EFFECT OF TRANSFUSION WITH SYNGENEIC UV-IRRADIATED BLOOD ON BONE MARROW HEMATOPOIETIC FUNCTION IN NORMAL MICE AND AFTER CRANIOCEREBRAL TRAUMA

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In patients with severe craniocerebral trauma (CCT) the development of early infectious complications resistant to antibiotic therapy often terminates in death. One cause of the weakening of resistance to infection under these conditions may be a disturbance of the ability of bone marrow to produce and supply hematopoietic stem cells (HSC), precursors of immunocytes. Inhibition of this function of bone marrow has been observed in local injury to hypothalamic and brainstem structures in experimental animals [2, 13].

Autotransfusion with UV-irradiated (UVI) blood for therapeutic purposes is known to stimulate hematopoiesis, and the available information is chiefly concerned with the maturing and functional pools of blood cells [6].

There have been sporadic studies of the effect of this therapeutic procedure on HSC also: a stimulating effect of plasma from UVI blood on medullary HSC in man has been detected in vitro [7]. Methods of quantitative analysis of HSC or, more precisely, their analogs (splenic colony-forming units – CFU-S), in inbred mice enable this problem to be studied not only in vitro, but also in the intact organism.

In the investigation described below these methods were used in an attempt to evaluate the colony-forming activity of CFU-S and UVI blood and the ability of medullary CFU-S to form colonies and their powers of differentiation after incubation with plasma from UVI blood, the effect of plasma from UVI blood on endogenous and exogenous colony formation, and the effect of transfusion with UVI blood on colony formation in mice with closed CCT.

EXPERIMENTAL METHOD

Experiments were carried out on male (CBA × C57BL)F_1 mice weighing 22-25 g. Freshly collected heparinized (25 U/ml) blood from mice was exposed to UV rays (wavelength 254 nm, flow rate 20 ml/min) on an "Izol'da MD-73M" apparatus by the standard method used under clinical conditions [5]. Syngeneic blood, passed through the irradiation system but without UVI, and physiological saline with heparin served as the controls. Blood plasma was separated by centrifugation (8000g, 7 min).

Different versions of endogenous and exogenous cloning were used [3, 14, 15]. In the case of endogenous cloning, mice were irradiated sublethally and uniformly (6.4 Gy) or with a lethal dose (9.0 Gy) and with protection of part of the bone marrow, whereas with exogenous cloning, recipient mice were irradiated with a total dose of 9.2 Gy. In the last case, the irradiated mice were given an intravenous injection of 10^6 cells from UVI blood or 5×10^4 syngeneic bone marrow cells in 0.5 ml of medium 199.

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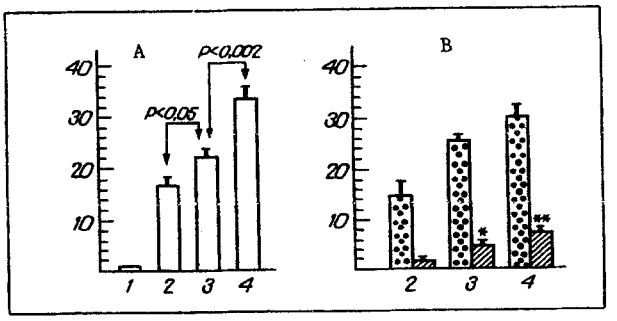

Fig. 1. Effect of incubation of plasma from UVI blood with bone marrow cells (HSC, 2 h, 37°C) on colony-forming activity (a) and direction of differentiation (b) of CFU-S. Abscissa: 1) medium 199; 2) HSC + physiological saline with heparin; 3) HSC + syngeneic blood plasma; 4) HSC + plasma from UVI blood. Ordinate: a) number of CFU-S per 10^5 cells; b) number of microcolonies per spleen. Columns with dots indicate erythroid, obliquely shaded columns — granulocytic cells. $*p < 0.05$ compared with group 2; $**p < 0.05$ compared with group 3, and $p < 0.001$ compared with group 2.

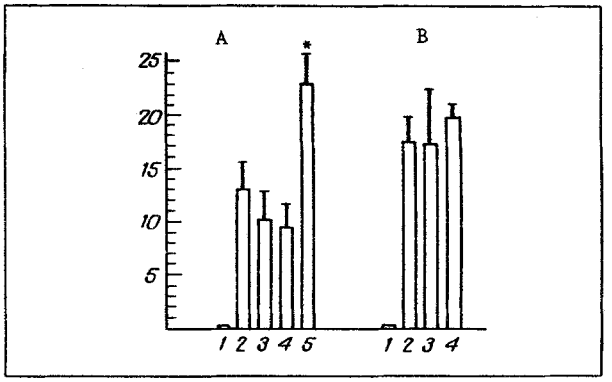

Fig. 2. Effect of plasma from UVI blood on endogenous (A) and exogenous (B) colony formation. A: 1) 9.0 Gy; 2) screening of one-third of tibia; 3) physiological saline with heparin; 4) syngeneic blood plasma; 5) plasma from UVI blood. B: 1) 9.2 Gy; 2) 5×10^4 HSC + physiological saline with heparin; 3) HSC + syngeneic blood plasma; 4) HSC + plasma from UVI blood. Abscissa, groups of animals; ordinate, macrocolonies on spleen. $*p < 0.01$.

A suspension of bone marrow cells from the femora was obtained by a single flushing out of the medullary cavity (500g, 7 min) and resuspended in 2 ml of medium 199. To 0.1 ml of the suspension, equal volumes (0.3 ml) of plasma from UVI blood, from plasma of blood without UVI, or physiological saline were added separately, and the samples were incubated for 2 h at 37°C. The cell concentration in each version was then adjusted to 10^5/ml, and 0.5 ml of the suspension was injected into irradiated mice.

These same substances were injected intravenously in a dose of 0.15 ml uniformly and twice into totally irradiated mice, namely 2 and 24 h after lethal irradiation, and as a single injection into sublethally irradiated animals.

Disturbances of medullary function in CCT were corrected by intravenous injection of UVI blood (0.2 ml per mouse), different numbers of times. Graded closed CCT was inflicted in the occipito-parietal region of the mouse's head by a falling weight (25 g), which slid vertically down a rod from a height of 65 cm. The severity of trauma corresponded clinically and morphologically to concussion of moderately severe degree, and the mortality was 17.6%.

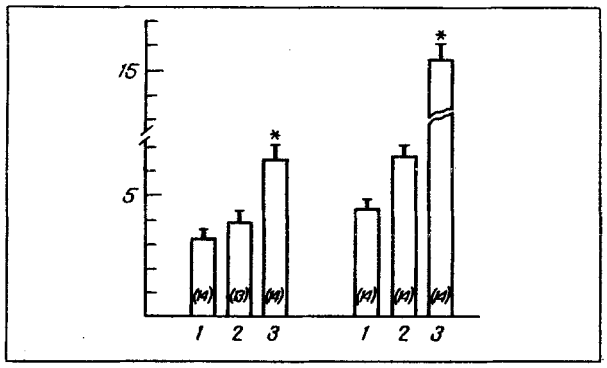

Fig. 3. Effect of plasma from UVI blood on colony formation in sublethally irradiated mice. 1) 6.4 Gy + physiological saline with heparin; 2) syngeneic blood plasma; 3) plasma from UVI blood. *$p < 0.05$ compared with group 2. Numbers in parentheses indicate number of animals in group. Abscissa, groups of animals; ordinate, macrocolonies per spleen.

During the first 4 days after CCT, including the day of injury, animals of different groups received from 1 to 4 sessions of UVI blood, but later mice of all the groups were irradiated sublethally, and this was followed by determination of the intensity of colony formation and the weight of the thymus of these animals.

In all cases the number of macro- or microcolonies was counted 8 days after irradiation, and the type of hematopoietic colonies was determined by the method in [10]. The results were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

The experiments showed that UVI blood, under the conditions used, did not significantly change the colony-forming activity of CFU-S present in the UVI-blood sample: the number of CFU-S per 10^6 cells in the experiment (5.9 ± 0.6) did not differ from the control (6.4 ± 0.9).

Conversely, incubation of the bone marrow cells for 2 h with plasma from UVI blood increased the yield of exogenous colonies considerably (by 1.5-1.9 times) and significantly ($p < 0.002$) compared with the action of syngeneic blood plasma or physiological saline (the number of CFU-S per 10^5 cells was 32.2 ± 2.4, 21.8 ± 1.4, and 17.4 ± 1.6, respectively) and it enhanced the granulocytic trend of CFU-S differentiation (Fig. 1).

A similar stimulating effect on endogenous colony formation was obtained in mice irradiated nonuniformly: injection of plasma from UVI blood into these animals increased the number of endogenous colonies by 1.8-2 times (significantly, $p < 0.05$) compared with the control value. Meanwhile, a similar injection of plasma from UVI blood into totally irradiated animals with preliminary transplantation of bone marrow cells was accompanied only by a tendency toward intensification of colony formation (Fig. 2).

Thus, treatment of blood by UV rays under therapeutic conditions potentiates the colony-stimulating properties of plasma without changing activity of UVI CFU-S of the blood. Stimulation of colony formation is realized both on direct contact of plasma from UVI blood with bone marrow cells in vitro and also on its injection into the intact organism. The effect is perhaps connected with the direct or indirect action of active peptides, whose appearance has been demonstrated after UVI of blood or plasma enriched with leukocytes [7], on proliferation of HSC. Considering the data obtained in the present investigation with endogenous and exogenous cloning, potentiation of migration of migration of HSC from the protected region of the bone marrow, under the influence of factors of UVI blood can be postulated in uninjured animals. Preliminary series of experiments showed that in mice with closed CCT maximal inhibition of endogenous colony formation is observed 4 days after trauma, against the background of a sharp decrease in weight of the thymus.

Increasing the number of sessions of UVI of the blood led to dose-dependent inhibition of the bone marrow function tested. The maximal reduction in the number of colonies was observed in mice (group 5) receiving 4 sessions of UVI-blood (Table 1), possibly due to the characteristic action of regulatory peptides in response to an increase in their dose [1].

TABLE 1. Colony Formation and Weight of Thymus from Sublethally Irradiated Mice with Closed CCT after Different Numbers of Intravenous Injections of Syngeneic UVI Blood

Group of animals	Procedure	Days ater CCT				8 days after irradiation		
		1	2	3	4	weight of thymus	number of colonies	number of spleens
1	CCT + physiological saline	—	—	—	6,4 Gy	20,5±1,7	11,7±3,0	10
2	CCT + UVI blood	—	—	—	6,4 Gy	23,9±1,1	26,5±3,7	10
3	CCT + UVI blood	UVI blood	—	—	6,4 Gy	25,4±1,7	11,3±0,9	10
4	CCT + UVI blood	UVI blood	UVI blood	—	6,4 Gy	22,5±1,7	8,4±1,5	10
5	CCT + UVI blood	UVI blood	UVI blood	UVI blood	6,4 Gy	22,4±0,9	5,2±1,4	10
6	Intact	—	—	—	6,4 Gy	22,1±1,9	22,0±2,0	7

Similar disappearance of the stimulating effect in relation to more mature blood cells in response to an increase in the number of sessions of UVI blood has been observed in experiments on dogs [4].

Some increase in the weight of the thymus in animals receiving UVI blood must be noted (it was particularly marked in the mice of groups 2 and 3), with a smaller increase in weight in response to an increase in the number of transfusions of UV blood. Meanwhile, no correlation was found between the weight of the thymus and the number of splenic colonies.

According to data in the literature, UVI stimulates production of interleukin-1 (IL-1) by macrophages in vitro [9] and potentiates the IL-1-like activity of blood serum in vivo [12]. Meanwhile, the stimulating effect of leukocytic pyrogen on granulocytopoiesis [8] and increased production of colony stimulating factors by fibroblasts under the influence of IL-1 have been demonstrated [11].

Injection of UV blood or its plasma has been shown to potentiate colony-forming activity of HSC, to stimulate granulocytopoiesis and to restore normal colony formation after CCT. A definite role in the mechanisms of hematopoiesis-stimulating action of UVI blood may perhaps be played by IL-1 — a regulatory peptide with both neurotropic and immunotropic activity.

Thus, the desirability of using autologous transfusion of UVI blood to correct disturbances of bone marrow function in brain pathology was demonstrated in experiments on mice.

A corrective effect was obtained after a single injection of UVI blood on the day of infliction of CCT. The dose of UVI blood used (0.2 ml/mouse or 8 ml/kg) corresponds in total to about 3 to 5 sessions of this treatment, if used for the treatment of human patients.

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