 1 mg/ml (Dorbene®, Laboratorios Syva, Spain) 0.5 ml i/v. All animals were intubated and anesthesia was maintained with a mixture of isoflurane 2% (Forane®, Abbott Laboratories Ltd, England) and pure oxygen with a flow of 11 ml/kg/min *(Riebold et al, 1995).* A Komesaroff (Medical Developements, Melbourne, Australia) inhalation anesthesia device was used. Lactated Ringers solution (Ringer-Lactat, B.Braun Melsungen AG, Germany) 11 ml/kg/h was administrated intravenously during the anesthesia. At the end of the operation, for antibiotic prophylaxis procainpenicillin 300 mg/ml (Norcilliin®, Norbrook Laboratories Ltd., North Ireland) 5 ml i/m was administered on the first and second postoperative day. Carprofen 50 mg/ml (Rimadyl®, Vericore Ltd., Dundee, Scotland) 2 mg/kg according to body weight s/c once a day was administered as ananalgesic for the two postoperative days.

Preparation for surgery

The left tibia was radiographed in mediolateral and craniocaudal projections by *Medlink URS Veterinary Portable X-ray SP-VET-4.0* (SEDECAL, Spain) (Fig 8/1). The f.f.d. (focal spot-film distance) was 100 cm and the exposure data were 50 kVp and 10.0 mAs. Radiographic screen *AGFA CR MD 4.0 General Plate* (AGFA-Gevaert, Germany) was used and images were loaded to computer by *AGFA ADC Solo Digitizer* (AGFA-Gevaert, Germany). The size of the plate and rods were chosen according to the bone size on a craniocaudal radiograph. The plate was accurately contoured to the shape of the bone. Curved rods had to correspond with the diameter of the medullary canal and to extend in maximal distance from the osteotomy site proximally and distally according to the radiograph. Intramedullary rods are curved and have to be in contact with the cortex at three points: at the end of the rod, at the centre of the rod arc, and at the point of rod and plate union. Before surgery, hair was clipped from the region of the jugular vein, the cephalic antebrachial vein and from the centre of the left femur to the centre of the metatarsus. Blood samples from the jugular vein were taken for biochemistry (and immunological analysis). Intravenous cannula was placed to cephalic vein.

Operative procedure

The animal was placed on right lateral recumbency (Fig. 2). Operation field was disinfected with 75° ethanol (Chemisept-G, Chemi-Pharm, Tallinn, Es-

Figure 2. Position of the animal on the table.

tonia) followed by covering with towels to get an aseptic working area (Fig. 3).
A skin incision about 12 cm in length was made in the middle third of the tibia at the lateral side. Fasciae were identified and cut in line with the skin incision.

Figure 3. Covered operation field.

The intermuscular septum between *M. extensor digiti quarti proprius* and *M. flexor digitorum profundus* (*May, 1964*) was identified and separated by blunt dissection. The peroneal nerve was identified and protected *(Dyce et al, 1987).*
Self-retaining retractors were used to maintain the exposure to the tibia. With a periosteal elevator, muscle attachments were freed from bone to about one third of the diameter of the bone and all the length of the skin incision (Fig. 4).
The plate was applied to the lateral side of the tibia and held in position by bone holding forceps. The plate was conformed to the bone contour, if necessary, using plate benders. The forceps holding the plate and the bone were placed in the centre of the plate. In this position they did not hinder the following manipulations.
Starting proximally, a 4 mm hole was drilled through near the cortex of the bone at approximately 45° to its longitudinal axis. The channel for the rod in the plate served as a guide ensuring the correct angle and position of the 4 mm hole (Fig. 5).

Figure 4. Access to the tibia.

Figure 5. Drilling 4 mm hole at 45 dregrees to the long axis of the tibia.

The rod was inserted through the hole into the medullary cavity holding the tail of the rod with forceps. If necessary the arc of the rod was changed according to the need not to apply too much pressure to the cortex of the bone. After the insertion of the rod, the distance between the tail of the rod and the plate should be about 2-3 mm. At the same time the holes for the cortical screws in the plate and in the rod's tail were aligned. For correct alignment of the holes, 2 mm Kirschner wire was used through both holes and the wire was moved in the required direction until both holes were perfectly aligned (Fig. 6).
A cortical screw was inserted through rod and plate holes to the bone. Screws were inserted according to the AO standard technique *(Brinker et al, 1984)*. Using a 2.5 mm drill bit, holes through both corticles were drilled. The length of the hole was measured, both corticles tapped and an appropriately sized 3.5 mm screw was inserted. Tensioning the screw pushed the rod's tail to the plate and gave tension to the bone corticles from the medullary canal. The

Figure 6. Plate on the bone with proximal intramedullary rod in its position.

same procedure was performed distally.

For osteotomy the fixator was removed and osteotomy was performed perpendiculary to the long axis of the bone. A Gigli wire saw was used for osteotomy, which was centered midway between the screw holes.

The fixator was placed back on the osteotomised bone using the same locations. In our modification of the RTP fixator, there are two additional screws for fixation of the rods to the plate.

Cortical screws were first inserted but not tightened. The next step was placement of the locking screws followed by the tightening cortical screws (Fig. 7).

Wound was closed in layer: the deep fascia using continuous suture and the subcutaneous tissue and skin layer by layer using absorbent material (Safil®1, Braun, Germany). After the closure Chlortetracycline spray 1 ml/20 mg (Pederipra Spray, Laboratorios Hipra, Spain) was applied to the wound. No bandages on the operated leg were used.

Figure 7. Fixator installed without distal locking screw.

The first postoperative radiographic examination of the operated leg was undertaken immediately after the surgery in the craniocaudal direction to describe the location of the fixator on the bone (Fig. 8/2). Follow-up examinations were performed after 2, 4, 6, 8 and 10 weeks (Fig. 8/3-7)

Free movement of the animals was allowed directly after the recovery from anesthesia. To grant similar handling of all operated animals all surgical procedures were performed by the same surgeon and one assistant. Average operation time was 1 hour and 40 minutes. After eight weeks from the beginning of the experiment fixators were removed. Removal of the implant took about 20 minutes. Clinical monitoring time of the animals after fixator removal was 2 weeks to ensure the absence of any possible complication.

The animals were euthanized after the follow-up time of ten weeks. Euthanasia was carried out after sedation with 20 mg/ml Xylazin hydrochloride 0.08-0.75 ml/100 kg according to the body weight i/v (Xsylapan®, Vetoquinol AG, Bern, Switzerland) by i/v administration of T61® (Embutramid 200 mg + Mebezoniumiodid 50 mg + Tetracain hydrochloride 5 mg, Intervet S.A., EU) 5 ml/50 kg.

Results

No complications after the surgery of the experimental animals were detected. Postoperative swelling of the operated leg was minimal and no clinical problems were noted. On postoperative radiographs, the osteotomy line was clearly visible, two weeks after the operation radiolucent periosteal callus formation was seen on radiographs and after four weeks a wider radiodense callus formation was noted. After six weeks the osteotomy line was still visible, but disappeared on eight-week radiographs (Fig. 8). No implant loosening was noted during the experiment. Animals started to use the operated leg on the third day after the operation except one sheep, who

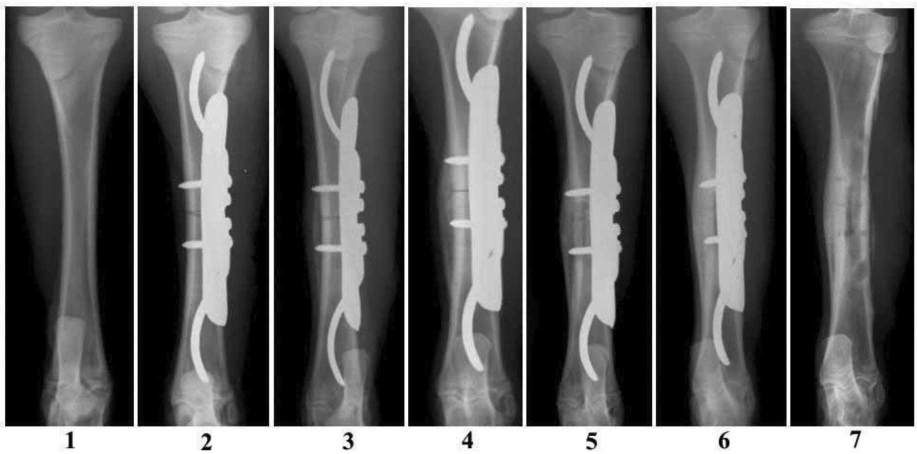

Figure 8. Radiographs of the left tibia. 1 – left tibia before surgery; 2 – radiograph after surgery, wk „0"; 3 – wk 2; 4 – wk 4; 5 – wk 6; 6 – wk 8; 7 – wk 10.

started to load weight on the treated limb on the seventh day.

Discussion

Although there are numerous methods for fracture fixation in small animals, operative methods are usually preferred. The choice of operative technique depends on the location and type of the fracture, but the purpose of the fixation is always stability of the fragments to allow quick consolidation of the fracture and more importantly to restore the function of the leg. The RTP fixator has been designed for internal fracture treatment in small animal surgery. The fixator was previously tested on femurs of rabbits *(Andrianov et al, 2007).* Rabbits are relatively inexpensive and widely used experimental animals. Leporine long tubular bones are big enough for internal fixation without the use of microinstruments *(Huber et al, 2006).* On the other hand tubular bones of rabbits have fragile structure with a thin cortical layer *(Crigel & Balligand, 2002),* which complicates experimenting with RTP. According to our experience the bone structure of small ruminants is more similar to the small animal bones and that was the reason to use sheep in this experiment. The second reason was the much bigger body weight compared to rabbits and more active movement of sheep giving the more loading to the fixator.

The tibia was chosen because approach to the bone is easy and radiographic examination is technically simple. The skin of the sheep is very thin and on the medial side there is not enough soft tissue to cover the fixator but on the lateral side we could hide the fixator under the soft tissues, while on the medial side it is simple to take bone biopsies during the healing process, if necessary.

This experiment is a part of the larger project, where bone healing is compared after applying either RTP fixator or the plate fixator. The plate fixator is also applied to the lateral surface of the bone *(Tralman et al, 2008).* In this experiment the RTP fixator is modified with the addition of two locking screws to fix intramedullary rods to the plate. Cortical screws can get loose and cause loosening of the rod and instability at the fracture site *(Andrianov et al, 2003).* Locking screws are inserted through holes in the rods and fixed to the plate, which has threaded holes in it. Locking screws are short enough not to penetrate the surface of plates contacting the bone. This type of construct should maintain stability at the fracture site even in case of cortical screw loosening. Transcortical screws penetrate through cortex medially after tightening and the ends of these screws serve as landmarks for bone biopsy.

Conclusions

The clinical and radiological findings in this experimental study in sheep show that successful consolidation and rigidity after long bone osteotomies can be achieved by the use of a RTP fixator

This novel type of fixator can be recommended to be applicable also in clinical cases of companion animal diaphyseal fractures. The technique for RTP fixator placement is simple and does not require special instrumentation.

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IV

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ORIGINAL ARTICLE

A Novel Combined Method of Osteosynthesis in Treatment of Tibial Fractures: A Comparative Study on Sheep with Application of Rod-Through-Plate Fixator and Bone Plating

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With 10 figures

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Summary

The study compares the efficiency of a new bone fixator combining periostal and intramedullary osteosynthesis to bone plating in treatment of tibial fractures in sheep. Experimental osteotomies were performed in the middle third of the left tibia. Animals were divided into two groups: in one group (four animals) combined osteosynthesis (rod-through-plate fixator, RTP fixator) was applied, and in the other group (three animals) bone plating was used. The experiments lasted for 10 weeks during which fracture union was followed by radiography, and the healing process was studied by blood serum markers reflecting bone turnover and by histological and immunohistochemical investigations. In the RTP fixator group, animals started to load body weight on the operated limbs the next day after the surgery, while in the bone plating group, this happened only on the seventh day. In the RTP fixator group, consolidation of fractures was also faster, as demonstrated by radiographical, histological, and immunohistochemical investigations and in part by blood serum markers for bone formation. It can be concluded that application of RTP fixation is more efficient than plate fixation in the treatment of experimental osteotomies of long bones in sheep.

Introduction

Although numerous fixation methods have been devised to treat bone fractures, the search for optimal fixation methods continues. The method has to be minimally invasive, preserve soft tissues, blood supply and trophics of the bone, but at the same time provide stable fixation (Stiffler, 2004). In veterinary practice, it is important that the fixation is strong and convenient allowing early loading of body weight to the affected limb, as the complete immobilization of animals is practically impossible and, furthermore, prolonged immobilization can alter the healing process (Doyle, 2004). To meet these requirements, the internal fixation of fractures has evolved in recent decades with a change of emphasis from mechanical to biological priorities (Palmer, 1999). Stable but still flexible fixation should enhance the formation of callus (Carter et al., 1998), and a less invasive technique will reduce operative trauma (Schmökel et al., 2007). This approach has been described as 'biological internal fixation' with minimized implant-to-bone contact, long-span bridging and fewer screws for fixation (Palmer, 1999). Formerly, internal fixation with a plate aimed at absolute stability to avoid micromovement which could result in loosening of the implant and a delay in healing (Uhthoff et al., 2006). The new approach to internal fixation even requires some degree of mobility of the interface of the fracture (Goodship and Kenwright, 1985; Perren, 2002; Jagodzinski and Krettek, 2007).

To test the efficiency of this approach, a new rod-through-plate fixator (RTP fixator) has been compared with the conventional plate fixator in treatment of experimental tibial fractures in sheep. The RTP fixator combines intramedullary and extramedullary osteosynthesis and is composed of a pair of curved rods, a connecting

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To test the efficiency of this approach, a new rod-through-plate fixator (RTP fixator) has been compared with the conventional plate fixator in treatment of experimental tibial fractures in sheep. The RTP fixator combines intramedullary and extramedullary osteosynthesis and is composed of a pair of curved rods, a connecting plate and two bone screws (Fig. 1). The simple construction of the RTP fixator makes its installation relatively easy and traumatization of soft tissues during the operation is minimal compared with conventional bone plating operations (Tralman et al., 2010). The design of the RTP fixator is intended to reduce the pressure of the plate on the bone cortex, while the dynamic fixation given by intramedullary rods allows micromovements to stimulate callus formation and avoids implant-induced osteoporosis (Andrianov et al., 2003). In this study, to compare bone fracture healing with application of bone plating or RTP fixator, several investigation methods were used: radiography, detection of serum markers reflecting bone healing, as well as histological and immunohistochemical analyses.

Materials and Methods

The experiments were performed on seven male sheep – four sheep with the application of the rod-through-plate (RTP) fixator (the RTP fixator group) and three sheep with the application of bone plating (the bone plating group). The combined new rod-through-plate internal fixator has two patents – European Patent No 1682008 and Estonian Republic patent No 04899.

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). The animals had free access to food and water. All animal procedures were approved by the University of Tartu Animal Care Committee in accordance with the European Communities Directive of 24 November 1986 (86/609/EEC). The experiments lasted for 10 weeks. In one animal from the RTP fixator group, biopsy under anaesthesia was taken 2 weeks and, in another animal, 4 weeks after the operation. At the end of the experiment, the animals were euthanized and bone biopsy was taken from the osteotomy site for morphological investigation.

Surgical procedures

A detailed description of the operative procedure for RTP fixator application has been recently published (Tralman et al., 2010). As a first step, the size of the plate and the rods were chosen according to the bone size on the craniocaudal radiograph. The plate was accurately contoured to the shape of the bone. Curved rods correspond to the diameter of the medullary canal and extended in maximal distance from the osteotomy site proximally and distally, according to the radiograph. Intramedullary rods were curved and were in contact with the cortex at three points: at the end of the rod, at the centre of the rod and arc at the contact point between the rod and the plate.

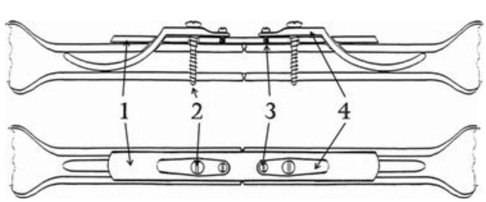

Fig. 1. Construction principle of the rod-through-plate fixator: 1 – support plate, 2 – cortical screws, 3 – curved rods, 4 – locking screws.

A skin incision was made in the middle third of the tibia on the lateral side. Fasciae were identified and cut in line with the skin incision. The intermuscular septum between the deep digital flexor muscle and the lateral digital extensor muscle was identified and separated by blunt dissection. Muscle attachments were separated from the bone from about one-third around the bone throughout the length of the plate using a periosteal elevator. The fixator was then applied to the lateral side of the tibia. The plate was conformed to the plate contour and the arc of the rod was changed not to apply too much pressure to the cortex of the bone. Using a Gigli saw, the osteotomy was made in the middle third of the tibia.

The wound was closed in layers, starting with deep fascia with continuous suture and subcutaneous tissue and skin with interrupted suture with absorbable material VICRYL® 1, CP-1. After closure, chlortetracycline spray 20 mg/ml was applied to the wound. Amoxicillin (Amoxy-kel 15%) 5 ml i/m was administered after surgery as a prophylactic antibiotic. Rimadyl 2 mg/kg (carprophenum 50 mg/ml) was administered for pain management. The operated leg was radiographed with Medlink URS Veterinary Portable X-ray SP-VET-4.0 device (Sedecal, Madrid, Spain).

The operative procedure for bone plating included the same initial steps as described for the application of the RTP fixator – the plate was similarly applied to the lateral side of the tibia and the osteotomy was performed using a Gigli saw. As the plate is larger compared with the support plate of the RTP fixator, then the skin incision was more extensive. The plate was attached to the bone by six screws. The wound was closed in layers as described above and similarly to the application of RTP fixator the same prophylactic treatment, pain management and radiography was conducted.

After 8 weeks, the fixator was removed and animals were observed for 2 weeks to ensure that no kind of complications occurred and that bone healing was complete.

Blood biochemistry

Blood samples were taken from jugular vein (*v. jugularis*). Hair was clipped from the region of the jugular vein and skin was disinfected with Chemisept. Blood samples were taken before surgery and then after every 2 weeks up to the end of the experiment, that is, on post-operative weeks 2, 4, 6 and 8. From blood samples, the level of alkaline phosphatase (U/I), osteocalcin (ng/ml), total protein (g/l) and C-reactive protein (CRP; mg/l) was evaluated.

Radiography

The operated leg was radiographed following the surgery and then after every 2 weeks up to the end of the experiment (AGFA CR MD 4.0 General Plate AGFA-Gevaert, Germany) in craniocaudal projections. Positioning of the bone fragments, position of the fixator and callus formation were evaluated from the radiographs. The height of the fracture callus on radiographs taken on post-operative weeks 2, 4, 6, 8 and 10 was measured on the medial side of the tibia using software CELL^ 2.5 (Olympus Soft Imaging Solutions GmbH). The nonparametric Mann–Whitney *U*-test was applied for statistical analysis.

Histology

Sections of the bone were fixed for histopathological evaluation with 10% buffered formalin solution, decalcified in the Sakura TDE™ 30 Decalcifier System with Decalcifier Solution (Code 1428; Sakura, Alphen aan den Rijn, the Netherlands) during 3 days, and embedded in paraffin with vacuum infiltration processor (Tissue-Tek® VIP™ 5 Jr; Sakura, San Francisco, CA, USA). Specimens were cut with microtome Ergostar HM 200 (Microm, Walldorf, Germany) at 7 μm thickness and stained using the H&E and van Gieson methods for general orientation to sections. Sections were examined using the Zeiss Axiophot-2 microscope (Zeiss, Munich, Germany).

Immunohistochemistry

Collagen I, collagen V, osteocalcin and osteopontin were evaluated in bone tissue immunohistochemically. Four-micrometre-thick paraffin sections were mounted on poly-L-lysine-coated SuperFrost slides (Menzel, Braunschweig, Germany), deparaffinized, and rehydrated. Peroxidase activity was removed by 0.6% hydrogen peroxide (Fluka, Lyon, France) in methanol (Fluka, Munich, Germany), then the 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 I (diluted 1:100; Abcam, Cambridge, UK), collagen V (diluted 1:100; Abcam), osteopontin (diluted 1:1000; Abcam) or osteocalcin (diluted 1:500; Abcam) overnight at 4°C. On the next day, sections were washed in PBS and incubated with diluted biotinylated secondary antibody (Vectastain; ImmunoVision Technologies, Co, Burlingame, CA, USA) for 30 min at room temperature. The sections were washed with PBS and incubated for 30 min with Vectastain ABC Reagent. Then, the sections were incubated in DAB solution (Vector Laboratories, Burlingame, CA, USA) for 4 min at room temperature, rinsed in the PBS, and cells nuclei were counterstained with haematoxylin. Sections were dehydrated through graded ethanols, cleared in xylene, and mounted with Eukitt (Fluka, Buchs, Switzerland). For osteopontin detection in the tissues, polyclonal antibody to osteopontin (ab14715, Abcam), diluted 1:1000, was used. A shorter version of the above-described protocol was applied, which includes incubation of sections with antibody to osteopontin during 45 min at room temperature. Staining intensity of collagen I, collagen V, osteonectin, osteocalcin and osteopontin was expressed by a subjective scale ranging from 0 to 3 (0 – no staining, 1 – weak staining, 2 – moderate staining, 3 – strong staining). Immunohistochemistry negative controls were performed by omitting the primary antibody. 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.

Statistical analysis

Significant differences between experimental groups were tested nonparametrically by the Mann–Whitney *U*-test. GRAPHPAD INSTAT3 software (GraphPad Software, Inc, San Diego, CA, USA) was used to perform statistical analysis. The level of significance was set at $P < 0.05$.

Results

In the RTP fixator group, animals started to load body weight on the operated limbs the next day after the surgery. In the bone plating group, animals started to use the operated limb on the seventh day (registered by regular daily follow-up). In both groups, no changes in food intake were recorded, physical activity of animals was normal and complications were not noted.

Blood biochemistry

Serum total protein (TP) levels of all animals in both groups were within normal limits and no changes were observed during the whole experimental period (Fig. 1). C-reactive protein values in all animals were either 0 or <1 throughout the experimental period. Bone formation marker alkaline phosphatase (ALP) levels showed a clearly different pattern during the post-operative weeks in the bone plating group and the RTP fixator group: on the second post-operative week, ALP levels decreased in the bone plating group and increased in the RTP fixator group ($P < 0.05$). In the bone plating group, ALP levels stayed at a lower level throughout all post-operative weeks as compared to the pre-operative period, but in the RTP group ALP levels increased significantly on week 2 and started to decline steadily during the following post-operative weeks achieving the pre-operative values on week 8 (Fig. 2). On the other hand, no significant differences between the experimental groups were noted in the levels of osteocalcin (Fig. 3), another bone formation marker.

Radiography

After the operation, osteotomy lines were clearly visible. In the RTP fixator group, the first sign of callus formation was noted on week 2 radiographs, and on week 4, radiographs callus is clearly visible. After 6 weeks, the osteotomy line is poorly visible, and after 8 weeks, it is not radiographically detectable (Fig. 4). On radiographs, all the fixator elements were in their position throughout the whole experiment, neither were any abnormal tissue reactions noted during the experiment. In the bone plating group, callus formation was minimal and was seen on radiographs starting from week 4 (Fig. 5). A gap between the fragments was visible even after 10 weeks, at the time of implant removal.

Extensive callus formation in the RTP fixator group and modest formation in the bone plating group were also confirmed by measurements of the height of callus

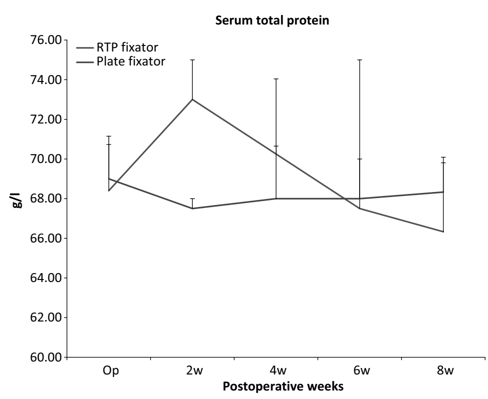

Fig. 2. Levels of serum total protein in experimental groups (RTP fixator – rod-through-plate fixator group; plate fixator – bone plating group; Op – values on operation day, on day 14 (2w), day 28 (4w), day 42 (6w) and day 56 (8w). Differences between groups are statistically not significant.

on radiographs. In the RTP fixator group, callus height (measured in pixels) on week 2 was 9.13 ± 1.08, on week 4 – 14.50 ± 1.06, on week 6 – 13.50 ± 0.38, on week 8 – 10.75 ± 0.45 and on week 10 – 10.38 ± 0.80. In the bone plating group, callus height was not measureable on week 2, on week 4 – 3.88 ± 0.35 ($P < 0.001$ versus week 4 in the RTP fixator group), on week 6 – 4.75 ± 0.31 ($P < 0.001$ versus week 6 in the RTP fixator group), on week 8 – 4.63 ± 0.63 ($P < 0.001$ versus week 8 in the RTP fixator group) and on week 10 – 1.00 ± 0.38 ($P < 0.001$ versus week 10 in the RTP fixator group).

No implant failure was found on radiographs during the monitoring time.

Histology

On week 2, bone biopsy was taken from one experimental animal in the RTP fixator group (Fig. 6a). In this sample, there was no remarkable widening of bone channels. In the cambial layer of the periosteum, one or two rows of osteoblasts were seen; the fibrous layer was thickened and dense. Periosteal callus and intermediate callus were formed by fine bone trabeculae that had mostly developed from the connective tissue. Still, in the intermediate callus, endochondral ossification was obvious with a smooth transition from the cartilage tissue to the bone. From the side of the periosteum, callus was lined by young bone tissue into which strands of loose connective tissue invaded. In deeper layers, a small amount of hyaline cartilage was found. Bone trabeculae were differently sized and scantly mineralized (contained young and large-sized osteocytes). The clear predominance of cells in the cells to matrix ratio revealed that the bone is newly formed.

On week 4, bone biopsy was taken from another RTP group animal (Fig. 6b). In this sample, bone channels were markedly widened. No changes indicating inflammation were found. Periosteum, especially its fibrous layer, was markedly thickened as compared to the week 2 biopsy specimen. Coarse collagen fibres were externally attached to the fibrous layer. Callus was massive and rich in cells indicative of active tissue. Trabeculae of the bony callus were massive, and formation of the bone occurred both by intramembranous and endochondral ossification. In the first case, bone trabeculae were surrounded by a regular chain of osteoblasts, or periosteal cells were involved in osteogenesis. Endochondral ossification in the current experiment occurred in two ways. The first way of ossification was characterized by the lack of a clear border between the hyaline cartilage and the bone tissue. During the bone formation, islets of cell-rich cartilage tissue were entrapped. In the second way, chondrocytes swelled, perished and bone tissue started to form around the areas of preserved ground substance. The main differ-

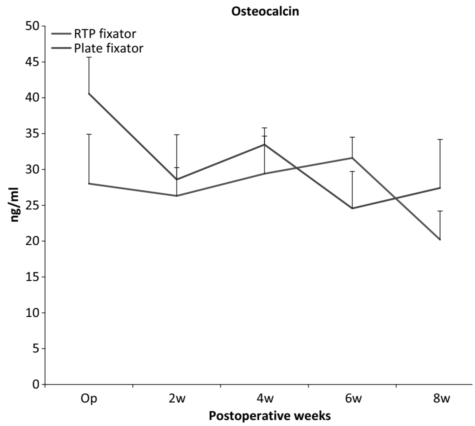

Fig. 3. Levels of osteocalcin in experimental groups (RTP fixator – rod-through-plate fixator group; plate fixator – bone plating group; Op – values on operation day, on day 14 (2w), day 28 (4w), day 42 (6w) and day 56 (8w). Differences between groups are statistically not significant.

ence in the newly formed bone, as compared to the week 2 biopsy specimen, was the development of the system of canals, which contained connective tissue and blood vessels, that is, the process of regeneration was more advanced, and in this, the set-up of the system of canals was in progress.

On week 10, all animals were sacrificed and osteotomy areas were studied histologically. In the RTP fixator group, normal osteocytes were seen in the bone fragments. Comparing to samples taken on week 2 and 4, the lacunae were less widened (Fig. 6c). Bone canals were enlarged, but widening of canals near the ends of fragments is a normal reaction to the trauma. As a rule, periosteal callus was very voluminous and contained a large amount of young cell-rich connective tissue. Bony callus contained differently sized bone trabeculae, the amount of which seemed to increase. This is indicated by the presence of osteoblasts around the trabeculae. Remodelling appeared to be limited, as no osteoclasts, resorption lacunae or macrophages were found.

Much less periostal callus formation and less connective tissue were seen in the bone plating group. In the connective tissue of the periostal callus, small bone trabeculae surrounded by chains of osteoblasts were seen (Fig. 6d). Cartilagineous periosteal callus contained hyaline and fibrous cartilage tissue, while in the bony callus, woven bone tissue dominated. Bone trabeculae were very different in size; osteocytes were normal and lacunae more narrow as compared to the RTP fixator group. On the bone trabeculae, chains of osteoblasts were seen. In bony callus, connective tissue was located between the trabeculae; osteoclasts were not present. Connective tissue and canals containing blood vessels had formed into bone trabeculae. In bone trabeculae, osteocytes in different developmental stages were localized. Intermediate and endostal callus contained a large amount of connective tissue; bone trabeculae were clearly expressed, but the few osteoblasts around them were placed irregularly.

Immunohistochemistry

Content of collagen I, collagen V, osteopontin and osteocalcin was detected in bone biopsies in the RTP fixator group on the second, the fourth and on the tenth post-operative week, and on the tenth post-operative week in plate fixator group. In the RTP fixator group, the level of the collagen I increased during the follow-up weeks: if on the second post-operative week collagen I content was low (Fig. 7a), then on the fourth week it was elevated (Fig. 7b) and reached its maximum on week 10 (Fig. 7c). In the bone plating group, collagen I content was statisti-

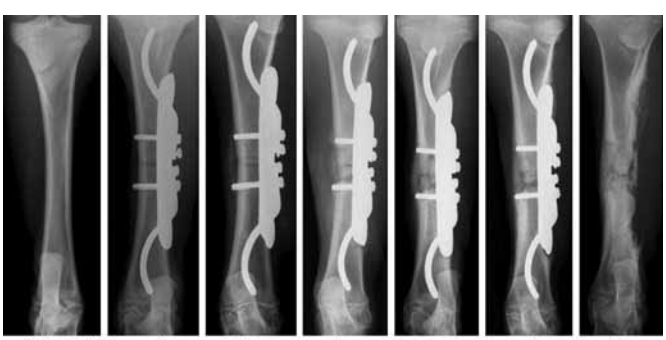

Fig. 4. Radiographs of sheep tibia before operation, after application of rod-through-plate (RTP) fixator, on post-operative weeks 2, 4, 6, 8 and on week 10, when RTP fixator was removed.

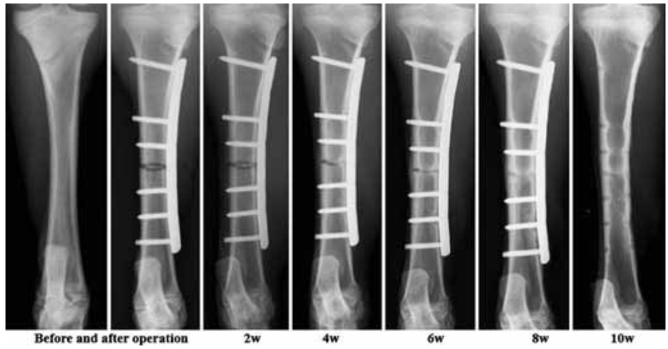

Fig. 5. Radiographs of sheep tibia before operation, after bone plating on post-operative weeks 2, 4, 6, 8 and on week 10, when plate was removed.

cally lower on week 10 ($P < 0.05$) compared with the RTP fixator group (Fig. 7d). The level of collagen V was low in the RTP fixator group on week 2, but was markedly elevated on week 4 and especially on week 10 (Fig. 7e). In the bone plating group, collagen V level was statistically lower ($P < 0.05$) on week 10 as compared to the RTP fixator group (Fig. 7f).

Osteopontin level was low in the RTP fixator group on weeks 2 and 4 (Fig. 8a,b), but on week 10 its level was equally high in both groups of animals (Fig. 8c,d). There were no clear differences in osteocalcin level between both groups, though on week 10 osteocalcin level was slightly higher in the RTP fixator group than in the bone plating group (Fig. 8e,f).

Discussion

In the current experiment, the intensity of bone regeneration after tibial osteotomy was investigated comparatively in two groups of animals. In one group, the tibial fracture was treated with the new RTP fixator and in the second group with the conventional bone plating. The new RTP fixator combining intramedullary and extramedullary osteosynthesis is designed to reduce soft tissue damage during the operation and to ensure stable fixation of bone fragments. Stable fixation of the fracture is definitely an important factor for effective reparative regeneration of the bone (Slone et al., 1991; Taljanovic et al., 2003). In the new RTP fixator, the support plate is relatively short and intramedullary rods extend the lever arm distally and proximally until metaphysis. Unlike bone plating, installation of the RTP fixator does not require excessive surgical approach, which reduces soft tissue damage and shortens the operation time (according to the records the operations where a RTP fixator was applied lasted for 84 ± 3 min on average, while the duration of bone plating operations was 95 ± 2 min). Limited tissue damage, stable fixation and early mobilization are key factors in

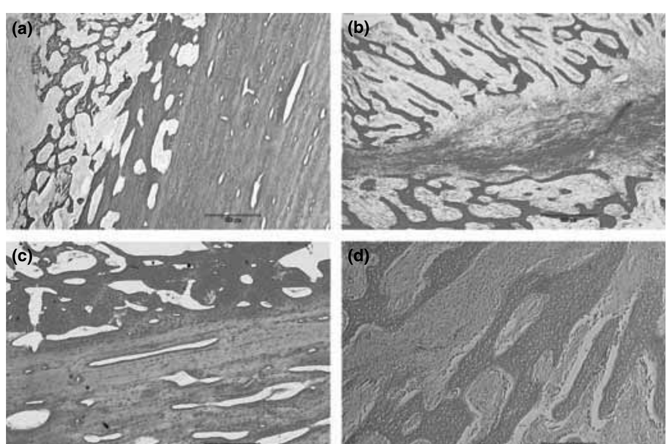

Fig. 6. Formation of bone in sheep. Rod-through-plate (RTP) fixator: (a) sample taken on week 2, intensive bone formation. (b) sample taken on week 4, formation of young bone tissue. (c) sample taken on week 10, complete healing of bone fracture. Bone plating: (d) sample taken on week 10, healing process in progress, amount of bone tissue still moderate. Haematoxylin and eosin staining (a, b, d), van Gieson staining (c).

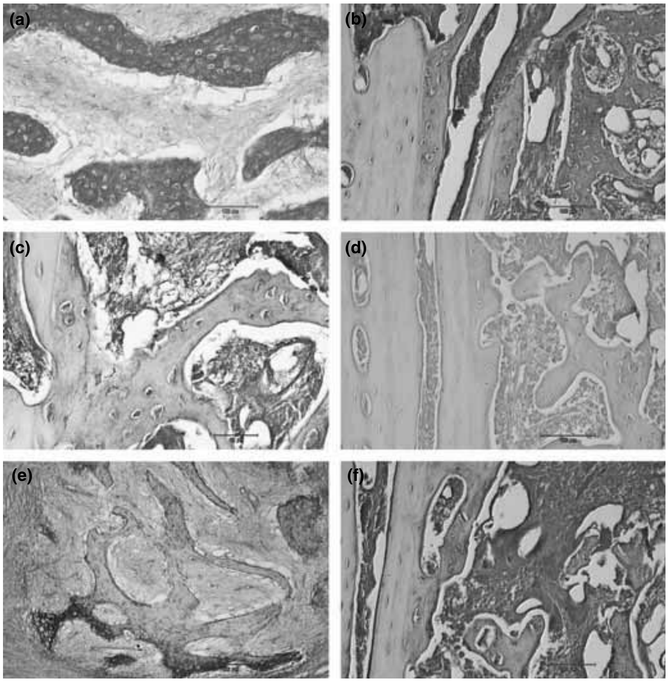

Fig. 7. Immunohistochemical detection of collagen I in samples of rod-through-plate (RTP) fixator group: moderate immunostaining on week 2 (a), strong staining on weeks 4 (b) and 10 (c), and moderate staining in bone plating group on week 10 (d). Strong immunostaining for collagen V in samples of RTP group (e) and bone plating group (f) on week 10. Staining DAB+haematoxylin.

restoring limb function and bone regeneration (Palmer, 1999). The stable construct of the RTP fixator was seen in radiographs, as all elements of the RTP fixator were in the same position throughout all the weeks of experiments. More intensive bone callus formation in the RTP fixator group compared with the bone plating group was confirmed by both radiographical and histological studies. By the end of the second week, histological findings of the bone biopsy of the RTP group samples matched the normal bone healing process (Murray et al., 1996). At that time, two stages (destruction and inflammation) of the bone healing were completed, and intense tissue regeneration and callus formation were seen on histological examination – fibrous connective tissue together with a smaller amount of cartilage tissue and young bone tissue were formed. After 4 weeks, the regeneration process was in progress, and in the newly formed bone, the characteristic formation of the canal system could be followed together with the increasing number of cells (McKibbin, 1978). Lack of processes typical of inflammation can be considered as a positive result, which is confirmed by low C-reactive protein levels. After 10 weeks, a small amount of cartilage tissue was seen in the RTP fixator group, which indicates a normal ossification process (Ekholm, 2001). A large amount of avascular cartilage tissue can lead to the formation of the fracture non-union (Harrison et al., 2003). As seen on radiographs and also histologically, a large amount of periosteal callus was found on week 10. At the same time, in bone plating group, the amount of callus was modest. The large amount of callus is obviously produced by the construction principles of the RTP fixator, more precisely by the method of its attachment to the bone cortex. The principle of the combined fixator is to create a so-called forceps effect between the support plate and the curved intramedullary rods. The intramedullary rods are in contact with endosteum at two points, and the third supporting point is on the plate. This special construction ensures pressure of the bone cortex between the plate and intramedullary rods when the screws are tightened. At the same time, the elasticity of the rods ensures micromovement between the fragments to avoid osteoporosis and refractures after implant removal (Field, 1997). Micromovement between the fragments stimulates callus formation and thus bone healing (Goodship and Kenwright, 1985; Jagodzinski and Krettek, 2007). If in the early stages of bone healing micromovements between bone fragments stimulate callus formation, then in the later stages in calcifying callus

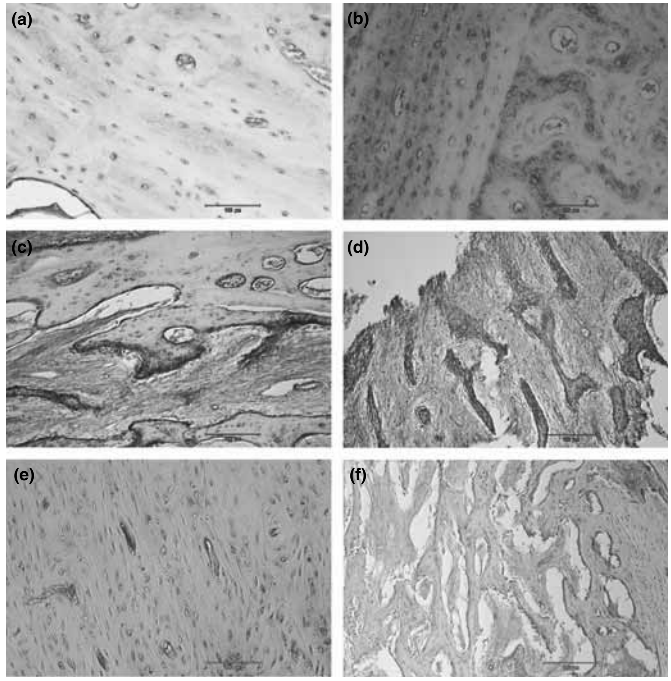

Fig. 8. Immunohistochemical detection of osteopontin in samples of rod-through-plate (RTP) fixator group: moderate immunostaining on week 2 (a), weak staining on week 4 (b), stronger staining on week 10 (c), and strong staining in bone plating group on week 10 (d). Strong immunostaining for osteocalcin in samples of RTP group (e) and moderate staining in bone plating group (f) on week 10. Staining DAB+haematoxylin.

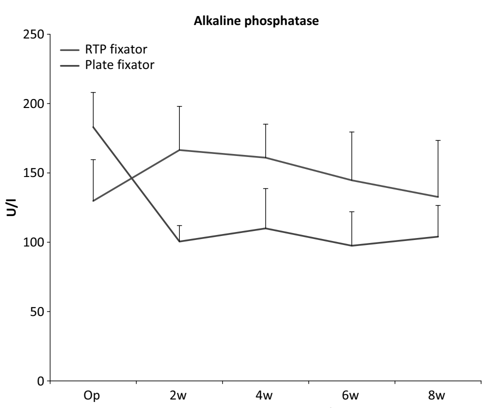

Fig. 9. Levels of serum alkaline phosphatase in experimental groups (RTP fixator – rod-through-plate fixator group; plate fixator – bone plating group; Op – values on operation day, on day 14 (2w), day 28 (4w), day 42 (6w) and day 56 (8w). $P < 0.05$ 2w RTP fixator group versus bone plating group.

movements should be minimal, but required stability is achieved naturally by increasing ossification of the callus (Jagodzinski and Krettek, 2007).

Although the levels of blood serum markers reflecting the process of bone regeneration fluctuated and the sample size was small, the dynamics of these parameters generally correlated with the morphological findings. While no changes were found in the serum total protein level in the bone plating group during the post-operative weeks, then in RTP fixator group, the levels were elevated on week 2, followed by a gradual decline during the next weeks. As deviations were high, the results were not statistically significant (Fig. 9). Clear dynamics could be seen in differences of the alkaline phosphatase (ALP) content. In the bone plating group, the levels of serum ALP decreased on week 2 and remained at the same level during the rest of the experiment. On the contrary, in the RTP fixator group, ALP levels were elevated on week 2 and decreased during the following weeks, reaching pre-operative levels on week 8. On week 2, serum ALP differences between the groups were statistically significant (Fig. 3). As the ALP is considered a parameter of intensity of bone formation, then this marker also refers to more intensive ossification in the RTP fixator group in comparison with the bone plating group. A similar increase in the level of serum ALP in sheep after experimental osteotomy during post-operative weeks 2–4 with a decline in the following weeks has been described by Klein et al. (2004). The same dynamics of the

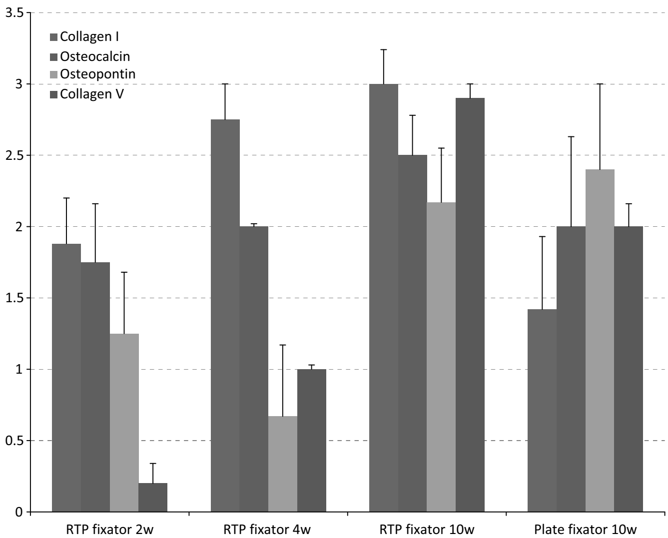

Fig. 10. Collagen I, collagen V, osteocalcin and osteopontin immunostaining in samples of fracture area of rod-through-plate fixator group animals (RTP fixator) on week 2 (w2), week 4 (w4) and week 10 (w10) or bone plating group animals (plate fixator) on week 10 (w10). Staining intensity was expressed by a subjective scale ranging from 0 to 3 (0 – no staining, 1 – weak staining, 2 – moderate staining, 3 – strong staining). Statistically significant differences ($P < 0.05$; the Mann–Whitney *U*-test): collagen I – RTP fixator 10w versus plate fixator 10w; collagen V - RTP fixator 2w versus RTP fixator 4w and 10w; RTP fixator versus plate fixator 10w; osteopontin – RTP fixator 4w versus RTP fixator 10w.

serum ALP level has also been reported in humans after femoral fractures – an increase during the second and the third week and a decrease during the next weeks (Ikegami et al., 2009). On the other hand, no changes between experimental groups were found in serum levels of osteocalcin (Fig. 10), another bone formation marker (Cox et al., 2010), although a declining tendency as compared to pre-operative levels was seen in both groups on week 8. A serious deviation of parameters reflecting bone formation in sheep has also been reported by other investigators (Klein et al., 2004), and thus, a larger number of animals should be involved to overcome the problem of dispersion of serum parameters.

The results of immunohistochemical investigation of bone biopsy samples indicated differences in the intensity of bone formation between the two experimental groups. The levels of collagen I, the main component of the extracellular bone matrix, were relatively high in the RTP fixator group on week 2 already, but still increased on week 4 and reached their maximum level on week 10. In the bone plating group, the content of collagen I was significantly lower on week 10 as compared to the RTP fixator group (Figs 10 and 7d). The results of collagen V immunostaining were relatively similar. Collagen V is a component of the bone matrix, which is found in a significantly smaller amount than collagen I. Collagen V is considered to regulate the formation and orientation of type I collagen fibres (Birk, 2001). In the RTP fixator group, the level of collagen V was very low on week 2, but on week 4 the levels elevated and on week 10 the collagen V content was almost equal with collagen I. In the plate fixator group, the content of collagen V remained low, similarly with the levels of collagen I (Figs 10 and 7f). Similarly to the osteocalcin levels in blood serum, no differences were found in immunohistochemical staining of osteocalcin in biopsy samples from the RTP fixator group. On week 10, osteocalcin immunostaining intensity was not different between the two experimental groups either (Fig. 8f). The tissue content of osteopontin, on the other hand, showed some differences. Osteopontin is one of the main non-collagen proteins of the extracellular matrix of the bone. The elevation of its level has been described during bone remodelling and mineralization (Yamazaki et al., 1999). In our study, an increase in osteopontin level on week 10 as compared to week 4 was also noticed in the RTP fixator group. At the same time, no considerable differences in the osteopontin level between the RTP fixator group and the plate fixator group were seen on week 10. (Fig. 8c,d). A similar increase in the content of osteopontin, but also of osteocalcin, has been described in rabbits 8 weeks after femoral osteotomy (Andrianov et al., 2007a).

Although the study was based on a limited number of animals, it can still be concluded that application of RTP fixation, which employs intramedullar and periostal osteosynthesis, is more efficient in early mobilization and faster bone regeneration than conventional bone plating in the treatment of experimental osteotomies of long bones in sheep. In the RTP fixator group, consolidation of fractures was faster, as demonstrated by radiographical, histological, and immunohistochemical investigations and in part by blood serum markers for bone formation. Similar results with the application of the RTP fixator have earlier been described in experiments with rabbits (Andrianov et al., 2007a) and in clinical cases with dogs (Andrianov et al., 2007b); hence, the RTP fixator with its simple construction and application can be recommended for treatment of diaphyseal fractures of long bones in small animals.

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Scientific publications of 5 last years and up to 5 major publications from an earlier period, if desired[1]	
1.1	**G. Tralman**, V. Andrianov, N. Talvi, A. Arend, M. Aunapuu. Standard surgical technique for applyingrod through plate (RTP) internal fixator.An experimental study in sheep. Scand J Lab Anim Sci, 2010, 37: 141-146.
1.1	Andrianov V., **Tralman G**., Hõim R., Haviko T., Lenzner A., Pääsuke M., Arend A., Aunapuu M. Rod-through-plate fixation of canine diaphyseal fractures. Vet Comp Orthop Traumatol 2007, 20(4): 308-11.
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1.2	**G. Tralman**, K. Nõupuu., D. Uksov, R.T. Kibur, P. Männik, N. Talve, V. Andrianov, P. Roosaar, A. Arend, M. Aunapuu. Using internal fixation for the treatment of long tubular bones in small animals: Evaluation of the plate fixator for tibial fractures in sheep. Papers on Anthropology 2008, XVII: 311-319.
Training activities (data about last 5 years' training activities and teaching aids compiled)	2009- 2013 lectures and practical training for veterinary medicine students in „Radiology " (3,5 points).

In-service training	06.04.-19.04.2004 Ludwig-Maximilian University in Munich, Germany 27.02.-29.02.2004 Soft tissue surgery courses, Helsinki, Finland 02.07.-04.07.2004 ECVS 13th scientific meeting, Prague, Czech Republik 01.07.2004 Clamp rod internal fixation seminar, Prague, Czech Republic 10.09.-12.09.2004 ESVOT congress, Munich, Germany 06.02.2005 Seminar in cat diseases, Jelgava, Latvia 24.09.2005 Oncologic surgery and reconstruction seminar, Vilnius, Lietuva 18.10.-21.10.2005 Courses in distal extremity surgery, Cremona, Italy 06.09.-07.09.2006 Cementless total hip replacement courses, Munich, Germany 08.09.-10.09.2006 ESVOT congress, Munich, Germany 28.10.2006 TTA courses, Zurich, Switzerland 08.09.2007 Elbow dysplasia film reading seminar, Munich, Germany 24.11.-25.11.2007 Dermatology seminar, Tallinn, Estonia 10.02.2008 Practical pharmacology seminar, Jelgava, Latvia 10.09.-14.09.2008 ESVOT congress, Munich, Germany 10.09.2009 Cranial cruciate ligament extracapsular stabilisation course, Munich, Germany 15.09.-18.09.2010 WVO Congress, Bologna, Italy 10.09.2011 Kyon TTA Master class, Zurich, Switzerland

22.09.-23.09.2011 Baltic Morphology 6th scientific meeting, Tartu,Estonia

12.09.-15.09.2012 ESVOT Congress, Bologna, Italy

30.11.2012 Kyon Proximal plated ulna osteotomy seminar and wet lab, Munich, Germany

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1.1	**Tralman G**., Andrianov V., Talvi N., Arend A., Aunapuu M. Standard surgical technique for applying rod through plate (RTP) internal fixator. An experimental study in sheep. Scand. J. Lab. Anim. Sci., 2010, 37: 141-146

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