EFFECT OF TRAUMA ON THE TIME COURSE OF MITOSIS IN RAT BONE MARROW CELLS

Yu. V. Red'kin and O. A. Miroshnik

UDC 616-001.36-07:616.832-018.1]-092.9-07

KEY WORDS: traumatic shock, bone marrow, mitosis, pathological mitoses, treatment.

Under the influence of a stimulus increasing its powers of adaptation, the level of pathological mitoses (PM) in the epithelial tissues of an animal rises sharply in association with an increased level of mitotic activity [7]. If this phenomenon is of a sufficiently marked degree, the aberrant distribution of genetic information to subsequent cell generations may significantly disturb the physiological competence of the tissues. It is particularly interesting to discover the prevalence of this phenomenon in hematopoietic tissue, whose cells, migrating into the blood stream, largely determine the state of homeostasis of the organism.

The aim of this investigation was to study the effect of traumatic shock, a condition giving rise to severe pathological changes in the body, on the pattern of mitosis of the bone marrow cells and also to attempt to correct the disturbances arising by pharmacotherapy.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male albino rats weighing 180-220 g. Traumatic shock was produced under pentobarbital anesthesia (3-4 mg/100 g body weight) by the method in [3]. The animals were divided into two groups: group 1 consisted of 96 rats not receiving any form of treatment after trauma; the mortality rate was 54.17 ± 5.09%; group 2 consisted of 69 animals treated with a combination of various preparations after trauma. The antianoxic agent sodium hydroxybutyrate was injected intraperitoneally in a dose of 300 mg/kg, the nonsteroid cell membrane stabilizer sodium oxyferriscarbonate intramuscularly in a dose of 1.75 mg/kg, and the immunomodulator levamisole perorally in a dose of 2.5 mg/kg. The first two compounds were given daily for 7 days after trauma, whereas leavamisole therapy consisted of three courses, each lasting 3 days, separated by an interval of 3 days. The mortality rate in this group was 43.48 ± 5.97%. Six intact rats served as the control. The animals were killed on the 1st, 3rd, 7th, 14th, 21st, and 35th days after trauma, always in the morning. Bone marrow was flushed out of the long bones into a centrifuge tube with Carnoy's fluid at the rate of 2 ml per femur. The resulting cell suspension was centrifuged 3 times for 5 min each time at 600 rpm, after which the residual cell suspension was treated with 2 or 3 drops of a 2% solution of acetoorcein. After 15 minutes films were prepared and stained by the Romanovsky–Giemsa method. Proliferative activity, estimated on the basis of a differential count (phase by phase) of the cells in mitosis, by examination of 1000 karyocytes, and the cytotoxic and cytogenetic effects were assessed by determining the number of PM per 100 mitotic figures, using the classification in [1] for guidance. The results were subjected to statistical analysis by Student's test.

EXPERIMENTAL RESULTS

The results indicated stimulation of the mitotic index of the bone marrow cells as a result of traumatic shock, accompanied by an increase in the number of PM (Table 1), characteristic features of proliferative processes in epithelial tissue in severe forms of stress in general [7]. However, it is in the corneal epithelium in traumatic shock that inhibition of cell division has been described [3]. This difference in the direction of proliferative processes in bone marrow and epithelial cells in response to severe trauma can evidently be

Central Research Laboratory, Omsk Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR, L. V. Poluéktov). Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 106, No. 9, pp. 353-355, September, 1988. Original article submitted October 27, 1987.

 0007-4888/88/0009-1332$12.50

TABLE 1. Changes in Pattern of Mitosis of Bone Marrow Cells of Rats Exposed to Traumatic Shock (M ± m)

Time after trauma, days	Group of animals	MI, promille	Ratio between phases of mitosis, %		PM level, %		
			prometaphase	anatelophase	associated with chromosomal aberrations	associated with damage to mitotic apparatus	total
1	1	12,43±1,36*	64,36±10,94	35,64±3,62	5,50±1,06	12,5±1,59*	18,00±1,59**
	2	13,00±1,33**	65,38±11,23	34,62±7,15	4,00±0,61	17,42±1,82**	21,71±1,81**
3	1	12,44±1,43*	64,31±7,64	35,69±3,86	4,44±0,95	15,33±1,67**	19,89±1,90**
	2	13,67±0,42*	63,42±6,14	36,58±6,15	3,00±1,06	11,75±1,59*	14,75±1,32
7	1	13,60±3,22	72,06±20,51	27,94±6,32	3,00±1,68	7,33±1,41	11,00±2,24
	2	15,00±1,79**	74,80±7,13	25,20±5,53	4,00±0,95	15,11±1,19**	19,33±1,43**
14	1	13,42±1,02**	64,58±6,94	35,45±8,28	5,40±0,87	13,09±2,19**	18,00±2,00**
	2	11,0±1,19	60,27±7,27	39,73±12,09	3,75±1,06	15,50±1,06**	19,25±2,20**
21	1	14,83±2,30*	74,17±7,15	25,83±8,36	9,33±0,84*	25,33±0,84**	37,00±1,26**
	2	14,28±1,81	73,02±8,47	26,98±5,31	5,75±1,33	9,50±1,33*	14,25±1,59
35	1	14,25±2,80	66,67±9,83	33,33±9,82	9,66±1,41*	13,67±1,06**	23,33±1,77**
	2	13,5±1,77	79,04±10,44	20,96±6,52	5,00±2,24	8,00±1,68	13,00±1,77
	Control	8,18±0,69	59,32±7,46	40,68±7,58	5,00±1,06	6,00±0,71	12,00±1,77

Legend. MI) Mitotic index. One and two asterisks denote significance of difference compared with control at levels of probability of 95 and 99% respectively.

explained on the grounds that different mechanisms must be involved in the change of their kinetics. Activation of cell division in bone marrow, a manifestation of long-term adaptation [4], is probably aimed not merely at maintaining natural tissue homeostasis, but also at maintaining all spheres of ensuring the necessary number of blood cells in order to maintain all spheres of vital activity of the animal under the new conditions.

Meanwhile, in animals in a terminal state, many factors toxic and pathogenic for the cell and its structures (free-radical products of lipid peroxidation, ischemic and bacterial toxins, high blood enzyme levels, etc.) accumulate [3, 5], and many of them may lead to disturbance of the normal course of mitosis.

The results of the investigation show that elevation of the PM level after severe trauma takes place mainly on account of PM which are connected with damage to the mitotic apparatus of the cell (mainly different forms of C-mitosis). In the late posttraumatic period, however, in the animals of group 1 there was a significant difference in the numbers of PM due to chromosomal damage compared with the control. Delay of the cells in prometaphase and their delayed transition into the final phases of mitosis (Table 1), moreover, are evidence of the stathmokinetic effect of traumatic shock. The increase in the mitotic index which was recorded may perhaps be connected to some degree with the increase in the duration of the mitotic cycle due to this process.

Some aberrant cells formed as a result of PM may complete the stages of subsequent proliferation and differentiation and be released into the peripheral blood and tissues. However, the altered genome of these cells predetermines their functional incapacity, and this may be a potential factor in the pathogenesis of various pathological processes in the blood system (anemia, immunodepression), which take place in traumatic shock [6].

The combination of preparations used to correct proliferative processes in the bone marrow had no significant effect on the value of the mitotic index, but led to a relative fall in the total number of PM and its earlier restoration to normal. The beneficial effect of treatment can evidently be explained by blocking of individual links of the pathogenetic chain developing after trauma. Under the influence of the experimental therapy used, anoxic and metabolic disturbances are evidently reduced, lipid hyperperoxidation is weakened, the systemic redistribution and functional activity of the lymphoid tissue is modulated, and operation of the pituitary-adrenal system is improved. This ultimately alleviates the course of the general adaptation syndrome and eliminates the factors disturbing mitosis.

LITERATURE CITED

1. I. A. Alov, The Cytophysiology and Pathology of Mitosis [in Russian], Moscow (1972).
2. G. M. Kalivetskaya, S. S. Timoshin, A. P. Lychkov, and N. B. Murzina, Byull. Eksp. Biol. Med., No. 4, 92 (1982).

3. V. K. Kulagin, The Pathological Physiology of Trauma and Shock [in Russian], Leningrad (1978).
4. F. Z. Meerson, Adaptation, Stress, and Prophylaxis [in Russian], Moscow (1981).
5. V. A. Negovskii, A. M. Gurvich, and E. S. Zolotokrylina, Postresuscitation Sickness [in Russian], Moscow (1987).
6. S. A. Seleznev and G. S. Khudaiberenov, Traumatic Shock: Current Aspects of the Problem [in Russian], Ashkhabad (1984).
7. S. S. Timoshin, Arkh. Patol., 45, No. 4, 83 (1983).

BLOOD SERUM AND GRANULATION TISSUE ASCORBIC ACID AND HYDROXYPROLINE LEVELS IN RATS WITH ASEPTIC AND INFECTED WOUNDS

A. N. Novikova, L. A. Mamedov,
N. D. Egorova, A. B. Shekhter,
A. V. Nikolaev, and V. V. Zakharov

UDC 617-001.4-022.7-07:616.153:577.164.2]-092.9-07

KEY WORDS: wound, inflammation, ascorbic acid, hydroxyproline.

Determination of the hydroxyproline level, an indicator of collagen accumulation, i.e., of scar healing of a wound defect, is used as an objective criterion in the quantitative evaluation of the course of wound healing [2, 3, 6]. Hydroxyproline formation is dependent on the presence of ascorbic acid, which participates in the oxidation of proline into hydroxyproline, and also influences the biosynthesis of other proteins [1, 9, 13]. The ascorbic acid level in granulation tissue also characterizes the state of oxidation-reduction processes in the wound [5, 8, 12, 15]. It also has a role in stress situations (response to pathogenetic factors, trauma, etc.) [10]. Meanwhile the dynamics of the ascorbic acid and hydroxyproline levels during healing of infected wounds, and its comparison with that during the healing of uncomplicated wounds, has been inadequately studied.

The aim of this investigation was to determine ascorbic acid and hydroxyproline levels in the blood serum and granulation tissue of rats with aseptic and infected wounds.

EXPERIMENTAL METHOD

Experiments (two series) were carried out on 220 male Wistar rats weighing 200-210 g. Aseptic and infected full-thickness wounds with an area of 400 mm^2 served as the experimental model. The model of an aseptic wound was created by the method described previously [4]. To obtain the model of an infected wound, the edges and floor of the wound were additionally traumatized with toothed forceps, and 0.5 ml of a suspension of a 24-h culture of a pathogenic staphylococcus ($1.5 \cdot 10^9$ bacterial cells in 1 ml physiological saline) was introduced into the wound surface. At intervals, on the 1st-10th, 12th, and 15th days after the operation concentrations of hydroxyproline and ascorbic acid were determined in the tissues in the region of the wound (granulation tissue), and the serum ascorbic acid level also was determined at the same times and before the operation. Hydroxyproline was estimated photometrically [14] and ascorbic acid titrometrically [11]. At each time point 8 to 10 rats were used, and killed by decapitation. The wound tissues of five animals also were studied daily histologically (staining with hematoxylin and eosin, toluidine blue, and by van Gieson's, venules, arterioles) in the course of all phases of wound healing was estimated in each animal on a 5-point system (from 0 to 4). The numerical results were subjected to statistical analysis.

EXPERIMENTAL RESULTS

Analysis of the data showed (Fig. 1a) that in both aseptic and infected wounds there was a significant increase in the ascorbic acid concentration in the granulation tissue

I. M. Sechenov First Moscow Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR, V. V. Kovanov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 106, No. 9, pp. 355-358, September, 1988. Original article submitted December 18, 1987.

 0007-4888/88/0009-1334$12.50