Histological and biological changes in the epiphyseal plate during fracture healing

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Abstract The alterations that the epiphyseal plate undergoes during fracture healing are well documented microscopically, yet there are no reports in the literature which discuss the cellular and molecular changes that accompany this process. We studied fracture healing in 49 Wistar rats (5 weeks old) in which we inflicted a fracture to the distal third of the femur of the right hind leg (experimental side). The rats were killed 2 weeks later, and we dissected both hind legs from the hip joint to the knee joint, detaching all the surrounding soft tissues. We manually detached the distal epiphyses and the epiphyseal plates from both femurs. A piece of the epiphyseal plate was removed from the epiphyseal side of the femurs. In 25 animals, we analyzed the DNA content. In 8 animals, the specimen was studied under an electron microscope, and in the remaining 16 animals, the control and experimental sides were studied histologically. We found that healing was accompanied by an increase in DNA content, by a change in cellular activity, and by greatly accelerated apoptosis.

Key words Fracture healing · Epiphyseal plate

Introduction

Since the time of Hippocrates it has been realized that in children, reduction of a fracture of the femur should not be perfect, because this would result in a longer limb on the affected side. More than a century ago, Bryant described the method for femoral fracture treatment, stressing that overlapping on the side of the fracture (and thus an initial shortening of the leg) is very important to avoid elongation of the affected side. This method of inducing healing of the fracture mainly restores axial deformities, as opposed to rotational ones.

Materials and methods

Five-week-old male Wistar rats (n = 49) were housed at a constant temperature (22°C) with a 12-h light-dark cycle, and allowed water and food ad libitum. The experiments outlined below were conducted in accordance with the Guidelines for Animal Experimentation at the Aristotelian University of Thessaloniki.

With the animals under general anesthesia, we used mosquito forceps to trap and twist the bone and thus create a comminuted fracture of the right hind femur. We used a lateral approach to create the fracture in the distal third of the femur (not including the growth plate), where the quadriceps femoris and hamstring muscles are attached by tendons, because exposure of
the bone in this region is easy and the incision is nearly bloodless.

The wound was closed with one absorbable stitch, and the animal was allowed to recover and to move freely about the cage. All animals began using the operated leg on the sixth day after surgery. Two weeks postoperatively, the animals were killed, and both hind legs were dissected at the hip level. The femoral bones from the hip joint to the knee joint were prepared after removal of the soft tissues, at which point we noted richly woven bone on the fractured side. The distal epiphysis of each femur was then visually identified and manually detached from the metaphysis via the epiphyseal plate. Our specimens therefore included the proliferating zone, with part of the maturing zone, as the other zones remained with the metaphyseal part of the plate.

In 33 of the 49 rats, a cartilaginous specimen of the epiphyseal side of the right (experimental) and left (control) epiphysis was removed. In 8 animals, the specimens were prepared for study under an electron microscope. In 25 animals, the DNA content of these cartilaginous specimens was studied in two ways: in 6 animals, pure DNA was extracted by the phenol-chloroform method and measured as described by Teixeira et al., and in the remaining 19 animals, the amount of DNA was assessed indirectly by fluorimetric methods. Briefly, the specimen as a whole was ground in a solution containing Hoechst 33258 dye (which binds only DNA by intercalating in the double helix), and the fluorescence of the mix was then determined in a fluorimeter. As the fluorescence emission value is proportional to the amount of DNA in each sample, the DNA amount (in micrograms) of each sample was determined by comparing its fluorescence emission with that of samples containing known amounts of DNA.

In 16 of the 49 rats, the right (experimental) and left (control) femurs were demineralized, and slides were prepared which included the epiphysis, epiphyseal plate, and metaphysis. The slides were stained with hematoxylin and eosin and prepared for histological examination under an optical microscope.

Results

Histology

Macroscopically, the thickness of the right femur distal to the fracture was greater than that of the control distal femur (Fig. 1a,b compare right-side [5-mm] and left-side [3-mm] images). This difference was readily apparent when the bones shown in Fig. 1 were examined histologically (Fig. 2a,b corresponds to the left and right bones shown in Fig. 1a,b). After the epiphysis was, detached the remaining epiphyseal plate on the metaphyseal side showed shallow notches on the experimental side and deep notches on the control side (Fig. 1b). With the camera at 5× magnification, we observed that the diameter of the epiphysis of the right femur was much larger than that on the control (left) side. This was expected, because one result of a fracture is an increase in blood supply which in turn increases the activity of the ossification center. Because of this enlargement of the epiphysis of the right femur, the folds of the epiphyseal plate on the right side were smooth, but on the left they were jagged (Fig. 2a,b). At higher magnification (25×), the above finding was more pronounced. We also observed that the right (experimental) epiphyseal...