EXPERIMENTAL INVESTIGATIONS

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A Comparative Electron Microscopic Study of Bone Repair After Internal Fracture, Osteotomy, and Perforation of Rat Tibia

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Key Words: *posttraumatic bone repair; electron microscopy; osteohistogenesis; osteotomy; perforation; tibia.*

Summary. *Background and Objective. Although previous studies have provided new information on bone repair, there are still gaps in knowledge about resorptive and formative processes during bone repair at the electron microscopic level. The aim of this study was to compare bone repair after the internal fracture, osteotomy, and bicortical perforation of the tibia by means of electron microscopy.*

Material and Methods. An electron microscopic study of bone repair after the internal fracture, osteotomy, and bicortical perforation of the tibia was performed on 72 male Wistar rats. Rats undergoing osteotomy and perforation were further subdivided into the control and immobilization subgroups. Bone repair was observed during the first posttraumatic weeks.

Results. Although bone repair in general had similar bone healing stages in all the groups, the repair process depended on the mode and degree of injury thus being different in the experimental groups. After the internal fracture, indirect ossification was observed; after osteotomy, primary periosteal, secondary endosteal ossification was noted; and after perforation, primary endosteal, secondary periosteal ossification was documented. Immobilization had an inhibitory effect on bone repair.

Conclusions. The results of the present study gave new information at the electron microscopic level about intracellular changes and intercellular matrix synthesis during different types of posttraumatic bone repair and confirmed our previous reports on similar posttraumatic bone repair in histomorphometric and immunohistochemical studies.

Introduction

Posttraumatic bone repair is one of the most complex and interesting phenomena in vertebrate biology (1). During fracture repair, unlike in the repair process of other mesenchymal tissues, a bone callus forms. The callus is divided histologically into mesenchymal, fibrous, chondrous, and bony (2).

In traumatology, contemporary surgery is largely based on a histomorphologic study of the callus in the healing of a bone fracture. Different types of injuries give rise to various results (3–4).

Although many experiments have been carried out to explain the factors of the strain environment that have an influence on the bone repair system (5–7), there are still no clear answers to it being a subject for extensive research.

As no literature can be found about comparative electron microscopic studies on repair osteohistogenesis originating from different types of trauma, the aim of the present study was to compare bone repair after the internal fracture, osteotomy, and bicortical perforation of the tibia by means of electron microscopy.

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Material and Methods

A total of 72 young male adult Wistar rats weighing 200–220 g were used in the study. The animals were kept at a constant temperature (22°C) and allowed water and special rat food (Dimela, Finland) *ad libitum*. The guidelines for the care and use of the animals were approved by the Ethical Committee of the University of Tartu.

The animals were divided into 3 groups: 1) internal fracture (34 rats); 2) osteotomy (15 rats); 3) perforation of the tibial cortex (28 rats). The second and third groups were subdivided into the following subgroups: 1) control animals (9 and 14 rats, respectively); and 2) immobilized animals (6 and 14 rats, respectively).

For immobilization, the rats were separated into narrow boxes, one animal in each to limit significant

movements of the rats. The internal fracture group was not subdivided into the control and immobilization groups as the internal fracture is considered as severe damage itself causing natural immobilization to the animal, and there is no need for additional immobilization.

Operative Technique. Pre- and postoperative prophylaxis of infection was carried out with ampicillin (7.5 mg/kg intramuscularly). Operative procedures were performed under strictly aseptic conditions, and anesthesia was induced with an intramuscular injection of ketamine (50 mg/kg body weight) and diazepam (5 mg/kg).

According to the study protocol, an internal nonfixed experimental fracture in the middle part of the tibia was done in the animals of the first group. In the second group, osteotomy 4 mm in length, 1–2 mm below the epiphyseal line of the tibia, followed by fiber fixation with the fibula was performed. In the third experimental group, a bicortical perforation hole 1.3–1.5 mm in diameter on the anterior surface of the tibia between the diaphysis and the proximal epiphysis was bored.

The animals were sacrificed with an overdose of ketamine and diazepam.

Transmission Electron Microscopy. For fixation, the specimen measuring about 0.5 cm was fixed in 3% glutaraldehyde solution (pH, 7.2), washed with 0.2-M phosphate buffer (3 times for 10 minutes), postfixed with 1% osmium tetroxide, and washed with phosphate buffer (3 times for 10 minutes). After dehydration (alcohol 70° → alcohol 90° → alcohol 100° → epoxypropan or aceton I → epoxypropan or aceton II, twice), the samples were embedded in epon in a thermostat at 60° for 24 hours followed by the sectioning of semithin (0.7 μm in thickness) and ultrathin (90 nm in thickness) sections with a Reichert-Jung Ultracut ultramicrotome. The semithin sections were stained with toluidine blue and observed under a light microscope. Ultrathin sections were contrasted with 2% uranyl acetate (for 2 hours) and examined and photographed with a transmission electron microscope JEOL 1200-EX II.

Results

The gaps between the fractured bones in the case of the internal fracture on the 4th posttraumatic day were filled with the mesenchymal and fibrous callus. Undifferentiated mesenchymal cells and fibroblasts synthesizing collagen fibers and extracellular matrix were observed (Fig. 1). During 7 posttraumatic days, the amount of collagen fibers synthesized by fibroblasts increased continuously. On the 14th day after the fracture, the islets of chondrocytes in the fibrous callus were noted; the fibrous callus was gradually replaced with the chondrofibrous, thereafter with chondrous callus. On the 28th posttraumatic day, osseous islets (osteoid, osteoblasts, osteocytes, and osteoclasts) appeared in the chondrous callus, an intensive synthesis of collagen was documented (Fig. 2). After the internal fracture, indirect, secondary osteohistogenesis was noted.

Fibroblasts, macrophages, and lymphocytes were first observed in the resection site 4 days after osteotomy (Fig. 3). Fewer fibroblasts and collagen fibers in the extracellular matrix were noted in the immobilization group compared with the control group (Fig. 4). At the end of the first postoperative week (7th day), the fibrous callus was replaced with the chondrofibrous and chondrous callus (Figs. 5 and 6). The synthesis of collagen fibers was less intensive in the immobilization group compared with

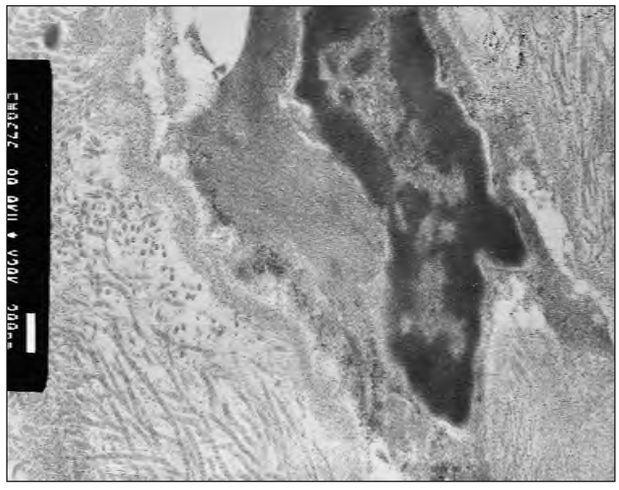

Fig. 1. Collagen type I synthesized by undifferentiated mesenchymal cells 7 days after the internal fracture (magnification ×20 000).

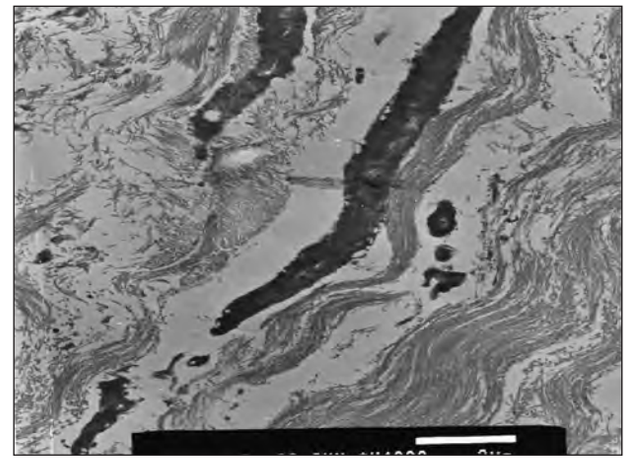

Fig. 2. Osteocytes and collagen type I 28 days after the internal fracture (magnification ×4000)

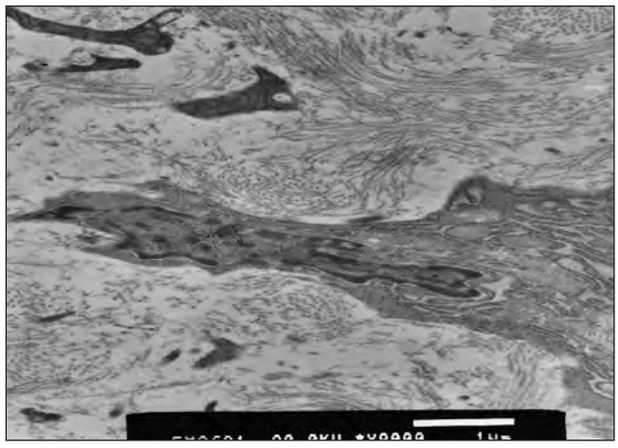

Fig. 3. Fibroblasts synthesizing collagen type I 4 days after osteotomy (magnification ×8000)

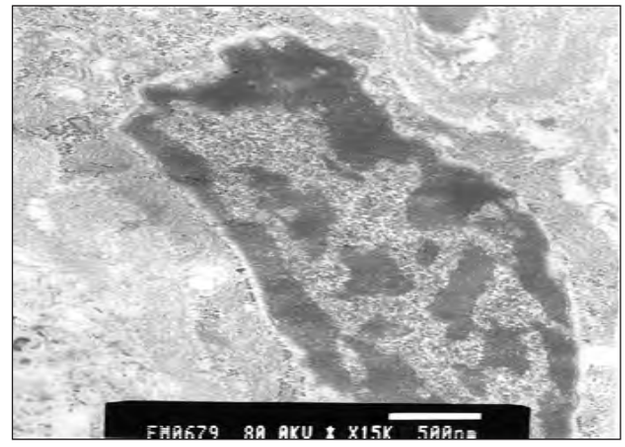

Fig. 4. Undifferentiated mesenchymal cells 4 days after osteotomy in the immobilization group (magnification ×15 000)

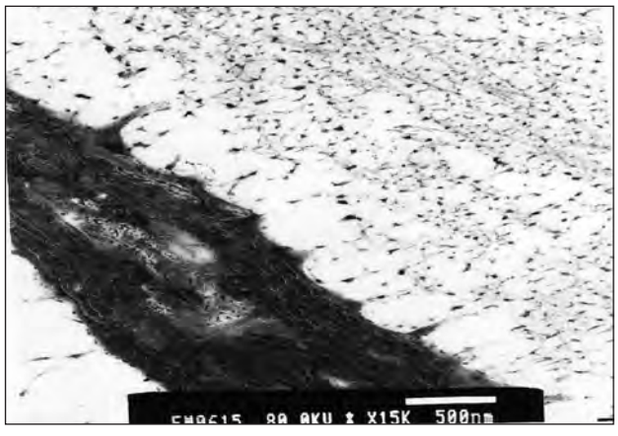

Fig. 5. Chondrocytes synthesizing collagen fibers 7 days after osteotomy (magnification ×15 000)

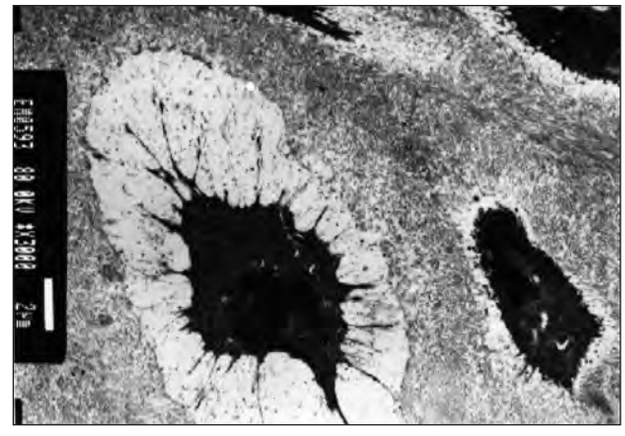

Fig. 6. Mature and immature chondrocytes 7 days after osteotomy in the immobilization group (magnification ×3000)

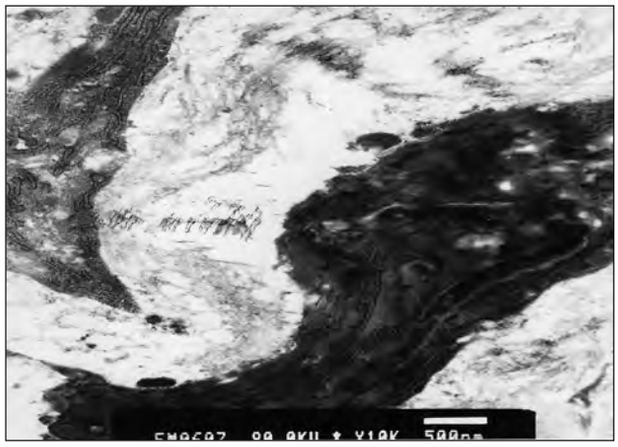

Fig. 7. Undifferentiated mesenchymal cells synthesizing collagen type I in the endosteal region 7 days after perforation (magnification ×10 000)

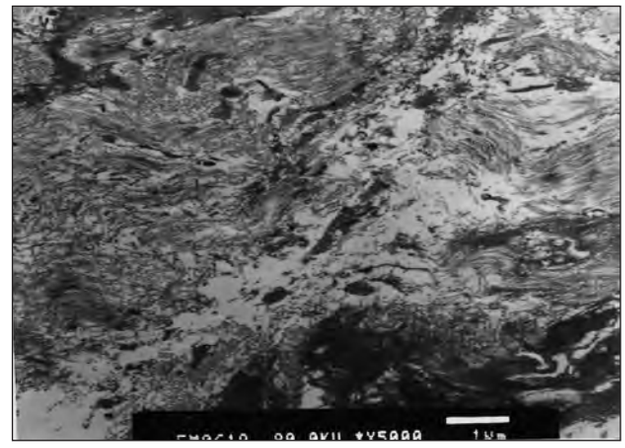

Fig. 8. Weak synthesis of collagen type I in the endosteal region 7 days after perforation in the immobilization group (magnification ×5000)

the control group. The repair after osteotomy was typical and similar to embryo histogenesis with direct/primary periosteal intramembranous and secondary endosteal ossification.

During 4 days after the bicortical perforation, the perforation site was filled with undifferentiated mesenchymal cells and fibroblasts. Until the end of the first posttraumatic week, no chondrocytes were

observed in the endosteal region; meanwhile, the chondrofibrous and chondrous callus was documented in the periosteal region. Compared with the control group, the synthesis of collagen fibers was weaker in the immobilization group (Figs. 7 and 8). The type of ossification was opposite to that in case of osteotomy, i.e., primary endosteal and secondary periosteal ossification was observed.

Discussion

Traumatology and orthopedics deal with the questions of bone healing as well as the recovery of limb functions during the treatment. Ossification processes are usually slow and frequently are accompanied by complications (pseudoarthrosis, osteoporosis, delayed repair). Posttraumatic bone repair is delayed by unstable fixation and several inhibitory external loadings (lack of movement, environmental factors, including different intoxications, etc.) and stimulated by physical activity, several osteoinductive factors, etc. Different types of bone injuries (external and internal bone fractures, osteotomy, etc.) have extensively been investigated by means of various experimental methods (8–12). However, data in the literature on the characteristics of histo- and organotypic posttraumatic bone repair depending on the size and localization of the bone defect, the influence of different external factors, etc. are scarce. Mesenchymal skeletal tissues as labile determined tissues easily turn into metaplasia with such clinical manifestations as easily developing hyperostosis, heterostosis, extraosseal heterotopic ossification, etc. To solve some of these clinically essential problems, morphological studies are needed.

Posttraumatic bone healing had similar repair stages (inflammation, callus formation, remodeling of callus tissues, etc.) in all experimental groups as reported previously. Still, based on our previous (basically histomorphometric and histochemical) and present electron microscopic studies, the repair process is generally dependent on the mode and degree of injury (13–15).

A lot of general (hypokinesis, intoxication) and local factors (blood supply, etc.) have an influence on bone healing (16–22). Studies employing different methods have shown that in trained mice, bone repair is stimulated (5), whereas it is inhibited in immobilized ones (23–26). Our present electron microscopic study showed that immobilization had an inhibitory effect on the regeneration of bone.

Conclusions

The results of our electron microscopic study on bone repair gave information about intracellular changes and intercellular matrix synthesis at the electron microscopic level and confirmed our previous histomorphometric and histochemical reports in posttraumatic bone repair studies. The formation of the chondrous and bony callus and speed of bone healing are dependent on the mode and degree of injury. Osteohistogenesis during bone healing is secondary (indirect) after the internal fracture; primary periosteal (direct and intramembranous), secondary endosteal (classical) after osteotomy, and primary endosteal (direct), secondary periosteal (replacing cartilage) after perforation. Immobilization had an inhibitory effect on bone repair, i.e., inhibition of collagen synthesis and later formation of the chondrous callus.

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Statement of Conflict of Interest

The authors state no conflict of interest.

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