ACTION OF PHYTOHEMAGGLUTININ ON TRANSPLANTABLE HUMAN CELLS

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Agglutinating and growth-stimulating properties of phytohemagglutinin (PHA) preparations were studied in experiments on transplantable human cells (clonal line HeLa k-41 and Cave). PHA and its γ-globulin fraction were found to possess weak hemagglutinating properties for human erythrocytes of groups A, B, and O, but strong cytoagglutinating properties with respect to HeLa k-41 and Cave cells. With large doses of PHA (100 and 500 μg/ml) proliferation and the mitotic index of the cells of the cultures were lower but the percentage of dead cells and the agglutinin titer in the preparations were higher (1:256). With smaller doses of PHA (5 and 25 μg/ml) growth was much more intensive and the percentage of dead cells was smaller. The agglutinin titer in the preparation fell to 1:16-1:32. The γ-globulin fraction of PHA had the strongest growth-stimulating action and gave the smallest number of dead cells. However, the agglutinin titer in the preparations was high (1:128). It is concluded that the inhibitory and growth-stimulating action of PHA preparations on transplantable human cells is directly linked with the agglutinin content in the preparations, for the γ-globulin fraction of PHA had the strongest cytoagglutinating and growth-stimulating action.

KEY WORDS: *Phytohemagglutinin; human cell cultures.*

Phytohemagglutinin (PHA) is known to agglutinate human erythrocytes and to stimulate mitotic division of lymphocytes in vitro [1, 3, 4, 11-13]. Investigations [5, 7, 10] have shown that the mitogenetic action of PHA and its hemagglutinating properties can be manifested independently of each other. One of us (M.T.Ts.) [7] previously found that the cytoagglutinating (for cell cultures) and mitogenetic (for lymphocytes) properties of PHA could coincide. Meanwhile there is a report in the literature [14] that PHA has a mitogenetic action on transplantable cells in culture. However, no parallel investigations of the mitogenetic and agglutinating action of PHA on transplantable cells has hitherto been carried out. Nevertheless this problem is of great theoretical and practical importance in connection with the study of the regulation of growth and cell division under normal and pathological conditions (including malignancy).

The object of this investigation was to study the agglutinating and growth-stimulating properties of PHA preparations (PHA and its γ-globulin fraction) on transplantable human cells in relation to the dose of agglutinins in the preparations.

EXPERIMENTAL METHOD

PHA Preparations were obtained from seeds of the bean *Phaseolus vulgaris* by the method described previously [8]. The cytoagglutinating and growth-stimulating properties of the PHA preparations were studied on cultures of cells of the clonal line HeLa k-41 [9] and of the Cave strain [2], obtained from tissues of carcinomas of the human uterus and stomach respectively. The method of agglutination of cells in culture was used for this purpose [6]. The hemagglutinating properties were tested on human group A, B, and O erythrocytes; the reaction was carried out in tubes by the classical method with a 3% suspension of human erythrocytes and with the PHA preparations in dilutions of between 1:2 and 1:1024.

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TABLE 1. Effect of PHA and its γ-Globulin Fraction on Growth and Viability of Cells of Clonal Line HeLa k-41 sal)

Dose of PHA preparation tested, μg/ml	CP	P	MI	P	Percentage of dead cells	P
500*	1,2±0,1	<0,01	19,8±0,8	<0,001	33,5±4,0	<0,001
100*	1,4±0,1	<0,05	23,7±0,3	<0,001	19,9±5,7	<0,02
25*	3,3±0,1	<0,001	38,9±2,5	<0,01	7,0±1,3	<0,5
5*	3,3±0,4	<0,01	41,3±1,5	<0,001	5,3±0,7	<0,05
4*	5,9±0,1	<0,02	59,8±3,1	<0,001	3,4±0,6	<0,05
20**	16,4±0,1	<0,001	81,7±1,4	<0,001	2,5±0,1	0,01
Control (without PHA)	1,9±0,01		27,5±0,7		6,1±1,0	

Legend. Here and in Table 2 one asterisk signifies the native preparation of PHA, two asterisks the γ-globulin fraction of PHA

TABLE 2. Results of Agglutination Reaction between Human Erythrocytes and Human Transplantable Cells under the Influence of PHA Preparations (agglutinin titers)

Dose of PHA preparation tested, μg/ml	Culture of human cells		Human erythrocytes		
	HeLa K-41	Cave	A	B	0
5*	1:16	1:16	N	N	N
25*	1:32	1:32	N	N	N
100*	1:256	1:256	1:32	1:16	1:16
500*	1:256	1:256	1:32	1:16	1:16
4**	1:128	1:128	1:4	1:4	1:8
20**	1:128	1:128	1:4	1:4	1:4

Legend. N) Negative result

The effect of the PHA preparations on growth of the HeLa k-41 cells was studied in vitro and the culture was seeded in a dose of 30,000 cells to each Wassermann tube. Three or four tubes were used in the experimental and control series. PHA was added in doses of 5, 25, 100, and 500 μg, and the γ-globulin fraction in doses of 4 and 20 μg/ml medium. The nutrient medium, added in a dose of 2 ml to each tube, consisted of 80% medium No. 199 and 20% bovine serum. The results of the tests were read on the 3rd day by quantitative study of growth of the cultures placed on the coefficients of proliferation (CP, the ratio between the number of cells taken and the number originally seeded) and the percentage of dead cells stained with 1% trypan blue solution, and also from the mitotic index (MI) in films fixed by Bouin's method and stained with Mayer's hematoxylin.

EXPERIMENTAL RESULTS

As the intravital observations showed, HeLa k-41 cells treated with the PHA preparations settled quickly to the bottom as conglomerates of different sizes, but later, until the 3rd day, they grew as medium-sized and large colonies, sometimes with the formation of a continuous cell monolayer. As Table 1 shows, growth of the cells was weaker with PHA in doses of 100 and 500 μg/ml (CP from 1.2 to 1.4) than in the control tubes (CP 1.9), but with doses of 5 and 25 μg/ml CP rose to 3.3. With doses of 100 and 500 μg/ml respectively the percentage of dead cells reached 19.9-33.5, whereas with doses of 5 and 25 μg/ml the percentage was 5.3-7. The percentage of dead cells in the control was 6.1. It is interesting to note that the γ-globulin fraction of PHA in doses of 4 and 20 μg/ml had the strongest growth-stimulating action (CP from 5.9 to 16.4) with a lower percentage of dead cells (3.4-2.5). Counting MI in 3000 cells showed that PHA, in doses of 5 and 25 μg/ml, stimulated cell division (MI from 38.9 to 41.3‰), but in doses of 100 and 500 μg/ml it inhibited cell division (MI from 19.8 to 23.7‰). In the control MI was 27.5‰. MI increased particularly (59.8-81.7‰) in experiments in which the γ-globulin fraction of PHA was added in doses of 4 and 20 μg/ml.

A study of the agglutinating properties of the PHA preparations showed that they had weak hemagglutinating properties for human group A, B, and O erythrocytes (the titers varied from 1:4 to 1:8), but they exhibited a stronger cytoagglutinating action against transplant-

able human cells (HeLa k-41 and Cave). In doses of 100 and 500 μg/ml, PHA caused agglutination of the cells in titers up to 1:256. The agglutinating titer of PHA was somewhat lower in doses of 5 and 25 μg/ml (1:32-1:64). However, the agglutinating titer of the γ-globulin fraction of PHA was high in doses of 4 and 20 μg/ml (1:128; Table 2).

The growth-stimulating action of PHA preparations on transplantable human cells was thus found to be directly linked with the agglutinin content in the preparations, for the γ-globulin fraction exhibited the strongest cytoagglutinating and growth-stimulating action. The titers of agglutinins in PHA preparations in doses of 100 and 500 μg/ml, incidentally, were sufficiently high, but they had no stimulating effect on the cells. This contradiction can evidently be attributable either to the dose of agglutinins or to impurities contained in the unpurified PHA preparations which had a toxic action on the cells in culture.

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