SOME ASPECTS OF THE EFFECT EXERTED BY MINIMAL PHYTOHEMAGGLUTININ DOSES OF PHASEOLUS VULGARIS, IN VIVO

M. Tzoneva-Maneva, Y. Vasilev, K. Perfanov

In the past several years, the interest in phytohemagglutinins (PHA) has greatly augmented (2, 3, 4, 10, 12, 13, 14, 15, 16) and more particularly, in PHA obtained from the seeds of Phaseolus vulgaris. This is due to the discovery that, in addition to hemagglutination properties, they are capable of stimulating the division of lymphocytes in tissue cultures of peripheral human blood.

Mac Kinney A. H. and assoc. (11) report that apart from the division under the action of PHA of Phaseolus vulgaris, transformation is likewise observed of the minute peripheral blood lymphocytes into large basophilic cells, the so-called „PHA cells" or „blast cells". These cells exhibit a homogeneous, round nucleus, with fine chromatin, one or more nucleoli, often with a pallid zone surrounding the nucleus and occasionally cytoplasmic vacuolation.

Some authors (5, 14) observed the occurrence of similar cells under the effect of other antigens (tuberculin etc.), in vitro as well as in certain morbid conditions in man, in vivo (10). Hence, the data reported render the denomination „PHA cells" or „blast cells" as conditionally accepted.

The transformed or so called „blast cells", according to investigations made by Elves M. W. and assoc. (5), represent a stage of the conversion of lymphocytes into plasmatic cells. The electron microscopic investigations show that occasionally, in the preparations of blood tissue cultures, the transformed cells bear the signs of primitive blast cells, which, in the course of ordinary microscopic study, might be easily identified as myoblasts, erythroblasts, lymphoblasts or reticular cells.

Lymphocytic transformation has been likewise confirmed by autoradiographic investigations (16).

These observations focused the attention of a great number of authors towards further studies on lymphocytic transformation. According to Joffrey, I. (10), the tiny lymphocytes normally participate in the bone-marrow and are being converted accordingly into young cells of the erythrocyte, granulocyte and thrombocyte order.

The evidence available about the stimulating effect exerted upon lymphocytes in vitro, provide sufficient ground for embarking on PHA application in the therapy of aplastic anemias (3,6) and as chemoprotector as well during massive cytostatic therapy (8, 9) of inoperable malignant neoplasms.

In some patients with aplastic anemia, subjected to treatment with PHA, the presence is noted of cells with a lymphocytic nucleus and granulated

cytoplasm in the peripheral blood as well as of neutrophil with bilobular nucleus. In other patients hyperplasia of the normal bone marrow elements is established by the same author. In addition, he describes mitotic figures in the peripheral blood and occurrence of lymph-like cells with nucleoli and finely granulated cytoplasm, with a positive peroxidase reaction, characteristic for neutrophil granulocytes. It is assumed that the application of PHA exerts a favourable effect in symptomatic arganulocytosis, but not in the idiopathic form of the same condition. The doses used by this author in clinical cases are 50 mg daily, form — M, applied intravenously for one week. Reticulocytosis (3) in patients is also observed, without changes in leukocytes and thrombocytes.

The results of the therapeutic application of PHA are as yet contrasting and insufficiently studied. This implies facing the problem of further investigation their effect in vivo. Therefore, we set ourselves the task to study the effect of small PHA doses from Phaseolus vulgaris in vivo.

Method

The experiment is carried out on 10 rabbits weighing from 1—3 kg. Before the treatment of experimental animals with PHA, the following examinations were made: Hb, erythrocyte, leukocytes, thrombocytes, differential blood picture. We used PHA, form — P, obtained from Phaseolus vulgaris. Doses were used, ranging from 20 to 100 micrograms per kg body weight i. v., daily for 10 consecutive days and thereafter, a single application every 10-day interval. Twenty four hours after every single injection, the listed above blood indices were verified. In addition, the myelogram of 5 rabbits (№ № 2, 3, 4, 7, 8) was traced up, performed on the 13th day after commencing the experiment. Bone-marrow material was obtained by means of perforation of the greater trochanter of the femur with a pneumothorax needle. The test animals № № 1—5 were followed up for 80 days, and №№ 6—10 — for 30 days. The myelograms of the middle third of the femur in all test animals were investigated after completion of the experiment on material obtained from two different points. The experimental background is schematically illustrated in table 1.

Results and discussion

The results of the experiments reveal no changes in erythrocytes and Hb of the peripheral blood of PHA — treated animals.

Alterations are established in the white blood picture and thrombocytes. Segmento-nucleated cells are concerned which are decreased as early as the first week of experimentation without change in the absolute number of lymphocytes. Relative lymphocytosis occurs (see diagram 1) during the period of maximum granulocytopenia, reaching 98 per cent in rabbits № № 8 and 9. The characteristic features of these changes are illustrated in diagram 1, displaying the changes in the absolute values of segmento-nucleated cells and lymphocytes in rabbit № 2.

Table 1

Table № 1. Illustrates schematikcally the experimental setting for investigation low PHA doses in rabbits, in vivo

Rabbit №	Dose kg/w gamma	Desensibilization	Duration of the experiment and investigations carried out on peripheral blood (PB) + bone marrow (BM)																			
			day investig.	1	2	3	4	5	6	7	8	9	10	13	20	30	40	50	60	70	80	
1	20	yes	PB	+	+	+	+	+	+	+	+	+	+	+	+×	—	—	—	—	—	—	autopsy
			BM	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	„
2	20	yes	PB	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	„
			BM	—	—	—	—	—	—	—	—	—	—	+	—	—	—	—	—	—	+	
3	20	yes	PB	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	„
			BM	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	
4	20	yes	PB	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	„
			BM	—	—	—	—	—	—	—	—	—	+	—	—	—	—	—	—	—	+	
5	20	yes	PB	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	„
			BM	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	
6	20	no	PB	+	+	+	+	+	+	+	+	+	+	+	+	+	autopsy					„
			BM	—	—	—	—	—	—	—	—	—	—	—	—	+						
7	20	no	PB	+	+	+	+	+	+	+	+	+	+	+	+	+×	„					„
			BM	—	—	—	—	—	—	—	—	—	—	—	—	—						
8	20	no	PB	+	+	+	+	+	+	+	+	+	+	+	+	+	„					„
			BM	—	—	—	—	—	—	—	—	—	—	—	—	—						
9	80	no	PB	+	+	+	+	+	+	+	+	+	+	+	+×	—	„					„
			BM	—	—	—	—	—	—	—	—	—	—	—	—	—						
10	100	no	PB	+	+	+	+	+	+	+	+	×—	—	—	—	—	„					„
			BM	—	—	—	—	—	—	—	+	—	—	—	—	—						

Note: The sign „x" indicates expulsion of the rabbit from the experiment on account of concomitant disease (rabbits №№ 1 and 9 — pleural empyema, № 7 — gluteal abscess and № 10 — shock with exitus lethalis)

Similar values are also osberved in the remaining rabbits irrespective of doses applied. On the 2nd day after PHA injection, granulocytopenia occurs in one third of the rabbits and on the 3-4th day — in two thirds. The maximum fall is marked between the 2nd and 7th day, most frequently on the 4th day, the absolute values being usually 2—5-fold lower than the initial.

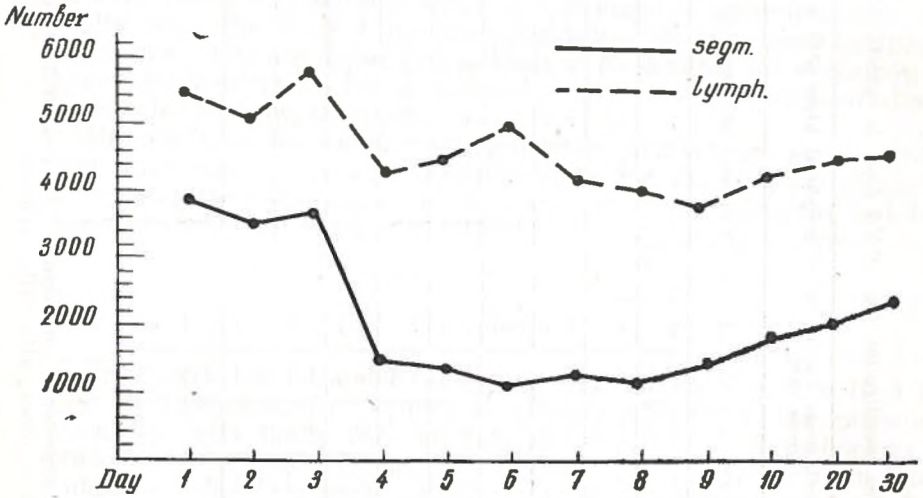

Fig. 1. The diagram illustrates the results of the changes in the number of segmented nucleated cells and lymphocytes under the effect of low pha doses

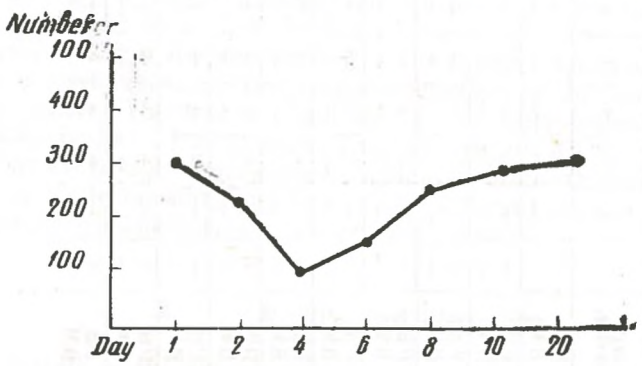

Fig. 2. The diagram shows the results brought about by changes in the number of thrombocytes under the effect of low pha doses

An increase in number of segmento-nucleated cells follows the suspension of daily injections on the 10th day, but nevertheless, it remains within limits, 2—3 times lower than the initial values. As illustrated in the diagram, the absolute number of lymphocytes is unchanged, all fluctuations being within the physiological limits for the rabbit.

Changes in the morphology of segmento-nucleated cells and lymphocytes in the peripheral blood are not found. Gumprecht's shadows are seldom

come across. „Blast cells", mitoses and basophilia are not met with in the peripheral blood.

The results of investigating the thrombocytes in the peripheral blood show a reduction in number about the fourth day after undertaking the experiment, coinciding in time with the granulocytopenia (see diagram 2). No megakaryocytes are discovered in the myelograms performed on the 13th day of experimentation whilst, on the 20th day, the megakaryocytes and thrombocytes are within normal limits both morphologically and quantitatively.

Further investigations of the same myelograms do not reveal changes in the white and red blood cells, nor statistically reliable hyperplasia of the reticular elements of the bone-marrow or changes in the morphology and maturation of cells.

No differences are detected in the myelograms of the rabbits, killed on the 30th and 80th day, each one previously treated with 20 micrograms per kg weight PHA (see table 2). Therefore, their cumulative myelogram is presented, duely elaborated statistically. It does not differ from the myelograms of the healthy rabbits, not treated with PHA. The latter evidence is in accordance with that displayed by the myelograms of rabbits №№ 8 and 10, treated with higher doses.

Table 2

Denomination	M	±	m	%	
Myeloblasts	0,70	0,52	0,20		
Promyelocytes	2,00	2,44	0,92		
Myelocytes	5,70	2,73	1,07		White cells : Red cells = 1,8 : 1
Metamyelocytes	3,50	1,73	0,65		
Nucleospindle	4,00	2,00	0,71		
Segmentonucleated	19,30	6,93	2,61	35,20	Index of maturation- 0,51 (l)
Proerythroblasts	1,85	2,00	1,37		
Macroblasts	2,20	1,41	0,92		
Normoblasts					
basophilic	3,40	2,61	1,36		
polychromic	11,90	4,00	1,50		
orthochromic	10,00	7,75	2,92	29,35	
Lymphocytes	3,30	2,54	0,96		
Monocytes	0,50	—	—	3,80	
Small reticulum cells	14,90	2,24	0,75		
Big reticulum cells	14,30	7,10	2,68		
Ferrata's cells	0,85	1,57	0,60		
Plasmocytes	1,43	0,70	0,26	31,48	
Megakaryocytes	0,30	—	—		

Cumulative myelogram of rabbits after injection of PHA — 20 gamma per kg/weight to each animal.

The experimental animals do not show specific changes in their behaviour during the injection and thereafter, except for rabbit 10, to which PHA 100 gamma/kg weight was applied without desensibilization. On the 8th day of the experiment it sustained a shock, immediately following the injection, with fatal outcome.

The results of the experiments performed on rabbits with very slight PHA doses, form P, show a development of granulocytopenia in the peripheral blood and initial thrombocytopenia of short duration. The absence of qualitative and quantitative changes in the myelogram is an indication that PHA in this instance does not account for the inhibition of granular cell formation, whilst the alterations observed appear to be of a different nature. No manifested stimulating effect of PHA upon the lymphocytes is established with the doses and method of application employed. In this connection, emphasis should be laid on the different results obtained with the application of massive doses (10—30 mg/kg weight) of the same preparation on rabbits, regardless of the presence of permanent neutropenia and lymphocytosis and „blast cells" occurrence (1). The analogy in the results of the two parallel experiments (conditioned by the action of minimal and maximal PHA doses, in vivo) is accounted for by the influence exerted on the segmento-nucleated cells in the sense of developing granulocytopenia.

The minimal doses applied in the experiment described do not display a stimulating effect on the lymphocytes and their „blast" transformation. It is assumed that the stated differences might be conditioned apart from the dosage, also from the technique of PHA application (in smaller doses daily, in the beginning and higher doses — weekly).

Another difference in the results of the experimental settings is like-wise the occurrence of anisocytosis and megalocytosis in higher doses, considered as indirect index for the affection of bone-marrow function; similar changes are lacking when minimal doses are applied.

In the experimental setting described, the desensitization obviates the anaphylactic shock during secondary PHA applications.

The results of the work presented prove the necessity of investigating the effect of average PHA doses; in the latter case stimulation of lymphocytes is very likely to be achived. The results claimed by Humble I. G. (6, 7), obtained after PHA application in man, are encouraging in this respect and should be experimentally studied in detail. Further elucidation of the problem will be the scope of future investigations.

REFERENCES

1. Цонева-Манева, М., К. Бошнакова. — *Трудове на ВМИ—Варна*, т. IV, св. I, 1965, 43—49.
2. Astaloi, G., L. Ratto, K. Airo. — *Schw. Haematol.*, Geselschaft, 1964, 94, 1345.
3. Baker, G. P., R. I. M. Oliver. — *Lancet*, 1965, II, 438.
4. Dujarrie de la Riviere, R., M. Saint. — Paull. — *C. R. Akad.* Sci., 1953, 337, 211.
5. Elves, M. W., J. Gougll, J. A. Chapman et al. — *Lancet*, vol. I, 1964, 7328, 306—308.
6. Humble, J. G. — *Nature*, 1963, vol. 198, 4883, 1313—1314.
7. Humble, J. G. — *Lancet*, 1964, vol. I, 7341, 1945.

8. Israel, L., J. Delobel., G. Bernard. — *Pathologie-Biologie*, 1965, vol. 13, 19—20, 887—890.
9. Israel, L., I. Delobel, D. Lambard. — *Soc. Méd. des Hôpitaux de Paris*, 116, 1965, 15, 1531 — 1534.
10. Joffrey, J. Quantitative cellular Haematology, III, 1960, Speing feld.
11. Mac Kinney, A. H. et ab. (цит. по M. W. Elves et al). — *Lancet*, 1964, vol. I, 7328, 306—308.
12. Marschall, Roberts. — *Quart. J. Exp. Phisiolog.* 1963, 48, 146.
13. Marschall, W. H., K. B. Roberts. — *Lancet*, 1963, vol. 1, 773—728.
14. Permain, G., R. R. Lycette, P. H. Fitzgerald. — *Lancet*, 1963, vol. I, 7283, 637—638.
15. Permain, G. E., R. R. Lycette. — *Lancet*, 1963, II, 1072.
16. Porter, K. A., E. H. Cooper. — *Lancet*, 1962, 6, vol. II, 7251, 317.

О ДЕЙСТВИИ МИНИМАЛЬНЫХ ДОЗ ФИТОГЕМОАГГЛЮТИНИНОВ ИЗ PHASEOLUS VULGARIS IN VIVO

М. Т. Цонева-Манева, Й. Василев, К. Перфанов

РЕЗЮМЕ

Сообщаются результаты многократного внутривенного введения фитогемоагглютининов (ФХА) из Phaseolus vulgaris кроликам. Использованы были небольшие дозы (20—100 гамма/кг веса) формы РФХА. Наблюдалась стойкая гранулоцитопения, релятивный лимфоцитоз и кратковременная тромбоцитопения у подопытных животных. При используемых дозах и способе применения, не установлено наличия стимулирующего действия ФХА на деление и бластную трансформацию лимфоцитов в периферической крови. Миелограмма была без изменений.