Academic Sciences Asian Journal of Pharmaceutical and Clinical Research

Vol 5, Suppl 2, 2012 ISSN - 0974-2441

Research Article

A COMBINATION OF PACLITAXEL AND DI ALLYL SULFIDE ON 7, 12 DIMETHYL BENZ (A) ANTHRACENE INDUCED LIPID PEROXIDATION AND ANTIOXIDANT SYSTEM IN WISTAR RATS

MUNINATHAN N[1], DR MALLIGA S[1], DR SELVAKUMAR C[1] *, KUMAR J[1]

[1] **Department of Biochemistry, Meenakshi Medical College and Research Institute, Enathur, Kanchipuram, Tamilnadu-631552, India.Email:** drselvakumarc@yahoo.com

Received:18 February 2012, Revised and Accepted:7 May 2012

ABSTRACT

The effect of paclitaxel along with Di allyl sulfide on 7, 12 Dimethyl benz (a) anthracene (DMBA) induced experimental skin cancer was investigated in wistar rats. Control rats were fed the basal diet throughout the experiment. Four other group received DMBA (5µg in 100ml of acetone) i.p to develop skin tumor and one another group of control rats treated with combination of paclitaxel and Di allyl sulfide. After the tumor induction period administration of paclitaxel and Di allyl sulfide effectively suppressed skin cancer which is revealed by the decrease in the extent of lipid peroxidation with concomitant increase in the activities of enzymatic antioxidants (SOD, CAT and GPx) and non enzymatic antioxidants (Reduced Glutathione, Vitamin C and E) levels when compared to skin cancer bearing animals treated with paclitaxel and Di allyl sulfide alone. It was therefore concluded that the combination of paclitaxel and di allyl sulfide on tumorogenesis might be casually related to its antilipidperoxidation, antioxidant and cytosolic activities that plays an important role in DMBA induced skin cancer.

Keywords: Paclitaxel, Di allyl sulfide, DMBA, Skin cancer

INTRODUCTION

Skin cancer is the most common form of human cancer. It is estimated that over 1 million new cases occur annually[1]. The annual rates of all forms of skin cancer are increasing each year, representing a growing public concern. It has also been estimated that nearly half of all Americans who live to age 65 will develop skin cancer at least once. Skin cancer patients have stage IV receive chemotherapy and /or hormonal therapy to suppress cancer cells and control the disease. The goal of chemotherapy is to destroy, shrink primary tumors, slow the tumor growth, and to kill cancer cells that may have spread (metastasized) to other parts of the body from the original tumor. Chemotherapeutic drugs elicit some toxicity towards normal cells also, that limits its usage.

Paclitaxel is 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[2]. 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. Paclitaxel is a most effective drug in skin cancer, it has several important side affects particularly neutropenia, peripheral neuropathy and hypersensitivity reactions[3]. Myelo suppression or neutropenia is the principal dose limiting toxicity of paclitaxel on all administration schedules. It is undeniable that the need for new agents with both improved activity and acceptable safety profile is urgent. Nausea, vomitting, thrombocytopenia, mucositis, decreased appetite and diarrhea are the less common side effects of administration of paclitaxel. Ongoing clinical trials suggest that combining paclitaxel with other anticancer drugs may be an effective treatment for patients with skin cancer. Researchers are exploring ways to reduce the side effects of treatment improve the quality of patients' lives, and reduce pain.

The chemotherapeutic and antitumor activity associated with garlic has been attributed to the presence of various organosulfide-based active compounds including Di Allyl sulfide [4]. A topical application of Di allyl sulfide is the most promising approach for treating skin tumors as it leads to a localized effect at the desired site with minimal side effects. Polycyclic aromatic hydrocarbons (PAHs) are commonly occurring environmental contaminants and are widely distributed in the environment as pollutants of air, water and soil [5]. Benzo (a) pyrene is the most toxic compound of PAHs [6].The purpose of the present study is to evaluate the combined effect of Paclitaxel and Di allyl sulfide against the DMBA induced skin carcinogenesis.

MATERIALS AND METHODS

Chemicals

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

Animals

Experiments were performed in male wistar rats weighing 150 – 200 gms. The animals were housed in groups at room temperature and provided with a balanced diet (Gold Mohur pellet feed) and drinking water *ad libitum*. Each group consisted of six animals. This research work on wistar 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.

At the end of experimental period the animals were sacrificed by cervical decapitation. Blood and tissues like skin and liver were collected. The tissues were immediately weighed and then homogenized in Tris HCl buffer 0.1 M (pH 7.4). A 10% homogenate of the tissues were used for following parameters.

Biochemical analysis

Total protein was estimated by the method of Lowry et al[7]. The antioxidant enzymes Superoxide dismutase was analyzed by Marklund and Marklund[8], Catalase and Glutathione Peroxidase by Sinha[9] and Rotruck[10] respectively. Nonenzymic antioxidants Reduced Glutathione was analyzed by Morom et al[11], Vitamin E and

C by Desai [12] and Omaye et al[13] respectively. Lipid peroxidation was analyzed by Ohkawa [14]

Statistical Analysis

For statistical analysis, one way analysis of analysis of Variance (ANOVA) was used, followed by the Newman-Keuls Multiple Comparison test.

RESULTS

The table 1 depicts the effect of paclitaxel and *Di allyl sulfide* on lipid peroxidation in plasma, skin and liver of control and experimental animals. Lipid peroxidation was found to be significantly increased in both plasma, skin and liver of cancer bearing group II animals when compared with control animals (G-I). This was more significantly decreased on treatment with combination of paclitaxel and *Di allyl sulfide* (G-V) when compared with paclitaxel(G-III) or *Di allyl sulfide* (G-IV) alone treated cancer bearing animals.

Table 1: Effect of Paclitaxel and Di allyl sulfide on lipid peroxidation in 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)
Plasma	1.58 ± 0.16	2.02 ± 0.21^{a*}	1.72 ± 0.17$^{b@}$	1.7 ± 0.17$^{b@}$	1.61 ± 0.16^{b*}	1.56 ± 0.16
Liver	1.07 ± 0.11	4.25 ± 0.43^{a*}	3.14 ± 0.31^{b*}	2.8 ± 0.27^{b*}	1.91 ± 0.2^{b*}	1.1 ± 0.11
Skin	0.61 ± 0.06	2.8 ± 0.28^{a*}	1.97 ± 0.15^{b*}	1.81 ± 0.15^{b*}	0.98 ± 0.09^{b*}	0.61 ± 0.06

Each value is expressed as mean ± SD for six rats in each group.

Units:

Plasma : nmoles of MDA liberated/mg protein
Liver : nmoles of MDA liberated/mg protein
Skin : nmoles of MDA liberated/mg protein
a: as compared with Group I
b: as compared with Group II
Statistical significance: * p<0.001 @ p<0.01 # p<0.05, NS- Not significant

The table 2 shows the levels of antioxidant enzymes like SOD, CAT and GPx and non-enzymatic antioxidants GSH, Vit E and C in skin of various experimental groups. Highly significant reductions in the activity of enzymic and non-enzymatic antioxidants in the tumor bearing rats (Group II) were observed. These adverse changes were reversed to near normal levels in paclitaxel and Di allyl sulfide treated animals (G-V) than the animals treated with Paclitaxel (G-III) and Di allyl sulfide alone (G- IV) alone.

Table 2: Effect of Paclitaxel along with Di allyl sulfide on enzymatic and non enzymatic antioxidants 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)
SOD	3.68 ± 0.36	2.11 ± 0.21^{a*}	3.12 ± 0.32^{b*}	3.23 ± 0.32^{b*}	3.59 ± 0.36^{b*}	3.62 ± 0.36
CAT	67.42 ± 6.52	39.97 ± 4.04^{a*}	51.15 ± 5.2$^{b@}$	58.12 ± 5.9^{b*}	65.41 ± 6.5^{b*}	66.45 ± 6.7
GPx	6.39 ± 0.58	3.67 ± 0.41^{a*}	5.83 ± 0.72^{b*}	5.92 ± 0.65^{b*}	6.15 ± 0.62^{b*}	6.37 ± 0.64
GSH	3.23 ± 0.32	1.34 ± 0.14^{a*}	2.98 ± 0.31^{b*}	2.77 ± 0.27^{b*}	3.1 ± 0.31^{b*}	3.22 ± 0.32
Vit-E	1.97 ± 0.17	0.86 ± 0.09^{a*}	1.52 ± 0.21^{b*}	1.72 ± 0.17^{b*}	1.86 ± 0.16^{b*}	1.95 ± 0.19
Vit-C	1.56 ± 0.14	0.66 ± 0.07^{a*}	1.22 ± 0.16^{b*}	1.35 ± 0.12$^{b@}$	1.45 ± 0.16^{b*}	1.57 ± 0.16

Each value is expressed as mean ± SD for six rats in each group.

Units:

SOD - Units/min/mg protein
CAT - µmoles of H_2O_2 liberated/min/mg protein
GPx - µmoles of GSH oxidised/min/mg protein
a: as compared with Group I
b: as compared with Group II
Statistical significance: * p<0.001 @ p<0.01 # p<0.05, NS- Not significant

The table 3 shows the levels of enzymic and non- enzymatic antioxidants in liver of various experimental groups. Significant decrease in antioxidant activities was observed in cancer bearing rats (group II). These changes were reversed in the treatment groups.

Table 3: Effect of Paclitaxel along with Di allyl sulfide on enzymatic and non enzymatic antioxidants in the liver 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)
SOD	6.25 ± 0.56	3.45 ± 0.33^{a*}	4.81 ± 0.45^{b*}	4.31 ± 0.42$^{b@}$	5.38 ± 0.52^{b*}	6.30 ± 0.61
CAT	34.01 ± 3.21	18.33 ± 1.77^{a*}	24.08 ± 2.20^{b*}	27.06 ± 2.01^{b*}	30.46 ± 3.22^{b*}	33.50 ± 3.30
GPx	5.36 ± 0.54	3.79 ± 0.32^{a*}	4.40 ± 0.41$^{b\#}$	4.62 ± 0.45$^{b@}$	5.23 ± 0.51^{b*}	5.42 ± 0.50
GSH	4.12 ± 0.41	2.03 ± 0.2^{a*}	3.21 ± 0.31^{b*}	2.91 ± 0.25^{b*}	3.98 ± 0.39^{b*}	4.07 ± 0.41
Vit-E	2.53 ± 0.22	1.24 ± 0.12^{a*}	1.8 ± 0.16^{b*}	1.65 ± 0.15$^{b@}$	2.23 ± 0.21^{b*}	2.49 ± 0.25
Vit-C	7.35 ± 0.72	4.06 ± 0.39^{a*}	5.13 ± 0.51$^{b@}$	4.82 ± 0.47$^{b\#}$	5.42 ± 0.51^{b*}	6.34 ± 0.59

Each value is expressed as mean ± SD for six rats in each group.

Units: µg/mg protein

a: as compared with Group I
b: as compared with Group II
Statistical significance: * p<0.001 @ p<0.01 # p<0.05, NS- Not significant

DISCUSSION

Oxidative stress especially lipid peroxidation is known to be involved in carcinogenesis[15]. Lipid peroxidation (LPO) is a chain reaction that involves the oxidation of polyunsaturated fatty acids in membranes induced by free radicals and is an indicator of oxidative cell damage[16]. The free radicals react with lipids causing peroxidation, resulting in release of products such as malondialdehyde (MDA), hydro peroxide and hydroxyl radicals. MDA has also been reported to cause mutagenesis in various tissues by forming DNA adducts. Increased levels of lipid peroxidation products play a role in the early phases of tumor growth[17]. Significant increase in lipid peroxidation associated with various forms of carcinogenesis has been documented widely and known to play an important role in cancer prevention or treatment. In the present study also significant increase in the levels of lipid peroxidation was observed in the plasma, skin and liver tissues of cancer bearing rats. This may be due to the enormous production of reactive oxygen species and free radicals, which are produced in response to exposure to the carcinogen. Ide and Lau (2001) (18) have shown that the patients with skin cancer have higher MDA levels when compared to controls. Paclitaxel eliminates the tumor cells and reduces the tumor burden in group III cancer bearing rats. Also Di allyl sulfide when given in combination with any drug increases the potential of the accompanying drug. Hence group V animals treated with both Paclitaxel and Di allyl sulfide show significant decrease in Lipid peroxidation by modulating the glutathione peroxidase- glutathione reductase system. The present study indicates that Di allyl sulfide with paclitaxel significantly suppress the LPO in plasma, skin and liver tissues of cancer bearing rats.

Naturally there is a dynamic balance between the amount of free radicals generated in the body and antioxidant defense system that quench or scavenge them and protect the body against their deleterious effects [19]. Hence antioxidant status has been suggested as a useful tool in estimating risk of oxidative damage induced carcinogenesis.

The antioxidant enzymes SOD, CAT and GPx play an important role as protective enzymes against reactive oxygen species in tissues and also comprise the cellular antioxidant defense system[20]. This is due to the increased levels of oxygen radicals which themselves reduce the activity and availability of antioxidants[21]. Several authors have cited the decreased activities of these antioxidant enzymes in various types of tumors [22]. Our present shows a reduction in the activities of antioxidants enzymes were significantly reduced in skin cancer group II rats. Cancer bearing animals treated with paclitaxel (Group-III) and Di allyl sulfide (Group – IV) alone showed increase in the activities of these enzymes when compared with group II. Combination treatment with paclitaxel and *Di allyl sulfide* (Group-V) caused a considerable significantly increased in their levels when compared with the cancer animals. There was no significant difference in the antioxidant levels between the control animals and the control animals treated with the combination of paclitaxel and *Di allyl sulfide* (G-VI).

Neoplastic cells may sequester essential antioxidants from circulation to supply the demands of growing tumor[23]. Vitamin C, E and reduced glutathione comprise the non-enzymatic antioxidant system that protects the cells against free radicals and ROS. Antioxidant vitamins have a number of biological activities such as immune stimulation, scavenging the free radicals and alteration in metabolic activation of carcinogens[24]. GPx the major antioxidant enzymes decomposes hydroxyl radicals at the expense of GSH. Hence any decrease in GPx concentration can attribute to the decline in glutathione concentration [25]. Ascorbic acid and alpha-tocopherol levels were reduced in tumor bearing animals. This may be due to the utilization of vitamin C and vitamin E to keep up the cellular levels for cell proliferation. When there is reduction in the levels of GSH, cellular levels of ascorbic acid and tocopherol are also lowered [26]. Combination treatment with paclitaxel and *Di allyl sulfide* (Group-V) caused a significantly increased in their levels when compared with the cancer animals.

CONCLUSION

In conclusion the antioxidant properties of paclitaxel and Di allyl sulfide could very well be attributed to their free radical scavenging activity against alkoxyl radicals. 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 increases the levels of free radical scavenging enzymes.

REFERENCE

1. American Cancer Society (ACS). "Report sees 7.6 million global 2007 cancer deaths." 2007.
2. Parkin DM, Pisani P, Ferlay J. Estimates of the worldwide incidence of 25 major cancers in 1999.Int.J.Cancer 1999;Int. *J Cancer*, 80:827-41.
3. Rowinsky E.K.and Donehower R.C.Paclitaxel (Taxol). N.Eng.J.Med. 1995;332: 1004-1014.
4. Agarwal KC. Therapeutic actions of garlic constituents. *Med Res Rev* 1999; 16:111–24..
5. Wan Lee, Hye Sang Shin, Jee Eun hang, Hesoopyo and Yunjgm . Studies on the analysis of Benzo (a) pyrene and its metabolites in biological samples by using high performance liquid chromatography. fluorescence detection and gas chromatography / mass spectrometry. Bull. Korean. Chem. Soc2003;24(5), 559-565. 2003
6. Elcheberger J.W., Kerns C.H., Ohyny K.P. and Buddle W.H. Anal. Chem1985; 55: 1471.
7. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ. Protein measurement with the folin-phenol reagent.J.Biol.Chem11951;93: 265-275.
8. Marklund S and Marklund G. Involvement of superoxide anion radical in the autooxidation of pyrogallol and a convenient assay for Superoxide dismutase.Eur.J.Biochem1974; 47:469-474.
9. Sinha AK. Colorimetric assay of Catalase.Anal.Biochem1972;47:389-394.
10. Rotruck JT,Pope AL,Ganther HE ,Swanson AB,Hafeman DG and Hoekstra WG.Selenium: biochemical role as a component of glutathione peroxidae purification and assay.Science1973;179:588-590.
11. Moron MS, Depierre JW and Mannervik B. Levels of glutathione, glutathione reductase and glutathione -S-transferase activities in rat lung and liver. Biochem. Biophys. Acta197;.58:.67-68.
12. Desai ID. Vitamin E analysis methods for animal tissues. Meth.Enzymol1984;105: 138-144.
13. Omaye ST, Turnbull JD and Sauberlich HE.Selected methods for the determination of ascorbic in animal cells tissues and fluids.Meth.Enzymol1971;62:3-11.
14. Ohkawa H,Ohnishi N and Yagi K.Assay for lipid peroxidation in animal tissues by thiobarbituric acid reaction Anal.Biochem. 1979;95:351-358.
15. Trush MA. and Kensler TW. An overview of the relationship between oxidative stress and chemical carcinogenesis. *Free Radical Biol.* 1991; *Med.* 10 : 201- 9.
16. Halliwell B. and Gutteridge JMC. Free radicals in biology and medicine.3rd ed. Oxford: Oxford University Press. 1999.
17. Rice - Evans C, Spencer JE, Schroeter H. and Rechner AR. Bioavailalililihy of flavanoids and potential bioactive forms invivo. *Drug. Metabot Drug Interact* 2000; 17: 1-4.
18. Ide N, Lau BHS. Garlic compounds minimize intracellular oxidative stress and inhibit nuclear factor—KB activation. *J Nutr* 2001;131:1020S-6S.
19. Kolanjiappan K, Manoharan S. and Kayalvizhi M. Measurement of erythrocyte lipids, lipid peroxidation antioxidants and osmotic fragility in cervical Cancer patients. *Clin. Chimica Acta* 2002; 326 : 143 – 149.

20. Jansen Y.M.W., Van Houten B., Borm, P.J.A. and Massman B.T. Cell and tissue responses to oxidative damage. Lab invest 1993;69: 261-274.
21. Sun Y. Free radicals antioxidants, enzymes and Carcinogenesis.Free Radical Biol Med1990;8: 583-599.
22. Daniel P.S and Joyce NJ.DNA adduct formation by 7,12 dimethyl benz (a) anthracene and its non-carcinogenic 2-fluro analogue in female Sprague dawley rats.J.Natl.Cancer Inst1983;70: 111-118.
23. Ruddon RW. Cancer Biology. Oxford : Oxford University Press. 1995.
24. Van Poppel G. and Vanden Berg H. Vitamins and Cancer. *Cancer Lett* 1997; 114**:** 195-202.
25. Hazelton AG, Lang CA.Glutathione content of tissues in the aging mouse .Biochem J 1980; 188:25-30.
26. Kagen Y., Sharinova A. and Park L. Generation and recycling of radicals from phenolic antioxidant. Arch. Biochem. Biophys 1990; 280: 33-39.