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Effect of Protein Depletion on Host and Tumor Response to Paclitaxel in Experimental Animals

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Abstract: The present study is aimed to examine the possible effects of Protein Malnutrition (PM) on the therapeutic activity and toxicity of paclitaxel in mice implanted with Ehrlich carcinoma cells. Mice that were fed either with standard or low protein diets were treated with a single dose of paclitaxel (10 mg kg\(^{-1}\), i.p.). Paclitaxel administration increased the tumor growth delay of Ehrlich carcinoma from 2.8 days in protein deficient animals to 4.9 days in normal feeding mice and this represented about 43% increase in tumor growth delay. Furthermore, protein deficiency also interfered with the antitumor activity of paclitaxel. The percent survival of tumor-bearing mice after paclitaxel treatment were 56 and 19% in normal fed and protein deficient animals, respectively. Moreover, Paclitaxel administration increased the serum level of creatine phosphokinase isoenzyme (CPK-MB) in both groups, with maximum effect appeared after 48 h. Seventy two hours later, the levels was reduced to the normal values in normal fed animals while in protein malnourished mice, the activity was found to be high. In addition, paclitaxel administration significantly increased the plasma histamine concentration after 10 min and persisted for 120 min in animals on protein malnourished diet, however, in animals on a normal fed diet, histamine concentration reached the normal level after 120 min of paclitaxel administration. In conclusion, Paclitaxel administration exerts its toxic effects on both protein malnourished and normal feeding animals, however, its toxicity is enhanced and therapeutic activity is reduced in the protein deficient animals.

Key words: Malnutrition, toxicity, paclitaxel, Ehrlich carcinoma

INTRODUCTION

The progressive protein malnutrition so frequently encountered in cancer patient is often referred to as cachexia (Puccio and Natanson, 1997). It has been noted that lean mass and visceral protein depletion are characteristics of patient with cancer cachexia and the degree of deletion may be associated with reduced survival (Nixon et al., 1980; Rosenbaum et al., 2000). The nutritional status of patients diagnosed with cancer entering the treatment process varies from patient to patient. Not everyone begins therapy with anorexia, weight loss and other symptoms of nutritional deficiency. For patients who have such symptoms, however, anticancer therapies can complicate the treatment and expected recovery (Holder, 2003; Mantovani et al., 2008).

Paclitaxel (tax-11-en-9-one,\(5\beta,20\)-epoxy-1,2\(\alpha\),4,7\(\beta\),13\(\alpha\)-hexahydroxy-4,10-diacetate-2-benzoate-13\(\beta\)-(benzoylanino)-\(\alpha\)-hydroxyl-benzenepropionate,\(C_{42}H_{57}NO_{14}\)) is the prototype of the taxane class of compounds, it is a diterpine plant product obtained from the needles and bark of the Western yew, Taxus Brevifolia (Wani et al., 1971). It has been described as the most active single agent evaluated by the Eastern Cooperative Oncology Group (ECOG) in advance non small lung cancer (NSCLC). The relation between malnutrition and the response to toxicity to chemotherapy is not very well known, so, the aim of this study was designed to assess the possible effects of Protein Malnutrition (PM) on the therapeutic activity and toxicity of paclitaxel in experimental animals implanted with Ehrlich ascites carcinoma cells.

MATERIALS AND METHODS

This study has been conducted during the period between 2004-2008. The study is partly conducted in Pharmacology Unit, National Cancer Institute, Cairo University and partly in Pharmacology Department,

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**Animals:** Female Swiss albino mice weighing 20-25 g were obtained from the Animal House of National Cancer Institute, Cairo University. Animals were allowed free access to standard diet and water ad libitum. Animal treatment protocol has been approved by the ethical and animal care committees of National Cancer Institute before starting the experiments.

**Drug:** Paclitaxel (Bristol-mayers Squibb Co., USA) was obtained from drug store, National Cancer Institute (NCI), Cairo University. Paclitaxel was supplied in vial contain 5 mL clear colourless viscous solution containing 30 mg paclitaxel formulated in 50% polyethylene lactate castor oil and 50% dehydrated ethanol (cremophor EL). All other chemicals used were of the highest analytical grade.

Ehrlich ascites carcinoma cell line was obtained from National Cancer Institute, Cairo University. The cell line was maintained in our center by weekly transplantation in female albino mice by serial i.p. passage at 7-10 days intervals.

**Dietary treatments:** Animals used in this study were maintained for 3 weeks on synthetic diets containing standard levels of all the required components (Bamji and Sharada, 1972). Two types of diet were utilized depending on the amount of casein, standard diet containing 20% casein and low protein diet contain 5% casein.

**Determination of serum total protein:** One hundred and thirty two female Swiss albino mice previously subjected to 2 kinds of nourishment (as mention before) were used in this study. Six mice from each group were sacrificed after 3 weeks feeding; blood was collected to obtain serum. Serum total protein and serum albumin were measured to ensure occurrence of protein malnutrition as described by Weissman et al. (1950) and Dumas et al. (1971), respectively.

**Tumor growth delay:** Eighty female Swiss albino mice divide into 2 groups one group (40 mice) maintained on standard diet, whereas, the other group (40 mice) were maintained on low protein diet for three weeks. Ehrlich carcinoma cells (2×10³) were transplanted S.C. in the right thigh of each animal. When the tumor were approximately 100 mm³ as measured by Caliper, the tumor bearing mice were classified into 4 equally groups (20 mice each). The first group maintained on standard diet and injected with a single dose of cremophor EL in normal saline (0.2 mL/20 g body weight) and served as normally fed control group. The second group maintained on a low protein diet and injected with a single dose of the same vehicle (0.2 mL g⁻¹ body weight) and served as protein deficient control group. The thirds and fourth group were injected with a single dose of paclitaxel (10 mg kg⁻¹, i.p.) and maintained on standard protein or on low protein diet, respectively.

The tumor volume of each animal was measured by Vernier Caliper (Optilab, Berlin, Germany) every other day until reached a volume of 500 mm³. Change in the tumor volume were determined by the following formula:

\[
\text{Tumor volume (mm}^3) = \frac{4\pi \cdot A/2^2 \cdot B/2}{3} = 0.52 \cdot A^2 \cdot B
\]

where, A is the minor tumor axis and B is the major axis (Osman et al., 1993).

**Survival of tumor-bearing mice:** Animals were grouped, inoculated i.p. by Ehrlich ascites cells (2×10⁶ cells) and treated as mentioned in paragraph 2.5 and observed for 45 days. Mean survival time and long term survivor were assessed up to the end of the experiment.

**Determination of CK-MB:** Mice from the normally-fed and protein deficient were equally divided into four groups and injected with paclitaxel (10 mg kg⁻¹, i.p.).

Six animals from normally-fed and from protein malnourished group were sacrificed at 24, 48 and 72 h after paclitaxel treatment. Blood samples were obtained from ophthalmic artery in the orbital rim prior to animal sacrifice. Serum was isolated from blood samples and used for CK-MB assay as described by Wu and Bowers (1982).

**Determination of White blood cells:** Another six animals from each group were sacrificed 72 h after treatment. Blood was collected on heparinized test tubes and blood white cells were counted using couter counter.

**Determination of plasma histamine concentration:** Plasma was obtained from 6 sacrificed animals from each group. Plasma was extracted by n-butanol to determine histamine concentration using a spectrophotometer (Perkin Elmer) according to the method of Shore et al. (1959).

**Statistical analysis:** Data were presented as Mean±SEM. Multiple comparisons were achieved by one way ANOVA followed by Tukey Kramer as post ANOVA test. p values of 0.05 or less were considered significant.
RESULTS

Serum protein and albumin concentration: Animals on a protein malnutrition diet for 21 days had reduced serum protein and albumin concentration to 68 and 54%, respectively compared to animals fed a standard diet (Table 1).

Antitumor activity: Data presented in Fig. 1 and Table 2 indicate that mice when fed a protein deficient diet prior to tumor inoculation and then treated with paclitaxel (single dose of 10 mg kg⁻¹, i.p.) produced tumor growth delay amounting to 2.8±0.7 days. In contrast, mice fed a normal diet and treated with the same dose of paclitaxel exhibited 4.9±0.2 days delay in tumor growth.

Protein malnutrition also resulted in poor survival rate after paclitaxel treatment. Data are shown in Table 2 that demonstrated mean survival time was 17.5 days for tumor bearing mice fed a protein deficient diet. However, the mean survival rate was 25.6 days for tumor-bearing mice that were fed on a standard diet.

Cardio- and hematotoxic activity: Twenty four hours after paclitaxel administration (10 mg kg⁻¹, i.p.) there was a significant increase in the serum CK-MB activity in both mice fed either a protein deficient diet or on a standard diet by 83 and 55%, respectively. However, after 72 h of drug treatment, further increase in CK-MB activity was observed in mice fed a protein deficient diet whereas levels of CK-MB remain unchanged in mice fed a standard diet (Table 3).

White blood cells count were reduced after 72 h of paclitaxel treatment by 74% and 69% in mice fed a protein deficient or a standard diet, respectively (Fig. 2).

Table 1: Effect of protein deficiency (three weeks feeding) on the serum protein and albumin concentration in mice.

<table>
<thead>
<tr>
<th>Concentration (g/L⁻¹)</th>
<th>Animals on standard diet</th>
<th>Animals on protein deficient diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin concentration</td>
<td>2.67±0.06⁹⁺⁺</td>
<td>1.24±0.08⁹⁺⁺</td>
</tr>
<tr>
<td>Total protein concentration</td>
<td>5.9±0.025²⁺⁺</td>
<td>1.9±0.12²⁺⁺</td>
</tr>
</tbody>
</table>
*Data is expressed as Mean±SEM of six animals, **Significantly different from corresponding control at p<0.05

Table 2: Effect of paclitaxel treatment (10 mg kg⁻¹, i.p.) on the survival of tumor-bearing mice.

<table>
<thead>
<tr>
<th>Groups</th>
<th>MST±SEM (days)</th>
<th>LTS (Long term survivor)</th>
<th>Tumor growth delay (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normally-fed</td>
<td>23.3±1.8</td>
<td>0.16</td>
<td></td>
</tr>
<tr>
<td>EC-bearing mice</td>
<td>17.5±1.7</td>
<td>0.16</td>
<td></td>
</tr>
<tr>
<td>Protein-deficient</td>
<td>25.6±1.6</td>
<td>9.16</td>
<td>4.9±0.2</td>
</tr>
<tr>
<td>Paclitaxel</td>
<td>18.1±1.8</td>
<td>3.16</td>
<td>2.8±0.7</td>
</tr>
</tbody>
</table>

*(Normal)-fold and protein deficient Ehrlich carcinoma bearing mice (ECE) were treated with paclitaxel (10 mg kg⁻¹, i.p.) and Mean Survival Time (MST), long term survivor and tumor growth delay were determined as described in the methods and materials. Groups having the same symbolic letters in the same column are significantly different from each other at p<0.05 using ANOVA followed by Tukey-Kramer as post ANOVA test.

Fig. 1: Effect Paclitaxel administration (10 mg kg⁻¹, i.p.) on tumor volume of Ehrlich carcinoma bearing mice in normally-fed and protein deficient animals. NF: Normally-fed group, PM: Protein malnourished group. Significantly different from normally-fed mice at p*=0.5

Fig. 2: Effect of single i.p. dose of paclitaxel (10 mg kg⁻¹) on the white blood cells of NF and PM animals 72 h after treatment. Significantly different from corresponding control at p*<0.05 using ANOVA followed by Tukey Kramer as post ANOVA test.
Clinical outcomes and quality of life. The main objective of this study was to investigate the possible modulatory effect of protein deficiency on the anticancer activity and the toxicity of one of the most active drugs (paclitaxel) which is used for treating different types of cancer. We performed a nutritional assessment in all animals and our results clearly indicate a decrease in both total protein and albumin concentrations in animals after feeding a protein deficient diet for 21 days (Table 1). Paclitaxel administration in protein deficient tumor-bearing animals did not result in reduction of the tumor volume. In contrast, administration of paclitaxel in normally fed tumor-bearing animals resulted in a significant decrease in tumor volume (Fig. 1). The poor response to paclitaxel in protein deficient animals may be secondary to depressed tumor cell metabolism and replication rate which resulted in a clear difference in tumor growth between protein deficient diet and standard diet fed animals, since taxol is a cell cycle specific drugs and to be active the cells must be in highly proliferating stages Hsu (2008). The tumor growth was delayed (4.9 days) in animals on a standard diet than those on a protein deficient treatment (2.8 days). Furthermore, more animals survived (56%) after paclitaxel treatment when fed a standard diet than those fed a protein deficient diet (19%) (Table 2). The weight loss and protein malnutrition in patients with cancer have been identified as an important factor for survival (Dewys et al., 1980; Costa et al., 1981). Nutritional status play an important role in survival during cancer progression as well as during cancer treatment. It is found that survival in non small cell lung cancer patients receiving chemotherapy was improved after addition of hydralazine sulfate which improved their nutritional status (Chlebowski et al., 1990).

A significant elevation of serum CK-MB activity (a marker of cardiotoxicity) was observed 24 h after paclitaxel administration and persisted till 72 h in protein deficient animals, whereas, in animals on a standard diet, Paclitaxel administration did not show any significant elevation of CK-MB. Paclitaxel treatment is known to increase the probability to open the L-type calcium channels in intact cardiac cells, which may lead to an increase in intracellular calcium level, as a result it cause cardiac cell damage and contraction failure (Gali and Defelice, 1994). Our results revealed that paclitaxel induced more pronounced cardiotoxicity in protein deficient animals. Paclitaxel administration also affected total white blood cells and histamine liberation, in both protein deficient and normally-fed animals. However, more pronouncing effect was observed in protein-deficient animals which showed 85 fold increase in histamine liberation compared with 26 fold increase in normal group.
(Fig. 3). It is not clear during present investigation how protein malnutrition affects paclitaxel toxicity in this manner. There are no data or clinical trials that could be compared or support our findings. It is possible that paclitaxel free and bound ratios may play a role in its toxicity. It is known that 97% of paclitaxel binds to plasma protein mainly albumin (Kumar et al., 1993) and since albumin content in the protein deficient animals was decreased (Table 1), thus the free fraction of paclitaxel will be high in this group leading to an increased free form that results in enhanced toxicity. In conclusion, paclitaxel administration exerts enhanced toxic effects in protein deficient animals compared to animals fed a standard well balanced diet. Furthermore, the therapeutic activity was lower in the protein deficient animals.

REFERENCES


