MECHANISM OF THE CLINICAL EFFECTS OF UV-IRRADIATED BLOOD: STIMULATION OF DNA SYNTHESIS BY HUMAN CELLS IN CULTURE

N. K. Belisheva and K. A. Samoilova

UDC 615.38.015.2:615.831.4]
.015.4:612.398.145.1.015.36

KEY WORDS: UV irradiation; blood; growth factors; cells in culture.

The widespread use of a new method of treatment, namely autotransfusion of UV-irradiated blood (AUVIB), in medical practice is largely attributable to its successful application in surgery [5, 6]. Among clinical effects induced by AUVIB may be mentioned stimulation of regeneration and hematopoiesis. To study the possibility that UV-irradiated blood may stimulate the proliferative activity of cells, the growth-stimulating properties of UV-irradiated donated blood have been studied in the writers' laboratory on models of different types of human cells in culture [9].

In the investigation described below DNA-synthetic activity of human embryonic cells (EC) cultured in the presence of supernatants from intact and irradiated cell fractions of blood or plasma was studied.

EXPERIMENTAL METHOD

Human EC, obtained by explanation of abortion material, were cultured in Carrel flasks in a mixture of Eagle's medium and medium 199 (1:1) with the addition of 10% of bovine serum. For the experiments cells were seeded on coverslips placed in flasks under antibiotics (1.5×10^4 to 2.0×10^4 cells per flask). After 24 h the medium was replaced by new and supernatants of cell fractions of blood or plasma were added to the nutrient medium in the ratio of 1:100. The cells were incubated in this medium for 24 h, long enough for initiation of DNA synthesis by serum and other growth factors in normal diploid cells *in vitro* and for their entry into the S phase [10-12]. At the end of incubation [^{3}H]thymidine (0.5 μCi/ml, 41.6 kCi/mole) was added to the growth medium for 30 min, after which it was replaced by medium with unlabeled thymidine (0.001% solution), the cells were fixed with a mixture of methanol and glacial acetic acid (3:1), coated with emulsion (from "Ilford"), and exposed in darkness for 10-15 days, developed, stained with Mayer's hematoxylin, and used to obtain permanent preparations. Freshly donated blood, stabilized with heparin (25 U/ml) was irradiated. The experimental material consisted of the following specimens of blood. 1) Supernatants of intact and irradiated blood obtained by sedimentation of its cells (here and in the other versions the supernatants were obtained immediately after irradiation of the blood, cells being sedimented by centrifugation at 1000 rpm for 10 min). 2) Intact and irradiated blood plasma, obtained by two methods: a) the blood was allowed to stand for 1.5-2 h at room temperature, the plasma above the erythrocytes was withdrawn and separated from cells by centrifugation; b) the blood was allowed to stand for 24 h at 4°C and the plasma withdrawn. 3) Supernatants of intact and irradiated buffy coat (BC), obtained by two methods: a) the blood was allowed to stand for 1.5-2 h at room temperature, and plasma with leukocytes and platelets was withdrawn; b) the blood was allowed to stand for 24 h at 4°C and the layer of leukocytes and platelets on the boundary between plasma and erythrocytes was withdrawn and resuspended in Hanks' solution (1:1). 4) Supernatants of intact and irradiated red cell suspension (RCS), obtained by diluting the packed red cells (residue of red cells after allowing blood to stand for 2 h) with Hanks' solution (1:1). The blood and its fractions, in a volume of 2 ml, were irradiated with continuous mixing in small Petri dishes with short-wave UV rays (254 nm), produced by lamps of two types: DRB-8, fitted into an "Isolda" appara-

Laboratory of Biochemical Cytology and Cytochemistry, Institute of Cytology, Academy of Sciences of the USSR, Leningrad. (Presented by Academician of the Academy of Medical Sciences of the USSR V. S. Savel'ev.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 98, No. 12, pp. 675-678, December, 1984. Original article submitted December 27, 1983.

0007-4888/84/9812-1651$08.50

TABLE 1. Incorporation of [^{3}H]Thymidine into Human EC Cultured in Presence of Supernatants (s) of Intact and Irradiated Samples of Whole Blood (sWB), Red Cell Suspension (sRCS), and Intact (A) and Irradiated (B) Plasma

No. of blood specimen	Dose of UV rays, J/m^2	LI. Absolute values ($\bar{x} \pm S_{\bar{x}}$) and in series with irradiated blood specimens, % of LI in series with intact specimens (in parentheses)								
		EC	EC + sWB		EC + plasma		EC + sBC		EC + sRCS	
			A	B	A	B	A	B	A	B
1	36	21,3±0,5	30,7±2,5	37,2±2,5 (121)	—	—	30,8±1,3	36,9±4,0 (120)	—	—
2		29,1±7,1	29,3±1,9	34,9±1,4 (119)	35,4±1,8	32,1±1,9 (91)	26,0±2,4	35,9±1,9	29,0±2,8	33,6±3,9 (116)
3		4,4±0,4	4,0±1,4	8,4±0,2 (210)	—	—	—	—	—	—
4		4,4±0,4	5,0±2,8	6,3±0,2 (126)	5,6±1,7	5,2±2,4 (93)	1,5±0,4	3,7±0,1 (247)	2,6±0,3	4,5±0,3 (173)
5		19,9±5,5	21,3±0,4	32,9±4,5 (155)	—	—	26,8±2,4	19,5±4,4 (73)	26,8±1,5	26,2±1,5 (98)
6		4,4±0,4	4,0±1,4	7,6±1,3 (190)	6,8±1,9	6,7±2,8 (99)	6,9±1,8	8,6±1,7 (125)	6,7±1,4	8,2±1,4 (122)
7		4,4±0,4	5,0±2,8	5,9±0,9 (118)	5,6±1,7	4,7±1,8 (84)	1,5±0,4	4,0±0,5 (267)	2,6±0,3	4,8±0,3 (185)
Level of P for differ. betw. rows of results			<0,01	<0,01				<0,05		<0,05
Mean:				148		92		162		140
8	743	—	23,8±0,9	27,3±1,8 (115)	—	—	23,9±0,5	25,4±1,7 (106)	—	—
9		31,4±5,6	38,2±2,1	43,7±2,3 (114)	—	—	—	—	—	—
10		26,5±3,1	32,1±2,7	39,9±0,8 (124)	32,6±3,3	34,5±3,9 (106)	39,3±3,2	34,0±1,5 (87)	33,6±1,3	31,8±4,8 (95)
11		30,5±1,1	34,0±2,2	31,3±1,1 (92)	—	—	—	—	—	—
12		21,3±0,5	30,7±2,5	30,1±2,2 (98)	—	—	30,8±1,3	34,5±3,2 (112)		
13		29,1±7,1	29,3±1,9	41,0±2,1 (140)	35,4±1,8	34,9±1,6 (99)	26,0±2,4	34,5±4,7 (133)	29,0±2,8	35,8±1,9 (123)
14		13,0±1,5	—	—	28,9±3,5	26,4±1,8 (91)	27,0±2,1	30,3±1,0 (112)	—	—
15*		21,3±0,5	—	—	26,2±1,4	32,9±5,7 (126)	26,5±2,4	27,9±0,7 (105)	33,1±3,0	38,5±3,4 (116)
Level of P for differ. betw. rows of results			<0,01	<0,05		>0,05		<0,05		<0,05
Mean:				114		105		109		111
Level of P for diff. betw. rows of results for two different doses of UV rays			<0,01	<0,01		>0,05		<0,01		<0,01

*Blood specimens obtained by methods 2a and 2b (see "Experimental Method").

tus, and DB-30. Spectral and power parameters reflecting the action of irradiation on the blood were closely similar to those obtained by irradiation in an "Isolda" apparatus as is used for AUVIB under clinical conditions. The doses of irradiation were 36 and 743 J/m^2 respectively. According to our previous data [7, 8], exposure to UV rays in these doses is not lethal for blood cells. The action of UV-irradiated blood on DNA synthesis in EC was judged from the change in arithmetic mean ($\bar{x}$) of the labeling index (LI, percentage of labeled nuclei in a cell population), calculated for each version (of experiment and control) on the basis of data obtained by microscopic analysis of 3-6 preparations of EC (at least 1000 cells). In each preparation 25-50 fields of vision (ocular 7, objectives 40 and 100) were examined. The significance of difference between results in the experiment and control was estimated by Wilcoxon's nonparametric matched pairs signed ranks test and by comparison of dependent populations with quantitative data on the basis of the mean difference between each pair of variants [4].

EXPERIMENTAL RESULTS

If LI for EC cultured on standard medium and on medium with supernatants from intact whole blood is compared (Table 1) it will be clear that addition of the supernatants was accompanied by an increase in LI in 10 of 12 experiments. Since the supernatant consists of intact blood plasma, the observed effect could be attributed to an increase in the concentration of "serum" growth factors in the incubation medium. Supernatants from irradiated whole blood had an even stronger stimulating action on DNA synthesis by EC: in this series of experiments LI was always higher than in EC growing on standard medium, and in 11 of 13 experiments it was 14-110% higher than for EC cultured in the presence of supernatants from unirradiated whole blood (Table 1). The maximal effect was obtained by irradiation of blood with the smaller of the two doses (36 J/m^2).

The important result was that irradiated plasma, by contrast with unirradiated, had no growth-stimulating effect (Table 1): in six of eight experiments LI of EC cultured in the

presence of irradiated plasma was actually lower than LI in EC cultured in the presence of intact plasma, and on the whole the series of results of experiment to compare the action of irradiated and intact plasma did not differ significantly ($P > 0.05$). It can accordingly be concluded that stimulation of DNA synthesis in EC growing in the presence of supernatants from irradiated whole blood is not connected with photoactivation of growth factors in the blood plasma, but takes place as a result of their release from the cells.

This hypothesis is in agreement with the results of experiments in which EC were cultured in the presence of supernatants from irradiated specimens of BC and RCS. In 10 of 12 experiments with irradiation of BC and in six of eight experiments with irradiation of RCS addition of supernatants from the irradiated blood cell fractions led to an increase of DNA-synthetic activity in EC compared with that observed in EC to which supernatants from unirradiated cell fractions were added (Table 1). The stimulating effect attained its highest values when BC and RCS were treated with the smaller dose of UV rays and its mean value was comparable with that following exposure of whole blood to the same dose. Nevertheless, the results do not yet permit discussion of the contribution of the different cell fractions of UV-irradiated whole blood to the effect of enhancement of its growth properties, for the concentration of and ratio between the different blood cells in BC and RCS did not correspond with those in intact blood in this series of experiments.

When the possible causes of UV-induced enhancement of the growth properties of blood are analyzed it must be recalled not only that the main contribution to this effect is made by its cell fractions, but also that the growth-stimulating properties of blood were modified immediately after its exposure to UV rays. Accordingly the possibility cannot be ruled out that one mechanism of the effect under discussion is linked with the phenomenon discovered in the writers' laboratory: the appearance of their surface components in the medium surrounding blood cells immediately after irradiation as a result of partial photochemical destruction of the outer juxtamembranous layer [1-3, 8]. This hypothesis seems all the more likely because, in agreement with existing data [13], components of the plasma membranes of leukocytes of donated blood are able to stimulate proliferation of human cells in culture.

Donated blood, irradiated with UV light of the same wavelength and wighin the same dose range as are used under clinical conditions (up to 1200 J/m^2), thus possesses growth-stimulating properties. Similar results were obtained previously by the writers when studying the effect of UV-irradiated blood on the colony-stimulating ability of human bone marrow cells in culture [9].

LITERATURE CITED

1. R. A. Artsishevskaya and K. A. Samoilova, in: Abstracts of Proceedings and of Display Materials of the First All-Union Biophysical Congress [in Russian], Vol. 2, Moscow (1982), p. 299.
2. R. A. Artsishevskaya and K. A. Samoilova, Tsitologiya, No. 12, 1387 (1983).
3. R. A. Artsishevskaya, A. P. Mironova, and K. A. Samoilova, Tsitologiya, No. 2, 212 (1984).
4. I. P. Ashmarin, N. N. Vasil'ev, and V. A. Ambrosov, Rapid Methods of Statistical Analysis and Planning of Experiments [in Russian], Leningrad (1971), p. 80.
5. L. V. Potashov and R. V. Cheminava, Vestn. Khir., No. 6, 130 (1982).
6. V. S. Savel'ev, N. P. Aleksandrova, E. B. Petukhov, et al., Vestn. Akad. Med. Nauk SSSR, No. 10, 72 (1981).
7. K. A. Samoilova, K. N. Klimova, L. S. Priezzheva, et al., Tsitologiya, No. 12, 1378 (1983).
8. K. A. Samoilova, A. P. Mironova, and R. A. Artsishevskaya, Tsitologiya, No. 1, 102 (1984).
9. K. A. Samoilova, N. K. Belisheva, A. Yu. Zaritskii, et al., Tsitologiya, No. 9, 978 (1984).
10. K. A. D. Ellen and I. F. Gierthy, J. Cell. Physiol., 92, 381 (1977).
11. A. Lindgren and B. Westermark, Exp. Cell Res., 99, 357 (1976).
12. A. Okuda and G. Kimura, Exp. Cell Res., 111, 55 (1978).
13. G. B. Price, E. A. McCulloch, and J. E. Till, Exp. Hematol., 3, 227 (1975).