TOXICITY OF PARA-AMINOBENZHYDRAZIDE AND ITS EFFECT ON NUCLEI ACID BIOSYNTHESIS IN CULTURES OF NORMAL AND TUMOR CELLS

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UDC 616.006-018.1-008-939.633.2-02:615.277.3]-07

KEY WORDS: para-aminobenzhydrazide, hydrazine sulfate, nucleic acid biosynthesis, cytotoxicity.

Hydrazine sulfate has been shown to potentiate the action of some antitumor preparations and, in some cases, to render them active against tumor models normally resistant to the action of these substances [4]. Certain acylhydrazines and, in particular, para-aminobenzhydrazide (PABH) have been found to have the same effect [3]. The study of the mechanism of this phenomenon has shown that hydrazine sulfate and its derivatives inhibit metabolism of xenobiotics, including antitumor preparations [5]. It may account for the effect of potentiation of antitumor activity, but in that case it would be expected that the toxicity of preparations would also be potentiated, although this has not been observed experimentally. The aim of the present investigation was to explain this contradiction, by studying, in experiments on cell cultures, on the one hand, the cytotoxic action of PABH and, on the other hand, its effect on nucleic acid biosynthesis. These effects were compared with the effects of a typical cytotoxic agent, namely iododeoxyuridine (IDU) and a known stimulator of cell division, methyluracil. Normal and tumor cells, and also normal cells stimulated by phytohemagglutinin (PHA), were used as cultures.

EXPERIMENTAL METHOD

Cell cultures were obtained from normal Papio hamadryas lymphocytes, monkey lymphoid culture 594-S/F9, and human lymphocyte cultures stimulated by PHA. PABH and IDU were used in concentrations corresponding to the lower limit of cytotoxicity, established beforehand on freshly obtained cultures of mouse embryonic fibroblasts, by the method in [1]. Methyluracil acted as the second control in experiments with normal human lymphocytes. The action on nuclei and biosynthesis was judged from the level of incorporation of the labeled precursors ^{3}H-thyimidine and ^{3}H-uridine; thymidine incorporation characterizes DNA, uridine incorporation – RNA synthesis. The determination was carried out 24 and 48 h after the beginning of contact between PABH, IDU, and methyluracil with the test cultures. The ^{3}H-thymidine or ^{3}H-uridine was added to the medium 2 h before the end of incubation, up to a concentration of 0.2 MBq/ml. Rate of nuclei acid synthesis was estimated from the level of incorporation of the labeled precursors, which was determined after destruction of the cells and subsequent fixation on nitrocellulose filters. The nitrocellulose filters were then transferred into flasks containing scintillation fluid, and radioactivity was measured on a Mark II counter [2]. Radioactivity was expressed in cpm/10^3 living cells.

EXPERIMENTAL RESULTS

All the results are given in Tables 1 and 2.

A comparative study of the aftereffects of exposure to PABH and IDU on nucleic acid biosynthesis in normal monkey cells showed that both preparations stimulated incorporation of ^{3}H-thyimidine significantly into DNA in the first 24 h; however, 48 h after the beginning of exposure the level of incorporation of the labeled precursor was the same as in the control. Neither preparation had any effect on RNA biosynthesis (Table 1). The effect of contact between the preparations and 594-S/F9 monkey lymphoid cells were different (Table 1).

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 0007-4888/89/0002-0236$12.50 © 1989 Plenum Publishing Corporation

TABLE 1. Toxicity and Effect of PABH and IDU on Nucleic Acid Biosynthesis in Normal P. hamadryas Lymphocytes and Culture of Monkey Lymphoid Cells

Cells	Preparation	Concentration, mg/ml	Incubation for 24 h			Incubation for 48 h		
			number of dead cells, %	^{3}H-thymidine	^{3}H-uridine	number of dead cells, %	^{3}H-thymidine	^{3}H-uridine
Healthy Rapio hamadryas lymphocytes 594-S/F9	Control		0,27±0,12	120±11	162±18	7,1±0,9	102±13	106±14
	IDU	0,05	0,3±0,1	230±16,2**	132±18**	12,4±1,3*	110±4	155±33
	PABH	1,0	0,3±1,2	152±4,9*	131±22*	7,7±1,2	83±9	86±22
	Control		32,7±1,2	1369±228	135±25	30,3±0,9	580±59	182±12
	PABH	1,0	52,7±1,8***	195±15,5	204±46*	60,7±1,5***	165±19*	175±19
	IDU	0,05	43,3±2,4*	113,2±21***	99±10	45±1,2*	102±10*	152±10

Legend. *p < 0.05, **p < 0.005, ***p < 0.002.

TABLE 2. Effect of p-Aminobenzhydrazide, Iododeoxyuridine, and Methyluracil on Proliferative Activity and Nucleic Acid Synthesis in Normal Human Lymphocytes, Stimulated by PHA

Preparation	Concentration of preparation, mg/ml	Number of transformed cells, %	Incubation for 24 h	
			^{3}H-thymidine	^{3}H-uridine
Control		23±1,03	1585±19	3221±320
PABH	1,0	26±1,7	862±132**	502±17**
IDU	0,05	41,2±1,7**	647±115**	268±52**
Methyluracil	1,0	23,7±1,17	3741±549*	3952±908

Legend. *p < 0.02, **p < 0.001.

For instance, throughout the experiment (i.e., for 48 h) PABH and IDU significantly inhibited DNA biosynthesis. Under these circumstances inhibition of DNA synthesis by IDU exceeded 90% compared with the control in the first 24 h, and amounted to up to 80% in the subsequent period, whereas under the influence of PABH the figures were 85 and 70%, respectively. Characteristically under the influence of PABH stimulation of RNA biosynthesis was observed 24 h after the beginning of exposure. Throughout the experiment IDU had no appreciable effect on RNA synthesis.

Investigation of the action of the preparations on lymphocytes from human blood donors, stimulated by PHA and in the S-phase of the cell cycle, showed that PABH and IDU inhibited incorporation of the precursors into DNA and RNA significantly. IDU considerably increased the percentage of transformed cells, but this was not observed in the case of PABH. Under these conditions methyluracil stimulated DNA synthesis (incorporation of ^{3}H-thymidine was over twice that observed in the control).

The results are evidence that PABH and IDU have a similar type of action on nucleic acid synthesis in all types of cell cultures: in normal baboon lymphocytes they stimulate DNA synthesis briefly, whereas in lymphoid cells they effectively inhibit this synthesis, and in PHA-stimulated human lymphocytes they inhibit both DNA and RNA synthesis. The only difference consists of brief stimulation of RNA synthesis in the lymphoid culture under the influence of PABH, but under these circumstances IDU does not affect RNA.

The situation is different with cytotoxic action. PABH is nontoxic for normal baboon lymphocytes and does not induce transformation of PHA-stimulated cells. Under these same conditions IDU exhibits marked cytotoxicity. The results of tests on a culture of tumor cells show that both substances are toxic, but PABH is significantly more toxic than IDU.

It follows from the results that PABH is highly toxic for tumor cells but completely nontoxic for normal cells, a feature which distinguishes it from the typical cytotoxic agent. Toxicity or its absence, to judge by our own observations, cannot be explained by any special action of PABH on DNA and RNA biosynthesis. Meanwhile, the special toxicity of PABH for tumor cells can easily be associated with the fact that this compound potentiates the antitumor

activity of preparations and with the absence of such potentiation of toxic side effect. This may be the explanation of this same action of other derivatives of hydrazine, notably hydrazine sulfate.

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SPONTANEOUS AND INDUCED PRODUCTION OF TUMOR-NECROTIZING FACTOR BY NEONATAL BLOOD CELLS

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UDC 616-006-002.4-018.1-053.
31-092:612.112.94.017.4

KEY WORDS: neonates; tumor-necrotizing factor; blood cells.

Tumor-necrotizing factor (α-TNF), a pleiotropic mediator with a broad spectrum of biological effects, not only possesses direct antitumor cytotoxicity, but it is also involved in the inflammatory and immune reactions of the body, interacting with other soluble factors and, in particular, with γ-interferon and interleukin 1 [5]. Participation of TNF both in protective reactions agains tumors or infections, and in reactions harmful to the body (hyperstimulation of macrophages under infectious or tumor conditions), is due mainly to its ability to increase expression of surface antigens during interaction with the specific receptor for TNF on a wide variety of cells [1, 3, 9]. An increase in the expression of HLA-antigens of the I and II classes may stimulate induction of a specific antitumor response as a result of increased ability to present an Ia-protein–antigen complex [3, 9]. Increased expression of the surface antigens on endothelial cells modulates their hemostatic properties, promoting adhesion of polymorphonuclear cells to endothelial cells, stimulating secretion of procoagulant factor, and reducing expression of thrombomodulin on endothelial cells [1].

The principal producers of TNF are activated macrophages [6, 8]. Increased sensitivity of newborn infants to infection have been linked with a defect of certain functions of the macrophages (ability to present antigens and to respond to lymphokines) [2, 7]. Accordingly the study of the ability of cells of newborn infants to produce TNF is of definite interest.

The aim of the work was to assess the level of TNF activity in the serum and to determine spontaneous and induced production of this factor by neonatal human blood cells.

EXPERIMENTAL METHOD

The test material consisted of mononuclear cells and blood serum from the umbilical cord of newborn infants. In some cases venous blood from infants aged 5-6 days also was investigated. The serum was frozen and kept at −20°C until required for testing. Mononuclear cells were isolated from heparinized blood with the aid of differential centrifugation on a Ficoll–Hypaque density gradient (α = 1.077) as described by Boyum [4]. Into four wells of a 24-well panel $1 \cdot 10^6$ mononuclear cells were introduced in 1 ml of medium RPMI-1640 with 10% calf embryonic serum, 1% glutamine, and antibiotics. After incubation for 1 h, cells not adherent to plastic were removed from two wells and the adherent cells were washed 3 times and treated

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(Presented by Academician of the Academy of Medical Sciences of the USSR D. S. Sarkisov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 107, No. 2, 2, pp. 222-223, February, 1989. Original article submitted July 13, 1988.

0007-4888/89/0002-0238$12.50

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