ANTI-OXIDANT EFFECT OF LENALIDOMIDE IN EXPERIMENTALLY INDUCED BREAST CANCER ON FEMALE WISTAR RATS

Shreelakshmidevi Singaravelu[1], Jaikumar S[2]

1.Research scholar, Bharath institute of higher education and research, Chennai, Tamil Nadu, India.

2. Bharath institute of higher education and research, Chennai, Tamil Nadu, India.

ABSTRACT

The role of antioxidants in preventing oxygen radical and hydrogen peroxide induced cytotoxicity and tissue damage in various human diseases is becoming increasingly recognized. Lenalidomide a derivative of thalidomide possesses anti-neoplastic, immunomodulatory and antiangiogenic properties. Oxidative enzyme studies estimated in the breast tissue showed an increase in enzyme levels especially catalase in group III while the other enzymes were found to be elevated in group IV. Low levels of oxidative enzyme in group I indicate severe enzyme depletion and tissue destruction which was further substantiated by histopathological studies. Oxidative enzyme in erythrocyte lysate in drug treated group II & III were lower than normal levels.

INTRODUCTION

At present, about 1.2 million new cases of cancer are detected annually in the US and about 600,000 people die of this disease every year. The role of antioxidants, vitamins, new diet and lifestyle modification in modulating human cancer incidence has drawn significant attention from basic and clinical scientists. This issue has been critically reviewed with respect to cancer prevention in a recent publication[1] [B2-1].Management of breast cancer relays on anti-angiogenic agents and surgical cure. Oxygen derived radicals have been implicated in the etiology of cancer development perhaps since the demonstration that ionizing irradiation caused cancer[2,3] (B4-3,6). It has been shown that free radicals have a mutagenic capacity as a result of the interaction between highly reactive chemical molecules and DNA[4]. Upon reaction with DNA, oxygen derived radicals produces base adducts and strand breaks. The former can cause mispairing lesions during DNA replication. It was also reported that oxyradicals can also cause cytotoxcity and stimulate changes in gene expression [4,5,6] The cells protect themselves against oxidative damage by enzymatic and nonenzymatic antioxidant systems. Superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase form primary enzymatic defense system[7]. SOD catalyses dismutation of superoxide radicals to hydrogen peroxide. Manganase containing form, MnSOD, is mainly found in mitochondria. Hydrogen peroxide is metabolized by catalase and GSH-Px via reducing into water and molecular oxygen. Anti-oxidants will reduce the oxidative damages

An experimental system is needed that closely mimics the human disease, and allows one (a) to elucidate the influence of host factors on the initiation of the neoplastic process, (b) to determine whether in the human the susceptibility of the mammary gland varies with age and reproductive history, and (c) to discover whether it can be manipulated by treatment of the host[8]. We consider that rat mammary gland carcinogenesis is a model that closely fulfills the above conditions. This is one of the most widely studied and useful models of mammary carcinogenesis[9]. Many strains of rats develop spontaneous tumors, and respond to a variety of chemical carcinogens and radiation with development of either hormone-dependent or hormone-independent mammary tumors. The most common chemical induced cancer is by using 7,12DMBA injection, a 100% rate of tumor incidence is assured if time elapses before necrolepsy[10]

Lenalidomide possesses anti-neoplastic, immunomodulatory and antiangiogenic properties. Lenalidomide inhibited the secretion of pro- inflammatory cytokines and increased the secretion of anti-inflammatory cytokines from peripheral blood mononuclear cells. Lenalidomide inhibits cell proliferation. Lenalidomide inhibits the growth of multiple myeloma cells from patients, as well as MM.1S cells (a human multiple myeloma cell line), by inducing cell cycle arrest and apoptosis.

MATERIALS & METHOD

48 wistar albino rats weighing 150-200 gms were used in the study. The study was approved by Institutional Animal Ethical Committee. Animals were housed at a temperature of 24 ± 2^0C and relative humidity of 30-70%. The animals were fed with staple pellet diet from Hindustan Lever Ltd., Mumbai. The animals were divided into five groups with eight animals in each. Mammary carcinogenesis was induced in rats in groups I through IV by injecting 7,12 DMBA in 0.1 ml subcutaneously to each rat at the mammary region. Group II & III received the investigational drug at two dose level of 10mg/day and 25mg/day for 16 weeks. The standard drug (Docetaxel 5mg/kg) was administered to rats in group IV. Group V was given the basal diet and water throughout the experimental period. At the end of 16^{th} week animals were sacrificed. Blood samples and tissue samples were sent for investigation.

RESULT

BREAST TISSUE OXIDATIVE ENZYME PARAMETER (Vide table 1)

The oxidative enzyme namely superoxide dismutase, catalase and glutathione peroxidase were estimated in the breast tissue. Group IV has the highest SOD level and Glutathione peroxidase levels whereas GroupI has the least SOD level, GroupIII statistically has the highest Catalase level when compared with Group I which had the least. Group I levels of anti-oxidant enzymes were the lowest when compared to group III and IV. The increase in anti-oxidant enzyme observed in group III & IV were comparable to the normal enzyme levels seen in control group V. The T BARS level showed a decrease in normal and drug treated groups, Group I has the highest T bar levels whereas Group III,IV,V has the lowest T bar level.

ERYTHROCYTE LYSATE OIDATIVE ENZYME PARAMETERS (Vide table 2): Group IV has the highest SOD, catalase, Glutathione peroxidase levels whereas Group I has the least. Oxidative enzyme in erythrocyte lysate in drug treated group II & III were however lower than normal levels. GroupI has the highest T bar levels whereas Group IV has the lowest T bar level.

LIVER FUNCTION TEST (Vide Fig.1,2,3): The results of the serum liver enzymes in animals which received DMBA was found to be elevated to a marked degree. The means values of AST and ALT in the animals treated with lenalidomide showed a moderate increase when compared with normal controls. The levels of alkaline phosphatase showed a minimal variation similar to the values of group IV(Docetaxel treated group).

TABLE:1 BREAST TISSUE OXIDATIVE ENZYME PARAMETERS

Groups	T.BARS	SOD	CATALASE	GPX	Mean
I	18.015	19.165	101.835	52.895	46.33
II	14.27	23.605	169.36	80.42	69.07
III	13.375	24.985	173.785	84.845	70.71
IV	11.275	40.01	155.88	96.94	69.05
V	12.435	37.325	149.83	90.89	66.53

TABLE:2 OXIDATIVE ENZYME PARAMETERS IN ERYTHROCYTE LYSATE

Groups	T.BARS	SOD	CATALASE	GPX	Mean
I	12.185	12.84	52.895	8.035	25.97
II	9.68	23.284	70.42	11.71	34.46
III	8.44	28.665	74.845	12.88	37.31
IV	5.395	33.69	89.94	18.365	43.00
V	6.605	31.005	90.89	17.235	42.83

FIGURE1: Liver Function Test (SQOT / AST)

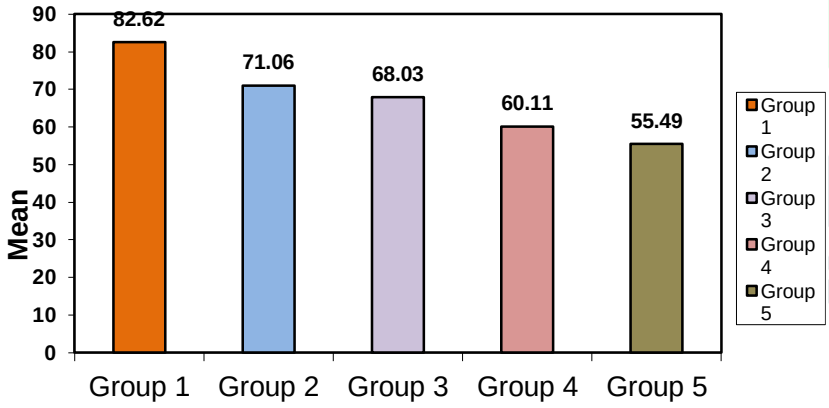


FIGURE 2: LIVER FUNCTION TEST (SQPT / ALT)

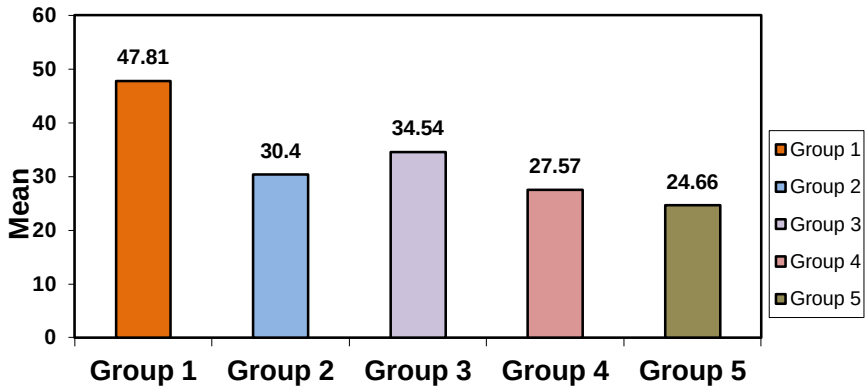
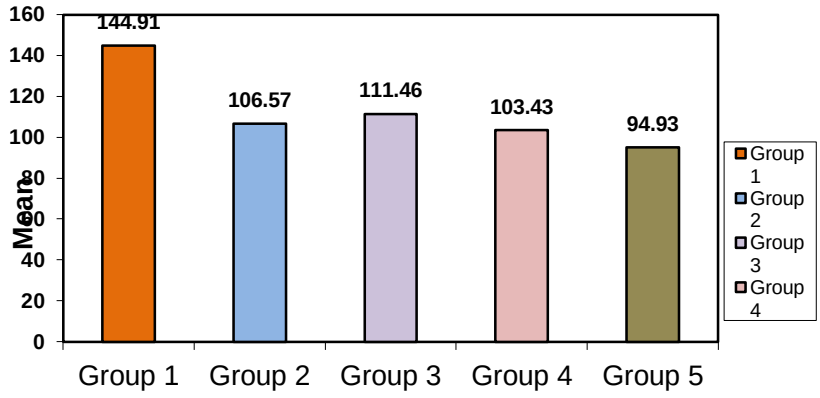


FIGURE 3: LIVER FUNCTION TEST (ALP)



DISCUSSION

The role of lenalidomide in the treatment of multiple myeloma [11] was tried on basis of its immunomodulatory properties. The mechanism of lenalidomide which includes an anti-angiogenic action probably acts through one of the following mechanisms hypothesized are Inhibition of activated EC (Endothelial cells), Inhibition of EC Intracellular signaling and Inhibition of TNF-α signaling.

Significant clinical experience has been acquired on the activity of thalidomide / lenalidomide in both, newly diagnosed and pretreated / refractory Multiple Myeloma patients. Preclinical data suggesting more potent activity than its parent compound as well as less toxicity and lesser tetratogenicity potential has made it a promising new drug in cancer chemotherapy.

At least four distinct but potentially complementary mechanisms have been proposed to explain the anti-tumor activity of lenalidomide.

1. Direct anti-proliferative / pro-apoptotic anti-tumor effects [12] probably mediated by one or more metabolites, they include inhibition of the transcription activity of NF-KB and its anti-apoptotic target

genes, including the caspase inhibitors FSIP, CIAP-2 or anti-apoptotic Bcl-2 family member AI /Bfl-1.[13]

2. Indirect targeting of tumor cells by abrogation of the protection conferred to tumor cells by their cell adhesion molecules or cytokine (IL-6) mediated interactions with bone marrow tumour cells.[12]
3. Inhibition of cytokine production, release and signaling, leading to anti-angiogenic effects.[14]
4. Immunomodulatory effects including enhanced NK cell mediated cytotoxicity[15] contributing to potential anti-tumour immune response.
5. Lenalidomide, inhibited the expression of COX-2 but not COX-1 in vitro.

The liver function tests reveal a marginal increase in AST/ALT enzyme levels of groups II, III & group IV. A statistically significant increase in the ALT & AST levels of group I, indicative of liver toxicity was observed in the DMBA treated group (vide Fig 1,2).

Oxidative enzyme studies estimated in the breast tissue showed an increase in enzyme levels especially catalase in group III while the other enzymes were found to be elevated in group IV. Low levels of oxidative enzyme in group I indicate severe enzyme depletion and tissue destruction which was further substantiated by histopathological studies.

CONCLUSION

The liver enzymes were moderately elevated compared to normal controls suggestive of reversible hepatotoxicity. The oxidative enzyme studies of erythrocyte lysate and the breast tissue showed an increase in mean values especially of enzyme catalase which coincides with the immunomodulatory effects of lenalidomide. Thus given this degree of complexicity certainly no single agent could ever be expected to manage or cure “breast cancer” in its entirety yet lenalidomide with its anti-neoplastic, anti-angiogenic and immunomodulatory role could help alleviate this condition. A better understanding of the underlying processes would enable the scientists to develop similar drugs therapies that will significantly enhance our ability to treat intractable diseases such as cancer.

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