EFFECT OF THYROCALCITONIN ON THE MINERAL COMPONENT OF REGENERATING BONE DURING HEALING OF FRACTURES

V. A. Landa and A. I. Briskin

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Changes in the state of the mineral component of bone tissue (hydroxyapatite) during reparative regeneration of bone after administration of thyrocalcitonin (TCT) were studied in experiments on rabbits. Methods of histology, quantitative microroentgenography, and x-ray diffraction were used. TCT has no marked stimulant effect on normal osteogenesis and mineralization of newly formed bone tissue. However, mineralization of the callus follows an optimal course and osteoporosis of the fragments is much less marked than in animals not receiving TCT.
KEY WORDS: regeneration of bone; thyrocalcitonin; mineralization of bone tissue; hydroxyapatite.

Thyrocalcitonin (TCT), a thyroid hormone produced and secreted by the "pale" or parafollicular cells [10], plays an important role in the regulation of mineral metabolism. Experimental results have shown that TCT not only inhibits the osteoclastic resorption of bone tissue [8], but also leads to an increase in the number of osteoblasts and stimulates the absorption of bone and the intracellular accumulation of calcium [9, 11].

The object of this investigation was to study the effect of TCT on the state of the mineral component of bone (hydroxyapatite) in newly formed bone tissue at various stages of reparative osteogenesis.

EXPERIMENTAL METHOD

An incomplete transverse osteotomy of the middle third of the radius was performed in rabbits. Observations were made from 7 to 30 days after the operation, i.e., in the period of the reparative reaction of the bone tissue and the initial stage of reconstruction of the callus.

For quantitative evaluation of the content of the mineral component in the microstructures of the bone tissue the method of quantitative contact microroentgenography [2-6] was used. Microroentgenography of the specimen for testing a microsection of bone tissue was carried out simultaneously with the filming of a standard aluminum step-wedge by the contact method on VR-1 plates with high resolving power (about 1000 lines/mm for the visible spectrum). The subsequent photometry, comparison of the intensities of absorption of x rays by points of the experimental specimen with the steps of the standard, and the appropriate calculations, gave the content of the mineral component in the microstructures of the test object in σ/cm^3 [2, 3, 5, 12].

According to the results of quantitative microroentgenography the extent of the mineralization of intact rabbit bone is 1.57 ± 0.03 g/cm^3.

To judge the dynamics of the crystalline structure (in which the size of the crystallites plays an important role) the method of x-ray diffraction was used, and changes in the width of the 002 diffraction line, i.e., in a direction coinciding with the longitudinal axis of the intact bone, were studied. The ratio $b_0/b = K$ (a relative index of the width of the diffraction line), in which b is the width of line on the roentgen-

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ogram of the specimen and b_0 the width of the line of the standard substance, was used as the parameter characterizing these changes. The width of the corresponding maximum of the roentgenogram obtained by filming intact bone tissue, for which K=1.0, was used as the standard.

To monitor the state of the regeneration process, the data of ordinary roentgenography and histological investigation of the region of the fracture, at the same times as the microroentgenographic investigation, were used.

The blood calcium level in the rabbits was determined by a spectrophotometric method. A highly purified long-acting Soviet preparation of TCT [1, 7], consisting of a complex of TCT with polyvinylpyrrolidone, was used and was given by subcutaneous injection in a dose of 20 units once a day, 5 times a week.

A similar operation (incomplete osteotomy of the middle third of the radius) was performed on the rabbits of the control group. The periods of observation on regeneration in these animals were the same as in the experimental series.

EXPERIMENTAL RESULTS

The hypocalcemic action of TCT was clearly manifested 21 and 30 days after the operation, when the serum calcium concentration reached 12.2 ± 0.75 and 12.5 ± 1.7 mg %, respectively; the initial level was 14.9 ± 0.5 mg %.

On the 7th day after trauma no significant difference was found in the state of osteogenesis in the animals of the experimental and control groups. The gap in the radius with the thin shadow of the callus at the ends of the fragments was clearly visible on the macroroentgenograms. The presence of trabelulae of bone tissue (mainly of woven bone, in some places parallel bone fibers in the endosteal and periosteal callus) was visible histologically. The variations in the mineralization of the newly formed bone tissue were from 1.19 to 0.82 g/cm^3 compared with 1.13 to 0.67 g/cm^3 in the control series. The defect between the fragments was filled with fibrocellular osteogenic tissue. The value of the index K was 0.5-0.6 in both the experimental and the control series.

During the next 2-3 days the fragments in the experimental and control animals were united by endosteal, periosteal, and intermediate callus. On the macroroentgenograms the gap between the fragments was less clearly differentiated than previously. Their ends were more compact. Newly formed bone tissue consisted of woven bone, parallel fibers and, in some places, lamellar bone. However, the bone tissue of the callus in the experimental rabbits was on the whole rather more mature than in the controls, and mineralization of the microstructures of the callus was at the upper limits of its level in the control animals. For example, in the endosteal callus of the experimental rabbits it was 1.3-1.51 g/cm^3 compared with 1.05-1.48 g/cm^3 in the controls. The relative width of the diffraction line (K) in the experimental group varied from 0.6 to 0.85, virtually the same as in the control (from 0.5 to 0.85).

By the 30th day the gap was ill-defined on the macroroentgenograms of both the experimental and the control animals. Its outlines were rather thickened. In the control rabbits rarefaction of the cortical layer was observed, but this could not be seen in the experimental animals. Histologically, maturation of the bone tissue of the callus had progressed further, and the less mature fibrous bone tissue was replaced by lamellar. The data of the microroentgenograms, like the results of histological investigation of the preparations, showed that the newly formed cancellous bone tissue had been replaced during regeneration by compact bone, especially in zones of the callus near the cortical layer. Zones of resorption of callus were seen at a distance from the cortical layer. The newly formed bone tissue in the experimental rabbits was on the whole rather more mature than in the controls. Mineralization of the microstructures of the bone tissue of the callus was increased in both the experimental and the control series, to (for endosteal callus) 1.36-1.55 and 1.27-1.41 g/cm^3, respectively, i.e., in the experimental animals it was at the upper limits of mineralization in the control animals or a little above them. A diffraction roentgenogram could be obtained at this period from only one preparation because of resorption of the callus, of which only small traces were left. The index K was 0.5. Similar values were found in the control, where K varied from 0.5 to 0.8. In the control animals marked osteoporosis of the fragments of injured bone were still present, having developed during healing of the fracture. This was shown chiefly by the presence of many dilated vascular canals in the cortical layer, which were particularly conspicuous on the 30th day. There were far fewer vascular canals in the experimental rabbits and they were less dilated.

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