AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

# Effect of Paclitaxel Along With Di Allyl Sulfide on Glycoprotein Changes in 7, 12 Di Methyl Benz (A) Anthracene Induced Skin Cancer Wistar Rats

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## ABSTRACT

The purpose of this study is to investigate the glycoprotein and efficacy of combination of paclitaxel along with Di allyl sulfide against skin cancer in experimental animals. Skin cancer is the most common form of human cancer. It is estimated that over 1 million new cases occur annually. The annual rates of all forms of skin cancer are increasing each year, representing a growing public concern. The most common warning sign of skin cancer is a change in the appearance of the skin, such as a new growth or a sore that will not heal. Skin cancer is caused by chemical carcinogens and Papilloma virus infection. Skin cancer was induced in rats by 7, 12 Di methyl benz(a) anthracene (DMBA) at the dosage of 5 µg was dissolved in 100µl and administered into experimental animals for 28 weeks. In this study, we demonstrated that combination of paclitaxel and Di allyl sulfide protects the rats from a lethal dose of DMBA for 30 days. The levels of glycoprotein in plasma, skin and liver were found to be increased in the cancer bearing animals when compared with control animals. Treatment of Paclitaxel along with Di allyl sulfide to cancer induced animals showed significantly decreased levels of glycoprotein levels when compared with cancer induced animals. The treatment with combination of paclitaxel and Di allyl sulfide effectively reduced glycoprotein levels. So, from the obtained results it is concluded that paclitaxel and Di allyl sulfide is capable of restoring the skin architecture.

**Key words:** Paclitaxel, Di allyl sulfide, DMBA, Skin cancer.

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Received 8 March 2012, Accepted 24 March 2012

Please cite this article in press as: Selvakumar C. et al., Effect of Paclitaxel Along With Di Allyl Sulfide on Glycoprotein Changes in 7, 12 Di Methyl Benz (A) Anthracene Induced Skin Cancer Wistar Rats. American Journal of PharmTech Research 2012.

## INTRODUCTION

Skin cancer is the most common form of human cancer. It is estimated that over 1 million new cases occur annually.[1] In India, skin cancers constitute about 1-2% of all diagnosed cancers. Basal cell carcinoma is the commonest form of skin cancer worldwide, but various studies from India have consistently reported SCC as the most prevalent skin malignancy. [2]

PAH's an important class of chemical carcinogens that are widespread in the ambient environment due to fossil fuel combustion for energy production, transportation and industry.[3] DMBA, a potent PAH recognized as an initiator of both skin and liver cancer. [4] The covalent binding of DMBA metabolites to DNA has been implicated as a critical step in the initiation phase of cancers.

Paclitaxel (Taxol), a naturally occurring antineoplastic agent has shown great promise in the therapeutic treatment of certain human solid tumors particularly in metastatic breast cancer, skin cancer, lung cancer and refractory ovarian cancer.[5] It is the original member of the taxane group of anticancer drugs derived from the bark and needles of the pacific yew tree "Taxus brevifolia".[6] Paclitaxel's antitumor activity was discovered in1960's during a large scale 35,000 plants-screening program sponsored by the National Cancer Institute (NCI), USA.

Garlic has been applied since time immemorial as a culinary spice and medicinal herb and is an important constituent of traditional Chinese medicine. The chief constituent of garlic is the sulfur compound allicin, produced by crushing or chewing fresh garlic, which in turn produces other sulfur compounds: ajoene, mono-, di-, and tri-allyl sulfides, and vinyldithiins .[7]

Advanced glycation end products (AGEs) modify galactose, fucose and sialic acid contents of specific cellular glycoproteins. Glycoproteins are a group of complex proteins containing covalently bound oligosaccharides attached to their polypeptide backbone. Hexose, fucose, hexosamines and sialic acid form the monosaccharide units of oligosaccharide. Glycoproteins are important components of intracellular matrix, cell membrane and membranes of the sub-cellular organelles. [8] They play a vital role in the maintenance of structural integrity of the membrane bilayer. Cell surface glycoproteins have important roles in the transport of vitamins and lipids, in signal transduction as hormone receptors and in immunological specificity. Glycoproteins are common components of animal cell surfaces, constituents of lysosomes and among the products exported by the cell. The cell surface glycoproteins have been found to play an important role in tumorigenesis and as mediators of immunological specificity. Carbohydrate moieties of glycoproteins have been implicated in the transport of metabolites across cell membranes, and

also a direct relation between glycoproteins and tumorigenesis is observed. The biochemical markers, hexose, hexosamine and sialic acid, have been measured. Many chemical changes in the glycoproteins are detectable before the onset of secondary physiological and nutritional changes that may be associated with the condition of tumor-bearing animals. [9]

## MATERIALS AND METHODS

**Chemicals:**

7, 12 Dimethyl benz (a) anthracene and Di allyl sulfide were purchased from sigma chemical company, USA. All the other chemicals used were of analytical grade.

**Animal care and housing:**

Male Wistar rats, 6-8 weeks of age and weighing 150-200g, were used. The animals were procured from Central Animal House Block, Meenakshi Medical College and Research institute, Kanchipuran, Tamil Nadu, India and maintained in a controlled environmental condition of temperature and humidity on alternatively 12 h light/dark cycles. All animals were fed standard pellet diet (Gold Mohor rat feed, Ms.Hindustan Lever Ltd., Mumbai) and water ad libitum. This research work on wistar male rats was sanctioned and approved by the Institutional Animal Ethical Committee (REG NO. 765/03/ca/CPCSEA).

**Experimental Design**

The animals were divided in to six groups of 6 animals each. Group I animals served as control, Group II as animals treated with DMBA (5 µg ) per animal in acetone (100 µL ), three times a week for 28 weeks to induce skin cancer. After tumor induction Group III animals were treated with Paclitaxel (33mg/kg b.wt) once in a week for 4 weeks. Group IV animals were treated with garlic extract of Di allyl sulfide (250µg/animal) for 30 days. Group V animals were treated with both Paclitaxel and Di allyl sulfide (as in group III and group IV) after the induction of skin cancer. Group VI Control animals treated with paclitaxel and Di allyl sulfide for 30 days.

After the experimental period of 18 weeks, the animals were sacrificed by cervical decapitation. Blood was collected in a dry test tube and allowed to coagulate at ambient temperature for 30 min. Serum was separated by centrifugation at 2000 rpm for 10 min for the estimation of serum assay. Skin and liver were immediately dissected out and washed in ice-cold saline to remove the blood and stored at -20°C until analyzed. Tissues was minced and homogenized (10% w/v) with 0.1M Tris-HCl buffer (pH 7.4) in ice cold condition. The homogenates were centrifuged at 1000 rpm for 10 min at 0 °C in cold centrifuge. The supernatants were separated and used for estimation of glycoproteins.

**Biochemical Analysis**

**Extraction of glycoproteins**

To 0.1 ml of plasma, 5.0 ml of methanol was added, mixed well and centrifuged for 10 min at 3000×g. The supernatant was decanted and the precipitate was again washed with 5.0 ml of 95% ethanol, re-centrifuged and the supernatant was decanted to obtain the precipitate of glycoproteins. This was used for the estimation of hexose and hexosamine. For extraction of glycoproteins from the tissues, a known weight of the tissue was homogenized in 7.0 ml of methanol. The contents were filtered and homogenized with 14.0 ml of chloroform. This was filtered and the residue was successively homogenized in chloroform-methanol (2:1 v/v) and each time the extract was filtered. The residue (defatted tissues) was obtained and the filtrate decanted. A weighed amount of defatted tissue was suspended in 3.0 ml of 2 N HCl and heated at 90˚C for 4 h. The sample was cooled and neutralized with 3.0 ml of 2 N NaOH. Aliquots from this were used for estimation of hexose, hexosamine and sialic acid. Estimation of hexoses by Niebes [10], Protein bound hexosamine by Wagner [11] and Sialic acid in plasma and tissues by Warren .[12]

**Statistical Analysis**

The data for various biochemical parameters were analyzed using analysis of variance (ANOVA) and the group means were compared by Duncan's Multiple Range Test (DMRT). Values were considered statistically significant when $p < 0.05$. [13]

## RESULTS AND DISCUSSION:

The table 1 represents the effect of Paclitaxel and Di allyl sulfide on the levels of glycoproteins in plasma of control and experimental animals. All the three glycoproteins hexose, hexosamine and sialic acid were found to be significantly increased in the cancer induced group II animals when compared with control (G- I) animals. Paclitaxel (G-III) and Di allyl sulfide (G- IV) caused a significant decrease in their levels when compared to cancer bearing animals but a combination treatment of both paclitaxel and Di allyl sulfide (G- V) caused a very much significant decrease in the level of glycoproteins when compared with cancer bearing animals. There was no significant difference in the glycoprotein levels between the control animals and the control treated with the combination of paclitaxel and Di allyl sulfide (G-VI).

Glycoproteins are common components of animal cell surfaces, constituents of lysosomes and among the products exported by the cells. [14] Carbohydrate moieties of glycoproteins have been implicated in the transport of metabolites across cell membranes and also a direct relation

between glycoproteins and tumorigenesis is observed. [15] Elevation of the glycoprotein components serve as a classical marker and an indicator in the progression of tumor growth. Altered levels of protein bound carbohydrates are well documented during neoplastic process[16] that alters the structure, rigidity and function of cell membrane. [17] In the present study also the levels of glycoproteins hexose, hexosamine and silaic acid was significantly increased in DMBA induced cancer bearing animals. This change in surface carbohydrate during cellular differentiation and neoplastic transformation suggests the importance in physiology and behavior of the cells.

**Table 1: Effect of Paclitaxel along with *Di allyl sulfide* on the level of glycoproteins in the plasma of control and experimental animals**

| Particulars | Group I (Control) | Group II (DMBA induced) | Group III (Paclitaxel treated) | Group IV (Di allyl sulfide treated) | Group V(Both Paclitaxel and Di allyl sulfide treated) | Group VI (Control rats treated with Paclitaxel and Di allyl sulfide) |
|---|---|---|---|---|---|---|
| Hexose | 218.15 ± 20.8 | 290.59 ± 28.21$^{a*}$ | 242.66 ± 24.1$^{b@}$ | 251.22 ± 24.3$^{b\#}$ | 238.24 ± 22.32$^{b@}$ | 216.88 ± 20.68 |
| Hexosamine | 28.76 ± 2.91 | 42.71 ± 4.05$^{a*}$ | 35.75 ± 3.6$^{b@}$ | 38.41 ± 3.76$^{b\#}$ | 30.82 ± 3.04$^{b*}$ | 29.01 ± 2.86 |
| Sialic acid | 44.61 ± 4.35 | 69.72 ± 6.95$^{a*}$ | 54.31 ± 5.38$^{b*}$ | 59.61 ± 5.91$^{b@}$ | 48.78 ± 4.87$^{b*}$ | 45.12 ± 4.45 |

Each value is expressed as mean ± SD for six rats in each group.
Units: mg/dl
**a**: as compared with Group I, **b**: as compared with Group II
Statistical significance: $^{*}$ p<0.001; $^{@}$ p<0.01; $^{\#}$ p<0.05.

**Table 2 Effect of Paclitaxel along with *Di allyl sulfide* on the level of glycoproteins in the skin of control and experimental animals**

| Particulars | Group I ( Control) | Group II (DMBA induced) | Group III (Paclitaxel treated) | Group IV (Di allyl sulfide treated) | Group V (Both Paclitaxel and Di allyl sulfide treated) | Group VI (Control rats treated with Paclitaxel and Di allyl sulfide) |
|---|---|---|---|---|---|---|
| Hexose | 1.42 ± 0.12 | 3.45 ± 0.33$^{a*}$ | 1.95 ± 0.14$^{b*}$ | 1.62 ± 0.17$^{b*}$ | 1.4 ± 0.16$^{b*}$ | 1.41 ± 0.16 |
| Hexosamine | 0.45 ± 0.05 | 0.98 ± 0.07$^{a*}$ | 0.76 ± 0.06$^{b*}$ | 0.66 ± 0.06$^{b*}$ | 0.42 ± 0.05$^{b*}$ | 0.44 ± 0.05 |
| Sialic acid | 0.31 ± 0.04 | 0.59 ± 0.04$^{a*}$ | 0.43 ± 0.01$^{b*}$ | 0.39 ± 0.03$^{b*}$ | 0.31 ± 0.05$^{b*}$ | 0.31 ± 0.05 |

Each value is expressed as mean ± SD for six rats in each group.
Units: mg/g of defatted tissue
**a**: as compared with Group I, **b**: as compared with Group II
Statistical significance: $^{*}$ p<0.001; $^{@}$ p<0.01; $^{\#}$ p<0.05.

The Table 2 displays the effect of paclitaxel along with Di allyl sulfide on the levels of glycoproteins in the skin of control and experimental animals. The levels of all the three glycoproteins were found to significantly increased in cancer bearing animals (G-II) when

compared with the control group (G-I). Treatment with paclitaxel (G-III) and Di allyl sulfide (G-IV), caused a significant decrease in the levels of these glycoproteins when compared with cancer bearing animals (G-II). However, treatment with paclitaxel and Di allyl sulfide (G-V) caused a significant decrease in their levels when compared with the cancer-induced group. There was no significant difference in the glycoprotein levels between the control animals and the control treated with the combination of paclitaxel and Di allyl sulfide (G-VI).

The levels of hexose and hexosamine were significantly increased in skin cancer conditions.[18] Sialic acid are acylated derivatives of neuraminic acid, mainly occur as non- reducing terminal residues of carbohydrate chains of glycoproteins and gangliosides in biological materials. Serum sialic acids are raised in certain cancers including malignant melanoma, cancers of brain, gastrointestinal and gynaecological systems thus it has been used as tumor markers. [19]

Figure.1 represents the effect of paclitaxel along with Di allyl sulfide on the levels of glycoproteins in the liver of control and experimental animals. The levels of all the three glycoproteins were found to be increased in cancer induced group (G-II) when compared with the control group (G-I). On treatment with paclitaxel (G-III) and Di allyl sulfide (G-IV) there found to be a significant decrease in the levels of these glycoproteins when compared with cancer bearing animals (G-II). Combined therapy of paclitaxel and Di allyl sulfide (G-V) caused a highly significant decrease in their levels when compared with the cancer-induced group. There was no significant difference in the glycoprotein levels between the control animals (G-I) and the control treated with the combination of paclitaxel and Di allyl sulfide (G-VI).

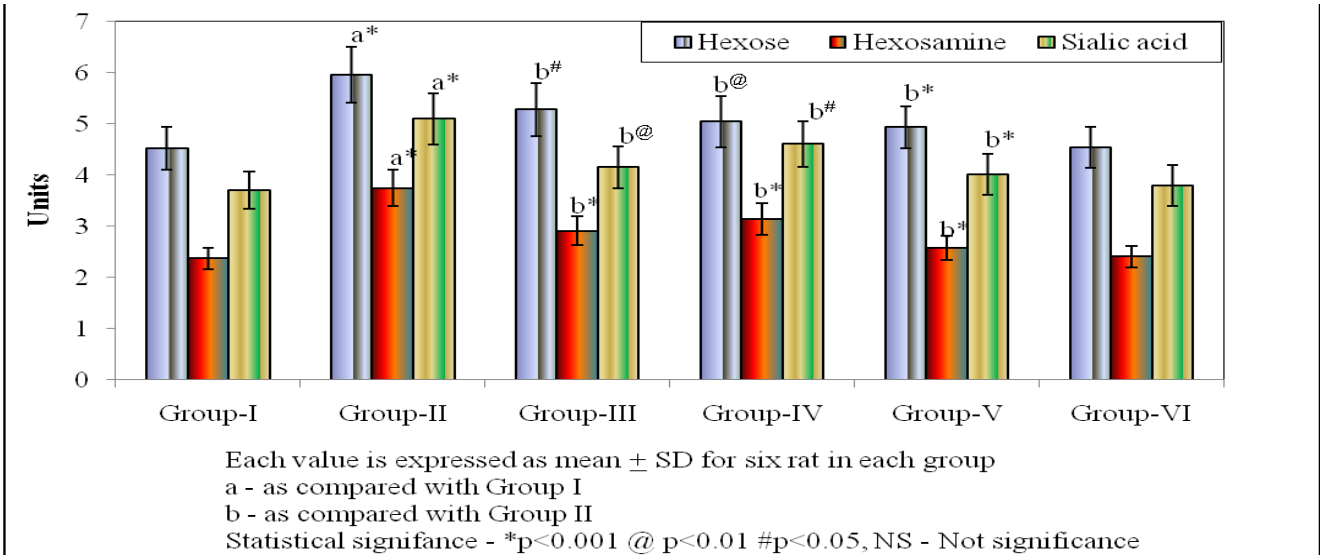


**Figure 1: Effect of paclitaxel along with di allyl sulfide on the levels of glycoprotein in the liver of control and experimental animals.**

Plasma sialyl transferase enzymes are involved in the transfer of sialic acid residues from cysteine monophosphate and their activities were elevated in variety of cancers. Bernacki and Kim have reported an increase in sialyl tranferase activity in metastasizing skin cancer bearing animals.[20] In Paclitaxel and DAS treated animals the levels of glycoproteins are decreased significantly. This reduction in the levels of glycoprotein components indicate that the drug has the ability to suppress malignancy by modulating cell transformation by controlling cell proliferation.

## CONCLUSION

The glycoprotein of paclitaxel and Di allyl sulfide could very well be attributed to their maintain the cell surface against polycyclic hydrocarbons. When given combination of paclitaxel and Di allyl sulfide it reduces the toxic side effects of the later by its immunomodulatory activity and improves the treatment strategy. Our data suggests that administration of Paclitaxel along with Di allyl sulfide significantly decrease the toxic implications of chemotherapy and decrease the levels of glycoprotein in skin cancer bearing animals.

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