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CHANGES IN THE BLOOD SYSTEM AFTER EXTRACORPOREAL IRRADIATION

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Whole-body irradiation of animals with comparatively small doses of radiation is known to be followed by an increase in the nonspecific resistance of the animal [8, 15], and by intensification of phagocytosis [3] and antibody formation [4]. The undesirable consequences of whole-body irradiation can be avoided by local and, in particular, by extracorporeal irradiation of blood (ECIB) [1, 9].

The aim of this investigation was to study the effect of a single ECIB on the cellular and biochemical composition of the blood and on activity of the factors of nonspecific immunity.

EXPERIMENTAL METHOD

Experiments were carried out on 16 mongrel dogs weighing from 12 to 28 kg. The animals were anesthetized by intravenous injection of a 2.5% solution of thiopental sodium and a bypass was created by means of a plastic tube between the femoral artery and vein. The development of thrombosis was prevented by injection of heparin in a dose of 200 U/kg body weight. The bypass tube was placed in a "Perenos" beta-apparatus, equipped with two IRUS-1 sources based on ^{90}Sr and ^{90}Y, with activities of $7.4 \cdot 10^{10}$ and $125.8 \cdot 10^{10}$ Bq. The blood was irradiated for 3 h to create a dose load of 6.0 to 12.0 Gy. The doses of irradiation of the blood were calculated by a mathematical method and by means of a chemical dosimetric system [5]. Blood was taken from a contralateral vein before formation of the bypass and in the course of 8 weeks after irradiation. The state of the blood system was assessed by reference to the following morphological and biochemical parameters: the erythrocyte and leukocyte counts, hemoglobin (Hb) concentration, hematocrit, ESR, activity of enzymes of the pentose phosphate pathway of glucose oxidation in erythrocytes (glucose-6-phosphate dehydrogenase (G6PDH) and transketolase) [12, 13], the concentrations of nucleic acids (DNA and RNA) [14], the total blood protein level (by Lowry's method), the concentrations of serum protein fractions, activity of factors of humoral and cellular nonspecific defense [6, 7, 10, 11], and also with reference to activity of benzylpenicillin, injected into the bloodstream in a dose of 20,000 U/kg body weight, by the agar diffusion method, using *Staphylococcus aureus* 209P as the test microorganism.

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TABLE 1. Changes in Blood Parameters of Dogs after ECIB (M ± m)

Parameter studied	Before irradiation	Immediately after irradiation	Duration of experiment: days 1	days 3	days 7	weeks 2	weeks 3	weeks 4	weeks 5	weeks 6	weeks 7	weeks 8
Hemoglobin (Hb), g%	13,7 ± 0,5	14,3 ± 0,6	15,7 ± 0,9	13,7 ± 0,7	14,0 ± 0,7	15,3 ± 0,7	15,9 ± 1,4	15,4 ± 0,7	15,6 ± 1,3*	15,9 ± 0,7*	16,1 ± 1,2*	16,3 ± 0,9*
Hematocrit,%	40,9 ± 1,1	40,3 ± 1,5	47,1 ± 2,2*	39,2 ± 2,2	42,3 ± 1,5	44,5 ± 1,3*	47,1 ± 2,0*	48,1 ± 1,2*	48,7 ± 0,9*	48,8 ± 3,2*	50,0 ± 3,0*	49,7 ± 0,9*
ESR, mm/h	20,0 ± 3,3	11,0 ± 3,2	19,5 ± 3,4	22,0 ± 2,8	27,0 ± 4,1	19,0 ± 3,5	14,4 ± 4,9	16,5 ± 3,0	13,4 ± 2,7	12,0 ± 4,0	10,9 ± 3,7	8,8 ± 1,8*
Number of leukocytes in 1 µl, mill.	9554 ± 1452	9833 ± 1979	15700 ± 1936*	15318 ± 1846*	11653 ± 1033	11755 ± 1417	9114 ± 1739	10540 ± 1180	10600 ± 1396	8275 ± 1121	12960 ± 3690	10267 ± 601
Number of erythrocytes in 1 µl, millions	4,81 ± 0,19	4,88 ± 0,21	5,24 ± 0,69	4,92 ± 0,29	5,15 ± 0,47	4,74 ± 0,28	5,47 ± 0,25*	5,26 ± 0,17	5,58 ± 0,36*	5,16 ± 0,23	6,31 ± 0,17*	5,26 ± 0,32
G6PDH, µmoles NADPH/g Hb/min	2,13 ± 0,22	2,2 ± 0,3	1,83 ± 0,36	3,5 ± 0,7*	2,87 ± 0,37	1,42 ± 0,16*	2,15 ± 0,84	2,27 ± 0,50	2,63 ± 0,63	1,72 ± 0,30	2,32 ± 0,23	1,73 ± 0,36
Transketolase, µmoles C-7/g Hb/h	8,69 ± 0,66	8,19 ± 0,61	14,78 ± 4,07	13,93 ± 2,45	9,74 ± 0,87	10,97 ± 1,61	9,94 ± 1,79	8,44 ± 0,85	10,88 ± 1,78	11,34 ± 0,85*	12,68 ± 0,19*	8,44 ± 1,01
DNA, mg/liter	14,12 ± 1,76	12,09 ± 1,49	15,98 ± 3,28	13,22 ± 2,58	17,35 ± 1,62	13,4 ± 1,3	16,09 ± 1,37	25,13 ± 3,82*	18,56 ± 3,58	13,34 ± 2,65	18,99 ± 4,35	16,87 ± 3,06
RNA, mg/liter	50,7 ± 6,4	78,28 ± 9,00*	49,08 ± 7,34	83,3 ± 16,4	59,53 ± 4,88	63,79 ± 7,35	59,6 ± 10,0	67,24 ± 7,06	81,54 ± 17,54	79,66 ± 22,00	78,42 ± 25,04	70,38 ± 16,60

Legend. Here and in Table 2, asterisk indicates $P < 0.05$.

TABLE 2. Activity of Factors of Nonspecific Immunity and of Penicillin in Blood of Dogs after ECIB

Parameter studied	Before irradiation	Immediately after irradiation	Duration of experiment: days 1	days 3	days 7	weeks 2	weeks 3	weeks 4	weeks 5	weeks 6	weeks 7	weeks 8
Lysozyme, µg/ml	2,97 ± 0,17	2,47 ± 0,21	1,85 ± 0,22*	2,40 ± 0,21	3,79 ± 0,25*	3,65 ± 0,21*	2,84 ± 0,19	2,65 ± 0,21	2,73 ± 0,20	2,82 ± 0,23	2,69 ± 0,23	2,87 ± 0,24
β-lysines, %: lytic activity	27,05 ± 2,04	25,13 ± 2,12	20,15 ± 1,93	31,12 ± 2,87	36,24 ± 2,96*	42,44 ± 3,15*	32,15 ± 3,01	35,67 ± 3,83	31,62 ± 2,08	30,05 ± 3,08	29,18 ± 2,97	32,17 ± 3,14
Static activity	36,16 ± 3,02	69,95 ± 5,97	54,18 ± 5,21	40,17 ± 4,06	0,05 ± 4,99	42,14 ± 4,03	39,20 ± 4,01	39,17 ± 3,84	35,18 ± 3,42	37,22 ± 3,67	33,48 ± 3,27	39,73 ± 4,11
Bacterial activity of serum, %	39,54 ± 2,97	36,19 ± 2,84	16,27 ± 1,95	24,19 ± 1,98	29,71 ± 2,13	35,44 ± 2,93	36,19 ± 2,99	40,05 ± 3,17	38,50 ± 3,05	32,17 ± 2,93	37,02 ± 3,17	41,24 ± 3,24
Complement Cn 50 units	42,44 ± 0,34	39,17 ± 0,37	40,75 ± 0,39	42,13 ± 0,42	45,86 ± 0,47	46,35 ± 0,41	44,05 ± 0,44	44,27 ± 0,42	41,75 ± 0,40	42,73 ± 0,43	40,27 ± 0,42	42,14 ± 0,44
Phagocytic neutrophils, %	37 ± 10	34 ± 9	40 ± 10	39 ± 13	46 ± 16	—	—	57 ± 21	—	—	—	—
Number of cocci per phagocyte	3,8 ± 0,8	3,8 ± 0,8	3,8 ± 0,9	3,4 ± 1,1	4,4 ± 1,3	—	—	5,1 ± 1,3	—	—	—	—
Penicillin activity 60 min after injection, µg/ml	1,3 ± 0,1	—	26,7 ± 2,6*	—	20,3 ± 2,2*	—	—	2,2 ± 0,4	—	—	—	—

EXPERIMENTAL RESULTS

According to the writers' previous investigations, formation of a bypass between the femoral vessels for 3 h is not accompanied by any significant changes in the blood parameters studied. Accordingly, results obtained on animals with bypassing of the vessels but before irradiation of the blood were used as the control. The hematologic parameters after ECIB changed in various ways (Table 1). The Hb concentration and hematocrit increased after irradiation. Their increase by the 8th week of the experiment was by 19 and 21%, respectively ($P < 0.05$). The ESR increased a little during the first 7 days but then fell, to reach 44% of its initial value by the end of the experiment. A marked leukocytosis developed during the 7 days after after ECIB. The erythrocyte count was significantly increased in the 3rd, 5th, and 7th weeks of the experiment by 15, 17, and 31%, respectively. The change in the number of erythrocytes was accompanied by changes in their energy metabolism, revealed as changes in G6PDH and transketolase activity. The DNA concentration was increased by the 4th week in the irradiated blood, not due to injury to the blood but reflecting intensification of destructive changes in the tissues. Meanwhile the RNA concentration in the blood of the experimental animals rose, due to activation of synthesis. Investigation of the parameters of protein metabolism after ECIB showed that it remained balanced, with few traces of disproteinemia.

After ECIB significant changes were found in the activity of several factors of natural immunity (Table 2). On the first day after irradiation the lysozyme activity in the blood was considerably inhibited. In the course of 7-14 days it rose sharply compared with its initial level, and then returned to normal. The bactericidal activity of the serum was sharply reduced 1 day after irradiation, but later it was indistinguishable from normal. During 7 days of the experiment opposite changes were observed in the serum β-lysine levels, with predominantly an increase in activity of β-lytic properties. Serum complement activity and phagocytic activity of the neutrophils showed little change. When penicillin was given 1, 7, and 30 days after irradiation of the blood a considerable increase inthe antimicrobial activity of the preparation was found in the blood, and this was particularly marked in the early period of the experiment. For instance, the penicillin concentration in the control dogs 3 h after injection was 0.3 ± 0.1 U/ml, whereas 1 day after ECIB it was 10.0 ± 1.6 U/ml. No trace of the antibiotics could be found in the dogs' blood 8 h after injection into the irradiated dogs, whereas in animals receiving ECIB its concentration was 1.7 ± 0.2 U/ml.

After ECIB none of the changes taking place during the development of radiation sickness were observed in the blood, although a single whole-body irradiation of dogs with a dose of 1.5 Gy itself leads to the development of anemia and leukopenia [2]. Conversely, with the dosage chosen erythrocytoses and leukocytoses developed, possibly on account of activation of hematopoiesis. The weak harmful action of radiation on blood biopolymers is confirmed by the resistance of the blood protein systems. Meanwhile potentiation of antibiotic activity in the irradiated blood is probably due to interaction between radiochemical products and the penicillin molecule. The increase in activity of some factors of nonspecific immunoreactivity and stimulation of repair processes indicate that the ECIB method is a promising one for stimulating nonspecific defensive mechanisms in the body.

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DIFFERENCES IN ACTION OF Ca^{++} IONS ON CUMULATIVE BLOCKADE OF SODIUM CHANNELS INDUCED BY TERTIARY AND QUATERNARY AMINES

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It was shown previously [1, 4, 7] in experiments on myelinated frog nerve fibers that tertiary amines (procaine and trimecaine) interact chiefly with inactivated Na channels, inducing a state of slow sodium inactivation (SI) in them, which is the cause of cumulative inhibition of the Na current (I_{Na}). An increase in the external Ca^{++} concentration counteracted the development of SI; the proportion of Na channels changed into a state of SI during prolonged (1 sec) membrane depolarization by Ca^{++} was reduced [1, 7]. Calcium ions had a similar action on SI induced by external K^+ [3].

Quaternary derivatives of tertiary amines QX-314 [9], QX-572, QT [1, 7], and N-propylajmaline (NPA) [2, 8, 10] also induced cumulative inhibition of I_{Na}, but under these circumstances they interact with the open Na channel. The fact that SI arises only after application of local anesthetics to the outer surface of the membrane, and that interaction with the open channel requires the presence of a blocker in the axoplasm, suggested that there are "binding sites" in the nerve fiber membrane that are responsible for cumulative blocking of I_{Na} [1, 7]. However, according to Hille's hypothesis [6], all types of cumulative blockade of I_{Na}, whether induced by tertiary or quarternary amines, are due to their interaction with one "receptor," located in the region of the inner opening of the Na channel. Differences in the phenomenology of blockade induced by tertiary and quaternary amines are due entirely to the fact that the former penetrate in the uncharged form into the inner opening of the channel through the lipid bilayer of the membrane, whereas the latter can enter the inner opening only from the axoplasm, after opening of the activation gates of the channel.

According to the single receptor hypothesis for tertiary and quaternary blockers, it would be expected that a change in the external Ca^{++} concentration would have the same effect on frequency-dependent blockade of Na channels induced by these compounds. Data obtained in the present investigation contradict this prediction of Hille's hypothesis.

EXPERIMENTAL METHOD

Experiments were carried out on the Ranvier node of isolated nerve fibers of *Rana ridibunda* by the voltage clamp method [5]. The ends of the fiber were divided on either side of the test node in isotonic CsCl solution, which completely blocked K currents. The experiments

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