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Biosynthetic Pattern of Liver Glycogen in Malignancy and Under Therapeutic Stress

Urmi SEN and J. Roy CHOWDHURY

Department of Tumor Biology, Chittaranjan National Cancer Research Centre, Calcutta: 700 026 INDIA

ABSTRACT

The hypoglycemic effect exerted by the tumor tissue causes an increased consumption of blood glucose from the surrounding medium and this in turn results in an elevated mobilization of host's liver glycogen. In the present paper [T-^{3}H] glucose has been injected in normal mice and those bearing S 180, and others treated with the chemotherapeutic agents 5-fluoro uracil and 5-fluoro deoxy uridine causing tumor regression. Information has been furnished in the present paper regarding the utilization pattern of the tritiated glucose in the liver glycogen along with the isotope clearance from the blood in normalcy, malignancy and under therapeutic stress. The check of the neoplastic process due to the therapeutic effectiveness of drugs exhibits parallelism with respect to the significant revival of liver glycogen and the blood glucose level in the treated groups. This is also reflected in the significant increase of incorporation of radioactive precursor in liver glycogen concomitant with decrease in the rate of utilization of isotope from the blood level in the treated series.

INTRODUCTION

The growth of malignant tumors is accompanied by marked metabolic changes which is reflected in the biosynthetic pathways of different macromolecules. Glycogen is known to be depleted with the development of malignant tumor and numerous workers have reported that there is disappearance of glycogen from the stratified epithelium of cervix uteri in malignancy, which is of diagnostic importance (1～5). The tumor tissues in animals also have been found to contain a very low level of glycogen, and this exhibits parallelism with the growth rate of the tumor cells (6～11). In our previous work it has been reported that consequent to transplantation of different tumor strains in mice there is a steady and gradual mobilization of host's liver glycogen and like tumor tissues, this mobilization is also proportional to the growth rate of the tumors. It was also observed that regression of tumor caused by potent chemotherapeutic

agents was associated with significant revival of liver glycogen (12).

Tumors act as a powerful hypoglycemic factor, and this leads to a lowering of the blood glucose levels in the tumor tissues and the surrounding medium compared to their normal counterparts (13～18). It has been suggested that the sharp difference in the concentration of glucose in the blood vessels surrounding the tumor and the tumor tissue itself, results in an elevated consumption of blood glucose by the tumor tissueswhich results in an increased mobilization of host's liver glycogen (15).

In the present paper information has been furnished with respect to variation of blood glucose level in the animals as a result of tumor development, and tumor regression studies. Utilization pattern of tritiated glucose in the liver glycogen with correlation of isotope clearance from blood level have been done under malignant condition as well as during control of neoplasia by therapeutic stress.

MATERIALS AND METHODS

Selection of Mice and Tumor Strain

Swiss mice weighing 18～20 mg were used in the present study. Tumor strain Sarcoma 180 was maintained in ascitic form by serial transplantation of 3.7×10^7 cells/0.5 ml administered intraperitoneally.

Chemotherapeutic Regime

5-Fluoro uracil obtained from Sigma Chemicals was dissolved in physiological saline and injected in the dosage of 25 mg/kg body weight for 7 consecutive days. 5-Fluoro deoxy uridine also obtained from Sigma Chemicals, was dissolved in physiological saline and injected in the dosage of 40 mg/kg body weight for 7 consecutive days (19). The treatments were started on the 7th day after tumor transplantation.

Isotope Administration

Uniformly labeled D-[1-^{3}H] glucose was obtained from the Radiochemical Centre, Amersham. It was diluted in physiological saline, and each mouse received 1 μci/g body weight. The animals were prepared for the experiment by depriving them of food but not water for 4 hour prior to the administration of the isotope so that the blood glucose values would be uniform in all the mice (20).

Isotope injection was given in the following groups:

I. A group of 20 normal mice were injected with the isotope to serve as controls.

II. Sarcoma 180 bearing mice of equal number were injected with

tritiated glucose at 3rd, 5th, 7th, 14th and 21st day after tumor transplantation.

III. Isotope was administered in equal number of treated mice. The days selected were 7th and 14th day after treatment with 5-fluoro uracil and 5-fluoro deoxy uridine in S 180 bearing mice. This indicated a study after just completion of treatment (7th day) and after a gap of 7 days subsequent to the completion of treatment (14th day).

Only those mice which responded to therapy by way of tumor regression were taken in this series for investigation.

In all the groups the animals were sacrificed at intervals of 1 hr, 4 hr and 24 hr following injection of radioactive glucose. The nature of tumor regression was determined by comparing the ascitic fluid volume of the treated and untreated mice.

Estimation of Blood Glucose and Measurement of Radioactivity in Blood

Whole blood was collected by decapitation from the mice. Anti-coagulant used was sodium oxalate. Sodium fluoride was added to prevent glycolysis. Blood glucose was measured according to the method of Somogye and Nelson (21).

For measurement of radioactivity in the blood, 1 ml of the filtrate obtained after addition of $Ba(OH)_2$ and $ZnSO_4$ to the whole blood, was added to a fluid composed of 1 : 4 dioxane containing 0.5% 2, 5- diphenyl oxazole and 10% napthalene in the ratio of 1 : 9, and radioactive counts per minute were recorded in the scintillation counter (22).

Glycogen Estimation and Measurement of Radioactivity

Liver tissues collected from the sacrificed animals were processed for glycogen extraction and estimation by the method of Plummer (23). The tissues were extracted in 5% TCA, and the optical density recorded after colour reaction with anthrone reagent in the spectrophotometer.

A portion of the extracted glycogen dissolved in water was mixed with the fluid, as described in the previous experiment, and radioactive counts were recorded in the scintillation counter.

RESULTS

The growth pattern of Sarcoma 180 in treated and untreated series followed the pattern as shown in Fig. 1. The control of neoplasia by 5-Fu and 5-FuDR in S 180 bearing mice was associated with inhibition of ascitic fluid volume as compared to the typical growth of untreated mice tumors.

It has been previously reported that along with tumor development there is gradual mobilization of host's liver glycogen.

The comparative values of total liver glycogen in mice bearing S 180 and those treated with both the chemotherapeutic drugs have been depicted in Fig. 2. It is evident from the figure that regression of tumors subsequent to successful therapy with 5-Fu and 5-FuDR resulted in significant elevation

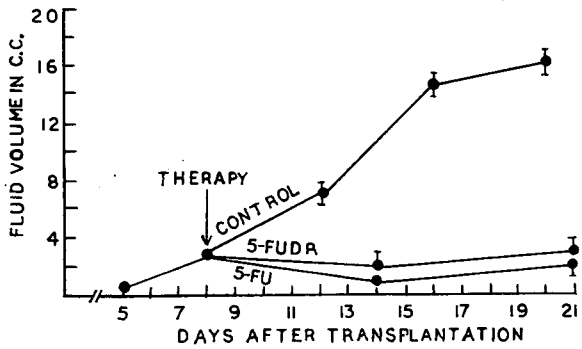

Fig. 1. Growth pattern of S 180 measured in terms of fluid volume in control and treated series. Each point in the curve is an average of 15 to 20 animals.

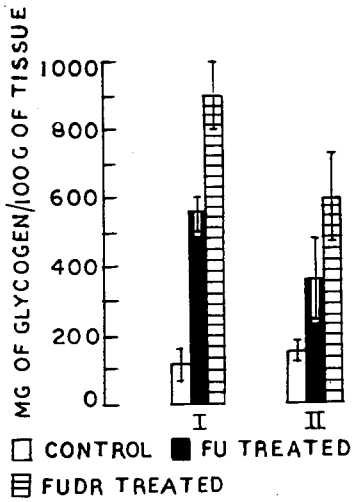

Fig. 2. Comparative values of liver glycogen in treated and untreated series of mice bearing S 180. I denotes the values where evaluation has been done on just completion of treatment (7th day after therapy) and II denotes the values where evaluation has been done after a gap of 7 days subsequent to the completion of treatment (14th day after therapy).

of liver glycogen as compared to low level of the same in the corresponding untreated groups. This is more pronounced in the 7th day of treatment shown as Group I as compared to that at 14th day of treatment shown as Group II.

The blood glucose values of the normal, tumor bearing and treated group of mice revealed that a steady decrease in the blood sugar values parallels tumor development. But in the treated group this phenomenon has been checked (Fig. 3).

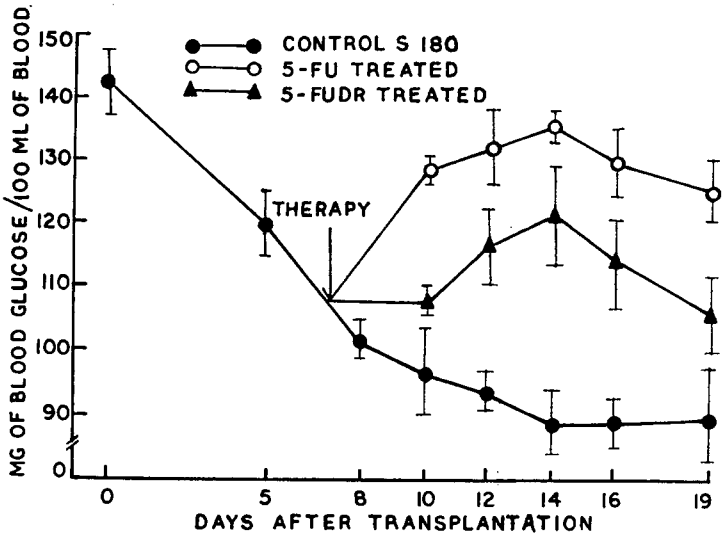

Fig. 3. Blood glucose values of the normal tumor bearing and treated groups of mice expressed as mg/100 ml of blood.

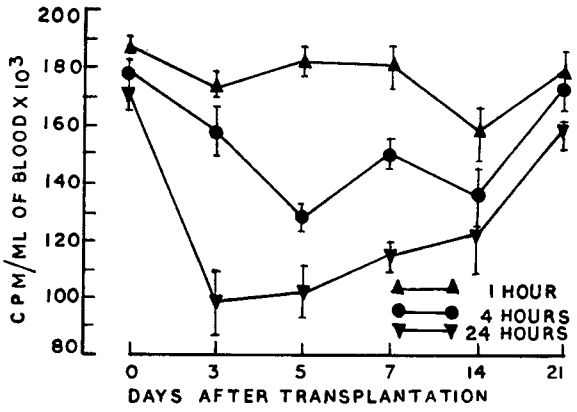

Fig. 4. The pattern of uptake of labelled glucose in the blood of the normal and tumor bearing mice expressed as CPM/ml.

The pattern of uptake of labelled glucose in the blood of the animals expressed as the radioactive counts per minute per ml (CPM/ml) of blood, were recorded at 1 hr, 4 hr, and 24 hr after isotope injection in normal and tumor bearing mice on 3rd, 5th, 7th, 14th and 21st day of transplantation (Fig. 4). Although the clearance of radioactivity was gradually increased with time in all the groups, but the rate of the clearance in case of tumor bearing mice was at a much greater pace than that of normal mice, particularly in the early active phase of growth.

The pattern of clearance of labelled glucose in the blood of treated groups of mice revealed inhibition from rapid rate of clearance which was evident in corresponding untreated control of 14th and 21st day of transplantation (Fig. 5 and 6).

In case of tumor bearing mice the rate of incorporation was significantly low as compared to that of normal mice, being lowest during the early log phase of tumor growth (Fig. 7).

With the regression of tumor after chemotherapy there was revival of the glycogen synthesis in liver as evident by an increase in the rate of incorporation of labelled glucose. Compared to the matched control series, the incorporation of H^3-glucose in the livers of the mice after 7th and 14th

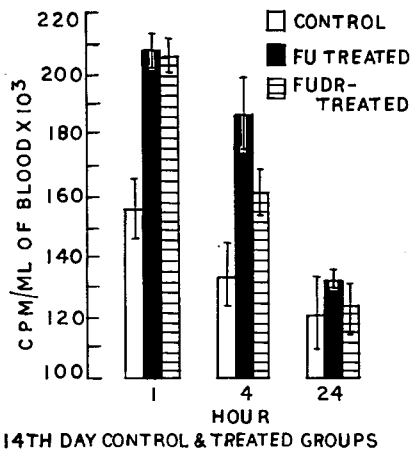

Fig. 5. The pattern of uptake of labelled glucose in the blood of treated groups of mice and their matched controls on 14th day of transplantation that is on 7th day after therapy.

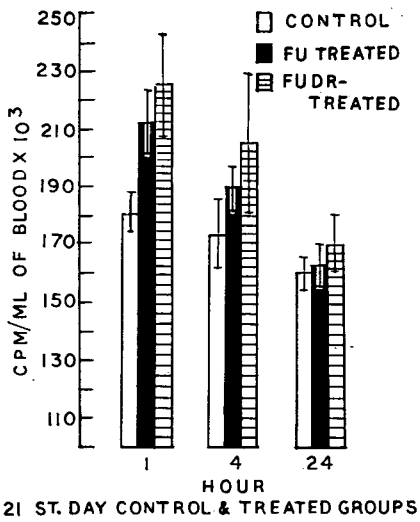

Fig. 6. The pattern of uptake of labeled glucose in the blood of treated groups of mice and their matched controls on 21st day of transplantation that is on 14th day after therapy.

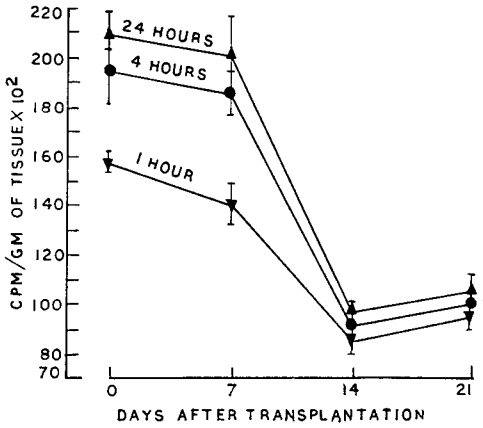

Fig. 7. The pattern of uptake of labelled glucose in the glycogen of the liver in the normal and tumor bearing mice expressed as CPM/gm.

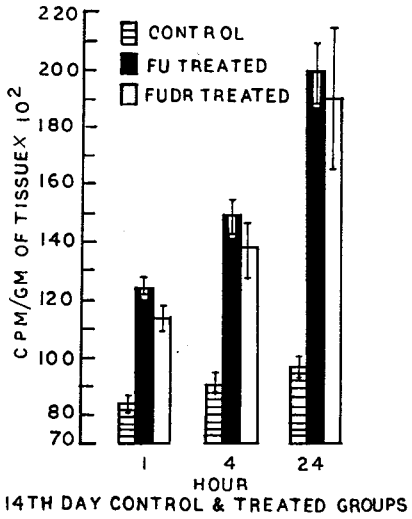

Fig. 8. The pattern of uptake of labelled glucose in the liver glycogen in treated groups of mice, and their matched controls on 14th day of transplantation that is on 7th day after therapy.

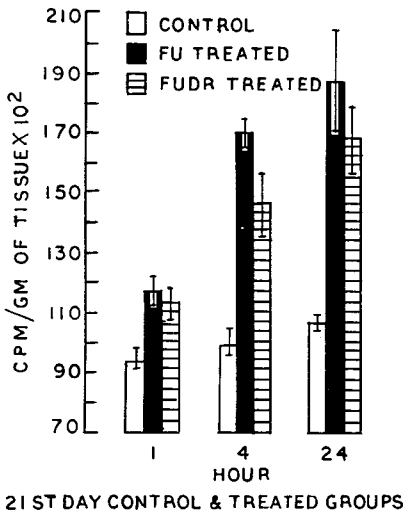

Fig. 9. The pattern of uptake of labelled glucose in the liver glycogen in the treated groups of mice and their matched controls on 21st day of transplantation that is on 14th day after therapy.

day of treatment were significantly higher (Figs. 8 and 9).

DISCUSSION

It has been established that there is significant mobilization of blood glucose as well as liver glycogen as a result of the growth of the transplanted tumors (24, 25).

As glycogen is known to participate in the energy requirement of the cell, its disappearance from the liver with the development of tumor can be well correlated with the high growth potentials of the tumor cells along with greater metabolic competence of the cells (26, 27).

As regards the mobilization of blood glucose, it has been stated previously that tumors growing in any part of the body behave as a powerful hypoglycemic factor, drawing glucose from the host's blood to meet the energy requirement of growing tumor cells. This in turn results in an elevated mobilization of glycogen from the liver and skeletal muscle of the host and causing reduction in the glycogen deposition.

The present result with respect to utilization of labelled precursor has also furnished evidence of direct utilization of blood glucose by the tumor tissue resulting in inhibition of glycogen synthesis in liver. The change of synthetic pattern of liver glycogen may also be due to alteration of enzyme system operating in the synthetic pathway particularly glycogen synthetase as a result of malignancy (28).

5-fluoro uracil and 5-fluoro deoxy uridine are potent anticancer drugs with proven record of tumor inhibition in a variety of tumor strains (19). The check of neoplastic process due to the therapeutic effectiveness of these drugs demonstrated in the present experiment exhibits parallelism with respect of the significant revival of liver glycogen. This is also reflected in the blood glucose levels which is revived in the treated groups, indicating that the hypoglycemic effect exerted by the growing tumor has been controlled as a result of tumor inhibition. The increase of incorporation of radioactive precursor in liver glycogen concomitant with decrease in the rate of utilization of isotope from the blood level in the treated series is thus highly significant.

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