GROWTH PATTERNS OF BLOOD CELLS IN CULTURE IN THE PRESENCE OF POLYETHYLENE OXIDE 400 CRYOPROTECTOR

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UDC 612.112:578.085.23

The effect of the cryoprotector polyethylene oxide 400 (PEO-400) on mitotic activity of human peripheral blood leukocytes was studied in cultures stimulated by phytohemagglutinin. The cytological features of growth of the blood cells when PEO)400 was added before the beginning of culture were expressed as continued ability of the cells to undergo transformation and mitotic division despite a significant decrease in the mitotic activity of the experimental cultures compared with the controls.

KEY WORDS: short-term blood culture; cryoprotector; mitotic activity; phases of mitosis.

During the last decade the need for prolonged keeping of biological objects in transplantology has led to the development of the method of keeping human cells and cells of certain animals by cryopreservation in liquid nitrogen.

However, the process of freezing and thawing and prolonged keeping at the temperature of liquid nitrogen, according to some data in the literature, may have an adverse effect on the proliferative ability of the preserved cells. For instance, according to Yakusawa et al. [7], each stage of the freezing and thawing process causes its own type of damage and in that way inactivates to a certain degree the proliferative capacity of the thawed cells. At all stages of cryopreservation the cells are in contact with cryoprotectors, essential components of the medium in which the cells are frozen. Contact between the cells and cryoprotectors, especially in the period of preparation for freezing, is relatively prolonged. Meanwhile the character of the effect of cryoprotectors on growth of blood cells in culture and on their proliferative capacity has been inadequately studied.

Strong et al. [6], for instance, showed that after the addition of cryoprotectors such as dimethylsulfoxide (DMSO), glycerol, and polyvinylpyrrolidone to the culture medium in various concentrations, a culture of mouse lymphocytes stimulated by phytohemagglutinin (PHA) actively incorporated [^{3}H]thymidine only in the presence of 1% solution of DMSO, whereas the remaining cryoprotectors caused inhibition of cell growth. Investigations by other workers have shown that even brief incubation (150 min) of tissue cultures in a medium with 5% glycerol solution and 10% DMSO solution, after rinsing and subsequent culture in fresh medium, led to various cytological injuries in the cultured cells [5]. Meanwhile Aswood-Smith et al. [4], who studied the cryoprotective properties of methanol, found that Chinese hamster cells can grow without any special injuries for 3 days in a culture medium containing methanol in a final concentration of 5%.

The contradictory nature of the data described above can be explained by the heterogeneity of the material used and also by the fact that different cryoprotectors were tested in different concentrations.

Consequently the study of the patterns of growth of a cell culture after the addition of cryoprotectors to the culture medium is an urgent problem. The cryoprotector polyethyleneoxide 400 (PEO-400), which is used for freezing nucleated blood and bone marrow cells and which, as the work of Pushkar' et al. [2, 3], has shown, does not need to be rinsed out after preservation, is particularly interesting in this respect.

In the investigation described below proliferative capacity of human peripheral blood leukocytes was determined in culture when PEO-400 was added to the culture medium in different concentrations.

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(Presented by Academician of the Academy of Medical Sciences of the USSR V. I. Struchkov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 85, No. 6, pp. 729-731, June, 1978. Original article submitted September 21, 1977.

0007-4888/78/8506-0799$07.50

TABLE 1. Mitotic Activity and Phases of Mitosis in Control and Experimental Cultures of Human Peripheral Blood (M ± m)

Series of experiments	Culture	Mitotic index, %	Phases of mitotic division					
			prophase	%	metaphase	%	anaphase	%
I	Control	34,77±5,24	19,11±3,93	54,9	14,22±2,74	40,9	1,44±0,54	4,2
	5% PEO-400 (n=9)	17,66±3,57 (P<0,02)	8,55±3,09 (P>0,05)	48,4	8,66±3,93 (P>0,05)	48,9	0,44±0,12 (P>0,05)	2,7
II	Control	32,14±6,60	15,43±4,65	48,0	15,14±3,45	47,1	1,57±0,59	4,9
	10% PEO-400 (n=7)	10,28±3,76 (P<0,02)	3,28±1,36 (P<0,05)	31,9	7,00±3,32 (P>0,05)	68,1	—	—

EXPERIMENTAL METHOD

A short-term culture of human peripheral blood leukocytes stimulated by PHA was used. A suspension of leukocytes was obtained from heparinized blood of clinically healthy subjects by allowing the blood to stand. Culture was carried out for 69 h in medium 199 containing 10% bovine serum and the optimal dose of PHA (Reanal). A sterile solution of PEO-400 in final concentrations of 5 and 10% was added to the cultures. The suspended cells were centrifuged and films were prepared from the residue; these were fixed with methanol and stained by the Romanovsky–Giemsa method. The proliferative power of the cells was studied by determining the mitotic index and phases of mitosis in 1000 transformed cells (blast and transitional forms), and was expressed in parts per thousand.

Two series of experiments were carried out. In series I the mitotic activity of the leukocytes was determined in the presence of PEO-400 in a final concentration of 5%, and in series II a 10% solution of PEO-400 was added to the culture medium (Table 1). The control to each series of experiments consisted of leukocytes cultured without the addition of the cryoprotector. The data for mitotic activity and the ratio between the phases of mitosis in the control and experimental cultures were subjected to statistical analysis by the Student–Fisher method.

EXPERIMENTAL RESULTS

Some differences were found in the ratio between the numbers of cell forms in the control and experimental cultures. In the experimental series, by contrast with the control, most cells were "small" lymphocytes, whereas the transformed transitional forms and blast cells, which predominated in the control, were found much less frequently, although they preserved their morphological individuality and their ability to undergo mitotic division.

As Table 1 shows, the mitotic index in the experimental cultures differed from that in the control. In the experimental cultures after addition of a 5% solution of PEO-400 to the culture medium, for instance, the mitotic index was reduced by half ($P < 0.02$), and after addition of a 10% solution of PEO-400 it was reduced by two-thirds ($P < 0.02$) compared with the control. When values of the mitotic index obtained in the experimental cultures with 5% (17.66 ± 3.57%) and 10% (10.28 ± 3.76%) concentrations of PEO-400 were compared, it will be noted that the differences were not significant ($P > 0.05$).

As regards the distribution of the phases of mitosis, as Table 1 shows the number of cells in the various phases of mitotic division was smaller in the experimental cultures than in the controls. Significant differences were found with respect to the decrease in the number of cells in prophase, the increase in the number of cells in metaphase ($P < 0.05$), and the decrease in the number of cells in anaphase ($P < 0.05$) during growth in medium with 10% PEO-400 solution ($P < 0.05$). In the experimental cultures a tendency was observed for the number of cells in prophase and anaphase to be reduced, whereas the number of cells in metaphase was increased.

Recent investigations have shown that PHA exhibits its transforming action by acting on the surface of lymphocytes [1]. It can accordingly be postulated that PEO-400, which is a cryoprotector of mixed types (exo-endocellular), acts in some way on the surface of the lymphocytes and also, perhaps, on various of its components, and in that way inhibits the normal course of transformation under the influence of PHA. Meanwhile lymphocytes which have undergone transformation divide by mitosis.

Biochemical and cytochemical investigations of nucleated blood and bone marrow cells, incidentally, show that cryoprotector PEO-400, despite its low toxicity, if incubated with these cells also leads to some decrease in the intensity of the biochemical and cytochemical indices [3].

It can accordingly be concluded from the results of cytological analysis that if PEO-400 is added to the culture medium before the beginning of culture in final concentrations of 5% or 10%, blood cells stimulated by PHA remain capable of transformation and of mitotic division, despite a significant decrease in the mitotic activity of the experimental cultures compared with the controls.

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MECHANISM OF ACTION OF BREAKDOWN PRODUCTS OF GRANULOCYTES ON GRANULOCYTOPOIESIS

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UDC 615.385.34.015.4:612.112.12

The leukopoietic activity of blood serum was studied after injection of the breakdown products of 12 and 60 million homologous granulocytes per 100 g body weight into intact Wistar rats, and the character of the action of the serum on proliferation and differentiation of hematopoietic stem cells was determined in the spleen of lethally irradiated mice. The accumulation of granulopoietins in the blood under the influence of the granulocyte breakdown products was greater after injection of material from 12 million granulocytes. Granulocytopoietins stimulate the proliferative activity of stem cells and their differentiation into granulocytes in the spleen of lethally irradiated mice. It is concluded that granulocytes breakdown products have a stimulating action on hematopoiesis through granulocytopoietins.

KEY WORDS: granulocyte breakdown products; granulocytopoietin; stem cell; leukopoietic activity of blood.

It was stated in previous communications [1, 2] that granulocyte breakdown products stimulate granulocytopoiesis in intact animals and that the intensity of this stimulation depends on the number of granulocytes injected. The most marked stimulating effect was given by a dose of 12 million lysed granulocytes per 100 g body weight of the rat: this dose increased the proliferative activity of the granulocytes and the absolute number of myelokaryocytes, mainly on account of granulocytes, as well as the number of granulocytes in the peripheral blood. Injection of 60 million lysed granulocytes led to some degree of inhibition of granulocytopoiesis in the first 3 days, followed by moderate activation.

To study the mechanism of action of granulocyte breakdown products on granulocytopoiesis, in the present investigation their effect was examined on the leukopoietic activity of the serum and the character of the action of serum on proliferation and differentiation of hematopoietic stem cells was determined in the spleen of lethally irradiated mice.

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(Presented by Academician of the Academy of Medical Sciences of the USSR N. A. Fedorov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 85, No. 6, pp. 731-733, June, 1978. Original article submitted December 8, 1977.

0007-4888/78/8506-0801$07.50