THERAPEUTIC EFFECT OF TRIDHAM AND 1,2,3,4,6-PENTA-O-GALLOYL-Β-D-GLUCOSE ON ALTERED ENERGY METABOLISM IN MAMMARY CARCINOMA BEARING RATS

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ABSTRACT

Objective: Emerging evidence indicates that cancer is primarily a metabolic disease involving disturbances in energy production, and hence, reprogramming of cellular energy metabolism is deemed to be one of the principal hallmarks of cancer. Tridham (TD) has been used by traditional practitioners of Siddha medicine against various ailments. Hence, the present study has been designed to evaluate the therapeutic effect of TD on altered energy metabolism in mammary carcinoma-bearing rats.

Methods: Adult female albino rats of Sprague-Dawley strain weighing 170-190 g were used and 7,12-dimethylbenzeneanthracene (DMBA) was used for induction of mammary carcinoma and rats were divided into seven groups. Group I - control rats, Group II - DMBA-induced rats, Group III - DMBA- and TD-treated rats, Group IV - DMBA- and 1,2,3,4,6-Penta-O-galloyl-β-D-glucose (PGG)-treated rats, Group V - DMBA- and cyclophosphamide-treated rats, Groups VI and VII are TD and PGG control rats.

Results: Significant (p<0.05) increase in the glycolytic enzymes, hexokinase, phosphoglucomutase and aldolase, was observed in tumor-bearing rats whereas gluconeogenic enzymes, glucose-6-phosphatase and fructose-1,6-biphosphatase, were significantly decreased. The activities of the mitochondrial Krebs cycle enzymes, isocitrate dehydrogenase, α-ketoglutarate dehydrogenase, succinate dehydrogenase, and malate dehydrogenase, and respiratory chain enzymes, nicotinamide dinucleotide dehydrogenase and cytochrome c oxidase, were significantly reduced in tumor tissue of the mammary carcinoma-bearing rats. These biochemical disturbances were effectively counteracted by TD and PGG which restore the activities of all these enzymes to the respective control levels.

Conclusion: TD and PGG effectively ameliorated the altered glucose metabolism resulting in the regression of breast cancer.

Keywords: Breast cancer, Cancer cachexia, Carbohydrate-metabolizing enzymes, Krebs cycle.

INTRODUCTION

Breast cancer is a serious public health concern, being the second most common of all cancers and by far the most frequent reason of cancer death among women throughout the world [1]. There is a continuing search for better and more effective herbal plants to treat breast cancer. Mitochondria play an important role in controlling the life and death of a cell. Mitochondria are central to oxidative phosphorylation and much of metabolism.

Energy metabolism is one of the most basic characteristics of living organisms. It is associated with the progress of metabolic reactions catalyzed by a variety of enzymes. The mitochondria are crucial in energy metabolism [2]. Impairment of energy metabolism in cancer cells has been a recurrent finding for many years. The current thought is that proliferating cells undergo a shift from oxidative to glycolytic metabolism, where the energy requirements of the rapidly dividing cell are provided by ATP from glycolysis [3]. One of the main characteristics of cancer cells is limitless replication potential which results in high energy requirement [4]. The high glycolytic rate under aerobic conditions is important for rapidly proliferating cancers not only as a major energy source but also to provide the cells with precursors for nucleotide and lipid biosynthesis [5]. This seems to be a general property of highly malignant tumors independent of their carcinogenic origin. High rates of glucose utilization with production of lactic acid are characteristic features of the neoplastic cell [6]. Lactic acid so produced may be utilized for energy purpose by other tissues or transported to the liver for resynthesis of glucose (Cori cycle). Utilization of lactic acid by peripheral tissues would normally provide the tumor-bearing patient with maximum energy available from glucose oxidation. Gluconeogenesis from lactate, on the other hand, is an energy-requiring process that, as pointed out by Fenninger and Mider [7], may play an important role in excessive energy expenditure of the host, thus contributing to mechanisms that promote weight loss [8]. In intact glycolytic tumor cells, only 10% of the pyruvate enters a truncated Krebs cycle [9]. This results in a decrease in the overall net ATP production in the host cells due to downregulation of the activities of Krebs cycle and respiratory enzymes, resulting in turn in a loss of body weight which occurs in cancer cachexia [10].

Tridham (TD) is a Siddha formulation prepared in our laboratory, consisting of Terminalia chebula, Elaeocarpus ganitrus, and Prosopsis cineraria in a definite ratio. Previous studies carried out in our laboratory have established its therapeutic effect against AFB1-induced hepatocellular carcinoma rat model as well as in cancer cell lines [11,12].

T. chebula is a deciduous tree, used in traditional medicines. It is reported to contain various biochemical compounds such as tannins, chebulinic acid, ellagic acid, gallic acid, punicalagin, and flavonoids. It has been reported to have antioxidant, anticancerous, anti diabetic, antibacterial, antiviral, antifungal, antiinflammatory, and wound-healing activities [13]. E. ganitrus is a widely used medicinal plant with various phytochemicals such as alkaloids, flavonoids, tannins, glycosides and ellagic acid derivatives. It has been known to have various pharmacological activities such as antioxidative, antitumor, anti inflammatory, antidepressant, antimicrobial, antidiabetic, antiviral, and anti hypertensive activities [14]. P. cineraria is a highly valued plant in the indigenous systems of medicine and it has been used for several ailments such as leprosy, dysentery, bronchitis, asthma, leukoderma,
piles, muscular tremors, asthma, rheumatism and inflammations. It is also known to possess antitumor, antihelminthic, antibacterial, antifungal and antiviral activities [15].

Phytochemical analysis carried out on the herbal formulation, TD, has established 1,2,3,4,6-penta-O-galloyl-β-D-glucose (PGG) as one of the active constituents present in this formulation. Tannins are predominantly found in plants as the core structure of the higher galloyl glucose. Tannins are good direct antioxidants. Even the tannin-protein complex can act as radical scavenger and radical sink. The amount of free PGG varies among different plant species but is present at sufficient levels to allow direct isolation from a number of oriental herbs and other plants such as T. chebula [16], Rhus chinensis Mill [17], Paonia suffruticosa [18], and Paonia lactiflora [19] and by solvent extraction, liquid-liquid partition, and chromatography separation.

Studies have shown the antioxidant potential of PGG, investigated by cell system experiments such as radical detection, antioxidant enzyme assay, lipid peroxidation detection, and cell viability assay [20]. With this background, the present study was designed to study the therapeutic effect of the drug TD and PGG, an active phytochemical constituent in the preparation in experimental mammary carcinoma.

METHODS

The three ingredients were collected and given for botanical authentication. The authentication was done by Professor M. Periyasamy (plant taxonomist) in the Centre for the advanced study in Botany, University of Madras, Guindy Campus, Chennai, India, and voucher specimens (CAS BH-16 T. chebula, CAS BH-17 E. ganitrus, and CAS BH-18 P. cineraria) of the plant are retained in the department herbarium.

Rats

Female albino rats of Sprague-Dawley strain, aged between 50 and 55 days, were used for the study. The rats were purchased from the Central Animal House Block, Dr. ALM PG IBSM, University of Madras, Tannamani Campus, Chennai - 600113. They were maintained at an ambient temperature of 25±2°C and 12/12 hrs of light/dark cycle. Rats were given standard commercial rat chow and water ad libitum and housed under standard environmental conditions throughout the study. The study has got the ethical committee clearance, and the experimental animals were handled according to the University and Institutional Legislation, regulated by the Committee for the purpose of control and supervision of experiments on animals, Ministry of Social Justice and Empowerment, Government of India (IAEC No. 01/03/13).

Sources of chemicals

7,12-dimethylbenz[a]anthracene (DMBA), PGG, and cyclophosphamide (CYCL) were obtained from Sigma-Aldrich Chemical Company, St Louis, USA. The rest of the chemicals were obtained from Sisco Research Laboratory Mumbai, India, and were of analytical grade.

Induction of mammary carcinoma

Mammary carcinoma was induced in rats by a single dose of DMBA dissolved in 1 ml of olive oil (25 mg/kg body weight) (Welsch, 1985) by gastric intubation. After overnight fasting, control rats were injected with the same volume of 1ml of olive oil. After 12 weeks, mammary carcinoma was confirmed by palpation and histopathological examination.

Experimental design

The rats were divided into seven groups of six rats each. TD and PGG dissolved in distilled water were administered orally using an intragastric tube for 48 days. Anticancer drug, CYCL, was dissolved in 0.5 ml distilled water and used as a reference drug.

Group I: Normal control rats (received 0.5 ml distilled water)
Group II: DMBA-induced rats (25 mg/kg body weight by single dose orally); vehicle olive oil
Group III: DMBA-induced rats treated with (TD: 400 mg/kg b.w./day)
Group IV: DMBA-induced rats treated with (PGG: 30 mg/kg b.w./day)
Group V: DMBA-induced rats treated with (CYCL: 30 mg/kg b.w./day)
Group VI: Normal rats treated with alone (TD: 400 mg/kg b.w./day)
Group VII: Normal rats treated with PGG alone (PGG: 30 mg/kg body weight/day)

The groupings of animals were done at the same time. Body weight changes were recorded throughout the period of study. After the experimental period (90 days of induction + 48 days treatment), the rats from all 7 groups were fasted overnight and sacrificed by cervical decapitation. Mammary gland were immediately excised from the rats and weighed. Small pieces of representative tumor tissue were fixed in 10% formalin for histopathological studies. Viable tumor tissue was then dissected free of grossly necrotic and hemorrhagic area and separated from connective tissue and normal tissue. Portions weighing approximately 500 mg were cut, placed into plastic snap-cap vials, and immediately frozen in liquid nitrogen. The vials were placed in a deep freezer at ~72°C and stored until assayed. At the time of assay, tissues were homogenized and used for biochemical assays.

Preparation of mitochondria from mammary gland

Mammary glands were removed and cooled in 0.3 M sucrose- ethylenediaminetetraacetic acid (EDTA), pH 7.4, on ice. All subsequent steps were done at 0–4°C. As much external connective tissue was removed as possible and the tissues were blotted dry, weighed, and minced very finely with small scissors. The tissue was then homogenized in a Potter-Elvehjem homogenizer using two passes with a loose pestle and two passes with a tight pestle. The homogenate was strained through four layers of 10-gauge cheesecloth and the filtrate was centrifuged at 900 g for 5 minutes. The supernatant was centrifuged at 11,500 g for 10 minutes to sediment the mitochondria. The mitochondrial pellet was washed twice with 5 ml of sucrose-EDTA medium. At each step, the upper fatty layer was carefully removed by aspiration and the walls of the tubes were carefully wiped clean. The final mitochondrial pellet was suspended at 10 mg mitochondrial protein per milliliter of sucrose-EDTA bovine serum albumin (BSA) medium. The purity of the mitochondria was assessed by estimating succinate dehydrogenase activity. Mitochondrial protein was estimated by the method of Lowry et al [21].

Preparation of mitochondria from tumor mammary gland

Tumor cells were identified by the appearance of a red mass under the epithelial layer of the mammary pad. Tumor mitochondria were isolated by the method of Senior et al [22]. A portion of tumor was removed carefully from the mammary pad and placed immediately in 0.3 M sucrose-EDTA, pH 7.4, at 0°C. All subsequent steps were done at 0–4°C. Necrotic tissue was removed and the tumor tissue was chopped into small pieces, weighed, and washed three times with 40 ml chilled sucrose-EDTA solution. The tumor tissue was then homogenized directly in five volumes of 0.3 M sucrose EDTA/1% BSA, pH 7.4, in a Potter-Elvehjem homogenizer. The homogenate was then centrifuged in the same way as the homogenate from a normal mammary gland (as above). BSA is necessary to obtain functional mitochondria from tumor.

Assays of carbohydrate-metabolizing enzymes

Hexokinase activity was measured with respect to the amount of glucose utilized after the addition of ATP [23]. Phosphoglucoisomerase activity was assayed according to the method of Horrocks et al [24]. Aldolase activity was assayed according to the method of King [25], with fructose 1,6-bisphosphate as a substrate and dinitrophenyl hydrazine as a coloring reagent. The activity of glucose-6-phosphatase and fructose 1,6-bisphosphatase [26] was assayed with respect to the amount of inorganic phosphorus liberated after the addition of their respective substrate, glucose-6-phosphate or fructose 1,6-bisphosphate. Serum lactate dehydrogenase (LDH) was assayed according to the method of King [27].

Mitochondrial Krebs cycle and respiratory chain enzyme assays

The activity of α-ketoglutarate dehydrogenase was assayed by the method of King [29]. The activity of α-ketoglutarate was assayed by the colorimetric
determination of ferrocyanide produced by the decarboxylation of α-ketoglutarate with ferricyanide as electron acceptor [29]. The activity of succinate dehydrogenase was assayed by the method of Slater and Borner [30], in which the rate of reduction of potassium ferricyanide was measured in the presence of potassium cyanide. The activity of malate dehydrogenase was assayed by the method of Mehler et al. [31]. The activity of nicotinamide dimonucleotide (NADH) dehydrogenase was assayed by the method of Minakami et al. [32]. The activity of cytochrome C oxidase was assayed by the method of Wharton and Tzagoloff [33].

Statistical analysis
Values are given as the mean ± standard deviation of six rats. The results were statistically evaluated using Student’s t-test using 16 Statistical Package for Social Sciences software and one-way analysis of variance. Values of p<0.05 were considered statistically significant.

RESULTS
Fig. 1 shows the initial and final body weights of the control and experimental rats. Initially, there was no significant change in body weight of the control and experimental rats. However, finally, there was a sharp drop in body weight of the mammary carcinoma-bearing rats when compared with the normal control rats. Drug-treated rats showed a gradual decrease in body weight compared with untreated rats. Drug control rats showed an increase in body weight, but the increase was not significant when compared with normal control rats.

Fig. 2 shows the activity of LDH in serum from control and experimental rats. LDH activity was found to be elevated significantly (p<0.05) in tumor-bearing rats compared with control rats. Drug-treated rats showed a gradual decrease in body weight compared with untreated rats. Drug control rats showed an increase in body weight, but the increase was not significant when compared with normal control rats.

The activities of the glycolytic enzymes, hexokinase, phosphoglucoisomerase, and aldolase, and the gluconeogenic enzymes, glucose-6-phosphatase and fructose-1,6-bisphosphatase, in the mammary gland of control and experimental rats are shown in Table 1. In tumor-bearing rats, the activities of the glycolytic enzymes were increased (hexokinase, phosphoglucoisomerase, aldolase), and the activities of the gluconeogenic enzymes were decreased (glucose-6-phosphatase, fructose-1,6-bisphosphatase) compared with mammary gland of control rats. The levels of glycolytic enzymes were significantly (p<0.05) decreased and the levels of gluconeogenic enzymes significantly increased in mammary gland tumor of drug-treated rats compared with untreated rats.

The activities of the mitochondrial Krebs cycle enzymes, isocitrate dehydrogenase, α-ketoglutarate dehydrogenase, succinate dehydrogenase, and malate dehydrogenase, and the respiratory chain enzymes, NADH dehydrogenase and cytochrome C oxidase, in the mammary gland (tumor and surrounding tissue) of control and experimental rats are shown in Table 2. The activities were significantly (p<0.05) decreased in both tumor tissue in carcinoma-bearing rats compared with control rats. Enzyme activities were increased in tumor tissue of rats treated with TD and PGG compared to untreated carcinoma-bearing rats. These enzyme activities were increased to a greater extent in rats treated with TD and PGG than in rats treated with CYC. In drug control rats, there was a slight increase in the activities of the mitochondrial enzymes but not to significant levels compared with normal rats.

DISCUSSION
The growth rate of mammary carcinoma cells and their carbohydrate metabolism are significantly correlated. Cancer cells generally have an elevated rate of glucose metabolism and abnormal pattern of energy metabolism compared to normal healthy cells. In the present study, we observed significantly higher activities of glycolytic enzymes in tumor of carcinoma-bearing rats. This indicates that an elevated rate of glycolysis is a characteristic of malignant cells. Through enhanced activity of key glycolytic enzymes and mitochondrial degradation, the rate of glycolysis in tumor cells is elevated to provide high levels of intermediates for enhanced synthesis of nucleic acids and lipids in rapidly proliferating tumor cells [34].

Hexokinase is the rate-limiting enzyme which catalyzes the conversion of glucose to glucose-6-phosphate in the first step of the glycolytic pathway. Hexokinase activity [35], m-RNA levels [36], and transcription rate [37] are increased markedly in rapidly growing tumors. To further potentiate the enhanced hexokinase activity achieved by overexpression, most of the enzyme is bound to the outer mitochondrial membrane, where it has direct access to mitochondrially generated ATP and is less sensitive to glucose-6-phosphate inhibition [38]. The proliferating cells undergo a shift from oxidative to glycolytic metabolism, where the energy requirements of the rapidly dividing cells are provided by ATP from glycolysis. This would direct mitochondrial

Fig. 1: Body weight changes in control and experimental animals. (Group I, control rats; Group II, tumor-induced; Group III, tumor-induced + TD treated; Group IV tumor-induced + 1,2,3,4,6-Penta-O-galloyl-β-D-glucose treated; Group V, 7,12-dimethylbenzeneanthracene-induced + CYC-treated; Group VI, control + Tridham; Group VII 1,2,3,4,6-Penta-O-galloyl-β-D-glucose + control). Comparison is made as a-Group I vs. Group II; b-Group II vs. Groups III-V; c-Group I vs. Groups VI and VII. Statistical significance: *p<0.05. NS: Non-significant.
Table 1: Activities of glycolytic and gluconeogenic enzyme in tumor tissue of control and experimental rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
<th>Group V</th>
<th>Group VI</th>
<th>Group VII</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexokinase</td>
<td>9.89±0.69</td>
<td>13.48±1.12</td>
<td>13.87±1.18</td>
<td>10.14±0.81</td>
<td>10.04±1.38</td>
<td>13.94±1.08</td>
<td>14.53±0.39</td>
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<tr>
<td>Phosphoglucomerase</td>
<td>387.1±29.1</td>
<td>19.6±1.68</td>
<td>21.36±1.82</td>
<td>7.79±0.54</td>
<td>10.0±3.95</td>
<td>12.81±0.98</td>
<td>15.0±3.95</td>
</tr>
<tr>
<td>Aldolase</td>
<td>13.87±1.18</td>
<td>19.6±1.68</td>
<td>21.36±1.82</td>
<td>7.79±0.54</td>
<td>10.0±3.95</td>
<td>12.81±0.98</td>
<td>15.0±3.95</td>
</tr>
<tr>
<td>Glucose-6-phosphatase</td>
<td>13.87±1.18</td>
<td>19.6±1.68</td>
<td>21.36±1.82</td>
<td>7.79±0.54</td>
<td>10.0±3.95</td>
<td>12.81±0.98</td>
<td>15.0±3.95</td>
</tr>
<tr>
<td>Fructose</td>
<td>13.87±1.18</td>
<td>19.6±1.68</td>
<td>21.36±1.82</td>
<td>7.79±0.54</td>
<td>10.0±3.95</td>
<td>12.81±0.98</td>
<td>15.0±3.95</td>
</tr>
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</table>

Values are expressed as nmol/minute/mg protein, means±SD, n=6. (Group I, control rats; Group II, tumor-induced; Group III, tumor-induced+TD-treated; Group IV tumor-induced+PGG-treated; Group V, DMBA-induced+CYC-treated; Group VI, control+TD; Group VII PGG+control). Comparison is made as a-Group I vs. Group II; b-Group II vs. Groups III-V; c-Group I vs. Groups VI and VII. Statistical significance: *p<0.05. NS: Non-significant. SD: Standard deviation

Table 2: Activities of mitochondrial Krebs cycle and respiratory chain enzyme in the mammary gland tumor tissue of control and experimental rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I</th>
<th>Group II</th>
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<th>Group VI</th>
<th>Group VII</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isocitrate dehydrogenase</td>
<td>538.8±35.1</td>
<td>307.1±29.1</td>
<td>472.1±27.8</td>
<td>491.2±31.8</td>
<td>501.2±31.8</td>
<td>530.9±37.2</td>
<td>532.9±37.2</td>
</tr>
<tr>
<td>α-Ketoglutarate</td>
<td>161.7±12.8</td>
<td>92.5±8.21</td>
<td>138.3±17.8</td>
<td>130.2±9.9</td>
<td>125.2±10.9</td>
<td>160.6±12.3</td>
<td>159.6±12.3</td>
</tr>
<tr>
<td>Succinate dehydrogenase</td>
<td>236.4±18.8</td>
<td>142.1±13.5</td>
<td>212.5±14.3</td>
<td>206.2±17.0</td>
<td>201.2±17.0</td>
<td>235.3±18.5</td>
<td>234.3±19.4</td>
</tr>
<tr>
<td>Malate dehydrogenase</td>
<td>3581±19.8</td>
<td>2316±1411</td>
<td>3213±1022</td>
<td>3198±1055</td>
<td>2989±1022</td>
<td>3499±124</td>
<td>3489±121</td>
</tr>
<tr>
<td>NADH–dehydrogenase</td>
<td>15.87±0.93</td>
<td>8.79±0.55</td>
<td>12.85±0.92</td>
<td>12.38±0.96</td>
<td>12.11±0.86</td>
<td>15.02±1.09</td>
<td>15.13±1.12</td>
</tr>
<tr>
<td>Cytochrome c oxidase</td>
<td>5.35±0.12</td>
<td>1.98±0.07</td>
<td>4.12±0.12</td>
<td>3.99±0.17</td>
<td>3.89±0.16</td>
<td>4.91±0.21</td>
<td>4.87±0.23</td>
</tr>
</tbody>
</table>

The data presented are the amounts of the following (mean±SD, n=6): α-ketoglutarate liberated (nmol/minute/mg protein) for isocitrate dehydrogenase; ferrocyanide liberated (mM/minute/mg protein) for α-ketoglutarate dehydrogenase; succinate oxidized (mM/minute/mg protein) for succinate dehydrogenase; NADH oxidized (mM/minute/mg protein) for malate dehydrogenase; NADH oxidized (μmol/minute/mg protein) for NADH dehydrogenase; cytochrome c oxidase (optical density 410nm/minute/mg protein) [Group I, control rats; Group II, tumor-induced; Group III, tumor-induced+TD-treated; Group IV tumor-induced+PGG-treated; Group V, DMBA-induced+CYC-treated; Group VI, control+TD; Group VII PGG+control]. Comparison is made as a-Group I vs. Group II; b-Group II vs. Groups III-V; c-Group I vs. Groups VI and VII. Statistical significance: *p<0.05. NS: Non-significant. SD: Standard deviation

Fig. 2: Activity of serum lactate dehydrogenase in control and experimental animals. (Group I, control rats; Group II, tumor-induced; Group III, tumor-induced+Tridham treated; Group IV tumor-induced + 1,2,3,4,6-Penta-galloyl-β-D-glucose treated; Group V, 7,12-dimethylbenzanthracene -induced + CYC-treated; Group VI, control + Tridham; Group VII 1,2,3,4,6-Penta-galloyl-β-D-glucose + control). Comparison is made as a-Group I vs. Group II; b-Group II vs. Groups III-V; c-Group I vs. Groups VI and VII. Statistical significance: *p<0.05. NS: Non-significant

ATP preferentially to glucose-6-phosphate synthesis and therefore would be expected to increase the biosynthetic pentose phosphate pathway. Hence, the glycolytic capacity of cancer cells depends totally on hexokinase activity for its metabolic fuel [35]. Fanciulli et al. [39] have demonstrated that increased hexokinase activity may be not only the consequence of altered metabolic requirements of cancer cells but also a modification per se to increase mitotic activity. Hence, alteration in hexokinase activity is a potential target for arresting tumor cell growth. Hennipman et al. [40] have also reported higher activity of hexokinase in breast cancer tissue. In this study, the observed increase in activity of hexokinase in tumor of animals with mammary carcinoma might have been due to the increased metabolic need of proliferating tumor cells for energy. Administration of TD and PGG to mammary carcinoma-bearing rats significantly reduced the enzyme activity, and this may be a positive indication of an antitumor effect.

Phosphoglucomoisomerase serves as a good index of tumor growth and is significantly elevated in cancerous animals. In agreement with our study, Campbell and King [41] have reported that phosphoglucomoisomerase is an indicator of metastatic growth and is elevated in patients with neoplasms, especially after metastasis. Aldolase, another key enzyme in the glycolytic pathway, has been found to be elevated in tumor-bearing animals and breast cancer [42]. The elevated activity of phosphoglucomoisomerase and aldolase may be due to cell impairment and necrosis. The activities of gluconeogenic enzymes such as glucose-6-phosphatase and fructose-1,6-bisphosphatase are inhibited significantly in tumor of carcinoma-bearing animals. This may be due to the higher lactic acid production of neoplastic tissue and it has been proved that tumor cells are able to utilize a large quantity of lactate for glycolysis in the form of glucose [via gluconeogenesis] and protein synthesis [43].

LDH is a tetrameric enzyme and is recognised as a potential tumor marker, especially for solid tumors in assessing the progression of proliferating malignant cells [44]. Elevation of serum LDH activity is common in myocardial infarction, hepatitis, and neoplastic disease [45]. The elevated activity of LDH may be due to over production by tumor cells, or it may be due to the release of isoenzymes from destroyed tissues [46]. Our findings are also consistent with the above reports. Lactate has been shown to progressively accumulate with increasing tumor burden [47]. The elevated LDH activity may be related to the increase in tissue lactate content with increase in tumor size. Treatment with TD and PGG caused a significant decrease in LDH activity. This clearly indicates the antitumor activity of the drug.

Drug-treated animals showed a significant drop in the activity of glycolytic enzymes and a concomitant elevation in the gluconeogenic enzymes and a concomitant elevation in the gluconeogenic enzyme activities.
enzymes in the mammary carcinoma cells, which indicates that the cells are converted from anaerobic to aerobic metabolism (i.e. tumor cells converted to normal cells). This modulation may be due to the antitumor activity of the drug either by inhibiting the glycolytic enzyme activities or by the suppression of tumor progression. The exact mechanism of carbohydrate metabolism altering the effect of TD and PGG remains obscure. However, Deshpande et al. [48] have proved that TD and PGG, by altering carbohydrate metabolism-related enzymes, retain malignant cells in the G1/G0 phase of the cell cycle. This process may inhibit tumor growth and hence lead to prolongation of disease-free survival.

In the present study, mammary carcinoma-bearing animals showed decreased activities of mitochondrial Krebs cycle and respiratory chain enzymes in tumor tissue of the mammary gland compared with normal control rats. Reductions in the activities of Krebs cycle and respiratory chain enzymes prove a defect in aerobic oxidation of pyruvate which might cause the low production of ATP molecules. Cancer cells have been reported to cause tissue hypoxia where there is increased oxygen demand. In hypoxia, mitochondrial Krebs cycle and respiratory chain enzyme activities are expected to be low [49]. Decreased activity of these enzymes might be due to alteration in the morphology and ultrastructure of cancer cells and the ability of mitochondria to undergo metabolic changes compared with normal cells, and furthermore, the number of mitochondria is drastically reduced in tumor cells [50]. The decrease in mitochondrial content might be due to the marked deficiency in one or more electron transport chain compounds. After treatment with TD and PGG, these enzymes reverted to near-normal levels.

The major mechanism by which cancer cells revert to aerobic metabolism and thus normal cells has three basic stages: Enhancement of the Krebs cycle, enhancement of the respiratory chain enzyme activities and enhancement of oxygen transport to the cells. On treatment with the combination therapy, the activities of these enzymes were significantly increased, which clearly indicates the reversal of cancer cells to normal ones. One would expect the anaerobic metabolism to cause breast cancer due to the lack of core enzyme Q10 and when it is replenished, anaerobic metabolism to restart and cancer cells to turn back into normal cells [50].

Mitochondria are involved in a variety of processes of which oxidative phosphorylation is the most important. Detoxification of oxygen via its reduction to H2O by the cytochrome oxidase system takes place in the mitochondria. Cytochrome C oxidase and NADH dehydrogenase are the enzymes involved in the electron transport chain and are located in the inner mitochondrial membrane. The process is ultimately linked to the production of useful energy-rich compounds such as ATP [51]. In this study, we observed a decrease in the activities of mitochondrial respiratory chain enzymes in mammary gland tumor of rats and these activities were significantly enhanced upon treatment with TD and PGG.

The surrounding normal tissue of the mammary glands showed lower activities of glycolytic, Krebs cycle, and respiratory chain enzymes and higher activities of gluconeogenic enzymes. This may have been due to an inadequate supply of glucose to the normal surrounding tissues. Usually, malignancies that commonly display a high rate of glycolysis exhibit a low rate of gluconeogenesis, Krebs cycle, and respiratory chain enzymes. The metabolic complications of this situation are sufficient to provide a cachexic mechanism [52]; in the anaerobic breakdown of glucose to lactic acid in tumors, two ATP molecules per glucose molecule are yielded net to the tumor, but synthesis of glucose from the resulting lactic acid (or from any other precursor) via gluconeogenesis requires the utilization of the equivalent of at least six ATP molecules derived from normal host sources. In the Cori cycle per se, the equivalent of at least 14 ATP molecules is, therefore, lost from the body economy with each specific recycling (based on two equivalents of lactate being recycled to glucose). 2 ATP molecules to the cancer cell and 12 ATP molecules from normal host tissues. As the tumor enlarges, consuming ever increasing amounts of glucose, vast energy reserves from the host can be depleted in maintaining the circuit of glucose presentation to the malignant cell, conversion of lactic acid and other precursors to glucose in host cells, and representation of the resulting glucose to the cancer cells. This systemic “metabolic circuit,” which is characterized by a malignancy’s utilization of glucose to produce relatively small amounts of energy for its own needs at the expense of relatively large amounts of energy from the host, gives every indication of being an operational biochemical mechanism for the production of cancer cachexia [52,53].

The functional significance of glycolysis in cancer appears therefore to be twofold: As a source of energy production (growth) for the tumor and as a source of lactate that initiates a progressive energy loss (cachexia) in the host through marked stimulation of gluconeogenesis. Inhibition of gluconeogenesis would not only cause inhibition of energy loss leading to cachexia but also, if tumor energy gain and host energy loss are functionally interrelated, as seems probable, provide a possible means of inhibiting tumor growth itself. The above-mentioned changes returned to their respective control limits in drug-treated animals. This may be assumed as the TD and PGG prevented host body weight loss by enhancing ATP production via the electron transport chain.

CONCLUSION

The overall projected mechanism of action being an inhibition of host energy loss (cachexia) caused by a directly augmented pathway of gluconeogenesis in cancer, the drug-treated animals showed a significant increase in body weight compared to the untreated tumor-induced animals. On the other hand, in tumor, the anticarcinogenic and apoptotic effects of TD and PGG inhibited cancer cell proliferation and led to apoptosis. No significant changes were observed in drug control animals, clearly indicating that the drug does not show any deleterious side effect. It may be assumed that the TD and PGG not only suppressed tumor growth but also enhanced ATP production in the host, thereby preventing cancer cachexia. Hence, TD and PGG could be of major therapeutic value.

REFERENCES