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

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ABSTRACT

Lenalidomide is an angiogenesis inhibitor with immunomodulatory properties and possess lesser toxicity when compared with Thalidomide and other teratogenic agents. The histopathological study reveals ductal carcinoma induced by 7.12DMBA and reduction in malignant cells in Lenalidomide and Docetaxel treated group. Breast cancer management with Lenalidomide will have less teratogenicity on par with Thalidomide. Along with its anti-angiogenic property Lenalidomide is also a potent antioxidant and Immunomodulatory agent which adepts it for Breast cancer treatment.

INTRODUCTION

Molecular genetic changes in breast carcinogenesis are due to the activation of dominant oncogenes which often involves specific mutation that changes the functional control of growth like promoting proteins or gene amplification that increases protein expression[1]. Overall, there has been a slight decline in mortality, which can attribute both to the success of early detection programs and to advances in treatment, particularly development in systemic therapy. The role of agents like Methotrexate[2], 5- Flurouracil, Tamoxifen citrate , Letrozole, Fulvestrant, Paclitaxel[3], Cyclophosphamide[4], Docetaxel in breast cancer is well established but their therapeutic benefits are masked by their toxic effects on various systems.

The therapeutic benefits and the immunomodulatory role of Thalidomide, as an angiogenesis inhibitor in the treatment of some cancers is well documented, but the major drawback highlighted was teratogenicity. **Lenalidomide** a newer class of Thalidomide derivative exhibits a constellation of pharmacological effects with lesser incidence of drug toxicity and lack of teratogenic effects. Hence this study of the effect of Lenalidomide in breast cancer was undertaken.

AIM & OBJECTIVE

Efficacy of lenalidomide in myelodysplastic syndrome has been proven by the inhibition of 1S cells (a human multiple myeloma cell line) by inducing cell cycle arrest and apoptosis. However, its role in the treatment of solid tumors such as breast cancer is yet to be studied. So this study is proposed to evaluate the anti-carcinogenic role of the drug and to compare its efficacy with other drugs being used in the treatment of breast cancer.

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^{0}C 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. GroupV was given the basal diet and water throughout the experimental period.

At the end of 16th week animals were sacrificed .Blood samples was send for Hb, WBC,RBC, Platelet count [5]. The tissue samples were sent for histopathology [6].

RESULT

HEMATOCRIT VALUES(vide Figure 1,2,3,4)

Peripheral cell count was estimated a month after the administration of lenalidomide and readings were recorded once a month for the next 3 months.

Lenalidomide resulted in a fall in hematocrit values indicating that lenalidomide has a myelosuppressive effect. The blood platelet count in the treated group was also lower than control values and so was the Hb%. This reduction was maximized in the third month of the study.

BODY WEIGHT OF RATS (Vide table:1)

The animals were weighed once a month and the mean weight gain / loss was estimated. The mean values of the body weight showed a gross reduction in group I, a moderate lowering of body wt in groups II & IV while group III was comparable to normal controls in group V.

HISTOPATHOLOGICAL STUDY OF BREAST TISSUE :

DMBA induced group I showed Infilterating Ductal Carcinoma with extensive Stromal involvement. Group II & III Lenalidomide treated groups showed a mild involvement of malignant cells in the stroma. Group IV showed reactive changes and few malignant cells. With regard to the above mentioned results, it was evident that Lenalidomide at doses of 10mg/day &25mg/day afforded significant protection in 7,12 DMBA induced breast cancer. The serological effects were further substantiated by histopathological studies.

TABLE 1: **BODY WEIGHT OF RAT (GM)**

Groups	I month	II month	III month	IV month	Mean
I	210	180	140	110	176.67
II	200	190	186	180	192.00
III	220	212	210	206	214.00
IV	200	196	192	186	196.00
V	210	214	220	231	214.67

Fig 1: PERIPHERAL CELL COUNT

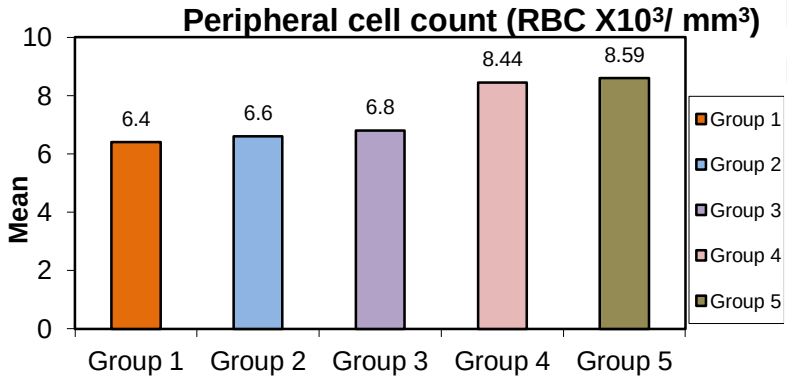


Fig 2: PERIPHERAL WBC COUNT

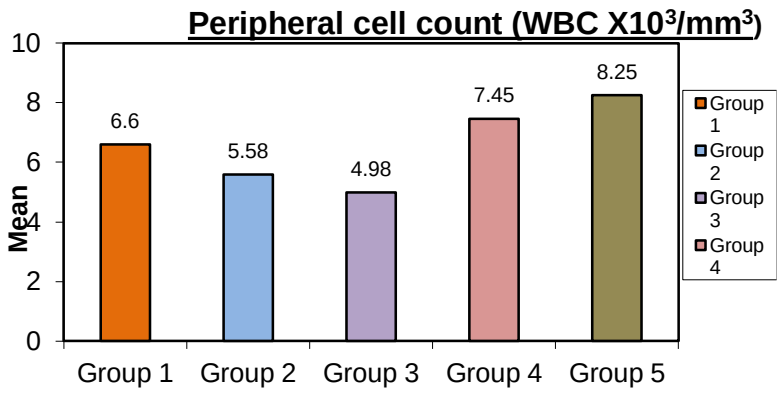


Fig 3: PERIPHERAL PLATELET COUNT

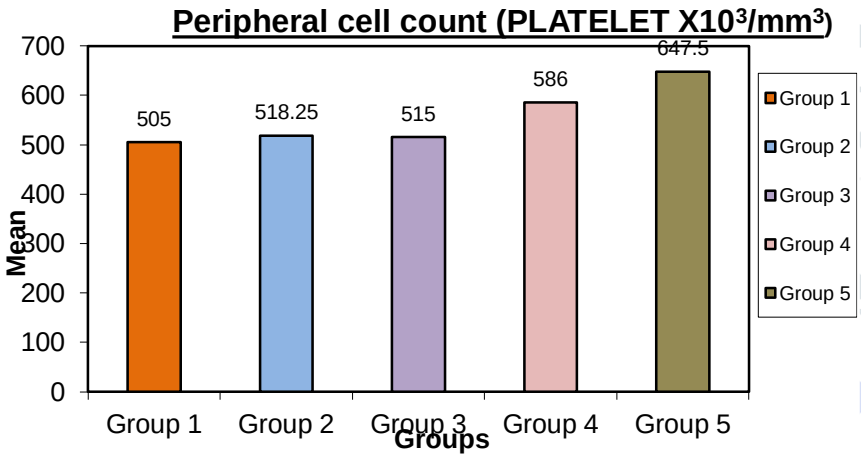
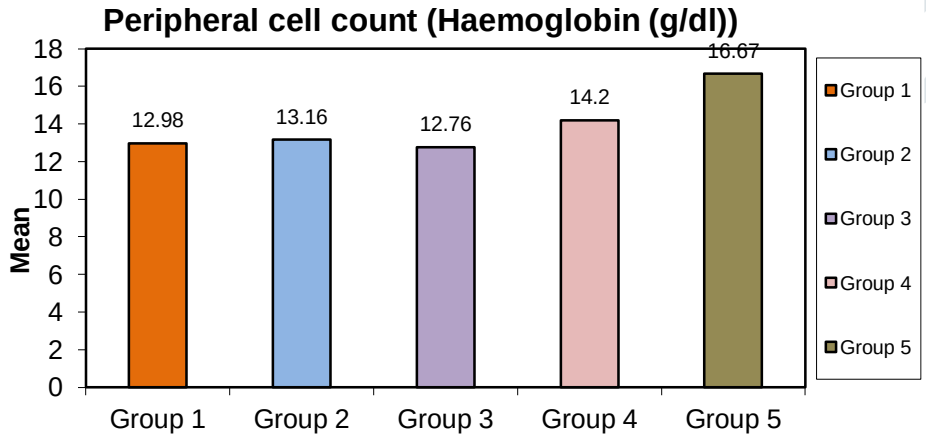


Fig 4: Haemoglobin(g/dl)



DISCUSSION

Lenalidomide, an analogue of thalidomide was created with the intention of improving the immunomodulatory properties as well as for reducing the toxicity of the preparation. Lenalidomide constitutes a lead compound in the new class of immunomodulatory thalidomide derivatives (IMID's) which exhibits a constellation of pharmacological properties, like stimulation of T-cells & NK – cells

[7], Inhibition of angiogenesis [8] and tumor cell proliferation [9] and Modulation of haemopoietic stem cell differentiation.

This orally administered agent though tested in myelodysplastic status is yet to be tried in other solid tumors. The role of lenalidomide in the treatment of multiple myeloma [10] was tried on basis of its immunomodulatory properties. Significant clinical experience has been acquired on the activity of thalidomide / lenalidomide in both, newly diagnosed and pretreated / refractory Multiple Myeloma patients.

From the results of this study we infer that lenalidomide at doses of 25 mg/day produced a significant effect in various parameters studies. There was a marked reduction in the hematological parameters especially peripheral count (WBC & RBC's platelet and Hb%) observed throughout the study period especially at the 3rd & 4th month. This adverse effect is accounted for the myelosuppressive effect of lenalidomide.

Histopathological studies revealed infiltrating ductal carcinoma with extensive Stromal involvement in group I, whereas groups II& III had mild involvement of malignant cells in the stroma, thus proving the anti-cancer effect of Lenalidomide. The Docetaxel group IV showed least evidence of malignant cells with reactive changes in the breast tissue.

Body weight in the DMBA treated group was significantly lower than others. Evaluation of the hematocrit values which were lower in the drug treated group clearly indicates that lenalidomide at both dose levels of 10 mg/d and 25 mg/d did have a myelosuppressive potential. The WBC could probably be treated by the addition of Filgrastim or Sargamostim colony stimulating factors which are used in cancer therapy.

Although the precise mechanism of antitumor activity remains to be fully elucidated, substantial insight has been generated by extensive preclinical studies.[11] 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.

CONCLUSION

From the above results it can be summarized that lenalidomide at dose of 25 mg /d was effective in reducing the tumor mass. The effect was less marked in doses of 10mg/d. The myelosuppressive teratogenic potential must be considered when prescribing the drug to women of child bearing age.

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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