DYNAMICS OF BLOOD NITRITE METABOLISM IN IRRADIATED RATS

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It has been shown that the methemoglobin concentration observed when sodium nitrite ($NaNO_2$) is injected after irradiation is significantly higher than the level observed when $NaNO_2$ is injected into unirradiated animals: the result of the combined action of the two factors is more than just additive [2, 3]. One cause of this effect may be a disturbance of nitrite metabolism in irradiated animals due to an increase in its rate of entry into the intravascular space, as a result of a change in water metabolism after irradiation [1, 6, 10], as well as a combination of radiation-toxic processes associated with disturbance of the internal structure of hemoglobin and of its resistance to oxidation-reduction factors, the additional formation of endogenous methemoglobin-forming substances, a decrease in the concentration of natural radioprotectors, and disturbance of intracellular generation of reducing agents [6-9].

In this investigation nitrite metabolism and the correlation between $NaNO_2$ metabolism and methemoglobin formation in the blood of irradiated rats was studied.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing 200-220 g. The rats were kept under animal house conditions on a standard Kombikorm (combined fodder) pellet diet. A single exposure to whole-body irradiation was given on the UGU-420 gamma-ray source in doses of 77.4 and 180.6 mC/kg with a dose rate of $1.64 \cdot 10^{-4}$ A/kg. Sodium nitrite (chemically pure) dissolved in physiological saline was injected intraperitoneally in doses of 3, 5, 7, and 9 mg/100 g body weight on the 1st, 3rd, 7th, and 15th days after irradiation. The animals were killed under superficial ether anesthesia by withdrawal of blood from the heart. The blood $NaNO_2$ concentration of the animals was measured 15, 30, 45, 60, 90, 120, and 180 min after injection by the method in [12]. The optical density of the solutions was measured on a VSU2-P spectrophotometer at 540 nm.

EXPERIMENTAL RESULTS

The blood $NaNO_2$ level in the control animals was $5.0 \cdot 10^{-8}$ to $9.0 \cdot 10^{-8}$ mg/ml. Injection of $NaNO_2$ caused its concentration in the animals' blood to rise with time, to reach a maximum 40-45 min after injection, and later it fell. With all doses studied, the time course of the change in blood $NaNO_2$ concentration in the animals was uniform in character. However, the coefficient of proportionality of the rise in the blood $NaNO_2$ concentration did not differ from that for methemoglobin. It was lower in the case of methemoglobin, due to oxidation of nitrite into nitrate by oxyhemoglobin and by other endogeneous oxidizing agents.

Irradiation caused the blood $NaNO_2$ concentration to fall. On the first day after irradiation in a dose of 77.4 mC/kg the blood nitrite concentration was 65% of the control, whereas after irradiation in a dose of 180.6 mC/kg, it was 50%. An increase in the time elapsing after irradiation led to normalization of the nitrite concentration, which reached the control level on the 15th day.

The fall in the blood $NaNO_2$ concentration of the irradiated animals in the early period was evidently due to oxidation of nitrite into nitrate as a result of additional formation of oxidizing agents in the irradiated animal.

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Dependence of nitrite metabolism in the blood of animals irradiated in a dose of 77.4 mC/kg was studied after injection of $NaNO_2$ in a dose of 7 mg/100 g. Unlike in intact animals, the blood nitrite concentration in these rats reached a maximum 30 min after injection (177.4%, 1st day), followed by normalization. The development of a radiation syndrome changed the course of nitrite metabolism in the rats. On the 3rd day after irradiation the blood $NaNO_2$ level was virtually the same as in unirradiated animals (105.6%). On the 7th and 15th days the nitrite concentration was lower, namely 85.3 and 59.5%, respectively.

With all dependences given above the blood $NaNO_2$ concentration on the 1st-7th days after irradiation reached its peak value after 30 min, whereas on the 15th, the peak was observed at the same time as in the intact animals, namely after 40-45 min. Irradiation thus led to an increase in the rate of entry of nitrite into the animal's blood, but only in the early stage (1st-3rd days). For all dependences on time and dose (1st, 3rd, and 15th days) the peak value of the blood $NaNO_2$ concentration was reached 30 min after injection, and later its level fell.

Peak values of blood $NaNO_2$ concentration of the animals amounted to $104.0 \cdot 10^{-8}$ and $124.0 \cdot 10^{-8}$ mg/ml on the 1st and 3rd days, respectively, and later it returned to normal, but on the 7th and 15th days the peak values were $119.0 \cdot 10^{-8}$ and $109.0 \cdot 10^{-8}$ mg/ml (i.e., 118.1, 140.9, 135.2, and 123.8%, respectively).

Irradiation thus reduces the time during which the animals' blood nitrate level reaches a maximum by 10-15 min, and increases the $NaNO_2$ concentration compared with that in intact animals by 1-3 days after irradiation in a dose of 77.4 mC/kg and by 1-15 days after irradiation in a dose of 180.6 mC/kg.

Calculation of the coefficient of combined action (K_{ca}) by the method in [4] leads to the conclusion that the influence of the two factors (radiation and injection of $NaNO_2$) on the blood nitrite metabolism of the animals is antagonistic, for $K_{ca} < 1$. When calculated relative to values obtained on the 1st day after irradiation in a dose of 77.4 mC/kg, the value of K_{ca} on the 3rd, 7th, and 15th days was 0.12, 0.16, and 0.20, whereas after irradiation in a dose of 180.6 mC/kg its value was 0.046, 0.034, and 0.011.

The uniform nature of the curves characterizing nitrite and methemoglobin concentrations [2, 3] after injection of $NaNO_2$ into unirradiated animals shows that methemoglobin formation is determined not only by the kinetics of the supply of nitrite into the animal's blood. For all doses studied, 80-100 min after injection of $NaNO_2$, the velocity constant of the reaction between oxyhemoglobin and $NaNO_2$ to form methemoglobin reaches values of $4.1 \cdot 10^{-7}$ to $4.3 \cdot 10^{-7}$, i.e., it is independent of the $NaNO_2$ concentration. It can be concluded from these results that methemoglobin formation is determined by the kinetics of the entry of nitrites into the body only in the initial stage, and later (after 40-60 min) the decisive factor in the above reaction is the blood level of oxyhemoglobin, and not of nitrite. A similar conclusion was drawn by workers [11] who studied the reaction of oxyhemoglobin with nitrites in vitro and expressed the view that O_2^-, bound with hemoglobin or with methemoglobin, is an autocatalyst of this reaction. As an autocatalyst of the effect we are studying, O_2^- cannot evidently be bound with methemoglobin, for the $NaNO_2$ concentration on the 3rd-7th day ought to have fallen, whereas experimentally it was observed to have risen. It can be concluded from our own data and evidence in the literature [1, 4-9, 11] that the autocatalyst of this reaction is O_2^- bound with hemoglobin.

The radiation-toxic effect of the combined action of irradiation and injection of $NaNO_2$ is exhibited as an increase in the rate of entry of nitrites into the blood stream in the early stage after irradiation, and also as a fall in the oxyhemoglobin concentration at these same times, for the time when the blood nitrite concentration reached a maximum was shifted by 10-15 min and the $NaNO_2$ concentration was higher on the 1st and 3rd days after irradiation in a dose of 77.4 mC/kg and on the 1st-15th days after irradiation in a dose of 180.6 mC/kg than in intact animals. This may be attributable to an increase in vascular permeability, an increase in the rate of water metabolism, and passive transport of nitrite into the animal's blood [1, 6, 10]. With a dose of 180.6 mC/kg this was also due to an increase in the methemoglobin concentration on the 3rd-7th day after irradiation, with a resulting fall of the oxyhemoglobin concentration, which reached a minimum on the 3rd-7th day after irradiation [2, 3].

Besides radiation damage to the dynamic oxyhemoglobin – methemoglobin system, other factors leading to intensification of methemoglobin formation in irradiated animals are an

increase in the rate of entry of $NaNO_2$ into the blood and additional endogenous methemoglobin generation.

LITERATURE CITED

1. L. V. Krichkovskaya, E. V. Peskareva, and N. A. Busygina, Med. Radiol., No. 10, 39 (1972).
2. A. F. Malenchenko, L. P. Asafova, and V. S. Kuchuk, Izv. Akad. Nauk BSSR, Ser. Biol. Nauk, No. 2, 48 (1984).
3. A. F. Malenchenko, V. S. Kuchuk, and L. P. Asafova, Byull. Éksp. Biol. Med., No. 11, 548 (1984).
4. Technical Recommendations on Quantitative Stochastic Evaluation of the Combined Effect of Factors of Radiation and Nonradiation Nature on the Living Organism [in Russian], Kiev (1981).
5. A. E. Myshkin, Usp. Khimii, 53, No. 6, 1045 (1984).
6. Yu. Yu. Osipov, "Effect of limitation of movement, ionizing radiation, and radioprotectors on parameters of water and salt metabolism in animals," Author's Abstract of Dissertation for the Degree of Candidate of Medical Sicences, Moscow (1974).
7. E. N. Panasyuk and B. I. Zdravko, Fiziol. Zh. (Kiev), 28, No. 3, 327 (1982).
8. B. F. Sukhomlinov, A. V. Savich, M. I. Shal'nov, et al., Ukr. Biokhim. Zh., 53, No. 6, 16 (1981).
9. M. I. Shal'nov and O. I. Olontseva, Problems in Radiobiology and the Biological Action of Cytostatic Preparations [in Russian], Vol. 5, Tomsk (1973), pp. 37-38.
10. N. I. Tsyran, A. A. Sveshnikov, and V. S. Nesterenko, Radiobiologiya, No. 4, 623 (1977).
11. B. Ehert, F. Jung, and G. Lassmann, Stud. Biophys., 89, No. 1, 65 (1982).
12. H. Oda, H. Tsubone, A. Suzuki, et al., Environ. Res., 25, No. 2, 294 (1981).

CHANGES IN BINDING CAPACITY OF HUMAN BLOOD ALBUMIN AFTER UV-IRRADIATION IN THERAPEUTIC DOSES

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Investigation of the primary mechanisms of the therapeutic action of autologous transfusion with UV-irradiated blood (ATUVB), which is widely used at the present time [2], showed that UV irradiation of blood in therapeutic doses gives rise to structural changes both in the blood cells and in the plasma proteins; of the latter, moreover, the thermolabile proteins are the most sensitive [9, 10]. Functional consequences of structural changes induced by UV irradiation have already been described for some such proteins (complement and IgM) [9]. It is only for albumin — the principal blood protein, a highly important transport system of the blood, a regulator of the colloid-osmotic pressure of the plasma, and a reserve protein of the body — that consequences of this kind have not been studied.

The aim of this investigation was to study the effect of UV irradiation of blood in the Izol'da apparatus, which is used for ATUVB in the clinics of the USSR, on the binding capacity of albumin. It is this which determines the transport of ions, physiologically active substances, and also many drugs (predominantly lipophilic) in the body [6, 12]. Since certain

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