

# SPLEEN STRUCTURAL DAMAGES AND TISSULAR BIOCHEMICAL PARAMETERS DISTURBANCES AT WISTAR RATS AFTER CHIMIO THERAPY ADMINISTRATION

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**ABSTRACT.** The Cyclophosphamide (CFA) is a frequently used citostatic drug in the anti-cancer chemotherapy. Unfortunately, the administration of the drug is accompanied by side effects which mainly affect the lymphoid organs. In this experiment, we have analyzed the Wistar rats spleen structural and ultrastructural changes for 24 hours after the CFA 3 consecutive administration and the ability of its morphology recovering after 10 days of finished treatment. The 24 hours spleen histopathological and electron microscopy examination after CFA last dose administration demonstrated the installations of some major structural changes especially at the white pulp level in a necrobiosis and apoptosis different lymphocytes stages, the loss of intercellular contacts. Spleen morphological alterations, although profound, are transitory. Since 10 days from the CFA therapy ending, the spleen starts to recover, a fact demonstrated mainly by the compensatory hematopoesis launching. In conclusion, the immune system normal morpho-functionality resume represents a drug therapy cancer reoccurring.

**Keywords:** chemotherapy, spleen morpho-functional damages, reversible side effects

## INTRODUCTION

The non-selectivity of the antitumor chemotherapy represents the major problem which constraints the use of these drugs, since they have effects both at the level of healthy and at the level of damaged cells. The harmful effects will interest especially the tissues whose cells divide fast and continuously, in specially in germinal and hematopietic tissues. (Morran P, 2000; Terezhalmly, 1996). The disturbances induced by the administration of the cancer drug are tightly connected to the setting of some serious structural and functional damages within central and peripheral lymphoid tissues. In the present paper, the investigations focused on the structural and ultrastructural alterations of the spleen.

## MATERIALS AND METHODS

### *Antitumoral drug*

Jenapharm, Ankerwerk-Germany  
Ciclofophosphamide (CFA), was before deluted in a 0,9% sterile physiologic Na Cl solution and intraperitoneal animals injected for 3 days.

### *Animals*

The experiments were made on white male Wistar rats, weighting 170-180g, kept under standard diet and conditions.

The following animal groups were used: control group (M); group treated with Cyclophosphamide (40 mg/kg body/day) administrated by intraperitoneal injections for 3 days;

The animals were sacrificed after finishing the treatment at 24 hours, respectively at 14 days Cyclophosphamide first dose given.

### *Histopathological analysis*

Spleen were collected into PBS and fixed overnight in 40 g/L paraformaldehyde in PBS at 4 °C. Serial 5- $\mu$ m sections of the organs were stained with hematoxylin and eosin (HE), and were examined histopathologically.

### *Biochemical investigation*

Alcaline phosphatase is determined from spleen tissular homogenate after Bergmayer' methods (1962) using pH=9 p-nitrophenil-phosphate and 37°C incubation.

Butyl cholinesterases from tissues are got after the Gorun method, using butirilcoline iodine as substrat. Cholinesterase makes hydrolysis butyl choline making up thiocoline and butyric acic. The resulted thiocoline reacts together with DTNB making up a yellow compound and the extinction is red at  $\lambda=405$  nm. The results are expressed in mU/g tissue or /mg protein or U.E./l ser.

## RESULTS

We can take notice that at CFA treated rats both alcaline phosphatase and BCE amount are a bit decreased in comparison with the control (table 1).

Table 1

EVOLUTION OF SOME BIOCHEMICAL PARAMETERS FROM SPLEEN OF CFA TREATED WISTAR RATS

Parameters	Control	CFA
Alcaline phosph. mU/g tissue	24,46±0,62 (7)	13,53±0,48 (7)
	-	-44,68
	-	p<0,001
BCE mU/g tissue	3618±95,47 (7)	865±116 (7)
	-	-48,45
	-	p<0,001

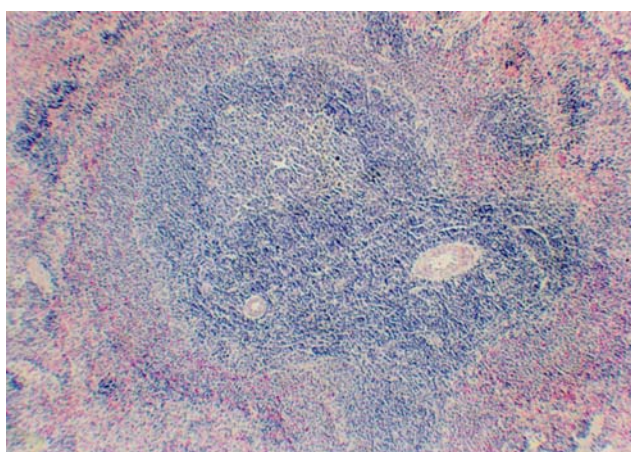
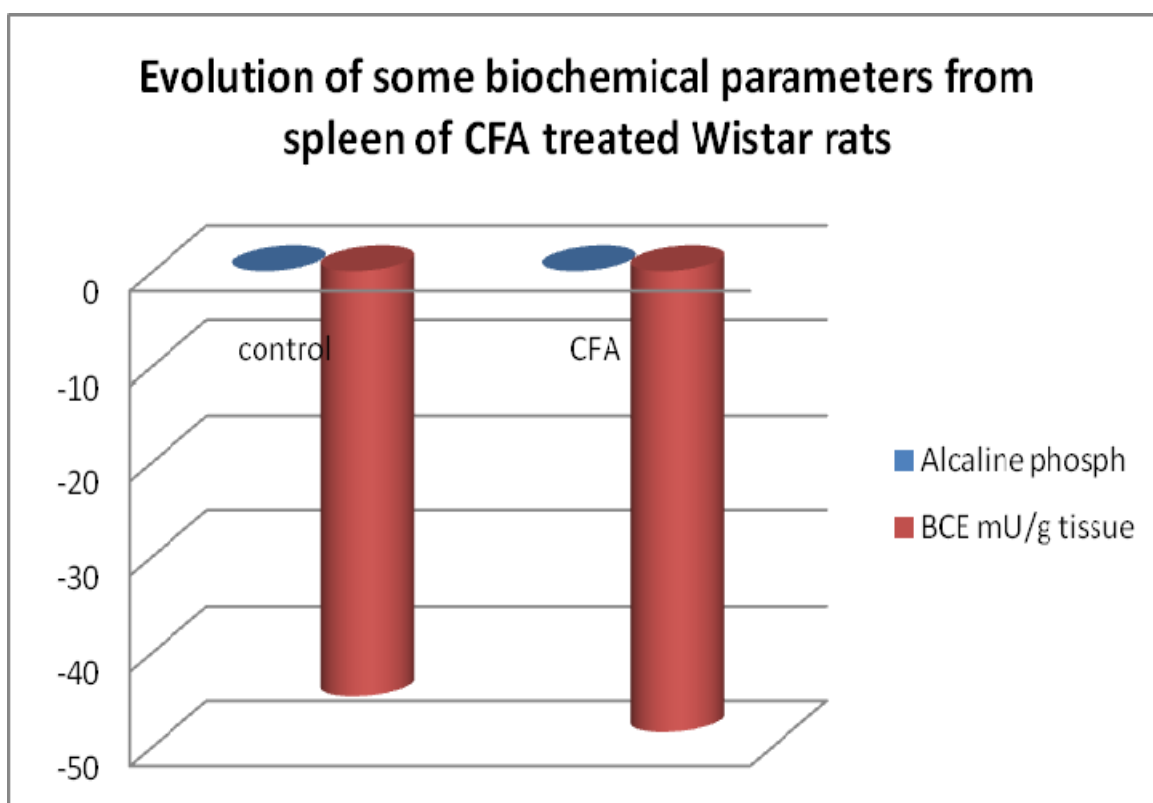


Fig. 1 The histological aspect of the spleen of the rats in the control group (col.H.E., 4x)

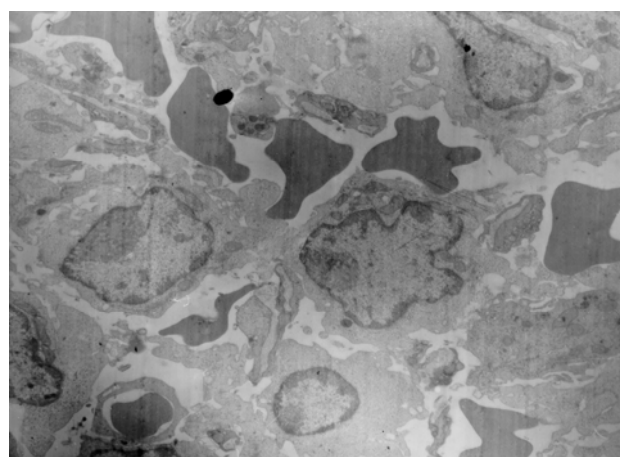


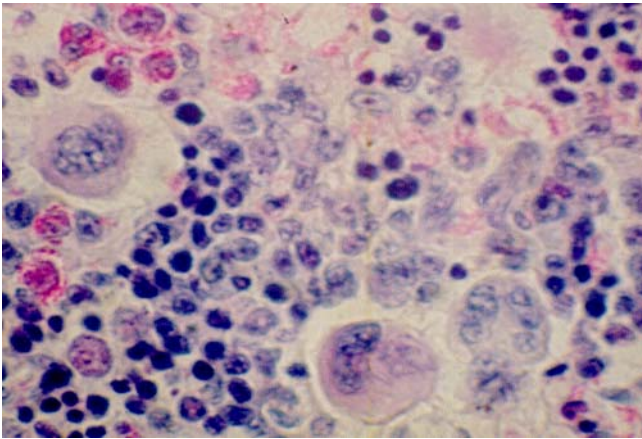
Fig. 2 The dilation of the intercellular spaces with a massive infiltration of red cells at the 24 hour CFA group (x7980)

The results of our study reveal a normal structure of white and red pulps within the spleen of rats belonging to the control group (Figure 1). The spleen follicles are big, centered on arterioles and their majority represent a well defined germinative center. The splenic sinuses are well loaded with red blood cells and lymphoid cells. The splenic belts (Billroth) are well evidenced and cells density that makes them up is found under normal limits. We find out very rare isolated megakaryocytes on the section surface.

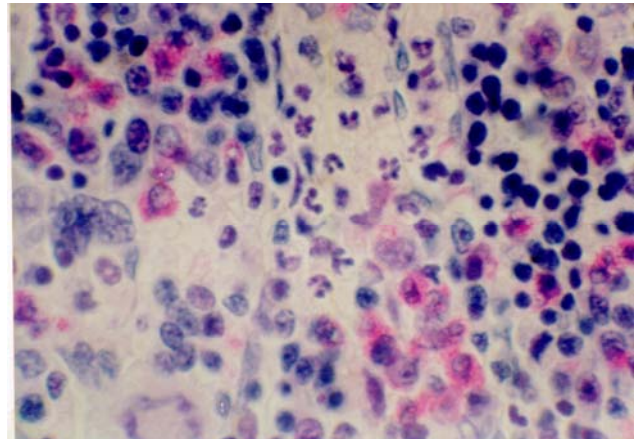
In the second test group we observed that the spleen structure is seriously changed. Comparing the second group with control we remark the following: most spleen follicles are almost equal in size with those of the control, even if they have a severe shifted structure; the density of the cells is much reduced in spleen follicles and none of these display a germinative center; a high number of lymphocytes have in their structure necrobiosis various stages; the loading of the sinuses with erythrocytes and lymphoid cells is very weak; the spleen belts are very thin and there are much less cells in their structure; at the electron microscopy

examination there is a severe cells number reduction in those rats spleen where Cyclophosphamide was applied. Also we can observe the wide intercellular occurrence (Figure 2).

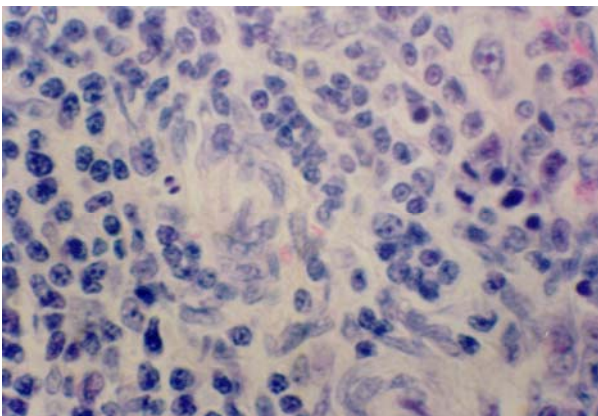
Concerning the third test group we have the following remarks: the action of the cancer drug seems to stop; the spleen is in a full process of structural recovery, at the level of both the red and the white pulp; at the level of the white pulp, the spleen follicles are still smaller; they have a relatively homogenous aspect, that is they are drawn on by entire young cells even if they do not display a germinative center; in the red pulp, the spleen sinuses are relatively well loaded with red blood cells and lymphoid cells; the spleen belts are well represented, they are thicker and contain a great deal of cells featured with emphasized polymorphism; there are numerous megakaryocytes all over the section surface (figure 3); many granulocytes, neutrophils and eosinophils have polymorphous structure, and they can be seen in layers (figure 4); numerous lymphoblasts in various stages of evolution (Figure 5).



**Fig. 3** The histopathological aspect of the spleen within 10 days after the end of CFA administration. (megakaryocytes with a polymorphous structure, nests of eosinophils, numerous lymphoblasts) (col. H.E., x73)



**Fig. 4** Detail aspect of the spleen within 10 days after the end of CFA administration, col. H.E., x63



**Fig. 5** Lymphocytes in various stages of evolution in spleen follicle at 10 days after the end of CFA administration, Col HE, x63

## DISCUSSIONS

After 24 hours since the last given dose, the histopathological examination of the spleen indicates that the cancer drug has a visible effect on the

lymphocyte populations, both to red and white pulp, and most of them were found out in different stages of apoptosis or necrobiosis. According to the previous studies, all cytostatic, including CFA, can block cell division that is grounded on tissue development and substitution, especially in germinal ones (Ardelean A et al, 2000). Similar results were obtained in vivo experiments when cyclophosphamide decreased almost all of peripheral blood cell counts and lymphocyte subset counts in the thymus and spleen at all ages (Blankenberg F et al, 2001; Michuyuki K, 2007). The electron microscopy micrographs confirmed findings obtained by means of optical microscopy. In addition to these, it also indicated serious changes of cells located within spleen at the reference moment. The severe cells number reduction can affect for a time the organ function.

During the 10 days period since the CFA last dose was given, spleen carries out a full recovery process. The great majority of the lymphocytes in the structure of the spleen follicles and the lymphoid

periarterial pods are young. That suggest that the lymphoid structures are in a continuous process of recovery. The presence of many dividing cells, at white and red pulps shows the same thing. The unusual polymorphism of the cells in red pup belts are more numerous as compared to the control. That suggests the body direct involvement to contribute to qualitative and quantitative recovery of populations treated with Cyclophosphamide . Many megakaryocytes with a marked polymorphism are present in the whole section surface. It means the beginning at the organ level, of a compensatory hematopoiesis process for a short time. Hematopoiesis reactivation is also present in the spleen and liver of the adult mice after giving them antiinflammatory compounds (Ardelean A et al, 1999). This phenomenon was noticed in case report also, in the case of human patients after the granulocyte colony-stimulating factor was given (Litam P et al, 1999). The presence of granulocitary precursors, at various stages of evolution, also confirm the development of some hematopoiesis processes.

The aspects noticed demonstrate the structures of the spleen recover after the treatment with Cyclophosphamide. Moreover, the spleen takes part in the general effort of the organism to recover the normal structures affected by the treatment with cancer drugs, by reactivating hematopoiesis, at least for the period when the medulla cannot provide all the necessary cells by itself.

## CONCLUSIONS

The histopathological examination of the spleen at the end of Cyclophosphamide administration, indicates the the cancer drug lead to spleen damages, both to red and white pulp, most of lymphocyte populations were found out in different stages of apophthosis or necrobiosis.

At ten days after cancer drugs therapy ending, the spleen is in a fully process of recovery. That proves that lymphoid tissues has capacity to recover, resuming the normal morpho-functionality of the imune system being an essential condition for the repetition of the therapy.

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