Effect of Paclitaxel and Paclitaxel-Di Allyl Sulfide Combination on 7, 12 Dimethyl Benz (a) Anthracene (DMBA) Induced Skin Cancer in Wistar Rats: A Comparative Study

N. Muninathan, C. Selvakumar, S. Malliga and J. Kumar
Department of Biochemistry, Meenakshi Medical College and Research Institute, Enathur, Kanchipuram, 631552 Tamil Nadu, India

Abstract: Cancer is a cellular tumor that unlike benign tumor can metastasize and invade the surrounding tissues. In the present study the anticancer effect of paclitaxel was evaluated on 7, 12 Di Methyl Benz (a) Anthracene induced skin cancer in Wistar rats and results were compared with normal, paclitaxel and paclitaxel-di allyl sulfide combined alternative chemotherapy. By analyzing the various biochemical parameters (lipid profile and lipid metabolizing enzymes) and marker enzymes (squamous cell antigen). Skin cancer was induced in rats by 7, 12 Di Methyl Benz (a) Anthracene (DMBA) at the dosage of 5 µg was dissolved in 100 µL and administered into experimental animals for 28 weeks. In this study, researchers demonstrated that combination of paclitaxel and di allyl sulfide protects the rats from a lethal dose of DMBA for 30 days. Total Cholesterol (TC), Free Cholesterol (FC), Phospholipids (PL) and Triglycerides (TG) were found to be significantly increased whereas Ester Cholesterol (EC) and Free Fatty Acids (FFA) were significantly decreased when compared with cancer bearing Group II animals. From the present study, the effect of Paclitaxel di allyl 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 the lipid profile and lipid metabolizing enzymes and marker enzymes.

Key words: Skin cancer, 7, 12 Dimethyl Benz (a) Anthracene (DMBA), paclitaxel, di allyl sulfide, India

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 cardiovascular 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 and Cory, 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 and Smith, 2003).

Exposure to ultra violet radiation is the single most important risk factor in the etiology of skin cancer (Al-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; Bates and Longo, 1987). 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). The present research is executed to compare the anticancer effect of Paclitaxel and Paclitaxel-di allyl sulfide combination on DMBA induced skin cancer in wistar rats. The serum and liver homogenate of the skin cancer rats were tested for lipid profile and lipid metabolizing enzymes. The results of the skin cancer induced rat were compared with controls for the study.

MATERIALS AND METHODS

Animals: Wistar rats (150-200 g) 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

Corresponding Author: C. Selvakumar, Department of Biochemistry, Meenakshi Medical College and Research Institute, Enathur, Kanchipuram, 631552 Tamil Nadu, India
12 h 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 on Wistar rats was sanctioned and approved by the Institutional Animal Ethical Committee (Reg NO. 765/03/cu/CPCSEA).

**Experimental protocol:** The animals were divided into six groups and each group consists of six animals:

- **Group I:** control animals
- **Group II:** acetone (100 µL) applied topically followed 1 h later by DMBA (5 µg) per animal in acetone (100 µL), three times a week for 28 weeks
- **Group III:** skin cancer bearing animals treated with Paclitaxel (33 mg kg⁻¹ body weight) weekly once for 4 weeks
- **Group IV:** skin cancer bearing animals treated with DAS for 30 days (250 µg/animal)
- **Group V:** skin cancer bearing animals treated with both paclitaxel and DAS for 30 days
- **Group VI:** control animals treated with paclitaxel and DAS 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).

**Biochemical analysis:** Total cholesterol was estimated by the method of Parekh and Jung (1970). The free cholesterol was analyzed by Leofler and McDougald (1963), triglycerides and free fatty acids by Rice (1970) and Hron and Menahan (1981), respectively. Lipid metabolizing enzymes lipoprotein lipase and lecithin cholesterol acyl transferase by Schmidt (1974) and Legraud et al. (1979), respectively.

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

**RESULTS**

Figure 1 shows the effect of paclitaxel and di allyl sulfide on lipid profile in the liver of control and experimental animals. Total Cholesterol (TC), Free Cholesterol (FC), Phospholipids (PL) and Triglycerides (TG) were found to be significantly increased whereas Ester Cholesterol (EC) and Free Fatty Acids (FFA) were significantly decreased when compared with cancer bearing Group II animals.

On treatment with Paclitaxel (G-III) lead to a significant decrease in the lipid profile and also a significant increase in the EC and FFA levels when compared with cancer induced animal (G-II). Di allyl sulfide (G-IV) also exhibited the same pattern of changes with a significant decrease in the overall lipid profile except EC and FFA which was significantly increased after treatment.

However, the effect shown by the combined treatment of paclitaxel and di allyl sulfide was more promising with significant decrease in the levels of TC, FC, PL and TG along with a significant increase in EC and FFA levels when compared with the cancer-induced group. There was no significant difference in the lipid profile levels between the control animals (G-I) and the control treated with the combination of paclitaxel and di allyl sulfide (G-VI).

Figure 2 represents the effect of paclitaxel and di allyl sulfide on lipid metabolizing enzymes in the liver of control and experimental animals. The activities of Lipoprotein Lipase (LPL) and Lecithin Cholesterol Acyl Transferase (LCAT) were found to be significantly decreased in cancer induced animals (G-II) when compared with control (G-I) animals.

On treatment with Paclitaxel (G-III) the activities of LPL and LCAT were significantly increased when compared with cancer induced group. Di allyl sulfide (G-IV) treatment also caused a significant increase in LPL and LCAT activities. However, combination treatment with both di allyl sulfide and Paclitaxel (G-V) caused a much significant increase in LPL and LCAT activities when compared with cancer bearing animals (G-II). There was no significant difference in the lipid metabolizing enzymes levels between the control animals (G-I) and the control treated with the combination of paclitaxel and di allyl sulfide (G-VI).

**Fig. 1:** Effect of paclitaxel along with di allyl sulfide on lipid profile in the liver of control and experimental groups. Each value is expressed as mean±SD for six rat in each group; a: as compared with Group I; b: as compared with Group II. Statistical significance *p<0.01, **p<0.01, ***p<0.05, NS = Not Significance
compared with cancer bearing animals (G-II). There was no significant difference in the enzyme activities in control animals (G-I) and the control animals treated with paclitaxel and di allyl sulfide (G-VI).

**DISCUSSION**

Cancer is associated with higher lipid metabolizing activity and cholesterol metabolism is regulated differently during tumor growth (Beek and Tisdale, 1981). Deregulated cholesterogenesis observed in tumors implicated an over production that could result in the enrichment of tumor cell membrane with cholesterol (Dessi et al., 1992). Abnormal levels of lipid profile and their changes in lipid metabolising enzymes are proportionate to the disease stage. Elevated cholesterol level precedes the observed changes in DNA and protein content suggested a link between cholesterol and DNA synthesis pathway.

In the present study it was observed the elevated levels of TC, FC and PL with a decrease in EC and FFA in cancer bearing (G-II) animals. Thirunavukkarasu et al. (2003) and Daciavitskaiia and Bergelson (1982) have also reported the same. The decrease in the levels of EC and increase in FC levels in cancer bearing animals may be due to multiplication of already proliferating cells (Rao et al., 1986).

The progression and proliferation of the tumor cells depends on the elevation of cholesterol and PL levels which modifies the lipid fluidity of the tumor cell membranes thereby increasing the rate of malignancy in tumorous conditions (Damen et al., 1984). Bergelson et al. (1970) have reported that the activation of various carcinogenic factors leads to alterations in membrane synthesis accompanied by changes in PL components of tumor cell membrane. Several reports also revealed the changes in the distribution of TC in animals observed during progression of tumor growth (Dessi et al., 1992). Ray et al. (1997) and Potschman et al. (1991) have observed significantly higher levels of TG’s in skin cancer patients when compared with controls. TG’s are metabolized by LPL and the reaction products FFA and glycerol may then recirculate to the liver from which they readily cross the cell membrane (Dessi et al., 1992).

LPL is the key enzyme responsible for the hydrolysis of TG’s that are shown to be significantly reduced during tumor growth (Damen et al., 1984). Hypertriglyceridemia in cancer bearing rats may be due to the clearance defect associated with lowered LPL activity. LPL have been observed in skin cancer bearing rats. This decrease in LPL activity may be due to HDL competing with the substrate for binding to the enzymes (Rogers and Hutchinson, 1981).
During hydrolysis of TG’s core of chylomicrons and VLDL reductant surface constituent, e.g., PL’s and cholesterol are transferred to HDL when the cholesterol is esterified by LCAT (Helmes et al., 1998). LCAT is the central enzyme responsible for the esterification of FC in various cells and tissues. The observed decrease in LCAT in cancer bearing animals may be due to the rapid multiplication of the already proliferating cells. This decrease in LCAT activity observed in skin cancer condition is responsible to maintain low EC level by reducing the efflux of FC from the proliferating cells and the influx of cholesterol ester in to the cells (Bergelson et al., 1970).

In paclitaxel and DAS treated animals the lipid profile and the lipid metabolizing enzymes were reduced to normal levels. A combination of paclitaxel and DAS is reported to positively affect the HDL and LDL levels of the rats (Kolanjiappan et al., 2002). It has been shown to stimulate various enzyme systems, cell metabolism and circulation. Stavrak and Matula (1992) have reported the effect of flavonoids in reducing TG level. The paclitaxel and DAS are powerful antioxidants and they have been shown to be capable of protecting against alterations in lipid metabolism.

CONCLUSION

From the present study, the effect of Paclitaxel-DAS combination proved to be a more significant chemotherapeutic agent against DMBA induced skin cancer in Wistar rats compared to that of paclitaxel by analyzing the various biochemical parameters, lipid profile and lipid metabolizing enzymes.

REFERENCES


