COMPARISON OF THE ACTION OF SOME SERUM PROTEINS ON TISSUE REGENERATION

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Serum albumin (SA) is known to have a common site of synthesis and very similar physiological properties with the basic protein of the early embryonic period, alpha-fetoprotein (AFP); the SA concentration, moreover, rises during prenatal development as the AFP level falls [7-9, 12]. In view of the indications in the literature that AFP participates in the immunogenesis system [6, 13] and that it performs growth-stimulating functions in embryogenesis [11, 13], experimental assessment of the action of ARP and SA on reparative regeneration is of great theoretical and practical importance. The reason is that commercial preparations of placental albumin, which are widely used in clinical medicine, contain high concentrations of AFP [3]. However, the biological role of this protein in the body has not yet been finally explained [1, 4, 10].

Traditional assessment of the effect of physiologically active substances, including protein preparations, on regeneration does not always characterize fully the ultrastructural changes in cells in the repair zone. By using radiospectroscopy, which has been applied on an ever-increasing scale in recent years in different branches of biology and medicine, new data can be obtained on the state of the cell membranes and the morphological and functional features of cells during regeneration [5]. Meanwhile no information could be found in the literature on the comparative results obtained by the study of reparative regeneration by the usual methods and by means of radiospectroscopy. To compare the action of ARP and SA on tissue regeneration, besides the histological and morphometric methods, we therefore also used the method of electron paramagnetic resonance (EPR).

EXPERIMENTAL METHOD

Healing of full-thickness skin wounds on the animal's back, with an area of 150 mm^2 (series I) and of closed diaphyseal fractures of the humerus (series II) was studied in experimental 145 (BALB/c × C57BL/6)F_1 mice weighing 20-25 g, of the same age and sex. In each series the animals of group 1 received a daily intramuscular injection of an AFP preparation containing 0.2 mg protein in 1 ml of physiological saline, obtained by various chromatographic methods from human fetal tissues. The sessional dose of AFP was 0.2 mg/kg body weight. Animals of group 2 received an injection of SA, isolated from blood from healthy blood donors, under the same conditions. Control animals received no protein preparations. The area of the skin wound was measured by a specially devised method (Certified Efficiency Suggestion No. 191 dated September 27, 1977) and the rate of contraction was calculated. Consolidation of the fracture was monitored by means of roentgenograms.

Samples of skin of equal weight (200 mg) were taken from the marginal zones of the wound surface after sacrifice of the animals on the 2nd, 5th, 8th, 11th, 14th, 17th, 20th, and 25th days of the experiment and the beginning of injection of the preparations, and suspensions consisting of mixtures of different types of cells in buffered physiological saline were prepared. Suspensions of callus cells, containing a considerable admixture of cartilage homogenate in the early stages of regeneration, were prepared similarly. The suspensions were prepared without the aid of enzymes [14]. The presence of small quantities of connective-tissue fibers and mineralized matrix as impurities in the suspensions was accepted.

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TABLE 1. Duration of Healing of Skin Wounds of the Dorsal Region and Fractures of the Humerus in Experimental Animals Receiving Protein Therapy (M ± m)

Character of trauma	Treatment	Number of animals	Time of healing, days	P
Full-thickness skin wound on the back, with an area of 150 mm²	AFP	30	16,0±0,5	<0,05
	SA	25	24,0±0,8	>0,1
	Control	20	26,0±0,6	
Diaphyseal fracture of humerus (without immobilization)	AFP	30	19,0±0,6	<0,05
	SA	25	26,0±0,5	>0,1
	Control	15	30,0±0,8	

The cell suspension prepared immediately before use was incubated in a volume of 1 ml with 0.02 ml of a 10^{-3} M solution of the spin-labeled benzocarbolene in dimethylsulfoxide (the benzocarbolene spin probe used was 5,6-benzo-2,2,4,4-tetramethylpiperidine-1,2,3,4-tetrahydro-γ-carbolene-3-oxyl). The biological material in a special quartz cuvette was placed in the resonator of a radiospectrometer of "Rubin" type, and the first derivatives of absorption lines obtained by scanning the strong magnetic field, in which the test object was placed, were recorded on an X-Y writer. The EPR spectra were processed as described in the literature [2]. Interaction between benzocarbolene and cells at different stages of tissue repair was assessed by analysis of the rotational mobility of the spin probe τ (the rotational correlation time of diffusion). Histological preparations obtained in parallel experiments from the repair zones were stained with hematoxylin and eosin, with toluidine blue, and by Van Gieson's and Mallory's methods.

EXPERIMENTAL RESULTS

The duration of healing of the skin wound and fractures in animals receiving and not receiving the protein is shown in Table 1. In the experimental animals of group 1 an increase in the quantity of fibrous structures in the granulation tissue, accompanied by stabilization, followed by a decrease in the concentration of fibroblasts, active macrophages, and vegetative surface microflora took place as early as on the second or third day of the experiment. In the animals of group 2 these processes began to develop one or two days later. The appearance of collagen fibers in wounds of the control animals was not observed until the beginning of the second week. The protein preparations thus accelerated wound healing, and administration of AFP led to a more intensive course of the regenerative process in the earliest stages. Unlike SA, ARP not only stimulated maturation of young connective tissues in the wound, but also stimulated proliferation of the epithelium. This led to hyperplasia of the epidermis around the wound. In the animals of group 1 on the 5th day of the experiment, for instance, the thickness of the epithelial layer adjacent to the wound was 10-12 times greater than that of the growing epidermis in the center of the wound, but in mice of group 2 and in the control animals it was only 5 or 6 times thicker. Graphic analysis of the parameters of the skin wounds demonstrated the nonuniformity of the healing process and activity of the proteins used, which was greater in the case of AFP (Fig. 1). This conclusion also was confirmed by the results of investigation of the cell suspensions by the EPR method.

Analysis of the spectrograms showed that in animals receiving AFP the value of τ was appreciably increased from the very first days, and reached a maximum by the end of the 2nd week (Fig. 2a). This corresponded to activation of the healing process, accompanied by reorganization and increased complexity of the tissues in the repair zone, both morphologically and chemically. In the second week a tendency was again observed for the value of τ to increase, which was a quantitative reflection of the qualitative changes in the regenerating skin, expressed as the formation of a connective-tissue scar. When SA was given the increase in the value of τ was not observed until the end of the first or beginning of the second week of the experiment, and it corresponded to activation of regenerative processes in the repair zone, in agreement with the histological picture. In the control animals the absolute values of τ were lower at all stages of healing than in the experimental animals, and they increased only toward the end of the period of observation, parallel with epithelization and scar formation. The results are thus evidence that the different stages of reparative changes in the tissues can be accurately recorded and assessed qualitatively.

In the experiments with fractures of the long bones in animals treated with AFP proliferative processes were observed in the zone between the fragments as early as on the first day,

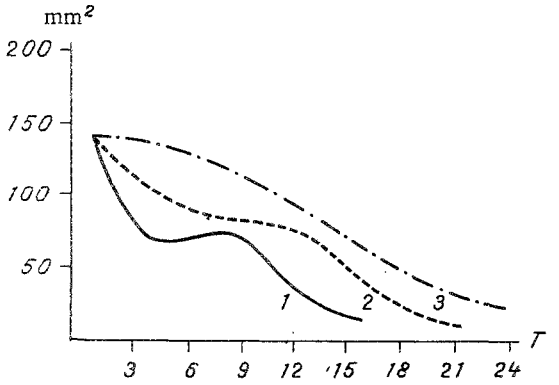

Fig. 1

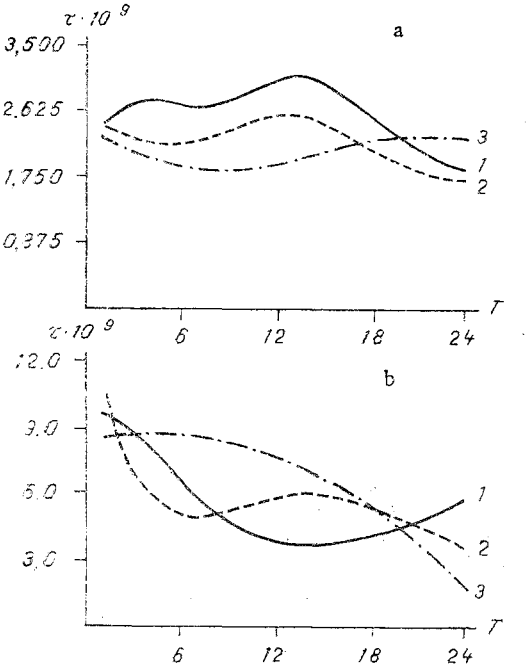

Fig. 2

Fig. 1. Changes in area of dorsal skin wound in mice during healing under the influence of serum proteins. Abscissa, Time of experiment (in days); ordinate, area of skin wounds (in mm). 1) AFP; 2) normal blood SA; 3) control (without injection of protein).

Fig. 2. Character of rotational mobility of skin probe in suspensions of tissue cells from regenerating skin (a) and callus (b) under the influence of skin proteins. Ordinate, rotational mobility of spin probe τ (in sec). Remainder of legend as in Fig. 1.

and after 2-4 days the periosteum of both fragments of the fractured humerus was thickened, with the formation of peristeal and endosteal callus with a cartilaginous structure. At the site of the fracture, in sections stained with toluidine blue, bright γ- and β-metachromasia was observed, and its intensity was greater than in sections obtained from the control animals. During the same period in the animals of group 2 connective-tissue cells formed a sheath surrounding the zone between the fragments and limited the newly formed callus with a cartilaginous structure. In the control, formation of the periosteum took place 5 to 6 days later than in the experimental animals of group 1. Resorption of cartilage with the formation of regenerating tissue with a bony structure, followed by its mineralization, were accompanied in all groups by a reduction in volume of the callus, with a simultaneous increase in its strength, although in animals not treated with the protein these processes took place appreciably more slowly than in the experiment.

The kinetics of rotational mobility of the spin probe (τ) in the suspensions of bony callus cells differed sharply from the character of the changes in this parameter during investigation of the skin (Fig. 2b). Before the beginning of the second week of repair a decrease in τ was observed when either AFP or SA was used. This corresponded to the accumulation of cartilage in the zone between the fragments and the formation of periosteal callus. The tendency for the value of τ to fall more slowly in the animals of the two groups coincided with increased vascularization, resorption of cartilage, and mineralization. The fall in the value of τ which continued after the end of the second week during administration of SA took place parallel to the steady rise of this parameter when AFP was used. As a result the absolute value of τ, just as in the experiments with skin, was greater in the animals receiving AFP. As the control experiments showed, the kinetics of τ did not reflect the phases of spontaneous reparative osteogenesis.

Injection of protein preparations thus appreciably modified the course of repair processes. Details of these changes recorded by the usual morphological methods are considerably enhanced and broadened by the use of the EPR method, by means of which the stages of

healing can be recorded quantitatively. Of the two proteins with similar physicochemical properties, AFP possess greater biological activity relative to reparative regeneration of the tissues than SA. Thus must be taken into account in clinical practice during parenteral administration of placental albumin preparations.

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