International Journal of Pharmacology

ISSN 1811-7775

science alert

ansinet
Asian Network for Scientific Information

International Journal of Pharmacology 5 (2): 173-177, 2009
ISSN 1811-7775
© 2009 Asian Network for Scientific Information

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

[1]Abdel-Moneim M. Osman, [2]Azza A. Abdel-Fatah, [2]Basent B. Hassan,
[3]Mahmoud M. El-Merzebani and [1]Zoheir A. Damanhouri
[1]Department of Pharmacology, College of Medicine, King Abdel Aziz University,
Jeddah, Saudi Arabia
[2]College of Pharmacy, Al-Azhar University, Cairo, Egypt
[3]National Cancer Institute, Cairo, Egypt

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,5β,20-epoxy-1,2α,4,7β, 13α-hexahydroxy-4,10-diacetate-2benzoate-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). 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 the period between 2004-2008. The study is partly conducted in Pharmacology Unit, National Cancer Institute, Cairo University and partly in Pharmacology Department,

Corresponding Author: Abdel-Moneim M. Osman, College of Medicine, King Abdel Aziz University, P.O. Box 80200, Jeddah 21589, Saudi Arabia

College of Medicine, King Abdel-Aziz University, Jeddah, Saudi Arabia.

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% 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 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 (2×10^6) 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 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 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:

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

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 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 coulter 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 spectrofluorometer (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 demonstrated that 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*

Concentration (g dL^{-1})	Animals on standard diet	Animals on protein deficient diet
Albumin concentration	2.67±0.06[a]	1.24±0.08[a]
Total protein concentration	5.95±0.0252[b]	1.95±0.12[b]

*Data is expressed as Mean±SEM of six animals, [a,b]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@

Groups	MST±SEM (days)	LTS (Long term survivor)	Tumor growth delay (days)
Normally-fed EC-bearing mice	23.3±1.8	0.16	
Protein-deficient EC-bearing mice	17.5±1.7[a]	0.16	
Paclitaxel (Normally-fed)	25.6±1.6[ab]	9.16	4.9±0.2
Paclitaxel (Protein deficient)	18.1±1.8[b]	3.16	2.8±0.7

@Normally-fed and protein deficient Ehrlich carcinoma bearing mice(EC) 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 materials and methods. 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

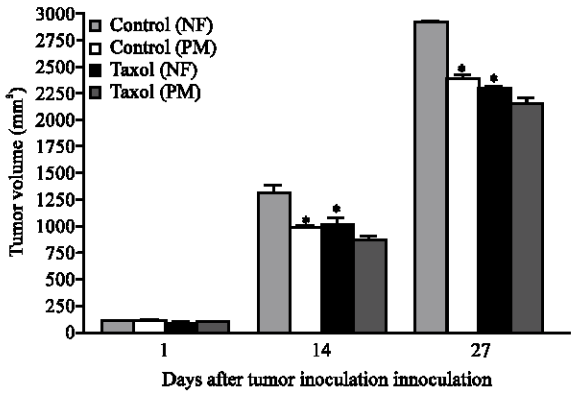

Fig. 1: Effect Paclitaxel administration (10 mg kg^{-1}, i.p.) on tumor volume of Ehrlich carcinoma bearing mice in normally-fed and protein deficient animals. NF: Normally-fed group, PM: Protein malnourished group. Significantly different from normally-fed mice at $p^*<0.5$

Table 3: Effect of paclitaxel administration (10 mg kg^{-1}, i.p.) on the cardiac isoenzyme CK-MB in normally-fed (NF) and protein malnourished (PM) mice*

Time after Paclitaxel administration (h)	CK-MB activity (U/L) Control (NF)	Control (PM)	Paclitaxel (NF)	Paclitaxel (PM)
24	2564±31[b,d]	4479±37[c,d]	3965±265[a]	8199±354[a,b,c]
48	2569±33[c,e]	4508±33[b,d,e]	2688±75[a,b]	8486±113[a,b,c]
72	2541±47[c,e]	4458±43[b,d,e]	2601±130[a,b]	8752±190[a,b,c]

*Data are presented as Mean±SE of 6 mice, 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

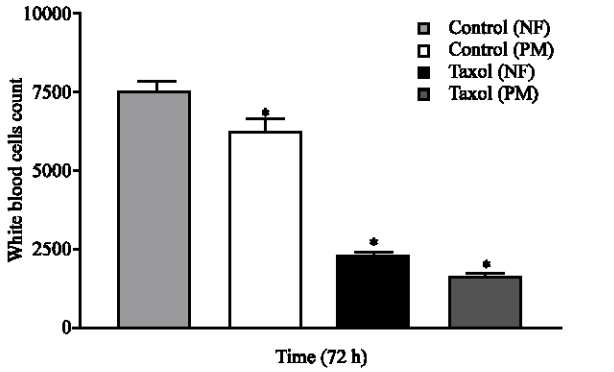

Fig. 2: Effect of single i.p. dose of paclitaxel (10 mg kg^{-1}) on the white blood cells of NF and PM animals 72 h after treatment. Significantly different from corresponding control at $p^*<0.05$ using ANOVA followed by Tukey-Kramer as post ANOVA

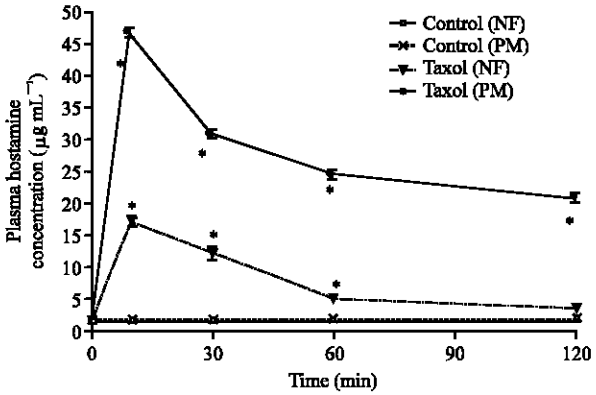

Fig. 3: Effect of paclitaxel (10 mg kg^{-1}) on the plasma histamine levels in normally-fed and protein deficient (malnourished) mice. Significantly different from normally-fed mice at p*<0.5

Plasma histamine concentration: Paclitaxel administration increased the plasma concentration of histamine in both protein deficient diet fed- and standard diet-fed animals at all the time points with maximum elevation achieving at 10 min (Fig. 3). There were a 85 and a 26 fold increase in histamine concentration in animals fed a protein deficient or standard diet, respectively.

DISCUSSION

Cancer is a major cause of morbidity and mortality throughout the world. It is the second most frequent cause of death in Europe. It is also becoming a leading cause of death in old age (Vainio *et al*., 1993; Tiwari *et al*., 2004). Anorexia resulting in malnutrition and consequently weight loss are common in cancer patients, which may be due the tumor, the host response to the tumor and/or anticancer therapies (Nelson *et al*., 1994; Barber *et al*., 1999). These symptoms are mediated via a number of mechanisms. Inadequate intake of energy and nutrients alone is unable to account for the substantial changes in nutritional status seen in patients with cancer. In advanced cancer, cachexia often occurs. This complex multifactorial syndrome is associated with metabolic abnormalities, anorexia, early satiety and reduced food intake, depletion of lean body mass, muscle weakness, edema, fatigue, impaired immune function and declines in attention span and concentration (Von Roenn and Knopf, 1996; Rosenbaum *et al*., 2000; Fujimiya and Inui, 2000). The development and implementation of screening and assessment tools is essential for effective nutritional intervention and management of patients with cancer. Proactive nutritional interventions should ideally form an integral part of cancer therapy, with the aim of improving 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

Bamji, M.S. and D. Sharada, 1972. Hepatic glutathione reductase concentrations in experimental deficiency of thiamine and riboflavin in rats. J. Nutr., 102: 443-448.

Barber, M.D., J.A. Ross and K.C. Fearon, 1999. Cancer cachexia. Surg. Oncol., 8: 133-141.

Chlebowski, R.T., L. Bulcavagem, M. Greosvenor, E. Oktay and J.B. Block *et al*., 1990. Hydralazine sulfate influences on nutritional status and survival in non-small cell lung cancer. J. Clin. Oncol., 8: 9-15.

Costa, G., W.W. Lane and R.G. Vincent, 1981. Weight loss and cachexia in lung cancer. Nutr. Cancer, 2: 98-103.

Dewys, W.D., C. Begg and P.T. Lavin, 1980. Prognostic effect of weight loss prior to chemotherapy in cancer patients. Am. J. Med., 69: 491-497.

Dumas, B.T., W.A. Watson and H.G. Biggs, 1971. Albumin standards and the measurement of serum albumin with bromcresol green. Clin. Chim. Acta, 31: 87-96.

Fujimiya, M. and A. Inui, 2000. Peptidergic regulation of gastroinstestinal motility in rodents. Peptides, 21: 1565-1582.

Gali, A. and L.J. Defelice, 1994. Inactivation of L-type calcuim channel in embryonic chick ventricle cells:Dependence on the cytoskeletal agents cholicine and taxol. Biophys. J., 67: 2296-2304.

Holder,H., 2003. Nursing management of nutrition in cancer palliative care. Br. J. Nurs., 12: 667-668.

Hsu, P., H.C. Hung, Y.F. Liao, C.C. Liu, G.J. Tsay and G.Y. Liu, 2008. Ornithine decarboxylase attenuates leukemic chemotherapy drugs-induced cell apoptosis and arrest in human promyelocytic HL-60 cells. Leukemia Res., 32: 1530-1540.

Kumar, G.N., U.K. Walle, K.N. Bhalla and T. Walle, 1993. Binding of taxol to human plasma, albumin and alpha1-acid glycoprotein. Res. Commun. Chem. Pathol. Pharmacol., 80: 337-344.

Mantovani, G., A. Maccio, C. Madeddu, G. Gramignano and R. Serpe *et al*., 2008. Clinical trial of five different arms of treatment for patients with cancer cachexia: Interim results. Nutrition, 24: 305-313.

Nelson, K.A., D. Walsh and F.A. Sheehan, 1994. The cancer anorexia-cachexia syndrome. J. Clin. Oncol., 12: 213-225.

Nixon, D.W., S.B. Heymsfield, A.E. Cohen, M.H. Kunter, J. Ansley, D.H. Lawson and D. Rudman, 1980. Protein-calorie undernutrition in hospitalized cancer patients. Ann. J. Med., 68: 683-690.

Osman, A.M., M.M. Sayed-Ahmed, M.T. Khayyal and M.M. El-Merzabani, 1993. Hyperthermic potentiation of cisp[latin on solid Ehrlich carcinmoma. Tumori, 79: 268-272.

Puccio, M. and L. Nathanson, 1997. The cancer cachexia syndrome. Semin. Oncol., 24: 277-287.

Rosenbaum, K., J. Wang and R.N. Pierson, 2000. Time-dependant variation in weight and body composition in healthy adults. J. Parenter. Enteral Nutr., 24: 52-55.

Shore, P.A., A. Burkhalter and V.H. Cohn, 1959. A method for fluorometric assay of histamine in tissues. J. Pharmacol. Exp. Ther., 127: 182-186.

Tiwari, R.C., K. Ghosh, A. Jemal, M. Hachey, E. Ward M.J. Thun and E.J. Fe-uer, 2004. A new method of predicting US and state level cancer mortality counts for the current calender year. Cancer J. Clin., 54: 30-40.

Vainio, H., E. Matos, P. Boffetta, M. Kogevinas and J. Willbourn, 1993. Occupational cancer in developing and newly industrialized countries. Ann. Acad. Med. Sigapore, 22: 170-181.

Von Roenon, J.H. and K. Knopf, 1996. Anorexia-cachexia in patients with HIV: Lessons for oncologist. Oncology, 10: 1049-1059.

Wani, M.C., H.L. Taylor, M.E. Wall, P. Coggon and A.T. McPhail, 1971. Plant antitumor agent. In: The isolation and structure of taxol, a novel antileukemic and antitumor agent from *Taxus brevifolia*. J. Am. Chem. Soc., 93: 2325-2327.

Weissman, N., E.B. Schoenbach and E.B. Arnistead, 1950. The determination of dulfhydryl groups in serum. I. Methods and results on normal sera. J. Biol. Chem., 187: 153-165.

Wu, A.H.B. and C.N. Bowers, 1982. Evaluation and comparison of immunoinhibition and immunoprecipitation methods for defferentiating MB and BB and macro forms of creatine kinase isoenzymes in patients and healthy indivioduals. Clin. Chem., 28: 2017-2019.