EFFECT OF EXOGENOUS RNA AND ULTRASOUND ON FRACTURE HEALING IN RATS

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Homologous RNA obtained from regenerating bone tissue stimulated healing of a fractured femur in rats. The tissue matured more rapidly, resistance to separation of the fragments was greater, and the collagen content higher. Injection of highly aggregated RNA had no stimulating effect on osteogenesis.

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The role of protein synthesis in bone regeneration has now been demonstrated [1, 3] and some aspects of mineralization and crystal formation have been made clear [4-6, 13]. Since one of the principal stages in osteogenesis is biosynthesis of the protein matrix, factors inducing this process must be included among the regulators of reparative regeneration of bone. Several workers have shown experimentally [1, 8-12] that the RNAs, with the ability to accelerate synthesis of synthetic proteins, are evidently inducing agents of this type.

Because of the organ-specific action of exogenous RNAs on protein synthesis, it was decided to investigate the effect of these RNAs on bone regeneration. The action of ultrasound and exogenous RNAs on the repair process in bone was also examined.

EXPERIMENTAL METHOD

Experiments were carried out on 240 albino rats weighing 180-200 g in which a fracture of the femur was produced and the fragments subsequently fixed by a metal pin. The animals of series I received homologous RNA obtained by Georgiev's method of thermal phenolic fractionation [2] within the range 0-10°. The RNA preparation was injected intramuscularly at a distance from the fracture site in accordance with the following scheme: 1 mg/100 g body weight on the 2nd day and 0.5 mg/100 g on the 4th, 6th, 8th, and 10th days. The animals of series II received a preparation of highly aggregated RNA obtained by the same method, but using dodecylsulfate and bentonite as detergent. The animals of series III received physiological saline intramuscularly in the same volumes (control). Some animals received the highly aggregated RNA by the same scheme with simultaneous irradiation of the fracture region with ultrasound on the UTP-1 apparatus, at a frequency of 890 kHz, with a special tube attachment, in a dose of 0.4 W/cm^2 (series IV). The animals were sacrificed 10, 15, 20, 30, and 45 days later. Regenerating bone was investigated histologically (stained with hematoxylin-eosin and by Van Gieson's method), for mechanical strength by means of the RM0.5 apparatus, and biochemically (collagen determined as hydroxyproline) [7].

TABLE 1. Effect of RNA on Fracture Healing

Series	Time of sacrifice (in days)	n	Hydroxyproline content in regenerating bone (in mg/g dry tissue)	Mechanical strength of regenerating bone (g/cm^2)
I		4	8,15±0,52	2 200±121,4
II	10	4	3,10±0,29	1 600±201,4
III		4	3,56±0,56	1 633±242,0
I		4	5,90±1,07	3 300±441,0
II	20	4	3,90±0,49	2 400±189,0
III		4	4,34±0,73	2 267±497,0
I		4	3,00±0,78	5 170±583,0
II	30	4	4,00±0,28	3 400±199,0
III		4	4,50±0,75	4 000±364,0
I		4	3,10±0,48	7 820±665,0
II	45	4	4,00±0,58	4 500±384,0
III		4	3,31±0,10	5 000±529,0

EXPERIMENTAL RESULTS

On the 10th day in the zone of the fracture in the control animals mainly a fibroreticular tissue was found, with osteoblastic cells near the ends of the fragments. In animals receiving injections of homologous RNA, the zone of the defect was filled mainly with chondroid tissue. In series II the fractured zone was filled partly with fibroreticular and partly with granulation tissue.

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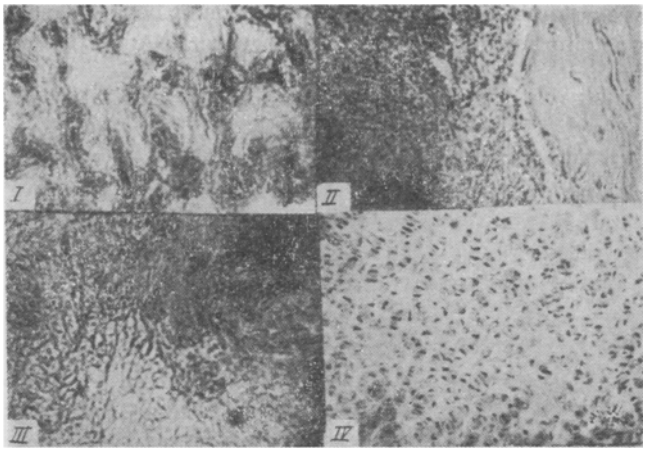

Fig. 1. Regenerating tissue predominating in zone of fracture on 20th day in different series of experiments (I-IV). Hematoxylin-eosin, 120 ×.

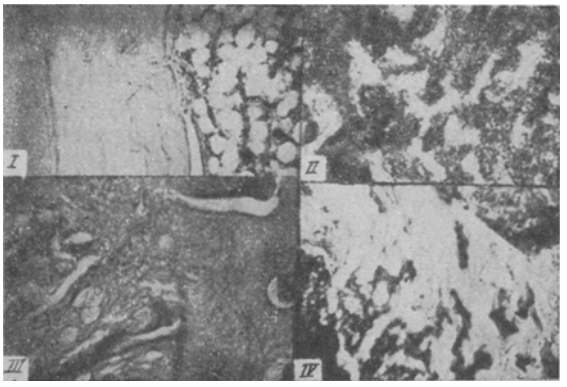

Fig. 2. Regenerating tissue predominating in fracture zone on 45th day in different series of experiments (I-IV). Hematoxylin-eosin, 120 ×.

As Table 1 shows, the mechanical strength of the newly formed regenerating bone was 35% less in the control on the 10th day than in the experiments of series I. In series II the strength of the regenerating bone to a separating force corresponded to the control results. The collagen content in the regenerating bone of the control animals was 3.56 mg/g dry tissue, and 8.15 mg/g in the animals of series I, i.e., 228% greater. In series II the hydroxyproline content was almost the same as in the control.

On the 20th day a significant difference appeared in the character of regeneration in all series of the experiments. At this time in the control animals the ends of the bone fragments were largely joined by zones of chondroid tissue. In the animals of series I the bone fragments were joined by tissue consisting of newly formed bone trabeculae, the areas between which were filled with fibroreticular tissue rich in blood vessels. In the animals of series II the zone of the fracture was filled with fibroreticular tissue, among which were zones of chondroid tissue. Deposits of newly formed bone tissue were present along the periosteal surface of the fragments (Fig. 1).

On the 30th day the mechanical strength of the regenerating bone (Table 1) in the experiments of series I was 129% greater than in the control. The collagen content on the 30th day in series I was 1.5 mg/g less than in the control.

On the 45th day differences persisted between the control and the experiments of series I. In the control and in series II, spongy bone tissue was present in the zone of the fracture, undergoing reorganization, whereas in series I the fragments of the femur were joined by newly formed compact bone tissue, with a developed medullary canal (Fig. 2). Significant differences also were observed in the strength of the regenerating bone. In the control and in series II the breaking force did not exceed 5600 g/cm^2, while in series I it was 7820 g/cm^2. The collagen content on the 45th day was back to normal in the experimental and control series, namely 3.5 mg/g.

As the experimental data show, different types of RNA obtained from bone tissue differ in their effect on healing of fractures. Homologous RNA stimulates fracture healing. This stimulating action is seen most clearly in the early stages of regeneration. The formation of differentiated structures and maturation of regenerating tissues in series I were observed 20-25 days earlier than in the control. The collagen content in series I rose sharply in the early stages of healing and anticipated the control series by 20 days.

Highly aggregated RNA did not accelerate regeneration of bone tissue, but actually slowed the process to some extent. Ultrasound, combined with highly aggregated RNA, in the dose used, likewise did not stimulate osteogenesis.

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