

INHIBITORY EFFECT OF CYCLOPHOSPHAMIDE ON THE BIOSYNTHESIS OF A PERCHLORO-SOLUBLE PROTEIN FRACTION OF EHRLICH ASCITES CARCINOMA CELLS *

R. RAPOSO ABREU and LUIZ A. ABREU

Instituto Oswaldo Cruz, Rio de Janeiro, Brasil

SUMMARY: The perchloro-soluble mucoprotein fraction was determined in the cells of Ehrlich ascites carcinoma on the 10th and 12th days post-inoculation of the tumor. After 3 days of a single subcutaneous dose of cyclophosphamide (200 mg/kg) the mucoprotein levels were found considerably lower. This difference was highly significant statistically.

A number of authors have reported about the presence of glycoproteins in malignant neoplasms. Acid glycoproteins were isolated from human cancerous ascitic fluid (12) and Yoshida ascites tumor (4, 7). A mucopeptide was obtained from the surface of Ehrlich ascites tumor cells (6). HOKKANEN et al. (5) studied the rising of mucoproteins in the ITB ascitic tumor of the rat and Weimer et al. (14) determined the perchloro-soluble histomucoid fraction in the Walker carcinosarcoma 256. ABREU & ABREU (1, 2) have studied the mucoprotein fraction present in rat liver and kidney.

Studies on the perchloro-soluble mucoproteins (16) of Ehrlich ascites carcinoma cells are lacking and since

we observed a significant inhibition of the biosynthesis of Ehrlich's mucoproteins by the cytotoxic alkylating drug cyclophosphamide (CY) the present paper will describe the results obtained in this connection.

MATERIAL AND METHODS

Male Swiss mice (26 g mean body weight) fed on a standard balanced diet and water *ad libitum* have been used throughout all experiments. All the mice were inoculated, by intraperitoneal route, with 0.1 ml of Ehrlich ascites carcinoma removed from a mouse injected similarly 10 days before. In two groups of mice ascitic fluid was removed by intraperitoneal puncture at 10 and 12 days post-inoculation, respectively. A third group was treated with CY at the 9th day after Ehrlich's inoculation. The drug was dissolved in 0.9 per cent sterile

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* Laboratory of Biochemistry, Department of Chemistry and Experimental Therapeutics, Instituto Oswaldo Cruz.

saline just before the injections by subcutaneous route at the dose of 200 mg/kg. The ascitic fluid of this group was collected at the 12th day after tumor inoculation. Only clear non-hemorrhagic ascitic fluids were used in the determinations.

The per cent counts of Ehrlich carcinoma cells in the ascitic fluids were evaluated by the examination of smears stained by the May-Grünwald-Giemsa method.

The packed cells volumes of the Ehrlich ascitic fluids were determined by centrifuging 30 minutes at 3000 r.p.m. using the Wintrobe hematocrit. The cells in 5 ml aliquots of the ascitic fluid were washed 3 times with 10 ml of isotonic saline, resuspended in 5 ml of the same solution and the perchloro-soluble mucoprotein fraction extracted and precipitated according to the method of Winzler⁽¹⁵⁾. The precipitate was dissolved in 3 ml of 0.2 N NaOH and the mucoproteins determined by the biuret method of Weichselbaum⁽¹³⁾ using crystalline bovine plasma albumin as standard.

By reference to volume of the packed cells the results were calculated in mg of mucoproteins per 100 ml of Ehrlich carcinoma cells. The differences between the groups were evaluated by standard statistical procedures⁽¹¹⁾.

RESULTS

From the data in Table I it is evident that there were not found significant differences neither in the mean per cent values of packed cells volumes nor on the mean relative numbers of Ehrlich cells in the 3 groups of mice. The statistical analysis of the results summarized in Table I indicate that there is no difference between the mucoprotein concentrations in the cells with 10 and 12 days after inoculation ($t = 0.957$, $P < 0.5$).

T A B L E I
MUCOPROTEINS IN EHRlich CARCINOMA CELLS

Days after inoculation	Number of mice	Packed cells volumes %	Ehrlich cells %	Mucoproteins mg/100 ml Mean \pm S.E.M. *
10	15	15	97	304 \pm 26
12	14	15	98	342 \pm 30
12 CY-treated	12	14	98	60 \pm 16

* S.E.M. = standard error of mean.

On the other hand the level of mucoproteins in CY-treated mice were considerably lower than those obtained in both groups of non-treated animals. These differences are statistically highly significant ($t = 7.992$, $P < 0.001$ and $t = 8.294$, $P < 0.001$, respectively).

DISCUSSION

Cyclophosphamide is activated primarily by a microsomal liver enzyme system^(3, 10). SCHMIDT & VERMATHEN⁽⁹⁾ have reported that the growth of Yoshida ascites tumor in rats induces a considerable hypoproteinemia with hypo-albuminemia.

These changes in blood plasma proteins were reversed in the main by cyclophosphamide. However, the drug has a potent immunosuppressive effect (8, 17).

Our experiments clearly show that cyclophosphamide has a strong blocking effect on the mucoprotein biosynthesis in the Ehrlich carcinoma cells. In view of the high production of the perchloro-soluble protein fraction in neoplasms (15, 16) the low levels obtained in Ehrlich carcinoma cells after cyclophosphamide administration are probably related with the therapeutic properties of the drug.

SUMARIO

Foi determinada a fração mucoproteica percloro-solúvel nas células do carcinoma ascítico de Ehrlich, no 10.^o e 12.^o dias após a inoculação do tumor. Nos camundongos injetados com uma única dose subcutânea de ciclofosfamida (200 mg/kg) os níveis desta fração foram encontrados consideravelmente mais baixos. A análise estatística demonstrou que as diferenças observadas entre os animais controles e os tratados com ciclofosfamida foram altamente significativas.

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