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EFFECT OF LEVOFALAN ON THE SPLENIC SYNTHESIS OF HAEMOLYSINS

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In addition to antitumoural antibiotics and other agents such as hexamethylmelamine and procarbazine, the alkylating agents represent a group of anticancerous drugs, of which the best known are: Busulphan, Chlorambucyl, Cis-Platin, Cyclophosphamide, Mechlorethamine, Melfalan (Levofalan), Nitrosoureas and Tio-Tepa (*Chiricuță*, 1983; *Bosanquet*, 1985).

In 1979 *Singer* pointed out that treating the cells with alkylating agents determined the formation of various adducts on the DNA molecule, of which O^6-methylguanine had an important biological role.

Since alkylation has definite cytotoxic effects, as demonstrated among others by *Boiteux* and *Lavel* (1985), who described the inhibitory effect of 7-methylguanine with split imidasole nucleus on DNA synthesis, we have asked the question how anticancerous therapy with alkylating substances affects the capacity of reaction of the immune system when stimulated with antigens. As a first step, we have started investigating the effect of Levofalan (Institute of Oncology, Bucharest) on the splenic synthesis of haemolysins with a view to clear up these interrelationships.

Material and Method

The researches were carried out on 50 CBA mice immunized with sheep erythrocytes harvested on Alsever medium in sterile conditions.

The animals were divided in 4 groups. Group I (15 animals) and Group II (10 animals) were treated with Levofalan in total dose of 0,4 mg/100 g. fractioned in usual doses of 0.1 mg 100 g given intraperitoneally at one week's intervals. Group I was stimulated antigenically by giving each animal 5.5×10^9 sheep erythrocytes 4 days before killing them. Group III (15 animals), untreated, was immunized under the same contitions as Group I, and Group IV (10 animals), untreated and non-immunized, also served as control group.

The erythrocyte count from the suspension in physiological saline solution suitable for immunization was checked spectrophotometrically by using a calibration curve constructed by means of series of erythrocyte dilutions, in which the erythrocyte count was determined haemocytometrically and the corresponding extinction spectrophotometrically in conditions suggested by *Levine*.

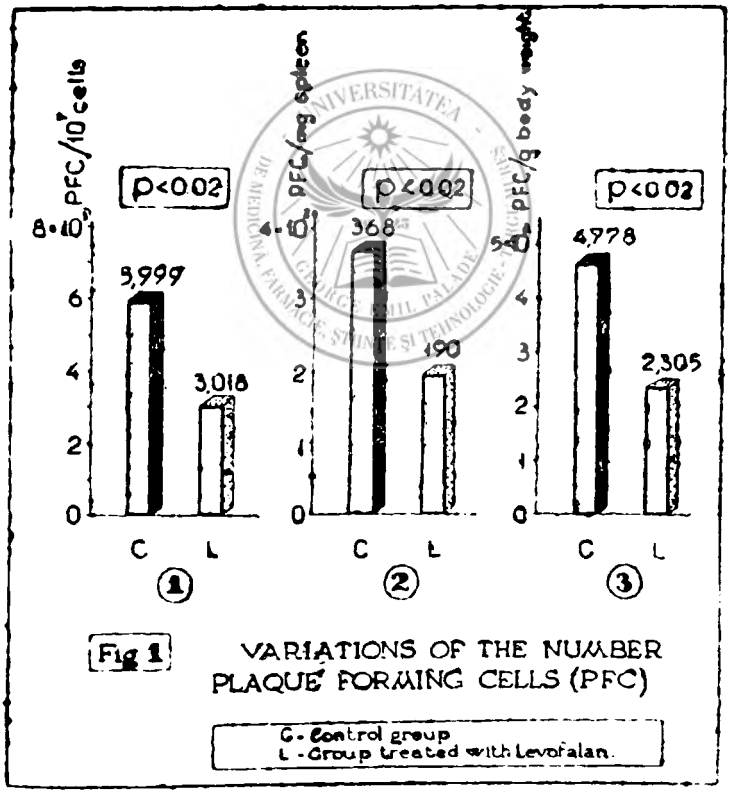
In the immunized groups, we determined the number of haemolysis plaques by Jerne's method, and the secretion of haemolysins was evaluated spectrophotometrically by a method described by van *Dijk-Bloksma*. We also determined, in all groups, weight curve, haemogram and mg spleen g body weight ratio.

According to the determinations we calculated: total number of LC/mg spleen and g body weight, number of plaques 10^7 cells per mg spleen and g body weight, LC50, in fine MHP/PFC per mg spleen and g body weight (expressed in HU50).

The data obtained were statistically processed by using Student's "t" test.

Results

Our investigations have shown that in the mice treated with Levofalan, both in those immunized and non-immunized, no significant weight losses were produced during the treatment as compared with the control



group. A good drug tolerance is also shown by the variations, within the physiological limits, of the figurative blood elements in the treated animals. After 4 weeks' tratament, the presence of neither anaemia nor thrombocytopenia was found, and the recorded leukopenia was discrete.

Levofalan treatment does not modify the total number of lymphoid cells in the immunized animals. However, if we investigate the variations of the number of lymphoid cells necessary to produce 50^{0}_{0} haemolysis, we find that LC50 (lytic concentration 50) increases from 6.88×10^{7} in the immunized and untreated animals to 13.99×10^{7} in the immunized animals treated with Levofalan ($p < 0.02$). Levofalan determines the significant fall ($p < 0.02$) of haemolytic plaque-forming cells (PFC) from 5,999 to 3,018: the degree of the significance of this fall is constantly maintained ($p < 0.02$) if it is related to mg spleen or g body weight (Fig. 1).

Correlating the data obtained by Jerne's method with the mean heamolysin production (MHP), we can state that in the Levofalan-treated animals the mean production of haemolysins per plaque-forming cell, expressed in HU50 (unit of 50^{0}_{0} haemolysin=amount of antibodies responsible for 50^{0}_{0} lysis in the test system with 5×10^{3} sheep erythrocytes)

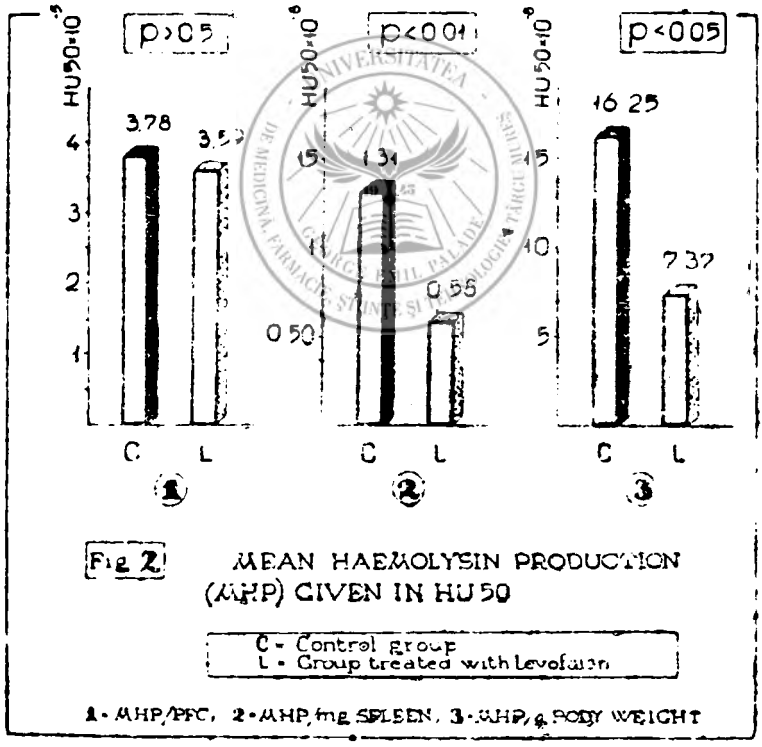


Fig 2 MEAN HAEMOLYSIN PRODUCTION (MHP) GIVEN IN HU 50

C - Control group
L - Group treated with Levofalan

1 - MHP/PFC, 2 - MHP/mg SPLEEN, 3 - MHP/g BODY WEIGHT

unsignificantly decreases from 3.78×10^{-5} to $3,59 \times 10^{-5}$ ($p > 0,50$). But related to mg spleen MHP significantly falls from 1.31×10^{-8} to 0.58×10^{-8} ($p < 0.01$), and from 16.25×10^{-8} to 7.37×10^{-8} ($p < 0.05$) if it is related to g body weight (Fig. 2).

Discussions

Our researches have revealed the inhibitory effect of alkylation mediated by Levofalan on the primary immune response. *Stezowski* et al. (1984) pointed out the stereochemical effects of alkylation by the study of crystalline structures of alkylated nucleosides, by making use of computerized molecular modelling. Thus, the structure of "compound 6" of the above authors — N^6 (12 methylbenzanthracenyl-7 methyl)2' — desoxyadenosine — shows a *syn* conformation for glycoside bond, in contrast with the *anti* conformation found in B-DNA.

The influence of alkylating agents including Levofalan on the hydrogen bonds in the formation of couples of complementary bases, as well as on the hydrophobic effect has an important role in establishing the duplex structure of DNA. Based on the investigations of *Harris* et al. (1983), *Shiloh and Becker* (1981) and on their own researches, *Karran* and *Williams* (1958) have concluded that O^6-methylguanine production due to the effect of alkylating agents determined human cell lesions that lead to the disorganization of infrastructures and to cellular death.

In concord with the data of the above mentioned authors, regarding the cytopathic effect of alkylating agents, our researches have revealed that the animals treated with Levofalan cannot synthesize a haemolysin amount equal to that recorded in the control group but by appealing to a double amount of lymphoid cells.

Levofalan administered for 4 weeks in a total dose of 0.4 mg 100 g reduces the capacity of efector cells to produce sheep anti-erythrocyte 19S antibodies, the degree of significance of this decrease becoming obvious if haemolysin production is related to g body weight or mg spleen ($p < 0.01$).

Conclusions

1. Levofalan therapy does not modify splenic lymphoid cell count implied in primary immune response in immunized animals.
2. Levofalan determines significant LC50 increase.
3. Levofalan therapy determines a significant decrease of the number of haemolysin plaques and reduces the mean haemolysin production.
4. Our results attest the inhibitory effect of Levofalan on the splenic synthesis of haemolysins.

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