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A STUDY OF EFFICACY OF PACLITAXEL ALONG WITH DI ALLYL SULFIDE ON LACTATE DEHYDROGENASE ENZYME ACTIVITY CHANGES IN 7,12 DI METHYL BENZ(A) ANTHRACENE INDUCED SKIN CANCER WISTAR RATS

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ABSTRACT

Aim: The purpose of this study is to investigate the changes in the levels of lactate dehydogenase enzyme (LDH) activity and efficacy of combination of paclitaxel along with Di allyl sulfide against skin cancer in experimental animals. skin cancer is the commonest cancer in all developed countries (except Japan) as well as in North Africa, South America, and southeastern and western Asia. While the incidence of skin cancer appears to be increasing, mortality rates are now declining in at least some western countries. 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. **Methods:** Skin cancer was induced in rats by 7, 12 Di methyl benz(a) anthracene (DMBA) at the dosage of 5 µg dissolved in 100µl acetone and administered into experimental animals for 28 weeks. In this study, we demonstrated that combination of paclitaxel and Di allyl sulfide revert the changes in the rats from lethal dose of DMBA within 30 days. **Results:** All the isoenzymes LDH1 – LDH5 were observed in cancer bearing animals. Expression of these isoenzymes were found be reduced in paclitaxel and Di allyl sulfide treated animals. **Conclusions:** The treatment with combination of paclitaxel and Di allyl sulfide effectively reduced LDH enzyme activity levels. So, from the obtained results it is concluded that paclitaxel and Di allyl sulfide is capable of restoring the Skin architecture.

KEYWORDS : Di allyl sulfide , DMBA, Paclitaxel, LDH and Skin cancer.

INTRODUCTION

Skin cancer is the most common form of human cancer. It is estimated that over 1 million new cases occur annually.1,2 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 once3. 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. 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 malignancy4. Although complete data of incidence is not available, various cancer registries in India reported cumulative incidence of skin cancer varying from 0.5 to 2 per 100 000 population5. Although, the incidence of skin cancers in India is lower as compared to the Western world, because of a large population, absolute number of cases is estimated to be significant. 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.

Polycyclic Aromatic hydrocarbons an important class of chemical carcinogens that are widespread in the ambient environment due to fossil fuel combustion for energy production, transportation and industry. DMBA, a potent PAH recognized as an initiator of both skin and liver cancer [5] 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 management of certain human solid tumors particularly in metastatic skin cancer and malignancy involves lung and refractory ovaries. 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". 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.

The chemotherapeutic and antitumor activity associated with garlic has been attributed to the presence of various organosulfide-based active compounds including Di Allyl sulfide. 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.

Materials and Methods

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 female rats was sanctioned and **approved by the Institutional Animal Ethical Committee (REG NO. 765/03/ca/CPCSEA).** 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.

Experimental Design

The animals were divided in to six groups of 6 animals each. Group I animals served as control, Group II, III, IV, V 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). These was Group VI Control animals treated with paclitaxel and Di allyl sulfide for 28weeks plus 30 days. After the experimental period of 32 weeks, the animals were sacrificed by cervical decapitation.

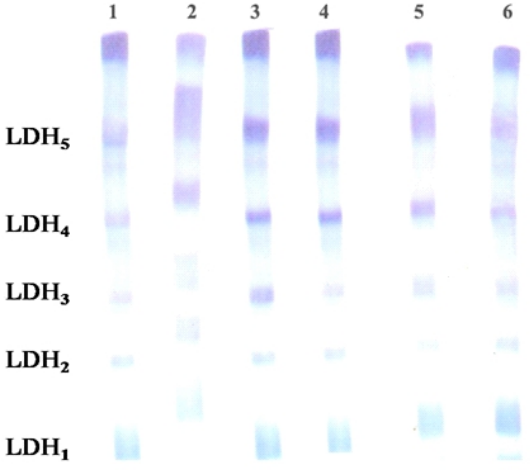
Biochemical analysis: The isoenzymes pattern of lactate dehydrogenase was separated by the method of Dietz and Lubrano (1967).

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

RESULTS

Plate 1 depicts the Isoenzyme pattern of lactate dehydrogenase in serum of control and experimental animals. All the isoenzymes LDH1 – LDH5 were observed in cancer bearing (group II) animals. Expression of these isoenzymes were found be reduced in paclitaxel (group III) and Di allyl sulfide (group IV) treated animals. However a much significant reduction in the LDH isoenzyme expression pattern was observed in-group V animals treated with both paclitaxel and Di allyl sulfide.

Lactate dehydrogenase (LDH) isoenzyme pattern in serum of control and experimental animals



Lane 1: Control
Lane 2: DMBA treated
Lane 3: Paclitaxel treated
Lane 4: Di allyl sulfide treated
Lane 5: Paclitaxel and Di allyl sulfide treated cancer bearing animal
Lane 6: Paclitaxel and Di allyl sulfide treated control animal

DISCUSSION

Lactate dehydrogenase (LDH) is the most common clinical enzyme used in the cancer patients for prognostic purpose [9]. It has an important role in germ cell functions and can predict responses to chemotherapy and the prospects of remission. Human cancer tissues typically exhibit 2-3 fold increases in glycolytic enzymes and LDH activity. Sandhya Mishra et al. (2004) have also reported the increased level of LDH in breast cancer patients. [10]

[11]reported greater LDH activity in skin cancer conditions. There was a significant increase in LDH level in serum of cancer bearing Group II animals. This might be due to the membrane disruptions that caused the release of these enzymes from the cancer cells or the overproduction by the tumor cells [12]. The elevated LDH activity may also have resulted from differences in the rate of synthesis, degradation or the excretion of the enzymes in the skin cancer bearing animals. [13] has reported that among the isoenzymes LDH5 was six times greater than LDH1 in mammary tumor cells.

Flavanoids have proved to possess antitumor effect on various animal models [14]. The biological and pharmacological activity of Di allyl sulfide was associated with phenolic compounds mainly to flavanoids, aromatic acids and esters [15,16]. Antioxidant activity of flavanoids may also be due to their structural features and its action on membrane[17,18,19]. Paclitaxel being rich in flavanoid content possess antitumor and antiproliferative activities that stabilizes the membrane permeability and reduces the release of LDH [20, 21].

Conclusion

From the present study, the effect of Paclitaxel- Di allyl sulfide combination proved to be effective chemotherapeutic agent against DMBA induced skin cancer in wistar rats compared to that of paclitaxel or Di allyl sulfide confirmed analyzing the LDH isoenzymes levels in serum.

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