ENZYMIC ACTIVITY IN RABBIT OSTEOBLASTS AFTER COMBINED TRAUMA

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Activity of NAD- and NADP-diaphorases, cytochrome oxidase, and alkaline phosphatase was studied by histochemical methods in osteoblasts 3-72 h after combined radiation trauma in experiments on rabbits. The results showed that a fracture of the bone leads within 12 h to an increase in the intensity of energy and other forms of metabolism in the osteoblasts. Irradiation of the animals inhibits the activation induced by fracture in all the enzymes studied.

KEY WORDS: x-ray irradiation; fracture of bone; osteoblasts; oxidative enzymes; alkaline phosphatase.

Investigations of the oxidation–reduction and hydrolytic enzymes of bone cells in the early period after a fracture have recently been published [2, 13]. However, the information on early changes in the activity of enzyme systems in osteogenic cells in response to combined (radiation and mechanical) trauma cannot be found in the literature.

The object of this investigation was to study the dynamics of activity of certain enzymes of osteoblasts in the period from 3 to 72 h after combined trauma; this could yield information of importance to the study of the pathogenesis of disturbances of reparative regeneration of bone tissue in acute radiation sickness.

EXPERIMENTAL METHOD

Experiments were carried out on 70 young rabbits aged 3 weeks. The animals were irradiated on the RUM-3 x-ray apparatus in a dose of 500 R, after which a closed fracture of the right humerus was inflicted. A similar fracture was produced in the control rabbits, but without preliminary irradiation. The animals were decapitated 3, 6, 12, 18, 24, 48, and 72 h after trauma. To study alkaline phosphatase activity, pieces of bone with periosteum were fixed (24 h) in cold calcium formol. All the test material was decalcified in cold 10% EDTA solution in 0.1 M phosphate buffer, pH 7.2, with constant mixing [4]. Sections, 15 μ in thickness, were cut on a freezing microtome. Alkaline phosphatase was demonstrated by the azo-coupling method with Naphthol AS-MX phosphate and the diazonium salt of Fast Red TR [1]. Cytochrome oxidase was determined by Burstone's method [1], and NAD- and NADP-diaphorases by Nachlas's method [11] in sections of unfixed tissue. A parallel series of histological preparations was obtained and stained with hematoxylin–eosin.

Since fractures in young rabbits involve the whole diaphysis, with the corresponding part of the periosteum covering it, it was also necessary to study the enzyme activity in osteogenic cells located 200-300 μ from the site of injury. Their activity was assessed by a semiquantitative method and the mean histochemical coefficient (MHC) was then calculated [3]. The results were subjected to statistical analysis.

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TABLE 1. Activity of Various Enzymes in Osteoblasts of Young Rabbits (MHC in conventional units) after Combined Radiation Trauma

Time after trauma (in h)	NAD-diaphorase			NADP-diaphorase			Cytochrome oxidase			Alkaline phosphatase		
	expt.	control	P	expt.	control	P	expt.	control	P	expt.	control	P
3	2 28	2,39	>0,2	2 05	2,15	>0,2	1,35	1,49	<0,02	1,64	1,64	>0,5
6	2 26	2,39	<0,01	2 20	2,19	>0,5	1,29	1,45	>0,1	1,58	1,66	<0,05
12	2 40	2,62	<0,01	2 30	2,35	>0,5	1,49	1,82	<0,01	1,59	1,89	<0,01
18	2 47	2,68	<0,01	2 31	2,39	<0,01	1,81	2,10	<0,01	1,57	2,07	<0,001
24	2 67	2,82	>0,1	2 40	2,59	<0,001	1,88	2,29	<0,001	1,59	2,17	<0,001
48	2 70	2,90	<0,001	2 57	2,73	<0,01	2,29	2,56	<0,01	2,07	2,28	<0,05
72	2 75	2,94	<0,001	2 70	2,81	<0,02	2,39	2,67	<0,001	2,13	2,33	<0,001

Legend. In all cases M > 3m; number of rabbits in all cases 10.

EXPERIMENTAL RESULTS AND DISCUSSION

The study of sections stained with hematoxylin and eosin showed that from 3 to 24 h after combined trauma no abnormality could be found in the damaged periosteum. The cells of the periosteum stained well with hematoxylin–eosin as far as the site of the fracture. Evidence of increased proliferation and differentiation of the cells of the periosteum 48 h after combined injury could be seen throughout its extent.

The study of enzyme activity in the experimental and control groups showed that none of the enzymes investigated could be detected at the site of rupture of the periosteum 3 h after trauma. This was evidently an indication of commencing aseptic necrosis of bone at the fracture site.

Tests showed (Table 1) that the fracture induced an increase in activity of all the oxidoreductases studied in the osteoblasts. The increase in activity became statistically significant 12 h after trauma and reached its maximum for all enzymes on the 3rd day. Similar results have been obtained in experiments on mice and rats [5, 13, 15]. The increase in the intensity of oxidation–reduction processes reflects an increase in the bone-forming activity of the osteoblasts [7, 8]. Combined trauma also caused an increase in activity of the mitochondrial enzymes. However, a statistically significant increase in NAD-diaphorase and cytochrome oxidase activity was discovered only 18 h after the fracture. So far as absolute activities are concerned, these were statistically significantly lower for all oxidoreductases than in the control. Irradiation thus inhibits to some degree the activation of oxidation–reduction reactions induced by the fracture. According to data in the literature, lowering of the activity of mitochondrial enzymes in osteogenic cells is accompanied by inhibition of their specific activity. Relations of this sort are evidently universal and are observed under various experimental conditions: the use of inhibitors of energy metabolism, vitamin C deficiency [6, 16]. The facts described above demonstrate the inhibitory action of ionizing radiation on the processes of synthesis and formation of the bone matrix.

Similar conclusions can be drawn by analysis of the dynamics of alkaline phosphatase activity. Combined trauma caused an increase in the activity of this enzyme, although it was observed later than in the unirradiated animals. Also, at nearly all times of investigation the enzyme activity was lower in the experimental than in the control group. The close connection between alkaline phosphomonoesterase and the formation of the bone matrix is now generally accepted [10, 12, 14]; the inhibitory action of irradiation on the activity of this enzyme must therefore be regarded as evidence of depression of osteoplastic processes [9].

The reaction of osteogenic cells to combined trauma is thus determined, first, by increased osteoplastic activity in response to the fracture and, second, by the depressive action of ionizing radiation. Inhibition of osteosynthetic processes begins within the first hours after irradiation. One of the mechanisms of the pathological action of x-ray irradiation on the repair process in bone tissue is evidently inhibition of energetic and other metabolic reactions in the osteogenic structures of the periosteum.

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