EXCELLENT PUBLISHERS

International Journal of Current Research and Academic Review

ISSN: 2347-3215 Volume 1 Number 3 (2013) pp. 52-57

www.ijcrar.com

Effect of Paclitaxel along with Di allyl sulfide on Electron Transport Chain Complex changes in 7,12 Di methyl benz(a) anthracene induced skin cancer Wistar rats

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KEYWORDS

Mitochondrial electron transport chain complex; Skin cancer ; 7,12 Di methyl benz(a) anthracene .

A B S T R A C T

The purpose of this study is to investigate the mitochondrial electron transport chain complex 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. 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 electron transport chain complex of skin were found to be decreased in the cancer bearing animals when compared with control animals. Treatment of Paclitaxel along with Di allyl sulfide to cancer induced animals showed significantly increased levels of electron transport chain complex when compared with cancer induced animals. The treatment with combination of paclitaxel and Di allyl sulfide effectively increased electron transport chain complex levels. So, from the obtained results it is concluded that paclitaxel and Di allyl sulfide is capable of restoring the skin architecture.

Introduction

Cancer is a cellular tumor that unlike benign tumor, can metastasize and invade the surrounding tissues. Cancer has been the major cause of death after cardio vascular disease. Humans of all ages develop cancer. Cancer is derived from latin means "CRAB". Cancer is life threatening. Neoplasms are abnormal

uncontrolled proliferation of cells. Neoplasms can be benign or malignant (Cancer). Cancer can be classified into three types. They are Sarcoma, Carcinoma and Lymphoma. Skin cancer is a type of Carcinoma. It is not a single disease. It is associated with oral cancer. Prevalence of skin cancer in Indian population is 1,460 members affected (Adams, 1991). Skin cancer has two most common forms of skin cancer - Basal Cell Carcinoma (BCC) and Squamous Cell Carcinoma (SCC), which together account for over one million new cases each year (Arias, 2003).

Exposure to ultra violet radiation is the single most important risk factor in the etiology of skin cancer (A1- Dawood, 2000).. Variations in the incidence of Cutaneous Malignant Melanoma (CMM) between similar populations living at similar latitudes suggest that other factors including diet may play a role (Arora, and Shukla ., 2002). Experimental studies on mice provide evidence that dietary fat in general and polyunsaturated fat in particular, may enhance the carcinogenic effects of ultra violet radiations (Bates, 1991).

Many patients seek out for alternative medicine after they have tried conventional medicine and found it to be ineffective or result in side effects (Black et al, 1992; Henderson *et al.*, 1991; Hunder *et al.*, 1996). The present. work is executed to compare the anticancer effect of Paclitaxel and Paclitaxel – Di allyl sulfide combination on DMBA induced skin cancer in wistar rats. The skin homogenate of the skin cancer rats were tested for electron transport chain complex. The results of the skin cancer induced rat were compared with controls for the study.

Materials and Methods

Animals

Wistar rats (150 – 200 gms.) were purchased from Meenakshi medical college and Research Institute, Kanchipuram, India and were used throughout the study. They were maintained in controlled environmental conditions of temperature and humidity on alternative 12 hr light/dark cycles. All animals were fed standard pelleted diet (Gold Mohr rat feed, Ms.Hindustan lever Ltd. Mumbai) and water ad libitum. This research work on wistar rats was sanctioned and approved by the Institutional Animal Ethical Committee (REG NO. 765/03/ca/CPCSEA).

Experimental protocol

The animals were divided into Five groups and each group consists of six animals.

Group I: Control animals treated with corn oil (vehicle) orally.

Group II: DMBA treated 5 µg) per animal in Acetone (100 µL), three times a week for 28 weeks to induce skin cancer

Group III: Skin cancer bearing animals (after 28th weeks of induction) treated with paclitaxel (33mg/kg b.wt, i.p) for four weeks.

Group IV: Skin cancer bearing animals treated with paclitaxel (as above) along with *Di allyl sulfide* (250µg/animal) for four weeks.

Group V: Control animals treated with paclitaxel along with Di allyl sulfide (as above).

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).

Biochemical analysis

The activity of Complex I was assayed by the method of Birch-Maching *et al.,* (1994). The activity of Complex II was assayed by the method of Birch-Maching *et al.,* (1994). The activity of Complex III was assayed by the method of Krahenbuchl *et al.,* (1994). The activity of Complex IV was assayed by the method of Capaldi *et al*., (1995) and Smith (1995).

Result and Discussion

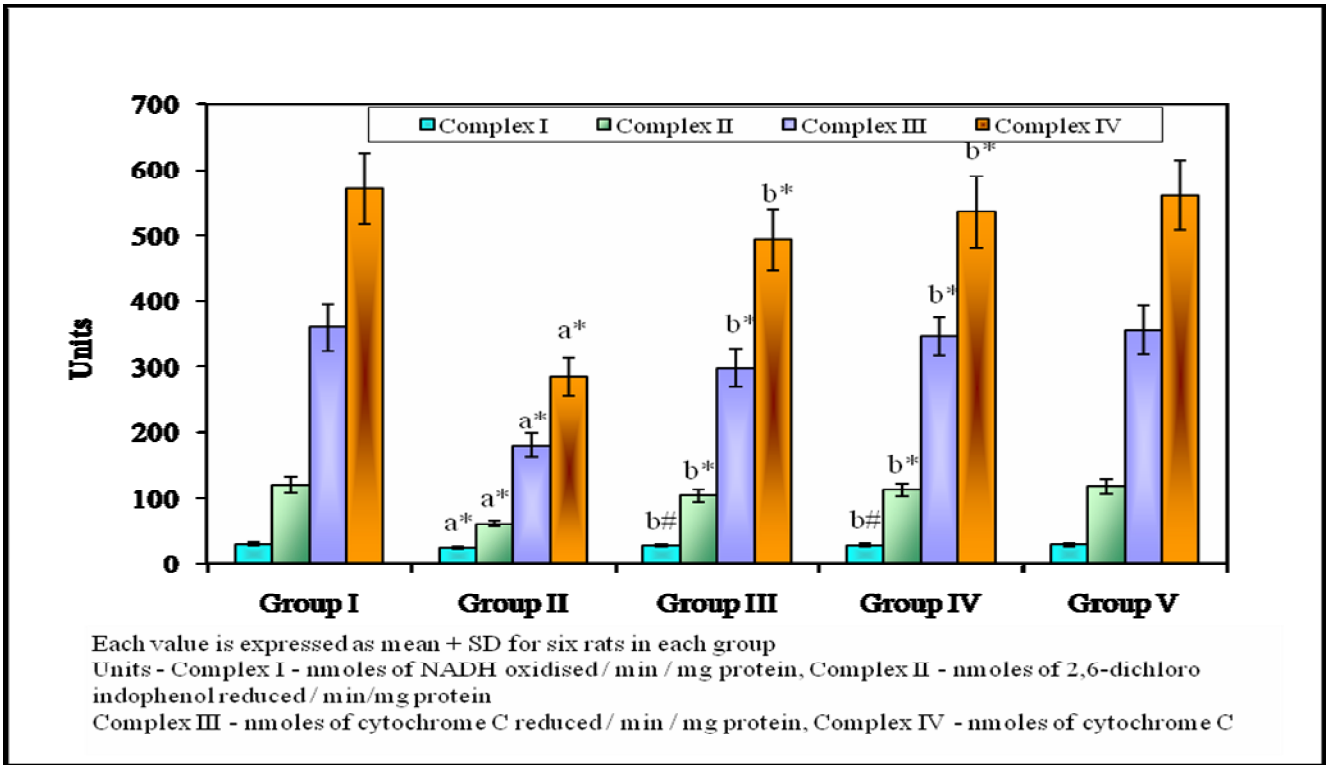
Figure.1 shows depicts the effect of paclitaxel along with *Di Allyl Sulfide* on the activities of electron transport chain complex enzymes in the lung of control and experimental animals. The activities of all the four complexes were found to be significantly ($p<0.001$) decreased in cancer induced group II animals when compared with the control animals (G-I). Paclitaxel (G-III) administration caused a significant ($p<0.05$; $p<0.001$) increase in the activities of these complexes when compared with the cancer bearing group.

Cancer bearing animals treated with a combination of paclitaxel and *Di Allyl Sulfide* (G-IV) showed a much more significant ($p<0.05$; $p<0.001$) increase in these enzyme activities when compared with the cancer bearing animals. However, there found to be no significant difference in the activities of the electron transport chain complexes between the control animals and the control animals treated with the combination of paclitaxel and *Di Allyl Sulfide* (G-V).

The respiratory chain, located in the inner mitochondrial membrane, provides molecular substratum for oxidative phosphorylation, a biochemical process based on the controlled oxidation of NADH or FADH2 through electron transfer from these donors via carriers (e.g. cytochromes) to molecular oxygen. Enzymes classically involved in NADH/FADH2 oxidation are dehydrogenases. According to molecular, structural and functional data, AIF plays a role analogous to the deyhdrogenases when it exerts its oxidaoreductase activity. Oxidative phosphorylation allows the pumping of protons out of the mitochondrial matrix, leading to the generation of a membrane potential (DCm) across the mitochondrial membrane. The energy liberated when the proton flow back across the gradient is stored as ATP produced from the enzymatic phosphorylation of ADP.

ROS are defined as redoxactive molecules able to oxidize a variety of cellular components including protein, lipids and DNA, leading to their structural and functional alterations. The vast majority of ROS are generation along the respiratory chain as a by-product of oxidative phosphorylation, during the incomplete reduction of oxygen to water. The family of ROS comprises free radicals such as superoxide anion ($O2^-$), nitric oxide (NO^-) and hydroxyl (OH^-) radicals as well as other reactive species (e.g. H_2O_2 and $ONOO^-$). The major site of ROS generated along the respiratory chain includes complex I (when the electrons are passed from NADH to oxidized coenzyme-Q, the first mobile electron acceptor) and complex III (when electrons are passed from

Figure.1 Effect of paclitaxel along with *Di Allyl sulfide* on the electron transport chain complex of skin in control and experimental animals



Each value is expressed as mean + SD for six rats in each group
Units - Complex I - nmoles of NADH oxidised / min / mg protein, Complex II - nmoles of 2,6-dichloro indophenol reduced / min/mg protein
Complex III - nmoles of cytochrome C reduced / min / mg protein, Complex IV - nmoles of cytochrome C

cytochrome c_1 to the second mobile electron acceptor, cytochrome c).

Electrons frequently escape along the electron transport chain (ETC), most usually at complex I (Barja., 1998) and complex II (Chen *et al* ., 2003). The reaction of the renegrade electron with molecular oxygen produces oxygen radical, which is normal converted into H_2O_2 or other ROS include hydroxyl radicals and superoxide anions, before being eliminated (Fleury et al., 2002)

The mitochondrion is the major oxygen-consuming organelle and, at least in some circumstances, is a major producer of oxygen radical species (Boveris and Chance, 1973) and as such it might be expected to play a central role in oxygen-sensitive processes of complex I of the electron transport chain and in cells lacking mitochondrial DNA. It has been proposed that complex III of the mitochondrial respiratory chain acts as an oxygen sensor and is a source of increased ROS. In this model, complex I inhibitors were proposed to act by blocking the electron flow proximally, so as to ablate such a signal. Mitochondrial function has also been implicated in other oxygen-sensing systems**.**

Mitochondrial electron transport complexes plays a critical role in generating cellular energy disorders that affect respiratory chain activity and can cause dysfunction of any organ system. It is formed and neutralized by the coordinate interaction of both the mitochondria and nuclear genome (Gregory M. Enns, 2003).

Electron transport between ETC complexes I-IV is coupled to the extrusion of protons across the inner mitochondrial membrane by proton pump components of the respiratory chain. Cytochrome C oxidase and NADH dehydrogenase are the mitochondrial enzymes involved in ETC complexes that plays an important role of producing energy rich compounds such as ATP. The decrease in the activity of NADH dehydrogenase can be due to the shortage of formation of reducing equivalents which in turn causes the depletion of mitochondrial glutathione accompanied by a functional loss of the activity of cytochrome C oxidase. Mitochondrial GSH plays a critical role in maintaining sulphydryl groups in the reduced state thus giving clue for the increase in the activities of NADH dehydrogenase and cytochrome C oxidase.

Statistical analysis

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

Conclusion

From the present study, the effect of Paclitaxel- Di Ally Sulfide combination proved to be a more significant chemotherapeutic agent against DMBA induced skin cancer in wistar rats compared to that of paclitaxel by analyzing transport chain complex levels.

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