EFFECT OF PHYSIOLOGICALLY ACTIVE SUBSTANCES ISOLATED FROM THE THYMUS ON WOUND HEALING AFTER CRYOSURGICAL DESTRUCTION IN RATS

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Experiments were carried out on 40 male albino rats after whole-body irradiation in a dose of 200 R to study the effect of thymarin, a preparation obtained from bovine thymus and consisting of a combination of three fractions of physiologically active polypeptides with a mean molecular weight of about 10,000 on the course of local wound healing after cryosurgical destruction of the mucous membrane of the hard palate. Thymarin was injected subcutaneously daily for 10 days after cryosurgical destruction. The experimental results reveal a beneficial effect of thymarin on the course of regeneration and, in particular, more rapid sloughing of necrotic tissues, a shortening of the period of epithelization of the wound surface, and prevention of the development of secondary infection.

KEY WORDS: *thymarin; mucous membrane of the hard palate; cryosurgical destruction; thymus.*

Cryosurgical destruction has found wide application in recent years as a component of the combined treatment of patients with maxillofacial malignancy [3, 6-8, 12-15]. Several combinations of cryosurgical destruction with other methods of treatment of the tumor have been suggested, but it is most frequently used as the final stage after radiotherapy or chemotherapy [9-11]. Meanwhile, ionizing radiation and cytostatics, which depress the general immunological reactivity of the body and the immunological status of tissues in the maxillofacial region, are known to cause considerable delay in the sloughing of necrotic tissues and epithelization of the wound surface after cryosurgery. Superadded infection aggravates the course of repair process still further and is accompanied by the appearance of pain, which interferes with the taking of food and with care of the oral cavity. To shorten the periods of treatment after cryosurgery and for the prevention of secondary infection, the use of stimulators of immunogenesis and reparative regeneration thus appears promising. In the search for such a stimulator the writers decided to study the effect of the substance "thymarin" on the course of wound healing after cryosurgical destruction in previously irradiated rats. Thymarin is obtained from bovine thymus [5] and is a combination of three fractions of physiologically active polypeptides with a mean molecular weight of about 10,000. Previous experiments have shown that thymarin stimulates regeneration and immunogenesis and increases resistance of the recipients to infection [4, 5].

EXPERIMENTAL METHOD

Experiments were carried out on 40 male albino rats subjected to whole-body irradiation on the RUM-17 apparatus in a single dose of 200 R (voltage 200 kV, current 15 mA, dose rate at entry 96 ± 9.6 R/min, at center 83 ± 8.3 R/min, at exit 72.1 ± 7.2 R/min).

The tissues in the region of the hard palate were frozen 24 h after irradiation in all the rats by means of the MD-23 cryoprobe, developed and made at the S. I. Vavilov State Optical Institute [2]. The diameter of the zone of freezing was 5 mm, the temperature of

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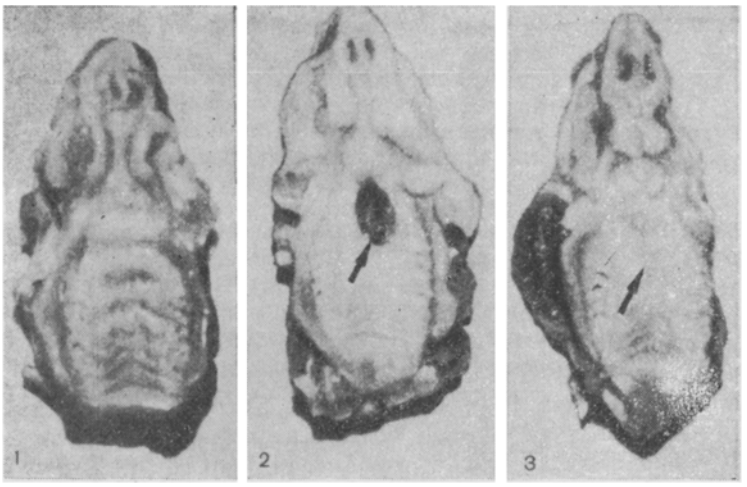

Fig. 1. Maxilla of rats. Macroscopic appearance of preparation from side of palatal surface. 1) Normal structure of mucous membrane of hard palate (before cryosurgical destruction); 2) perforating defect of hard palate of rat not receiving thymarin (28th day after cryosurgery); 3) epithelized scar on mucous membrane of hard palate of rats receiving thymarin (28th day after cryosurgery).

TABLE 1. Changes in Peripheral Blood Indices of Irradiated Animals of Control and Experimental Groups after Cryosurgery

Index studied	Background before beginning of experiment	Group of animals	Time after beginning of thymarin administration, days			
			7	14	28	35
Number in 1 mm³ blood:						
red cells, millions	6,78±0,20	Control	4,15±0,10	4,44±0,20	4,74±0,24	5,90±0,37
		Experimental	3,58±0,09*	3,34±0,23*	5,45±0,16*	6,30±0,29
white cells, thousands	11,4±0,95	Control	9,44±0,20	8,64±0,70	10,24±0,37	10,58±0,21
		Experimental	7,48±0,33*	15,4±0,85*	15,60±0,74*	15,60±0,31*
lymphocytes, absolute number	8960±770	Control	6780±520	6 620±490	6,920±430	7 220±305
		Experimental	5480±440	12 810±370*	11 420±510*	11 950±390*
platelets, thousands	721,6±706	Control	212,0±220	612,8±73,5	298,2±67,0	424,1±53,0
		Experimental	521,7±83,5*	850,8±76,0	759,0±58,0*	850,6±45,0*
Mean histochemical index of peroxidase activity	1,39±0,04	Control	1,14±0,05	1,15±0,02	1,17±0,04	1,16±0,03
		Experimental	1,14±0,04	1,45±0,05*	1,58±0,07*	1,50±0,04*
Phagocytic number	75,2±2,8	Control	38,4±1,4	41,4±4,9	57,6±3,3	61,8±3,9
		Experimental	30,6±0,8*	73,8±3,8*	76,0±3,9*	79,0±3,4*
Phagocytic index	11,0±0,9	Control	5,2±0,9	4,4±0,9	6,6±0,7	7,2±0,4
		Experimental	4,0±0,8	8,4±1,1*	12,4±1,3*	11,6±1,2*

*Difference between values for control and experimental groups statistically significant ($P < 0.05$).

the cryoprobe −190°C, and the duration of freezing 1 min. The rats were then divided into two equal groups, with 20 animals in each group. The rats of the experimental group started to receive thymarin 24 h after cryosurgery in a dose of 2 mg in 0.5 ml physiological saline by subcutaneous injection once a day for 10 days. The rats of the control group received 0.5 ml physiological saline at the same time.

The effect of thymarin on the course of wound healing was judged by measuring the zone of necrosis of mucous membrane of the hard palate and underlying bone, the time of removal of necrotic tissue from the wound, and the rate of wound epithelization, as well as on the basis of histological examination. For the latter purpose, two rats of each group were killed 1, 2, 4, 6, 9, 12, 20, and 35 days after the beginning of thymarin administration, the maxillae were removed and fixed in 10% neutral formalin solution, and transverse sections cut through them were stained with hematoxylin–eosin and by Van Gieson's method. Hematological investigations to determine the red and white cell and platelet counts in 1 mm³

peripheral blood, the leukocyte formula, peroxidase activity (by Cato's method [1]), and phagocytic activity of neutrophils (by the method of Almazov and Ryabov [1]) also were carried out before irradiation and 3, 7, 14, 28, and 35 days from the beginning of injection of thymarin.

EXPERIMENTAL RESULTS

On the 3rd day after cryosurgery necrosis of the mucous membrane of the hard palate developed in all rats of the control group. By the 7th-9th day sloughing of the necrotic soft tissues exposed the underlying bone. Later, as a result of sequestration of the injured bone tissue, perforating defects developed in the hard palate in all rats of the control group (Fig. 1). Later and until the end of the experiment, marked inflammatory changes were observed at the edges of these defects and the wound surface was not completely epithelized.

A different picture was observed in the rats receiving thymarin. The area of the zone of necrosis revealed on the 3rd day after cryosurgery was 33-50% smaller than in the rats of the control group. Sloughing of the necrotic tissues was complete on average by the 6th day, and the wound surface appearing after separation of the scab was covered with granulation tissue. Immediately at the edges of the wound signs of active epithelization appeared and this process was complete by the end of the 3rd week. No perforating defects developed in the region of the hard palate at the site of cryosurgery in any of the rats receiving thymarin.

On macroscopic examination of the specimens 24 and 48 h after cryosurgery no significant differences could be found between the rats of the control and experimental groups. During this period the following picture was characteristic of the animals of both groups: The epithelial cells were swollen and lacked clear boundaries, their cytoplasm was granular, and the tissues were infiltrated with neutrophils. Signs of infiltration and inflammation were observed also in the bone tissue adjacent to the zone of cryosurgical destruction, and the blood vessels in it were sharply dilated and packed with blood cells.

Differences in the course of wound healing began to be clearly apparent on the 6th day. In the rats of the experimental group, the picture of inflammation at this stage was still dominated by destructive changes with infiltration of the tissues mainly by neutrophils, indicating necrosis of the bone tissue over a wide area. Meanwhile, in the rats receiving thymarin the destructive changes in the zone of application of the cryoprobe were less severe, as also was the infiltration by neutrophils, whereas the macrophagal reaction was more active.

The results of the hematological investigations are interesting. As Table 1 shows, whole-body irradiation of the rats of the control group followed by cryosurgery was accompanied by a marked decrease in their red and white blood cell counts (mainly on account of a decrease in the number of lymphocytes) and platelet count and by a reduction in functional activity of their neutrophils. Meanwhile, the hematological disturbances were less severe in the rats receiving thymarin. Soon after the end of the course of thymarin injections most of the parameters studied had returned to their original levels or exceeded them (e.g., total white blood cells, lymphocytes, and platelets, mean histochemical index of peroxidase activity in the neutrophils, phagocytic index of the neutrophils; see Table 1). These findings are evidence of the beneficial effect of thymarin on the course of defensive and adaptive processes. Thymarine evidently acts on the course of local wound healing indirectly through general adaptive mechanisms; in particular it causes more rapid separation of necrotic tissues, shortens the time required for epithelization of the wound surface, and prevents the development of secondary infection.

There is reason to suppose that thymarin has a similar effect on the development of general adaptive reactions and on the course of local wound healing in man also. If this hypothesis is confirmed by clinical tests, it will be possible to use thymarin in the treatment of patients with tumors of the mucous membrane of the mouth, tongue, and elsewhere after cryosurgical destruction as the final stage of combined treatment in order to shorten the duration of treatment, to prevent infectious and inflammatory complications, and also perhaps to stimulate antitumor immunity.

LITERATURE CITED

1. V. A. Almazov and S. I. Ryabov, Methods of Functional Analysis [in Russian], Leningrad (1964).

2. V. V. Balakov, B. V. Klyucharev, N. A. Pankratov, et al., Optiko-Mekhan. Prom., No. 2, 33 (1970).
3. I. L. Balieva, Stomatologiya, No. 2, 49 (1974).
4. G. A. Belokrylov, V. G. Morozov, V. Kh. Khavinson, et al., Byull. Eksp. Biol. Med., No. 2, 202 (1976).
5. V. G. Morozov and V. Kh. Khavinson, Eksp. Khir., No. 2, 49 (1974).
6. A. I. Paches, T. P. Ptukha, and V. V. Shental', in: The Diagnosis and Organization of Treatment and Prophylaxis for Patients with Malignant Neoplasms of the Maxillofacial Region [in Russian], Moscow (1974), pp. 57-59.
7. L. I. Trushkevich, R. N. Kondratskaya, and V. A. Trushina, Stomatologiya, No. 1, 37 (1976).
8. V. V. Shental', in: The Diagnosis and Organization of Treatment and Prophylaxis for Patients with Malignant Neoplasms of the Maxillofacial Region [in Russian], Moscow (1974), pp. 61-62.
9. R. Barke and H. Sieler, Strahlentherapie, 115, 453 (1961).
10. J. Cherry, Arch. Otolaryngol., 91, 548 (1970).
11. I. S. Cooper, Fed. Proc., 24, 237 (1965).
12. A. A. Gage, S. Koepf, D. Wehrle, et al., Cancer (Philadelphia), 18, 164 (1966).
13. R. L. Goode and T. R. Spooner, Trans. Am. Acad. Ophthalmol. Otolaryngol., 75, 968 (1971).
14. P. J. Leopard, Br. J. Oral Surg., 13, 128 (1975).
15. R. W. Pearson, Laryngoscope, 78, 623 (1968).

DURATION OF MITOSIS IN THE CORNEAL EPITHELIUM AND SPLEEN CELLS OF MICE WITH LEUKEMIA La

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The diurnal rhythm of mitosis and its duration were investigated in the corneal epithelium and spleen cells of mice with leukemia La. Correlation was found between changes in the mitotic index and duration of mitosis during the 24-h period. It is suggested that the more rapid course of mitosis in tissues with intensive cell proliferation and its slower course in tissues with low proliferative ability are reflections of a general rule.

KEY WORDS: *leukemia La; diurnal rhythm of mitosis; corneal epithelium; spleen.*

Investigations have shown differences in the duration of mitosis at different times of the 24-h period in normal animal tissues and in tumors [1, 5, 7, 8, 10]. At times of day when the mitotic index reaches a maximum, the duration of mitosis has been found to be shortest and, conversely, a fall in the mitotic index is accompanied by a rise in the duration of mitosis. Definite correlation thus exists between the number of cells starting mitosis and the duration of mitosis. The relationship between mitotic activity and the duration of mitosis in tissues with different levels of cell proliferation is an interesting topic for study.

This paper describes the results of an investigation of the duration of mitosis in two different tissues from the same mice: in the corneal epithelium, in which the mitotic cycle lasts 3-4 days [9] and in spleen cells in leukemia La, with a mitotic cycle of 12 h [3].

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