TENSOMETRIC STUDY OF THE EFFECT OF EXOGENOUS FIBRONECTIN ON SKIN WOUND HEALING

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A regular increase in the fibronectin (FN) content in a wound on the 2nd-3rd day after tissue injury is evidence that this protein participates in reactions taking place in the early stages of healing [3]. Involvement of FN in the wound process is due to its ability to take part in the composition of the blood clot, to induce adhesion and spreading of the cells on fibrin and collagen, to stimulate phagocytosis, and to exhibit chemotaxis and various other biological properties [4]. The concept of FN as an endogenous stimulator of repair processes suggests that preparations of FN cound be used for therapeutic purposes.

In the investigation described below the effect of exogenous soluble FN on the healing of skin wounds was studied experimentally, with various methods of administration. As an integral parameter of the intensity of repair processes, wound tensometry was used, for it enables the various qualitative changes that lie at the basis of wound healing to be estimated quantitatively.

EXPERIMENTAL METHOD

Experiments were carried out on male rats weighing 150-200 g. Under sterile conditions and under ether anesthesia, standard linear incisions 50 mm long, at a distance of 2m from the spine through the whole thickness of the skin, were cut by means of a special instrument, after which interrupted Kapron sutures 10 mm apart were used to close the wound.

The preparation was administered in three different ways: local application, intravenous injection, and a combination of both. In the case of local application the preparation was introduced into the wound cavity at sites of the sutures in a dose of 0.5 ml per wound, twice a day for 5 days. The test preparation and the placebo were injected into the same animal, into wounds inflicted on both sides of the spine. In the case of intravenous injection the preparation of FN and the placebo were injected into different animals in a dose of 1.5-2 ml into the caudal vein once a day for 5 days. In the case of combined administration a solution of the protein was injected locally and intravenously in the same dose and the same number of times as when administered separately. The animals were killed with ether on the 3rd, 5th, 7th, and 11 days after the operation and four areas were cut out of each wound together with adjacent skin flaps for tensometry. The strain gauge transforms the mechanical force of stretching into an electric signal which, after amplication, is recorded graphically in the form of a tensogram. The breaking tension, i.e., the ratio of the breaking force to the area of the wound surface, was determined from the amplitude of the peak of a calibration tensogram.

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TABLE 1. Breaking Strain of Wounds (in g/mm^2) with FN Administered by Different Methods and at Different Times of Healing (M ± m)

Method of administration	Time after operation, days							
	3	n	5	n	7	n	11	n
Combined								
Albumin	5,7±0,8 (1,8—13,6)	12	17,1±1,6 (8,9—25,3)	11	38,8±4,0 (18,8—79,6)	14	71,9±10,9 (22,9—115,6)	10
FN	12.0±1,1 (7,2—20,7)	12	25,2±2,7 (16,6—43,3)	11	50,6±4,7 (31,4—82.2)	14	93,2±17,7 (31,3—145,0)	6
p	<0,001		<0,05		<0,05		>0,05	
Local								
Albumin	6,9±0,7 (3,7—12,7)	12	14,6±1,7 (4,7—28,8)	13	37,3±2.3 (27,3—50,8)	11	73,4±11,3 (22,4—115,6)	9
FN	11.8±1,0 (7,0—15.6)	10	21,0±2,4 (9,1—34,7)	10	46,7±3,0 (36,1—69,0)	11	93,6±10,6 (57,1—150,0)	6
p	<0,001		<0,05		<0,05		>0,05	
Intravenous	6,9±1,1	14	13,8±1,9	11	26,2±4,6	15	82,1±9,6	12
Albumin	(1.1—14,6)		(3,9—25,9)		(6,2—45,3)		(42,0—143,0)	
FN	10,7±1,5 (4,0—24,5)	13	17,9±2,4 (8,3—37,9)	12	23,5±2,7 (12,6—41.2)	10	87,4±9,3 (45,1—155,2)	14
p	<0,05		>0,05		>0,05		>0,05	

Legend. Here and in Table 2, limits of individual variations shown in parentheses.

The FN tested was obtained from fresh pooled citrated human blood plasma by affinity chromatography on immobilized gelatin. The sterile 0.1% solution of FN in phosphate-buffered physiological saline wih pH 7.3-7.4 was used. A sterile 0.1% solution of human serum albumin in the same solvent was used as the placebo in the control. The purity of the FN preparation was estimated according to the results of SDS electrophoresis and linear immunoelectrophoresis with antiserum to human blood serum proteins. The biological activity of the FN used was confirmed by its ability to bind with gelatin and to interact with phagocytes in vitro. A homogeneous preparation of FN obtained from the same pool of plasma was used in all the experiments.

The results were subjected to statistical analysis by parametric methods of variance analysis, on an Élektronika D3-28 computer. The significance of differences between mean values was determined by Student's t test.

EXPERIMENTAL RESULTS

The results of wound tensometry are given in Table 1. They show that after combined administration of the FN preparation on the 3rd day after wounding, the strength of adhesion of its edges under the influence of FN was significantly greater than under the influence of albumin. On the 5th, 7th, and 11th days after the operation, the difference in favor of FN was still found, but there was a gradual equalization of the tensometric parameters in the experiment and control as the wound healing process moved into the stage of scar formation. After local application, the stimulating action of FN on wound healing was confirmed. The time course of the tensometric parameters was similar in character, but the absolute values of the breaking strain were close to those observed in the case of combined administration. In the case of intravenous injection of FN alone, there was a significant increase in the strength of adhesion of the wound edges, but only on the 3rd day after the operation. Starting with the 5th day, a small difference in favor of FN was still found, but it was no longer significant, and it disappeared on the 7th and 11th days. The results indicate directly that FN stimulates reparative regeneration in the inflammatory stage of wound healing. The residual effect of FN on the following days was evidently largely secondary relative to its action in the stage of "traumatic inflammation" [1], since the duration and intensity of the effect depend directly on the quality of primary wound adhesion in the first days after trauma. With scar formation toward the 11th day after the operation the strength of adhesion of the wound edges ceased to depend to any great degree on the initial stages of the wound process, for it was determined by other mechanisms, mainly those responsible for the formation of collagen fibers.

Incidentally, the results differ from data in the literature [2] on the increase in strength of wound adhesion under the influence of FN at the times of the observed effect. In the study cited, FN induced a significant increase in the tensometric data only with

TABLE 2. Comparative Assessment (in %) of Efficacy of Various Methods of Fibronectin Administration (M ± m)

Method of administration	Time after operation, days			
	3	5	7	11
Combined	210,9±20,0 (127—363)	148,3±15,9 (97—255)	130,7±12,1 (81—212)	129,7±24,7 (43—202)
p_L	>0,05	>0,05	>0,05	>0,05
p_V	>0,05	>0,05	<0,05	>0,05
Local	166,8±13,7 (101—220)	143,5±16,7 (62—238)	125,2±8,0 (97—185)	127,0±14,6 (85—204)
p_C	>0,05	>0,05	>0,05	>0,05
p_V	>0,05	>0,05	<0,05	>0,05
Intravenous	155,6±22,4 (58—355)	129,1±17,6 (60—275)	89,6±10,5 (48—157)	106,5±11,3 (55—189)
p_C	>0,05	>0,05	<0,05	>0,05
p_L	>0,05	>0,05	<0,05	>0,05

Legend. Efficacy calculated by the formula $(x_i/\bar{y})\cdot 100\%$, where x_i denotes individual values of variants (breaking strain) in the experiment, $\bar{y}$ the arithmetic mean of the control data within each series. p_C) Compared with combined method of administration, p_L) compared with local, p_V) compared with intravenous method.

effect from the 7th day, and the effect lasted until the 21st day after the operation, whereas in the present investigation the action of oxogenous FN was limited to the inflammatory stage of wound healing. This difference can most probably be explained on the grounds that in the study cited above FN was applied locally in the form of a suspension of a protein residue, whereas in the present experiments it was used in the form of a solution, which is essential for manifestation of opsonic and chemotactic activity and the full and effective participation of FN in hemostatic reactions taking place in the wound. The mechanism of the effect of FN in an insoluble form on healing may be different and may be manifested differently from the mechanism of the effect of soluble FN. The contradictory nature of the results may also be explained by differences in the technique of wound tensometry.

The efficacy of the methods of administration of FN used can be judged on the basis of absolute values of breaking strain (Table 1), but not quite correctly because experiments with different methods of injection of the preparation were carried out at different times on different batches of animals, and for that reason within every series there was a control with a placebo. If, however, the mean values of the breaking strain in the control for each series are taken as 100%, the experimental mean values expressed in relative units become comparable with each other, and can be used to assess the efficacy of the different methods of administration of FN objectively (Table 2). Table 2 shows that the combined and local methods of administration of FN do not differ significantly in their efficacy. As regards intravenous injection of FN, this was less effective than local and, in particular, than the combined method of administration of the preparation. The difference became significant only on the 7th day after the operation, although it was close to significant on the 3rd day also ($t_C = 1.830$, $p_C < 0.1$). These results suggest that to stimulate repair processes the combined or local administration of FN is preferable. Intravenous injection is not the method of choice. However, the importance of the results obtained with intravenous injection of FN is that, for the first time, they prove that the course of the wound healing process is dependent not only on local production of FN in the wound, but also on its blood concentration.

Exogenous FN can thus significantly increase the strength of adhesion of the edges of a skin wound when administered locally, intravenously, and by a combination of both. The intensity of the stimulating action of soluble FN on wound healing is maximal during the first 3 days after injury and this is direct proof of the important role of FN in the early stages of wound healing. These results are evidence that FN preparations can and should be used in order to stimulate repair processes.

LITERATURE CITED

1. V. V. Serov and A. B. Shekhter, Connective Tissue [in Russian], Moscow (1981).
2. P. A. Falcone, M. Bonaventura, D. C. Turner, and D. Fromm, Plast. Reconst. Surg., 74, 809 (1984).
3. F. Grinnel, R. E. Billingham, and L. Burgess, J. Invest. Dermatol., 76, 181 (1981).
4. F. Grinnel, J. Cell. Biochem., 26, 107 (1984).