EFFECT OF 4-METHYLURACIL AND CARNOSINE ON HEALING OF SKIN WOUNDS IN RATS

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Trauma to organs during wounding or as a result of burns is characterized by disturbance of tissue respiration and by accumulation of active forms of oxygen and of acyl hydroperoxides. Products of free-radical reactions are found in such cases not only in the focus of injury, but also in distant organs. For instance, in rats with burns the intensity of NADPH-dependent lipid peroxidation (LPO) in the liver microsomes is doubled [7]. Being highly reactive, these products disturb the structure of macromolecules, namely membrane lipids, DNA, RNA, and proteins, and delay repair of the injuries to organs and tissues. The use of preparations with antioxidative activity to accelerate repair processes is a promising trend. In the present investigation the antioxidative properties of 4-methyluracil and carnosine and their ability to inhibit LPO and to accelerate healing of full-thickness skin wounds in rats were studied.

EXPERIMENTAL METHOD

Experiments were carried out on albino rats weighing 180-200 g. Wounds were inflicted under ether anesthesia in the interscapular region of the back. The size of the wound, with an area of about 3 cm^2, was standardized by inserting Teflon rings, 2 cm in diameter, into it by Slutskii's method [10]. Above the rings was placed a polyethylene cover with pores, through which therapeutic substances could be applied to the wound surface from a syringe once a day in a volume of 0.2 ml. In the course of treatment animals of the experimental groups received preparations of carnosine and of 4-methyluracil, made up in physiological saline, and an oily solution of α-tocopherol acetate, whereas animals of the control groups received physiological saline or sunflower oil. The above compounds were applied to the wound surface in a dose of 20 μmoles 4 or 5 times. In experiments to study the effect of the preparations on complete healing, the rings were removed from the region of the wound on the 5th day after the operation and the rate of connective tissue formation was determined planimetrically. The dimensions of the wounds were recorded every 2-3 days and their area estimated. The state of LPO was judged in homogenates of granulation tissue and in blood serum of the treated and control animals by determining the decrease in the concentration of conjugated dienes and ketodienes [3]. The reaction with thiobarbituric acid (TBA) [12] was used to estimate the content of TBA-active LPO products. Superoxide-scavenging activity of the biological material was determined by studying inhibition of reduction of nitro-BT in an O_2^- generation system [11]. Tissue and blood were collected on the 3rd day after wounding, at the peak of the inflammatory reaction.

The antioxidative properties of 4-methyluracil and carnosine also were estimated in experiments in vitro, using a cumene model, by the method of Kudrin et al. [4]. In this series of experiments the ability of alcoholic solutions of the two preparations, in concentrations of between 1 and 20 mM, to delay oxidation of cumene in the presence of the LPO initiator, azo-*bis*-isobutyronitrile, was studied. The period of induction of oxidation was measured in minutes. The numerical results were subjected to statistical analysis by Student's method [1].

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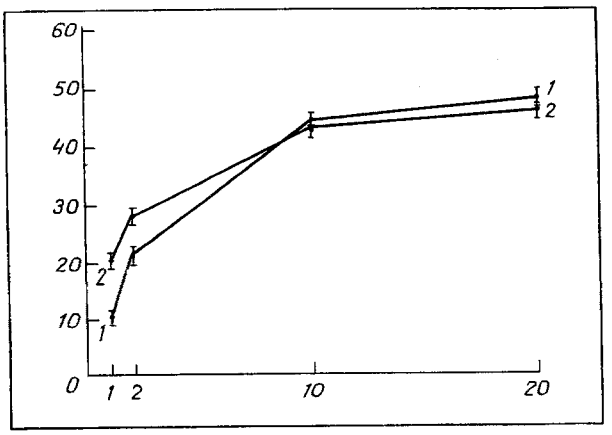

Fig. 1. Dependence of antioxidative activity of carnosine (1) and of 4-methyluracil (2) on their concentration. Abscissa, concentration of test preparations (in mM); ordinate, time of delay of oxidation of cumene (in min).

TABLE 1. Formation of Free-Radical Oxidation Products in Granulation Tissue and Blood Serum on 3rd Day of Wound Treatment with Antioxidants

Products of free-radical oxidation	Preparations (in % of control)*							
	vegetable oil		4-methyluracil		carnosine		α-tocopherol acetate	
	1	2	1	2	1	2	1	2
TBA-active products	82±13	82±10	63±7	72±6	61±8	80±5	79±14	82±3
Conjugated dienes	82±11	76±4	61±9	42±11	60±8	50±6	70±9	63±11
Ketodienes	60±15	76±15	25±10	30±10	28±12	32±13	50±13	40±16
O_2^- radical	110±10	110±12	150±10	140±12	155±7	145±7	140±8	130±12

Legend. 1) In tissue homogenate, 2) in blood serum. *) Results obtained for animals to whose wounds physiological saline was applied were regarded as the control.

EXPERIMENTAL RESULTS

The anti-inflammatory properties of 4-methyluracil, which are determined by its antioxidative activity, have been reported in the literature [5, 6]. However, these properties of the compound have received little study: there are no quantitative data on its interaction with free-radical products or comparison with activity of other antioxidants. Accordingly, the antioxidative activity of 4-methyluracil was determined initially in experiments in vitro in a water-free medium and compared with that of the natural water-soluble antioxidant of muscles, carnosine (β-alanyl-L-histidine) [2, 8, 13]. The curves in Fig. 1 show that in a cumene model of LPO, with active oxygen supply, the two preparations delay cumene oxidation. In a concentration of 1 mM carnosine protects cumene against oxidation less strongly by half than 4-methyluracil: the delay of oxidation amounted to 10 and 20 min, respectively. However, in concentrations of 10 and 20 mM the compounds had about equal antioxidant action, preventing oxidation of cumene for 44 and 48 min. The results are evidence that both compounds can react with free radicals, formed in a water-free homogenous model system.

The ability of the test substances to reduce the formation of LPO products in the wound tissues and blood serum was studied in experiments in vivo after applications of equimolar amounts of these preparations to the wounds. The experimental results are given in Table 1 and compared with the corresponding values obtained in animals treated with α-tocopherol acetate. As these data show, treatment of the animals with carnosine and 4-methyluracil reduced the accumulation of primary LPO products with conjugated diene and, in particular, with ketodiene groups, the content of the latter being reduced by 3-4 times compared with the control. The formation of TBA-active LPO products was reduced by 40%, and the ability of the tissue homogenate and blood serum to scavenge the toxic O_2 anion was increased by the same degree. The effectiveness of antioxidative protection of tissues treated with carnosine and with 4-methyluracil under these experimental conditions was greater than that of

TABLE 2. Action of Antioxidants on Wound Healing

Preparations	Size of wounds, mm²			Time of complete epithelization, days	Acceleration of epithelization, % of control
	days after operation				
	7	10	15		
Physiological saline (n = 10)	268,6±16	164,2±31	37,5±14	24,3±1,4	—
Vegetable oil (n = 6)	265,9±12	152,0±41	37,1±3	26,0±1,2	93,7±15
4-Methyluracil (n = 6)	246,4±17	140,3±34	34,7±11	22,0±2,2	113,0±10
Carnosine (n = 9)	255,8±18	100,7±21	29,7±6	19,4±1,9	125,0±9,7
α-Tocopherol acetate (n = 5)	260,7±13	126,3±49	35,3±2	23,0±1,5	106,0±6,5

Legend. *n*) Number of animals used in experiments.

treatment with α-tocopherol acetate. It can be concluded on the basis of these results that equimolar amounts of carnosine and 4-methyluracil in experiments in vivo and in vitro exhibit a very similar antioxidative effect. Lowering of the level of products of free-radical oxidation is observed not only in tissues of the wound, but also in the blood serum, evidence of the generalized antioxidative action of these preparations.

The results of a study of the time course of healing and of eventual cure of the animals are given in Table 2. After treatment for 5 days, during which 100 μmoles of antioxidative preparations was applied to the site of injury, the rings were removed and subsequent healing took place under open wound conditions. The most marked effect of treatment was observed on the 10th-15th day after the operation. For instance, on the 10th day of treatment with 4-methyluracil the size of the wounds was 20% less, and in the case of treatment with carnosine, 40% less, than in the control animals. In the later stages the difference in the rate of wound healing in the experimental and control animals became less, indicating that anitoxidative preparations are effective only in the initial stages of this process. By protecting the tissue against active forms of oxygen and LPO, they thereby reduce the intensity of destructive processes in the wound and shorten the duration of the inflammatory phase [9].

It will be clear from the results in Table 2 that the greatest degree of acceleration of wound healing was given by carnosine, although its antioxidative activity is virtually equal to that of 4-methyluracil. The differences observed are evidently attributable to the fact that the effect of carnosine on repair processes in the tissues are not confined simply to a decrease in the formation of free-radical products.

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