EXPERIMENTAL METHOD OF INDUCING A BONE DEFECT IN RAT AND HISTOLOGICAL MONITORING OF THE EVOLUTION OF THE HEALING PROCESSES

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Summary

Finding ways to stimulate bone healing is a major concern for researchers in orthopedics and maxillofacial surgery. In order to test these ways of stimulating bone healing, experimental models are necessary to ensure method reproducibility and homogeneity of experimental groups. Our paper describes an experimental model of this kind, namely drilling femoral shaft defects in rats. We used Wistar rats, in which, under neuroleptanalgesia, we created a defect in the femoral shaft cortex using a dental drill of 1.6 mm diameter. Subsequently, samples were taken for histological examination at 2, 4, 6 and 12 weeks. Samples were fixed with 10% formalin, included in paraffin, sectioned at 5 µm and stained by Goldner’s Trichrome method. At 2 weeks postoperatively we found early elements of the bone healing process, represented by a thin layer of proliferated bone on the surface of the defect. This layer of bone thickens progressively, external (a collar of periosteal bone) and internal strengthening structures (trabeculae) concurrently appear which fill the medullary canal at 12 weeks. Our study highlights the fact that the first objective in bone healing is to restore continuity in the cortical, only after this step strengthening structures appear in order to provide resistance to the injured area. The method ensures precise control of defect size, which allows it to be replicated on a large number of rats, resulting in homogeneous composition of experimental groups. Also by this method, the bone healing process can be fairly evaluated even when different methods for stimulation of healing are applied.

Key words: bone, healing, rat.

Introduction

Although some animals’ bones (mouse, rat, sheep) have a different structure compared to human bones (have simpler and less osteons), they were frequently used in orthopaedic studies (Miller et al., 1995, Sistema, 1995, An & Friedman, 1999). Fracture healing was studied on diafisary lesions in animals of different species (mouse, rat, rabbit, dog, sheep, goat, cat and calf). Animals most commonly used were: rat, rabbit, dog and sheep. To assess the healing processes there have been used four methods to induce experimental fractures: manual fracture (Penttinen 1972), three-point bending methods (Greiff 1978), a guillotine-like fracture apparatus (Bonnarens & Einhorn 1984) and osteotomy (Nyman et al. 1996). Marked variation in the methods used in studies of bone healing showed that experimental procedures are difficult to standardize. Another way of experimental study of bone regeneration is drilling holes in the shaft of long bones (Monfoulet et al. 2009). They used mice as experimental animals and drilled a 0.9-mm-diameter through-and-through cortical hole in the mid-diaphysis of the femur. Such study is much easier to standardize, surgery may be
repeated in comparable circumstances. Since the practice of a hole in the cortex of the shaft of a long bone can provide a more accurate monitoring of healing processes, we considered it appropriate to experience such a method on rat femur.

**Material and methods**

The biological material used in this experiment was represented by 20 white Wistar rats, males, aged 6 months, clinically healthy, average weight of 230 g. To achieve the bone defects we have chosen the femoral shaft. For the surgeries, the animals were subjected to neuroleptanalgesia, using xylazine 8mg/kg and ketamine 40 mg/kg. Bone defects were made using a dental drill of 1.6 mm diameter, adapted to a dental micromotor at a speed of 3500 rpm. Irrigation with saline was used to avoid overheating. The defect was made through the whole thickness of the compact, reaching the medullary canal. In order to conduct histological investigations samples were taken at 2, 4, 6 and 12 weeks after the surgery. We chose the piece size in such way that the defect was framed by perilesional healthy bone tissue. The fixation of the samples was done using formalin 10%. The samples were then decalcified using trichloroacetic acid. After inclusion in paraffin, serial sections of 5 µm thickness were performed. The histological slides were then stained by Goldner’s Trichrome method.

**Results and discussion**

Microscopic examination of serial sections from the intervention site, performed 2 weeks after the start of the experiment showed early processes of bone healing. In the area of the experimental defect, thin bone trabeculae are present, disposed in such a way to attempt to cover the defect area, to interrupt communication between the medullary channel and the outside (Fig. 1). The trabeculae were formed on account of periosteum surrounding the bone defect. Other trabeculae became detached from the internal part of the bone wall at the periphery of the defect and also tend to expand, parallel to the defect rather than towards the medullary canal. They are formed mostly on account of endosteal lining of the medullary canal, but there are also portions of continuity with the proliferated periosteal bone on the surface of the experimental defect. Moreover, the medullary canal is taken by congestive bone marrow and does not contain even discrete bone trabeculae.

At 4 weeks after the beginning of the experiment, the layer of proliferated bone at the surface of the experimental defect is significantly thicker and reinforced so that it ensures restoration of bone continuity in the intervention area (Fig. 2). Moreover, this periosteal bone tissue extends laterally for a certain distance forming a sleeve between the wall disposed on the periphery of bone defect and the periosteum. It represents an external structure to strengthen the area of intervention. Also, from the layer of bone at the surface of the defect, extending on the inner walls of the cortical bone there are bone formations similar to those outside the cortical bone, but they are shorter and thinner. A few short trabeculae are also leaving this layer to expand towards the medullary canal. All these bone formations are internal strengthening structures, even though they are more discreet than the external.

At 6 weeks after the beginning of the experiment the bone layer at the surface of the defect is thicker than at 4 weeks, but still far from the thickness of the wall in the vicinity of the defect. As a structure, it is in the process of proliferation and remodelling to compact bone. External strengthening structures are somewhat thicker and wider than at 4 weeks and the inner structures are significantly more developed, although they still occupy no more than 30% of the marrow cavity. They are young bone trabeculae arranged mostly in the external half of the bone defect, but here too, the intertrabecular spaces are large and polymorphous (Fig. 3).
At 12 weeks after the beginning of the experiment, all the structures described at 6 weeks are more developed. The evolution of the bone layer at the surface of the defect and of the external structures of consolidation has been slow, but the internal strengthening structures have evolved more rapidly, reaching to occupy virtually all the medullary cavity (Fig. 4). They are represented by cancellous bone found in the process of proliferation and remodelling, but have the aspect of a scaffold made of bone trabeculae arranged in the direction of force lines.

During the healing processes, restoring bone wall continuity is the first priority, the bone layer at the surface of the defect being the one that is formed at the highest speed, so 2 weeks after the surgery this objective is partially achieved. Strengthening structures are formed somewhat slower, changes in the external ones are different from the changes in the internal structures, namely the outer strengthening structures appear faster, being relatively well represented at 4 weeks after the beginning of the experiment. The inner structures appear to flood the whole bone cavity only at 12 weeks, but up to complete remodelling needs to pass a period of time that we can not accurately assess. The fact is that at 12 weeks after commencement of the experiment the two objectives related to bone healing (restoration of bone continuity and the formation of strengthening structures) are largely achieved.

Monofoulet et. al (2009) compared the kinetics and healing pattern of bone lesions in mice using two protocols that consisted of making holes in the femoral shaft and distal epiphysis. The cortical defect from the femoral shaft was covered.
with bone tissue after 2 weeks and 4 weeks after surgery bone healing was finalized, while epiphyseal defect healing occurred only after 13 weeks.

This comparative study (Monfoulet) indicates that the shaft defect is a model of cortical bone healing and the epiphyseal defect is a model for cancellous bone healing.

The results we obtained are comparable with those found in the consulted papers. Early bone healing processes in rats are observed 2 weeks after the creation of the bone defects, as evidenced by the presence of thin bone trabeculae especially coming from the periosteum. Bone healing processes are constantly evolving, but are not completed even after 12 weeks of creating the defects.

Conclusions

Using the rat as experimental model in assessing bone healing processes allows the drilling of a large enough defect in the femoral shaft, which ensures running all stages of bone callus formation.

The diameter of the rat femur bone allows the inclusion of the whole surface of the section in a histological slide, both bone defect and bone compact on the opposite side. This allows assessment of the healing process over the entire lesion and at the periphery.

This method of inducing bone defects maintains continuity of the compact on the most part of the circumference, not being necessary to apply any technique of immobilizing the area.

The method ensures precise control of defect size, which allows it to be replicated on a large number of rats, resulting in homogeneous composition of experimental groups. Also by this method, the bone healing process can be fairly evaluated even when different methods for stimulation of healing are applied.

References


