“IMMUNOMODULATORY ROLE OF HISTONE H1 IN CISPLATIN INDUCED IMMUNE SUPPRESSION IN EXPERIMENTAL BREAST CANCER”

Treatment for breast cancer has primarily been surgery, chemotherapy and radiation therapy. Until recently the involvement of hormones in the development of this disease has also lead to the use of hormone in therapy. A combination of these therapies is used to annihilate the growth of cancer cells. But the side effects rendered by these treatments are deleterious than the disease itself. Hence it is necessary to employ agents that swing the balance between tumor cell invasion and host immune cell defense in favor of the latter. This necessitates the employment of biologically active agents that are cytotoxic to a cancer cell but are not to normal cells.

Breast cancer has been chosen for the present study, as principles of therapy derived from this experimental study may be applicable in general. In breast cancer the usual routine of treatments results in tumor regression in 50% to 80%, but complete responses occur in only 11-20% and majority of patients even though attain complete response, have progression of their disease leading to death. Chemotherapeutic drugs used are antimetabolites, analogues or antitumour antibiotics that kill cancer cells and also affect the growth of normal cells. During tumorigenesis profound dysregulation of cytokine production by various lymphoreticular cells have documented.

Cis-platinum diaminedichloride (CDDP) is a heavy metal compound used as an anticancer chemotherapeutic agent and has a spectrum of unique biologic effects [1]. In aqueous solution, it forms stable inter- and intrastrand cross-links with DNA [2]. In addition, it could induce oligonucleosomal DNA fragmentation typical of apoptosis in malignant cells [3–7]. Moreover, the interaction of this drug with DNA produces reactive oxygen species [8].

This study aims in the use of HISTONE H1 as a therapeutic agent in cancer therapy, taking advantage of its hormone-like and immunostimulating functions. Histones are freely moving in circulation and lymphatic system and hence will bring biological response due its specific interaction with cancer cells. Histone being a component of Homeostatic Thymic Hormone (HTH) has multiple inter-relationships with several endocrine glands and suppresses immunological consequences of thymectomy.

- To evaluate the efficacy of HISTONE H1 as an anticancer agent by modulating cell mediated immune responses.
- its relationship to CDDP-induced immunosuppression and DNA damage in peripheral blood mononuclear cells (PBMC).

**Present knowledge:**

Treatment for breast cancer includes chemotherapy, radiation therapy, surgery and hormonal therapy in combination. Surgery causes destruction of normal tissues by surgeon's knife. Immunotherapy involves use of antibodies and lymphocytes against tumor tissues that causes spontaneous regression of tumor. In cancer conditions defective immune system fails to
generate killer lymphocytes, but on activation they acquire ability to generate killer lymphocytes, one such activating agent is Interleukin 2 (IL-2). Very large doses of IL 2 are required to achieve regression, but the side effects rendered are severe. This study focuses on highlighting HISTONE H1 as a protein of immune system that has an anticancer activity and stress the need to its use as a therapeutic carrier in a quantity having therapeutic effect taking advantage of its biologically active role.

Histone H1 is a biologically active protein that is cytotoxic to leukemic cells in vivo [1], ascites tumor cells [2] and exogenous histone stimulates normal peripheral lymphocytes in vivo [3]. Histone H1 is a component of the homeostatic thymic hormone, which co-ordinates immune homeostasis [4]. Internalized immuno-conjugates of histone significantly inhibit growth of breast cancer cells [5]. Our previous study revealed that histone H1 suppressed growth of mammary tumor [6], modulated the activation of macrophages in mice [7] and modulated the immune status in breast cancer [8].

Techniques involved:
1. Tissue culture techniques
2. Spectrophotometry
3. ELISA
4. Electron microscopic studies
5. Radioactive studies
6. PCR, RT-PCR studies

In Vivo Studies:

Virgin female Wistar rats 50 - 60 days old maintained on standard pelleted diet and water ad libitum are grouped as follows:

Group 1: Normal rats maintained as control group.
Group 2: Normal rats treated intraperitoneally with HISTONE H1.
Group 3: Normal rats treated intraperitoneally with cisplatin
Group 4: Rats induced for breast cancer with 9,10 dimethylbenz(a)anthracene
Group 5: Rats with breast cancer treated with HISTONE H1.
Group 6: Rats with breast cancer treated with cisplatin.
Group 7: Rats with breast cancer treated with cisplatin and HISTONE H1.

After experimental period of 30 days and the following studies are planned.

- Body weight, spleen and thymus weight
- Histopathological examination of spleen and thymus
- Hematology
- Natural killer cell activity
• Antibody dependent Cellular Cytotoxic activity
• Antibody dependent complement mediated cell lysis
• Lymphocyte blastogenesis
• α-naphthyl acetate esterase in bone marrow cells
• FACS assay of CD4/CD8 lymphocytes
• ELISA of Interleukin 2, Interleukin 10 and Tumour Necrosis Factor – α
• Electron microscopy of spleen
• Molecular analysis of IL-10, IL-2 and TNFα mRNA in lymphocytes

Expected Outcome:
• The cytotoxicity of histone H1 can be rationalized to a larger biological context
• Understand mechanism of action of histone H1

What is aimed?
• Assess the efficacy of histone H1 on the immune status after cisplatin treatment

How is it likely to advance and add to the existing knowledge in relation to human knowledge?

Preliminary studies have shown the efficacy of histone H1 in breast cancer as an antitumour agent. As with any drug development studies have planned in a transplantable animal model in a view to extrapolate the results to human trials. The association between cancer and immune suppression has long been recognized. It is difficult to identify which precedes the other. Based on the “immunosurveillance theory”, defective immunological control of potential neoplastic cells allow their proliferation and thus lead to the development of cancer. As cancer becomes widespread, anti-tumor immunity becomes more defective. It is essential to monitor immunological status in management of cancer. Currently, immuno-restorative drugs are designed with comparatively low side effects. In light of available literature, histone H1 is believed to be involved as an immuno-restorative agent in breast cancer.
References

9. Michael Baum, Christobel Saunders and Sherner Meredith in `Breast Cancer : A guide for every woman' Oxford University Press