JOINT INSTITUTE FOR NUCLEAR RESEARCH

Dzhelepov Laboratory of Nuclear Problems

FINAL REPORT ON THE SUMMER STUDENT PROGRAM

Evaluation of the Effect of a New Chemotherapy Against Breast Cancer

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Abstract

Breast cancer is one of the most prevalent cancer in women worldwide, and is the second leading cause of cancer deaths in women. Here we evaluate the efficacy of a novel drug containing a bithiophene nucleus and compare the results with the result of tamoxifen which is the commercially used drug. We induced the breast cancer using dimethylbenza (a) anthracene. Bithiophene compound showed significant decrease in the cancer induction ratio, number of tumors and oncogene expression over tamoxifen. Also, bithiophene restored the gene expression levels of some key genes responsible for cancer induction and treatment.

introduction

Breast cancer is the most prevalent cancer in women worldwide, excluding nonmelanoma skin cancer, and is the second leading cause of cancer deaths in women (following lung cancer) [1]. Once metastasis has occurred, the survival rate is drastically reduced to a median of 2–3 years; therapy is then aimed at controlling symptoms, prolonging survival and improving quality of life [2].

Cancer vaccine immunotherapy requires time to induce an immune response. The HER-2/neu E75 vaccine is a peptide vaccine consisting of amino acids 369–377. It has been most thoroughly studied as a single peptide vaccine combined with various adjuvants as trastuzumab and rituximab to stimulate class I cytotoxic CD8 T cell responses against cancerous cells [3].

Surgery is the most successful conventional treatment. It is very effective in removing localized tumors. If the cancer has spread to other parts of the body, it is far less successful. Debulking by surgical means is also very effective in treating life-threatening and advancestage cancers. In breast cancer, a good surgical excision can cure it when combined with non-toxic natural therapies together with detoxification and/or drugs [4].

Drugs used in chemotherapy were derived from mustard gas experiments during World War I and II. There is no doubt that cancer cells are easily destroyed during chemotherapy. Unfortunately, cancer cells rely on processes that are similar to those used by normal cells. The difference between cancer cells and normal cells lies in their activities and not in their functions. Hence, chemotherapy gives rise to complications as normal cells that exist alongside the cancer tissues are also damaged during the process [5].

Another negative side effect is that many chemotherapy drugs have mutagenic properties that cause abnormal changes in DNA. If the patient is pregnant, it may affect the fetus or embryo, leading to birth defects. Some drugs also cause localized skin irritations. It is a sad fact that many patients

often die from these side effects or from the drugs themselves due to their high toxicity. chemotherapy is not very effective in the treatment of about 80% of malignant tumors. It lowers the person's natural resistance to diseases as it suppresses the immune system [6].

radiation treatment, has similar side effects as chemotherapy. The radiation's effective use in palliative treatment for selected cancers cannot be doubted, but generally speaking, radiation treatment should only be administered very selectively [7].

Thiophene belongs to a class of heterocyclic compounds containing a five membered ring made up of one sulphur as hetero-atom with the formula C4H4S. In medicinal chemistry, thiophene derivatives have been very well known for their therapeutic applications.

Thiophenes have been reported to possess interesting biological and pharmacological activities and several derivatives with this ring are used as antibacterial (8-11), anti-inflammatory [12], anticancer [13-19], and antiviral agents [20]. Moreover, from the literature survey it was found that aniline, pyridine, nicotinamide, pyrimidine, triazolopyrimidine derivatives showed wide spectrum of biological activities, especially anticancer activities [21-26].

Recent advances in interdisciplinary field of nanobiotechnology have led to the development of newly inventive therapeutic strategies and drug delivery alternatives taking advantage of the architectural geniality of systems based on nanoscale devices particularly tailored to deliver drugs to a selected tissue [27–29]. In this sense, nanoparticles, and the associated nanomedicine tools, are becoming the most appealing answer to chemotherapy problems, such as low drug solubility, degradation, fast clearance rates and nonspecific toxicity [30].

Estrogen receptor (ER) signaling plays a pivotal role in breast cancer and has far reaching therapeutic implications as >70% of breast tumors express ER [31]. ER is best characterized as a transcription regulator activated by its ligand estrogen binding, which in turn binds directly or indirectly to

DNA in the genome and modulates gene expression involved in normal cell function and tumor progression [32-33]. Limiting estrogen binding to ER has been a successful strategy to treat ER-positive (ER+) breast cancer, specifically in an adjuvant setting for the prevention of tumor recurrence.

Tamoxifen is a selective estrogen receptor modulator (SERM) that is used in the treatment and prevention of breast cancer. In both men and women, tamoxifen is used to treat metastatic breast cancer [34].

Tamoxifen acts on the estrogen receptor (ER) and has both estrogenic and anti-estrogenic actions, depending on the target tissue. In the breast tissue, it acts as an anti-estrogen (inhibitory effect) and competitively inhibits cancerous ER+ cells from receiving the estrogen they need to grow [35-36].

Tamoxifen increases the risk of thromboembolic events, such as deep vein thrombosis (DVT) and pulmonary embolism. The risk of tamoxifen-associated thromboembolic events (TTE) is further increased when tamoxifen is co-administered with chemotherapy.

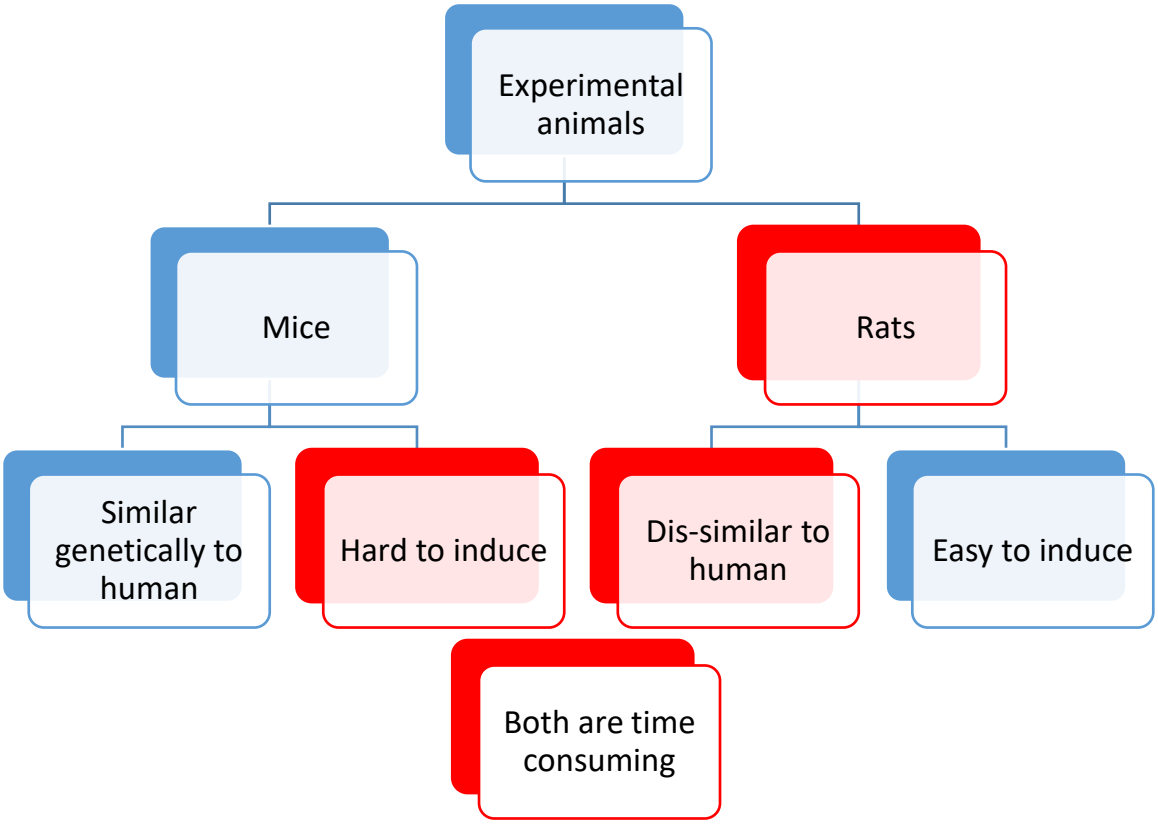


Fig (1) illustration of the advantage and disadvantages of using experimental animals.

The aim of the work is revealing the underlining mechanisms behind breast cancer induction and assessing the effects of the new compound Bithiophene.

Experimental Design

1. Five groups of albino mice, each consists of 40 individuals will receive DMSO, DMBA, Biothiophene, DMBA+Bithiophene and DMBA+Tamoxifene respectively.
2. 7, 12-Dimethylbenz (a) anthracene will be used to induce breast cancer and tamoxifen as reference treatment.
3. Bithiophene will be used as possible treatment.

4. Histology sections will be taken and stained with hematoxylin&eosin for light microscope examination to demonstrate the pathology and possible enhancement of breast cancer treatment due to bithiophene use.
5. **Sequencing** of the key genes of breast cancer as (BrCa1 and P53).
6. **Microarray analysis** of the total $_{m}$RNA and $_{LNC}$RNA.
7. **Determination of expression of following genes by quantitative real-time PCR.**

    - Glyceraldehyde 3-phosphate dehydrogenase [**GAPDH**]
    - Cytochrome p450 1A1 [**CYP 1A1**]
    - Cytochrome p340 1B1 [**CYP 1B1**]
    - Cyclin-dependent kinase 1 [**CDK1**]
    - Breast cancer 1 [**Brca1**]
    - Breast cancer 2 [**Brca2**]
    - Estrogen receptor 1 [**ESR1**]
    - Human epidermal growth factor receptor 2 [**HER2**]
    - Arhyl hydrocarbon receptor

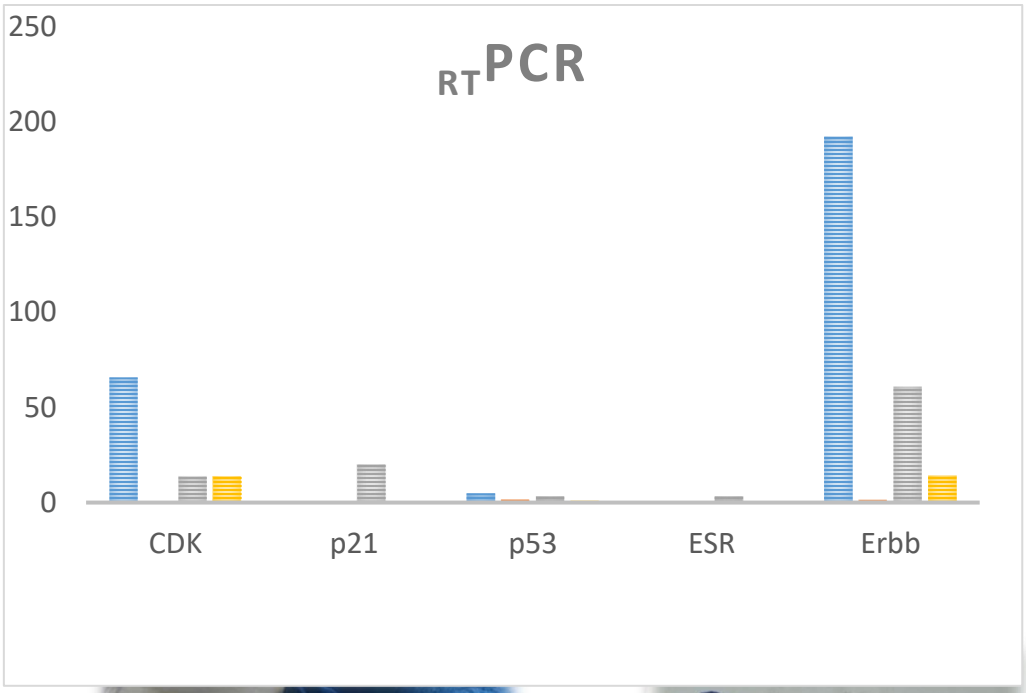
Sequencing Device

Affymetrix System

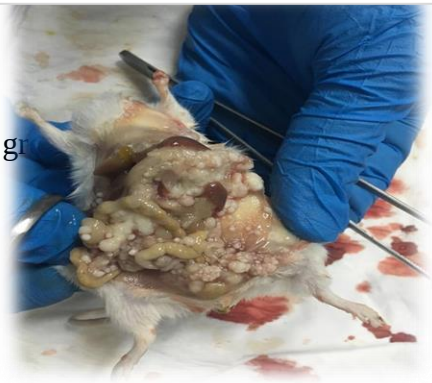
Results:

The use of bithiophene compound reduced the mortality rate of mice, incidence and multiplicity of cancer and restored the gene expression of some key genes in the breast cancer pathways as shown in fig (2). The results of sequencing technique give the types of mutations that resulted in cancer induction. The results of microarray analysis resulted in the discovery of some unknown pathways involved in the cancer induction and treatment.

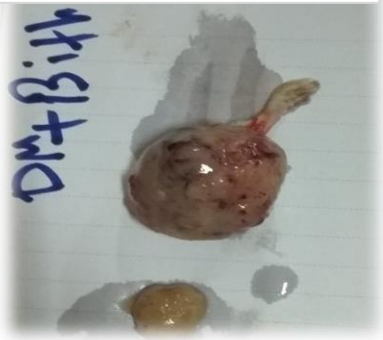
(A)



(B) cancer induced gr



(C)



(D) Normal mice after bithiophene treatment

Fig (2) (A) RTPCR results, (B, C and D).

Conclusion:

The use of bithiophene compound gave the opportunity to discover a new drug for breast cancer without the adverse side effects of chemotherapy and radiotherapy. The next step will be the pre-clinical studies which require more and further analysis.

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