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Protein Undernutrition in Tumor-Bearing Mice, Response and Toxicity to Paclitaxel

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Abstract: Malnutrition involving protein deficiency, which commonly occurs in cancer patient receiving anticancer drugs, is considered to be a risk factor for the development of toxicity. Therefore, current study is directed to investigate the possible effects of protein deficient diet on the therapeutic activity and toxicity of paclitaxel in mice bearing Ehrlich ascites carcinoma cells. Administration of a single dose of Paclitaxel (10 mg kg^{-1} , i.p.) decreased the tumor volume significantly in normally-fed animal (23%), while animals on deficient protein diet, the tumor volume decreased non significantly amounting to 13%. The long term survivors were increased by 58 % in normally fed tumor bearing animals compared to only 20 % in protein malnourished bearing animals after treatment with the same dose of paclitaxel. Moreover, 24 h after paclitaxel administration the serum level of lactic dehydrogenase activity significantly increased by 42% in protein deficient animals in compare to 12% in normally-fed ones. The activity of lactic dehydrogenase return to normal level 72 h after paclitaxel administration in normally fed-animals. In contrast, animals on protein deficient diet the activity still high (40%). In conclusion, paclitaxel administration exerts less therapeutic activity and more toxic effects in protein malnourished animals compared with normally-fed animals.

Key words: Protein malnutrition, tumor, anticancer activity, toxicity, paclitaxel

INTRODUCTION

Cancer cachexia and malnutrition decrease the quality of life of the affected individuals (Puccio and Nathanson, 1997). 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 the expected recovery (Holder, 2003; Mantovani *et al.*, 2008). Moreover, There is a clinical evidence that malnutrition is a risk factor for chronic anticancer toxicity (Olson *et al.*, 1988). Osman *et al.* (2009) reported increase toxicity of anticancer drugs in protein malnourished animals bearing tumor cells.

Paclitaxel (tax-11-en-9-one, 5β , 20-epoxy-1, 2α , 4, 7β , 13α -hexahydroxy-4, 10-diacetate-2 benzoate- 13β -(benzoylamino)- α -hydroxyl-benzenepropionate, $C_{47}H_{51}NO_{14}$) 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 Easter Cooperative Oncology Group (ECOG) in advance Non Small Lung Cancer (NSCLC). Paclitaxel causes several types of cardiac rhythm disturbance (Rowinsky *et al.*, 1991) in addition to a transit asymptomatic bradycardia (McGuire *et al.*, 1989) The relation between malnutrition

and the response and toxicity to chemotherapy is not very well known, so, the aim of this study was designed to asses 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 period between 2009-2010.

Animals: Female Swiss albino mice weighing 20-25 g were obtained from animal house of King Fahad Research Center, King Abdulaziz 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 King Abdulaziz University before starting the experiments.

Drug: Paclitaxel (Bristol-mayers Squibb Co., USA) was obtained from drug store, King Abdulaziz Hospital. Paclitaxel was supplied in vial contain 5 mL clear colorless viscous solution containing 30 mg paclitaxel formulated in 50% polyethoxylated 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 observed for a period of one week to ensure that they are in good health prior to their inclusion in the study. They were enter then into the protocol receiving either standard diet containing 20% casein or modified diet containing 5% casein for 4 weeks according to Bamji and Sharada (1972) with slight modification (Cusack *et al.*, 1992).

Determination of serum total protein: One hundred-thirty two female Swiss albino mice previously subjected to 2 kinds of nourishment were used in this study. Six mice from each group were sacrificed after 4 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 Lowry *et al.* (1951) and Dumas *et al.* (1971), respectively.

Tumor growth delay: Eighty female Swiss albino mice divided 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 (2×10^6) were transplanted S.C. in the right thigh of each animal. When, the tumor were approximately 100 mm^3 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 E1 in normal saline ($0.2 \text{ mL}/20 \text{ g b.wt.}$) 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 ($0.2 \text{ mL g}^{-1} \text{ b.wt.}$) 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 a Vernier Caliper (Optilab, Berlin, Germany) every other day until reached a volume of 500 mm^3 . Change in the tumor volume were determined by the following formula:

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

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 (2×10^6 cells) and observed for 45 days. Mean survival time and long term survivor were assessed up to the end of the experiment.

Determination of LDH: 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 LDH assay as described by Wacker *et al.* (1956).

Statistical analysis: Data were presented as Mean \pm SEM. Multiple comparison were achieved by one way ANOVA followed by Tukey Kramer as post ANOVA test. The $p < 0.5$ were considered significant.

RESULTS

Effect of different concentration of casein treatment: Mice on 5% casein diet for 28 days had reduced serum protein and albumin significantly by 67 and 53%, respectively compared to animals fed on standard diet (20% casein) (Table 1). Moreover, body weight of the malnourished animals have been decreased significantly by about 38% after 4 weeks feeding with diet contain 5% casein (Table 2). Treatment of protein malnourished animals with paclitaxel (single dose of 10 mg kg^{-1} , i.p.) showed about 60% decrease in serum protein content compared to only 4% in normally fed animals one week

Table 1: Effect of protein malnutrition (four weeks feeding) on the serum total protein and albumin concentrations in mice

Groups	Albumin concentration (g dL ⁻¹)	Total protein concentration (g dL ⁻¹)
Normally-fed	2.70 \pm 0.05	6 \pm 0.03
Protein malnourished	1.24 \pm 0.09 [#]	2 \pm 0.10 [#]

Data is expressed as Mean \pm SE of six animals. [#]Significantly different from normally-fed mice at $p < 0.05$

Table 2: Effect of protein malnutrition (four weeks feeding) on body weight of Swiss albino mice

Time	Body weight (g)	
	Normally-fed	Protein malnourished
Initial weight	21.0 \pm 0.33	21.00 \pm 0.40
One week	21.8 \pm 0.29	20.20 \pm 0.26
Two weeks	22.0 \pm 0.30 ^a	15.90 \pm 0.38 ^a
Four weeks	24.0 \pm 0.60 ^a	13.20 \pm 0.41 ^a

Data is expressed as Mean \pm SE. Mean values having the same symbolic letters in the same row are significantly different from each other at $p < 0.05$ ANOVA followed by Tukey Kramer as post ANOVA test

Table 3: Effect of paclitaxel (single dose of 10 mg kg⁻¹, i.p.) on serum total protein and serum albumin concentrations in normally-fed and protein malnourished Swiss albino mice

Parameters (g dL ⁻¹)	Control		Paclitaxel	
	NF	PM	NF	PM
Total protein	5.4±0.2 ^{ab}	1.3±0.06 ^{cd,e}	5.2±0.23 ^{cd}	0.52±0.04 ^{b,c,e}
Albumin	2.7±0.05 ^{ab,c}	1.1±0.1 ^{b,e,f}	2.4±0.05 ^{ab,d,e}	0.67±0.05 ^{d,f}

Total protein and albumin concentration is determined one week after drug administration. Data is expressed as mean±standard error of six animals. Mean values having the symbolic letters in the same row are significantly different from each other at p<0.05 using ANOVA followed By Tukey Kramer as post ANOVA test

Table 4: Effect of Paclitaxel (10 mg kg⁻¹, i.p.) on the percentage survival of Ehrlich ascites carcinoma-bearing mice

Groups	MST±SE (Days)	CLS (%)	LTS	Tumor growth delay (days)
Normally-fed	23.1±1.9	-	0/16	
EAC-bearing control				
Protein-malnourished	17.5±1.77 ^a	-	0/16	
EAC-bearing mice				
Paclitaxel (NF)	25.60±1.62 ^{ab}	9.6	9/16	4.9±0.2 ^a
Paclitaxel(PM)	18.10±1.86 ^b	3.6	3/16	2.8±0.7 ^a

MST: Mean survival time, CLS: Percentage change in life span = T-C/C x100, where T = mean survival time of treated EAC-bearing mice and C = mean survival time of control untreated EAC-bearing mice. LTS: long term survivors (mice survived to the end of the experiment (45 days). 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

Table 5: Effect of paclitaxel administration (10 mg kg⁻¹, i.p.) on tumor volume in normally fed and protein malnourished Swiss albino mice

Days after paclitaxel administration	Tumor volume (mm ³)*			
	Control		Paclitaxel	
	NF	PM	NF	PM
1	109±4.5	110±4.8	89±4.8	97±5
15	1306±69 ^{b,c}	980±12 ^b	1005±57 ^a	864±28 ^c
28	2893±11 ^{a,b,c}	2373±24 ^b	2278±10 ^a	2128±48 ^c

*Mean±SE. Mean values having the same symbolic letters in the same row are significantly different from each other at p<0.05 using ANOVA followed by Tukey Kramer as post ANOVA test

after drug administration. However, serum albumin in protein malnourished and normally fed animals showed 40 and 11% decrease, respectively (Table 3). Tumor volume was decrease by 10 %, 28 days after paclitaxel treatment in malnourished bearing animals while, normally fed bearing mice showed 20% decrease (Table 5).

Cytotoxic activity of paclitaxel: Administration of a single dose of paclitaxel (10 mg kg⁻¹, i.p.) in tumor bearing mice increased the tumor growth delay amounting to 4.9 in normally-fed animals. In contrast, animals fed with a protein deficient diet treated with the same dose of paclitaxel exhibited 2.8 days delay in tumor growth (Table 4). On the other hand, the long term survivors were increased by 58% in normally fed tumor bearing animals compared to only 20% in protein malnourished bearing animals after paclitaxel administration (Table 3, Fig. 1).

Table 6: Effect of paclitaxel (10 mg kg⁻¹, i.p.) on serum LDH activity in normally-fed and protein malnourished Swiss albino mice

Time after paclitaxel administration (h)	LDH activity (μ L ⁻¹)*			
	Control		Paclitaxel	
	NF	PM	NF	PM
24	2187±110 ^b	3020±3 ^c	2560±185 ^a	4290±354 ^{ab,c}
48	2155±92 ^{b,d}	3026±1 ^e	3208±283 ^{ab}	5621±227 ^{ade}
72	2328±16 ^e	3023±2.1	2045±631 ^a	3834±235 ^{ab}

*Mean±SE of six animals Mean values having the same symbolic letters in the same row are significantly different from each other at p<0.05 using ANOVA followed by Tukey Kramer as post ANOVA test

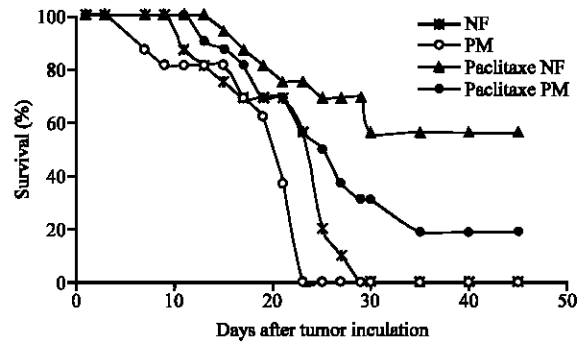


Fig. 1: Effect of paclitaxel (10 mg kg⁻¹, i.p.) On the percentage survival of ehrlich ascites carcinoma bearing mice

Cardio-toxicity of paclitaxel: Twenty four hours after paclitaxel administration (10 mg kg⁻¹, i.p.), there was a significant increase in serum LDH activity in mice with protein deficient diet amounting to 42%. In contrast, animals on standard diet showed only 12% increase in LDH activity. However, after 72 h of drug treatment, there was still increase in LDH activity in mice fed a protein deficient diet (40%) whereas level of LDH return to nearly normal level (Table 6).

DISCUSSION

Cancer is a worldwide public health problem accounting for the increasing proportion of all death as it is the second cause of death after heart disease (Tiwari *et al.*, 2004). Malnutrition and consequently weight loss are common in cancer patient, which may be to tumor, the host response to the tumor and/or anticancer therapies (Nelson *et al.*, 1994; Barber *et al.*, 1999). So, investigation of the 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 was our main targeting.

Administration of paclitaxel in protein deficient tumor bearing animals showed 20% survivors at the end of experiments while normally-fed bearing animals showed 58% survivors (Fig. 1). The poor response to paclitaxel in

protein deficient animals may be due weight loss (Table 2) and protein malnutrition (Table 1) since, Dewys *et al.* (1980) and Costa *et al.* (1981) have reported that the weight loss and protein malnutrition on patient with cancer have been identified as an important factor for survival. 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). Also, Copeland *et al.* (1977) showed that in malnourished cancer patients the parental nutritional repletion restored serum protein levels, improved host immunocompetence and increased tolerance to chemotherapy.

A significant elevation of serum LDH activity (Biological marker for cardiotoxicity) was observed 24 h after paclitaxel treatment and persisted for 72 h in protein deficient animals, whereas, animals on standard diet, the LDH activity return normal on day 3 after treatment. Paclitaxel treatment is known to open the L-type calcium channels in intact cardiac cell, which may lead to increase the probability of opened the L-type calcium channels in intact cardiac cells. This leads to an increase the in intracellular calcium level, as a result it causes cardiac damage and contraction failure (Gali and Defelice, 1994). Although, it is not clear during our study how protein deficient diet affect paclitaxel toxicity in such manner, Osman *et al.* (2009) reported that paclitaxel administration increased the serum level of CK-MB activity (biological marker of cardiotoxicity) and plasma histamine concentration in tumor bearing mice with protein deficient diet. Also, Kannan *et al.* (1980) showed that the rate of fatty acid synthesis was depressed in fasted Ehrlich ascites carcinoma bearing mice compared to controls. There is no explanation from the clinical trials point of view that could be compared or support our finding, but 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 protein deficient animals was decreased with more decrease after paclitaxel treatment (Table 1 and 3), thus the free fraction of paclitaxel will be high in protein deficient group leading to an increased free form that results in enhance toxicity. Although, Osman *et al.* (2009) has published a similar report with good agreement to my study but it was lacking the rate of survival of tumor bearing animal and long term survivors before and after treatment with paclitaxel which is a critical point in the study.

In conclusion, Paclitaxel treatment under our experimental conditions exerted effects on both animals

with protein deficient diet and normally-fed one, however its toxicity is higher and therapeutic activity is lower in the malnourished animals. More experimental studies on other anticancer drugs should be done to make such phenomenon clearer.

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