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ACTIVATION OF IMMUNOSORPTION PROPERTIES OF BLOOD BY UV IRRADIATION IN THERAPEUTIC DOSES

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The writers showed previously that UV irradiation (UVI) of human blood in therapeutic doses, such as are used in the treatment of various diseases by autologous transfusions of UV-irradiated blood (AUVIB), induces structural and functional changes in the erythrocyte surface [1, 4-6]. This paper describes an attempt to discover whether these changes in the erythrocyte surface are accompanied by potentiation of the immunosorption properties of Rh^+-blood in relation to anti-Rh_0 (D) antibodies. It is important to obtain data of this kind in connection with the search for ways of making the basic method of treatment of hemolytic disease of the newborn (HDN), namely exchange blood transfusion, more effective. In the treatment of HDN due to an Rh conflict, when the fetal tissues absorb a large quantity of material anti-Rh antibodies, the number of exchange transfusions of Rh^--blood sometimes reaches five or six [2].

EXPERIMENTAL METHOD

Immunosorption properties of 18 specimens of blood from Rh^+-donors, stabilized with "Gluyugitsir" solution, eight specimens of Rh^+ packed red cells (PRC), and two specimens of Rh^- blood and Rh^- PRC were studied. The blood and PRC were treated with UV radiation (254 nm) in the "Izol'da" MD-73M mass produced apparatus intended for AUVIB for therapeutic purposes. A standard therapeutic dose of UVI of the blood (1 D) was used, or half that dose (0.5 D) and two or three times that dose (2 D and 3 D). The same blood samples before UVI

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TABLE 1. Effect of UVI of Blood in Therapeutic Doses (0.5-3 D) on Its Ability to Adsorb Anti-Rh Antibodies from Sera of Sensitized Women when Mixed with Their Sera in the Ratio of 1:4 and 1:9

Parameter	Before addition of blood	After addition of blood		After addition of blood irradiated in different doses							
				0.5 D		1 D		2 D		3 D	
Antibody titer in serum	1:84	1:4	1:9	1:4	1:9	1:4	1:9	1:4	1:9	1:4	1:9
Frequency of lowering of titer, % of total number of serum specimens	—	1:39*	1:32	1:24	1:30	1:18**	1:30	1:26**	1:26	1:26	1:32
		70	66	50	44	80	33	72	44	40	13

Legend. Here and in Table 2: *) significant difference ($p < 0.01$) from initial value, **) significant difference ($p < 0.05$) from variant with addition of unirradiated blood; remaining differences not significant.

TABLE 2. Effect of UVI of Blood and PRC in Doses of 1 and 2 D on Their Ability to Adsorb Anti-Rh Antibodies from Preserved Blood of Sensitized Women when Mixed with Blood in the Ratio of 1:4

Parameter	Before addition of blood or PRC	After addition of Rh^+-blood			After addition of Rh^+-PRC			After addition of Rh^--blood			After addition of Rh^--PRC		
		UB	1 D	2 D	UB	1 D	2 D	UB	1 D	2 D	UB	1 D	2 D
Titer of antibodies in serum immediately after UVI of blood and PRC	1:91	1:30*	1:13**	1:12**	1:11*	1:12*	1:10	1:91	1:91	1:91	1:91	1:91	1:91

Legend. UB) Unirradiated blood, 1 D, 2 D) irradiated in the doses stipulated.

served as the control. Testing the immunosorption properties was carried out immediately and 1-48 h after UVI. The blood samples were mixed in the ratio of 1:4 and 1:9 with 19 specimens of stabilized blood or native serum containing anti-Rh antibodies. This blood was obtained from Rh^--sensitized women. On mixing the stabilized blood with serum, heparin was added (4 U/ml) to prevent the blood from clotting. The mixture was incubated for 45 min at 37°C with constant shaking, after which it was centrifuged at 100 g and the antibody concentration was determined in the supernatant. Antibody titers were determined before and after sorption. Geometric mean titers were calculated by the usual method [3]. The significance of the results was assessed by means of a nonparametric signs test.

EXPERIMENTAL RESULTS

The initial titer of anti-Rh antibodies in the test sera varied from 1:6 to 1:256. As Table 1 shows, the addition of unirradiated blood to the sera in the ratio of 1:4 and 1:9 led to reduction of their titer by more than half (from 1:84 to 1:39 and 1:32 respectively). This indicates sorption of anti-Rh antibodies by the Rh^+-blood. Preliminary UVI of the blood considerably enhanced its sorption properties, but this could be detected only when blood was added to the sera in the ratio of 1:4 (Table 1). The highest and most stable effect (twice the effect of unirradiated blood, recorded in 80% of cases) was achieved by the use of the standard therapeutic dose of UVI (1 D). In the other cases the antibody titer was lowered by not more than 1.5-1.6 times, and only in 13-50% of cases, except a dose of 2 D, when the effect was found in 72% of cases.

In the next series of experiments the effect of UVI (1 D and 2 D) on the ability of the blood and PRC to adsorb anti-Rh antibodies from preserved blood of sensitized women was studied. The titer of anti-Rh antibodies in the blood samples tested varied from 1:64 and 1:128, with a mean value of 1:91. Addition of unirradiated Rh^+ blood lowered the antibody titer by 3 times, whereas addition of irradiated blood lowered it by 7-7.5 times. Unirradiated Rh^+-PRC adsorbed antibodies almost 3 times more intensively than blood, reducing their titer by 8.1 times. This proves that erythrocytes are the chief sorbent of antibodies. This conclusion is in good agreement with the fact that Rh^- blood and Rh^- PRC did not affect titers of anti-Rh antibodies either before or after UVI (Table 2). UVI of Rh^+-PRC caused virtually no

change in their immunosorption properties. The absence of effect is probably connected with the fact that, as a result of the high concentration of erythrocytes, absorbing UV radiation intensively, the dose of that radiation calculated per erythrocyte fell and was not sufficient to ensure the rapid appearance of the test effect.

Thus Rh^+-blood, irradiated in therapeutic doses, effectively adsorbs anti-Rh antibodies not only from the serum, but also from the blood of sensitized women; moreover, a leading role in the enhancement of the immunosorptive properties of blood is played by erthrocytes. The mechanisms of this phenomenon are probably linked with structural changes in the surface of the irradiated erythrocytes and increased expression of surface antigens including Rh antigen [5, 6].

Considering data showing that expression of blood antigens increases 1-2 days after UVI [5, 6], we tested changes in the immunosorptive properties of Rh^+-blood and Rh^+-PRC 1, 12, 24, and 48 h after UVI indoses of 1 D and 2 D. The results showed that after 1 and 12 h the immunosorptive properties of the blood remained the same as immediately after UVI, but longer exposure (24 and 48 h) led to enhancement of the immunosorptive properties of the blood, the antibody titer being lowered by half compared with the effect of 1 D, and complete disappearance of antibodies took place when PRC were used as the sorbent.

In the next series of experiments the effect of UVI was studied on the immunosorptive activity of two blood samples from newborn infants with HDN. The direct Coombs' test was strongly positive. Two blood samples from sensitized women, Nos. 16 and 17, which had been used to study the immunosorptive properties of blood from normal donors, and which had a titer of anti-Rh antibodies of 1:128 and 1:64 respectively (Table 2), served as the test system. Addition of both intact blood of infants with HDN and blood irradiated in doses of 1 and 2 D did not cause any reduction of the antibody titer in the test samples. The absence of ability to adsorb anti-Rh antibodies in unirradiated blood is probably due to the fact that in this disease the erythrocyte surface, when saturated to the limit with maternal Rh antibodies, interacting with Rh^+-antigens of the erythrocyte membrane (which is confirmed by the positive Coombs' test with these blood samples), has no vacant receptors for anti-Rh antibodies. The presence of immune complexes on the erythrocyte surfce is evidence of a serious disturbance of its structure, and in turn this may be the cause of a whole range of functional abnormalities and, in particular, loss of the ability of membrane antigens to react to UVI. The discovery of this fact is in good agreement with our earlier observations, according to which diseases very different in their pathogenesis are accompanied by structural and functional disturbances of the surface of the blood cells, including erythrocytes [1, 7, 8].

Data on enhancement of the sorptive capacity of Rh^+-erythrocytes in relation to anti-Rh antibodies under the influence of UVI in therapeutic doses open the way for the use of UV-irradiated Rh^+-blood for the treatment of HDN, due to Rh-confict. The quantity of the blood to be used must be not less than one-quarter of the patient's blood. Preliminary injection of this blood into the patient, followed by exchange transfusion of Rh^--blood ought to reduce the number of exchange transfusions greatly and speed up the treatment process.

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