EFFECT OF ADMINISTERING CYCLOPHOSPHAMIDE AND VITAMIN E ON THE LEVELS OF TUMOR-MARKER ENZYMES IN RATS WITH EXPERIMENTALLY INDUCED FIBROSARCOMA

Ramachandran VINITHA, Muthusamy THANGARAJU and Panchanatham SACHDANANDAM

Department of Medical Biochemistry, Dr. A. L. M. Post - Graduate Institute of Basic Medical Sciences, University of Madras, Taramani Campus, Madras, 600113, India

(Received January 6, 1995. Accepted May 16, 1995)

SUMMARY: Cyclophosphamide, an antineoplastic drug, and vitamin E, the common antioxidant present in the diet, were administered in separate dosages and in combination to animals (rats) with fibrosarcoma, metastatic tumor of the connective tissues, induced. The anticancer drug (20 mg/kg body weight) and the vitamin-E (400 mg/kg body weight) was administered for a period of 28 days from the day of tumor transplantation. The individual and the combined effects of these two substances were investigated by checking the growth of the tumor. Tumor markers like lactate dehydrogenase (LDH), serum glutamate pyruvate transaminase (SGPT), serum glutamate oxaloacetate transaminase (SGOT), acid phosphatase, and alkaline phosphatase were analyzed for the changes in their concentration in serum, liver, and kidney to assess the success of the therapy. The increased level of the enzymes in the fibrosarcoma-suffering rats (GP II) was reduced by cyclophosphamide treatment (GP III) and vitamin E administration (GP IV). Among the treated groups, the combination therapy (GP V) showed greater efficacy in the treatment of fibrosarcoma than did individual administration, as there was more reduction in the levels of enzymes in Group V than those in Groups III and IV. The enzyme levels were brought to near the normal level.

INTRODUCTION

The anti-tumor activity of cyclophosphamide, a chemotherapeutic agent of considerable interest, is attributed to its alkylating property (1). Cyclo-
phosphamide is a nitrogen mustard which possesses a cytotoxic action and interferes with normal mitosis and cell division in all rapidly proliferating tissues (2). The efficacy of this drug in treating fibrosarcoma, a type of connective tissue malignant tumor, was studied.

Tocopherols act as antioxidants, breaking free radical chain reactions as a result of their ability to transfer a phenolic hydrogen to a peroxyl free radical of a peroxidized poly unsaturated fatty acid (3). Vitamin-E deficiency was found by Arrhenius (4) to cause a decrease of N-demethylase activity that would be expected to reduce the detoxification of certain carcinogenic aromatic amines. Vitamin E induces the microsomal mixed-function oxidase which destroys carcinogens (5). Large excess of dietary α-tocopherol given to C57 Leaden strain female mice reduced the yield of sarcomas due to subcutaneous injection of 3-methylchololantherene (6). The chain breaking antioxidant activity of α-tocopherol was also tested in fibrosarcoma-suffering animals.

In this article, we have attempted to present the changes in the tumor-marker enzymes in fibrosarcoma-induced animals. Changes were also observed in the cyclophosphamide-treated, vitamin E-administered group of animals. Combination therapy consisting of both cyclophosphamide and vitamin E were administered to a group of cancer-induced animals and changes noted.

**MATERIALS AND METHODS**

Adult albino male rats of the Wistar strain, weighing between 100-120 g, were used initially to induce fibrosarcoma by the method of Mohana and Purushothaman (7). A 10% tumor cell suspension (0.5 ml) obtained from tumor locus was injected into the auxiliary region through a 16-gauge needle. The animals were maintained on standard commercial pelleted diet and water provided ad libitum.

*Maintenance of cell line:* Fibrosarcoma cell line (20 - methyl cholantherene-induced) maintained in 8-10 weeks old, Wistar male rats by regular s. c. passage of 10⁶ cells, was used. A 1.0-ml of a 10% cell suspension in physiological saline, containing the same number of cells as mentioned above, was injected into the axillary region through a 16-gauge sterile needle. The transplanted tumor becomes palpable after one week (7).

*Experimental set up:* Each group contained six animals.
Group I: These animals were kept as controls, and food and water given ad libitum.

Group II: Animals were transplanted with 20-methylcholanthrene-induced fibrosarcoma cell line, and given food and water ad libitum. They were orally given the drug vehicle, sunflower oil, alone for 28 days from the day of tumor transplantation.

Group III: These animals were transplanted with 20-methylcholanthrene induced fibrosarcoma cell line and given food and water were ad libitum. In addition, they were given orally 20 mg/kg body weight of cyclophosphamide dissolved in 6 ml of sunflower oil for 28 days from the day of tumor transplantation.

Group IV: These animals were transplanted with 20-methylcholanthrene induced fibrosarcoma cell line, and given food and water were ad libitum. These animals were given orally 400 mg/kg body weight of vitamin E dissolved in 4 ml of sunflower oil for 28 days from the day of tumor transplantation.

Group V: These animals were transplanted with 20-methylcholanthrene-induced fibrosarcoma cell line, and provided with food and water ad libitum. In addition, they were given cyclophosphamide at 20 mg/kg body weight and vitamin E at 400 mg/kg body weight dissolved in sunflower oil (6 and 4 ml, respectively) separately through a stomach tube for 28 days from the day of tumor transplantation.

The animals were killed on the 29th day after the treatment with drugs for 28 days, by cervical dislocation, blood samples were collected and the tissues were dissected from the concerned organ of study, namely, the liver and kidney. Tissue protein and enzymes were estimated with a 10% tissue homogenate. The homogenate was prepared in Tris-HCl buffer. Extraction of proteins from the tissues was not carried out.

**Biochemical parameters investigated:** Total protein was estimated by the method of Lowry et al (8). The activity of lactate dehydrogenase (LDH) was measured by the method of King (9). Alkaline phosphatase activity in serum was assayed by the method of Moog (10) as modified by King (9). The procedure adopted for the assay of acid phosphatase was similar to that of alkaline phosphatase except that citrate buffer was used in the place of carbonate buffer and Mg ions were omitted from the incubation mixture. The activity of serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT) were assayed by the method of King (9).
RESULTS

Table I shows the level of serum changes LDH, alkaline phosphatase, acid phosphatase, SGPT, and SGOT in the five groups of animals. Group I (control) animals were compared with Group II (induced) animals. Group III (cyclophosphamide-treated) and IV (vitamin E-administered) animals were compared with Group II animals. The level of the tumor markers increased in Group II animals, while a significant decrease was observed in Group III, IV, and V animals. Among the latter groups, Group V animals had the maximum lowering of enzyme levels closer to control values.

The content of Table II reflects the changes in the marker enzyme levels in liver homogenate of the five groups of animals. There is a marked increase in the level of enzyme in the induced animals compared with control animals. A reduction in the level of enzymes is observed in the three treated groups. Maximum lowering of the enzymes level is noted in the combination therapy-extended animals (Group V), the values being nearer to those in control animals than those in Group III and IV animals.

Table III depicts the changes in enzyme levels (the five tumor markers) in the kidney homogenate of the five groups of animals. Increase in the level of marker enzymes in fibrosarcoma-induced animals as compared with normal animals decreased in Group III, IV and V animals. Among the Group III, IV, and V animals, the enzyme level in the Group V animals is lowered to a greater extent, approaching the level of control values, than the other two treated groups. The restoration of the enzyme levels correlates with the antitumor efficacy of the drugs, vitamin E and cyclophosphamide, when we analyze these findings with other supportive observations like weight gain and histopathology (not presented here).

Figure 1 shows the changes in body weight in control, fibrosarcoma-induced, and treated groups. Cachexia is characterized by generalized weakness, malnutrition and loss of body weight. The observed weight loss in fibrosarcoma-induced group may have been due to cachexia condition characteristic of cancer. Treatment with vitamin E, cyclophosphamide and combination therapy has prevented loss of body weight, leading to progressive weight gain in the treated groups. The study of Fig. 1 reveals that the major beneficial effect of combination therapy is the prevention of body weight loss. The greater decrease in the LDH level by the
<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
<th>Group V</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactate Dehydrogenase (IU/dl)</td>
<td>1.52±0.51</td>
<td>3.49±0.61a*</td>
<td>2.61±0.64b*</td>
<td>2.27±0.50c#</td>
<td>1.91±0.41d*e@</td>
</tr>
<tr>
<td>Alkaline Phosphatase (IU/dl)</td>
<td>3.075±0.58</td>
<td>6.06±0.09a*</td>
<td>4.01±0.67b*</td>
<td>3.81±0.50c*</td>
<td>3.34±0.34d*eNS</td>
</tr>
<tr>
<td>Acid Phosphatase (IU/dl)</td>
<td>1.64±0.57</td>
<td>3.36±0.41a*</td>
<td>2.43±0.21b*</td>
<td>2.15±0.61c*</td>
<td>1.86±0.40d*e@</td>
</tr>
<tr>
<td>SGOT (IU/dl)</td>
<td>11.23±1.63</td>
<td>21.31±2.57a*</td>
<td>17.78±4.95bNS</td>
<td>16.87±2.16c#</td>
<td>13.39±3.84d*eNS</td>
</tr>
<tr>
<td>SGPT (IU/dl)</td>
<td>6.68±1.21</td>
<td>13.18±1.60a*</td>
<td>10.49±1.81a@</td>
<td>8.83±0.99c*</td>
<td>7.13±0.70d<em>e</em></td>
</tr>
</tbody>
</table>

Values are expressed as Mean±S.D. For statistical evaluation of variations, aGroup II is compared with Group I, bGroup III, cGroup IV and dGroup V with Group II and eGroup V with Group III.
Statistical alterations are expressed as, @P<0.05, #P<0.01, *P<0.001. NS: Not significant.
Table II. Enzyme levels in liver homogenate of control and experimental groups at the end of the experimental period

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
<th>Group V</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactate Dehydrogenase</td>
<td>0.955±0.07</td>
<td>2.915±0.61a*</td>
<td>2.13±0.28b*</td>
<td>2.10±0.76c@</td>
<td>1.462±0.46d*e@</td>
</tr>
<tr>
<td>(µmoles of pyruvate liberated/mg of protein /hr)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alkaline Phosphatase</td>
<td>0.151±0.05</td>
<td>1.53±0.42a*</td>
<td>0.685±0.28b*</td>
<td>0.542±0.23c*</td>
<td>0.235±0.06d*e#</td>
</tr>
<tr>
<td>(µmoles of p-nitro phenol liberated/mg of protein/hr)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acid Phosphatase</td>
<td>0.29±0.10</td>
<td>1.23±0.16a*</td>
<td>0.51±0.10b*</td>
<td>0.51±0.10c*</td>
<td>0.432±0.13d*eNS</td>
</tr>
<tr>
<td>(µmoles of p-nitro phenol liberated/mg of protein/hr)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SGOT (µmoles of pyruvate liberated/mg of protein/hr)</td>
<td>23.02±3.58</td>
<td>36.38±5.02a*</td>
<td>30.14±3.30b@</td>
<td>27.47±2.28c*</td>
<td>25.85±3.60d*eNS</td>
</tr>
<tr>
<td>SGPT (µmoles of pyruvate liberated/mg of protein/hr)</td>
<td>10.56±1.66</td>
<td>22.08±3.23a*</td>
<td>16.01±1.13b#</td>
<td>13.83±3.25c#</td>
<td>12.27±1.87d*e#</td>
</tr>
</tbody>
</table>

Values are expressed as Mean±S.D. For statistical evaluation of variations, aGroup II is compared with Group I, bGroup III, cGroup IV and dGroup V with Group II and eGroup V with Group III.

Statistical alterations are expressed as, @P<0.05, #P<0.01, *P<0.001. NS: Not significant.
Table III. Enzyme levels in kidney homogenate in control and experimental groups at the end of the experimental period

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
<th>Group V</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactate Dehydrogenase (µmoles of pyruvate liberated/mg of protein/hr)</td>
<td>0.942 ± 0.26</td>
<td>2.685 ± 0.37a*</td>
<td>2.055 ± 0.19b*</td>
<td>1.63 ± 0.37c*</td>
<td>1.385 ± 0.72d*e@</td>
</tr>
<tr>
<td>Alkaline Phosphatase (µmoles of p-nitro phenol liberated/mg of protein/hr)</td>
<td>1.29 ± 0.44</td>
<td>3.12 ± 0.13a*</td>
<td>2.27 ± 0.58b#</td>
<td>1.86 ± 0.33c*</td>
<td>1.51 ± 0.34d*e@</td>
</tr>
<tr>
<td>Acid Phosphatase (µmoles of p-nitro phenol liberated/mg of protein/hr)</td>
<td>0.412 ± 0.08</td>
<td>1.74 ± 0.26a*</td>
<td>1.20 ± 0.24b#</td>
<td>0.95 ± 0.09c*</td>
<td>0.847 ± 0.16d*e@</td>
</tr>
<tr>
<td>SGOT (µmoles of pyruvate liberated/mg of protein/hr)</td>
<td>8.47 ± 1.09</td>
<td>17.79 ± 3.37a*</td>
<td>12.53 ± 1.90b#</td>
<td>11.20 ± 1.85c#</td>
<td>8.45 ± 1.24d*e#</td>
</tr>
<tr>
<td>SGPT (µmoles of pyruvate liberated/mg of protein/hr)</td>
<td>2.82 ± 0.22</td>
<td>7.85 ± 0.86a*</td>
<td>5.20 ± 0.59b*</td>
<td>3.82 ± 0.58c*</td>
<td>3.12 ± 0.60d<em>e</em></td>
</tr>
</tbody>
</table>

Values are expressed as Mean ± S.D. For statistical evaluation of variations, aGroup II is compared with Group I, bGroup III, cGroup IV and dGroup V with Group II and eGroup V with Group III. Statistical alterations are expressed as, @P<0.05, #P<0.01, *P<0.001. NS: Not significant.
combination therapy may have been due to recovery from cachexia of tumor-bearing rats.

**DISCUSSION**

Enzymatic changes reflect the overall changes in metabolism that occur in malignancy (11). The rapid increase in the turnover of malignant cells modulates the enzyme levels in blood circulation. A number of enzymes, SGOT, SGPT, LDH, alkaline phosphatase, and acid phosphatase, are released into the surrounding medium. Enzymes present in the cellular and sub cellular organelles are also released. The presence of cancer may induce the release of enzymes from the surrounding normal tissue (12).

LDH has been recognized as a potential tumor marker for various types of cancers (13). Increased activity of the enzyme is also seen in leukemia and in generalized carcinomatosis (14). When the activity of LDH is taken for investigation,
the cachexia condition, characteristic of cancer, should be given grave consideration. In cachexia, glucose is catabolized through the Embden-Meyerhoff pathway and an increase in the turnover of blood lactate results in lactic acidosis. This explains the increased level of serum LDH in Group II animals. During cyclophosphamide and α-tocopherol administration, the utilization of glucose is significantly reduced and, hence, the catabolism of glucose moderately decreased in Group III and IV animals. This may be the reason for the decrease in LDH enzyme observed in Group III and Group IV animals. The adverse effects of cachexia were slowly reversed by the coadministration of tocopherol with cyclophosphamide (Group V animals), the LDH activity tended to return to control levels (Group I animals). In an advanced stage of cancer, cachexia is a major cause of death (15). Any agent that would suppress cachexia would help repress and cure the disease by increasing the survival rate.

Another important observation is the relation between LDH level and tumor mass, and prognosis in adult and pediatric non-Hodgkin’s lymphoma (16,17) and in a variety of solid tumors including sarcoma (18). Tumor size and LDH levels show a direct proportion in different sarcomas (KOS-1, KOS-2, KOS-3 and OST) (19). Hence, an increase in LDH level in the induced group correlates with that in tumor mass in the sarcoma-induced animals, while treatment with vitamin E and cyclophosphamide could have resulted in decreased LDH level probably indicating proportionate decrease in tumor mass.

Alkaline phosphatase is a membrane-bound enzyme. Rapidly growing tissues such as a variety of carcinomas show greatly elevated alkaline phosphatase activity and in such cases there is usually a concomitant elevation in total serum alkaline phosphatase (20). Damage to the membrane as in necrosis results in increased alkaline phosphatase activity in Group II animals. Changes are more pronounced in serum due to ulceration and necrosis in the tumor site. Administration of vitamin E, a membrane antioxidant, prevents free radical attack on the lipid layer of cell membrane, thereby bringing about a check in the rate of membrane damage and cell destruction and hence the healing of the ulceration. Hence, a decrease in the level of enzymes was observed in Group IV animals.

Adverse effects of cachexia were slowly reverted due to the administration of cyclophosphamide (Group III) and the combination therapy (Group V).

Acid phosphatase is a known lysosomal enzyme. Due to neoplastic transformation, disruption of lysosomes results in altered extracellular connective tissue components. The abnormal fragility of the lysosomes, increased extracellular activity of the lysosomal enzymes and the continued release of lysosomal enzymes
are attributed to phagocytosis of immune complexes (21). The reduction in the level of enzyme in vitamin E-administered animals (Group IV) may be due to the free radical quenching action of vitamin E. Damage to the lysosomal membrane by free radicals generated in tumor cells reduced to a great extent by tocopherol administration. The decrease in the level of acid phosphatase in cyclophosphamide-treated animals (Group III) may be due to anti-mitotic activity possessed by the anti-tumor drug. It interferes with the rapid proliferation of the sub-cellular organelle, the lysosomes.

Combination therapy of cyclophosphamide and vitamin E achieved greater reduction in the acid phosphatase level in the serum, liver and kidney due to the above-mentioned activity of the two drugs.

In fibrosarcoma, the liver, lungs, and bones are the chiefly affected organs (22). Transaminases are marker enzymes in liver and kidney cancers. Thus, a significantly increased level of both the transaminases suggest that there may be some liver derangement in the neoplastic host. The administration of vitamin E and cyclophosphamide brings about a corrective change at the cancer locus, retards the release of stimulants from the cancer site. Hence, the liver recovers back to the normalcy because of the administration of cyclophosphamide and vitamin E in combination.

From the above observations, it can be conveniently inferred that even though cyclophosphamide and vitamin E can act individually as antitumor agents, the efficiency is much more improved when they are given in combination (from the values obtained in Group V animals) which may be due to complementation of aspects of each other.

REFERENCES


