To Study the Effect of Genistein & Conventional Anticancer Drug (Docetaxel) Combination on Breast Cancer

Mohd Zafar, Preeti Singh

Abstract: Cancer is a group of diseases characterized by uncontrolled growth and spread of abnormal cells. Breast cancer, lung cancer, prostate cancer and colon cancer are responsible for more than 50 percent of total deaths due to cancer. Many naturally obtained compounds (e.g. Genistein, diazin etc.) are closely linked to cancer, and many natural antitumor compounds are used to treat cancer. In the current work Genistein was extracted and isolated from soy plant (Glycine max.) plant, percent yield of Genistein was found 1.0% of crude extract and extract was standardized through TLC, UV-Visible spectrophotometry, FT-IR, NMR etc. Drug-Drug interaction studies were done with the help of FT-IR and DSC before testing them on breast cancer cell lines. The present work is an attempt to study the effect of Docetaxel in combination with Genistein on both estrogen receptor (ER) positive cell lines and ER negative cell lines. The study through Combination index values demonstrated that Genistein and Docetaxel show synergistic effect on ER positive cell lines MCF-7 over all effective dose ranges. But it showed antagonistic effect when tested on ER negative cell lines MDAMB-231 over all effective dose ranges.

Keywords: Genistein, docetaxel, breast cancer, aromatase, aromatase inhibitors, combination of genistein and docetaxel

1. Introduction

Cancer is the uncontrolled growth of abnormal cells in the body. Cancerous cells are also called malignant cells. Cancer known medically as a malignant neoplasm, is a broad group of various diseases, all involving unregulated cell growth.

Accordingly, the threat of cancer will be of major concern for the foreseeable future for those in both developed and developing countries. Cancer chemotherapy is an important alternative to surgery and radiation to treat successfully some types of solid tumors, lymphomas, and leukemia’s, and many clinically approved cytotoxic and anti-proliferative anticancer drugs are available, both of synthetic and natural product (microbial and plant) origin (1). However, much progress needs to be made to overcome the problems of resistance to and toxicity of existing cancer chemotherapeutic agents. Breast cancer is an important public health problem worldwide. In the United States, breast cancer represents the most common neoplasm and second most frequent cause of cancer death in women (2).

Worldwide the geographical variation in cancer incidence has shown a relation with differences in the dietary habits of populations at high and low risk of cancer. Thus the role of diet in the control of cancer risk has drawn widespread attention. The age-adjusted death rates from breast cancer are two- to eight-fold less in Asian countries than in the United States and Western Europe (3).

In epidemiological studies, the consumption of soy in Asian women has been associated with decreased rates of cancer including breast cancer (4). Experimental studies support the view that soy foods prevent cancer (5). Soy contains large amounts of isoflavones, among which, genistein is the most active growth inhibitor in a variety of tumors. Although epidemiological and experimental data suggest that the suppression of breast cancer is related to the ingestion of genistein (6), little is known about the mechanisms of action of this molecule in counteracting breast cancer cell growth.

Estrogens have been implicated in the etiology of breast cancer and have been added to the list of known human carcinogens (7). Estrogens are suggested to cause breast cancer by stimulating cell growth and proliferation through receptor-mediated processes and via their genotoxic metabolites (8) therefore, inhibition of estrogen production/ effect is nowadays a common practice for breast cancer treatment (9). The general strategies to inhibit estrogen action are to block estrogen receptor (ER) binding to its specific ligand or to disrupt estrogen production by altering the aromatase gene expression or enzyme activities (10). ER antagonists can block estrogenic actions; however, estrogen production can be inhibited by aromatase inhibitors (AI).

Aromatase is a cytochrome P450 enzyme and is responsible for catalyzing the biosynthesis of estrogens (Estrone and estradiol) from androgens (androstenedione and testosterone) (11). Aromatase has been found in numerous tissues throughout the body including breast, skin, brain, adipose, muscle, and bone (12). The concentration of estrogens has been shown to be as much as twenty-fold higher in breast cancer tissues than in the circulating plasma, suggesting locally increased aromatase expression for estrogen biosynthesis near or within the cancerous tissues (13).

Inhibition of the aromatase enzyme has been shown to reduce estrogen production throughout the body to nearly undetectable levels and is proving to have significant effect on the development and progression of hormone-responsive breast cancers. As such, aromatase inhibitors (AIs) can be utilized as either anticancer agents or for cancer chemoprevention (14).

It seems that Natural product compounds have substantial structural diversity and frequently afford new mechanisms of biological activity. As a result, natural products are used widely in cancer chemotherapy (15).

Genistein with conventional anticancer agents (Docetaxel) in order to decrease their toxicity and enhance their activity.
Docetaxel is semisynthetic derivative of taxoid family of antineoplastic family. Docetaxel has been effective against breast, ovarian, lung, head and neck cancer ([16]).

In the present work we are combining anticancer drug (Genistein) with anti-cancer drug (Docetaxel) to observe the synergistic / preventive/ therapeutic potential of combination against ER positive cell lines MCF-7 and ER negative cell lines MDAMB-231. The cell viability has been tested with the help of MTT Bioassay. The current study describes in detail the critical link between inflammation and cancer and Optimization of the doses for anticancer combination has been done with the help of MTT bioassay (ED50, ED75, ED100, ED95).

2. Materials and Methods

Cell lines: Cell Lines MCF-7, MDA-Mi 231, NCCSPune, India.

Chemicals: Genistein, docetaxel

Genistein (4, 5, 7-trihydroxyisoflavone), purified from soy beans.

Genistein has been successfully extracted and isolated from Soybean (Glycine max.) seed with help of preparative HPLC Soybeans were purchased from local market of Greater Noida (India) were ground to flour. The flour was defatted using hexane. The mixture of 100 mL of hexane and 50 g of soy flour was shaken at room temperature for 1 h. The supernatant was removed after the mixture was centrifuged at 1000g for 20 min. The defatted soy flour was dried under a hood overnight. Methanol was used as solvent to extract isoflavones from the defatted soy flour. The defatted soy flour was mixed with 80 mL of methanol. The extraction conditions and procedure were the same as that in defatting. The supernatant was placed under nitrogen flow to evaporate the solvent to a final volume of approximately 10 mL. ([17]). Docetaxel, gift sample from Dr. Reddy’s Lab, Hyderabad

FT-IR Spectroscopy

Taken about 1 part of the sample and 5 parts of potassium bromide. Mixed thoroughly in a mortar while grinding with the pestle. The powder mixture is then loaded into the press cell, and pressed at 5000-10000 psi to form a translucent pellet. Carefully remove the pressed sample from die and place in the FTIR sample holder through which the beam of the spectrometer can pass. As the same way combined sample of Genistein and Docetaxel pellets were prepared and the drug-excipient interaction study was carried out by FT-IR.

NMR:
The NMR spectra of Genistein was taken in the Department of Chemistry in IIT Delhi,

UV Spectroscopy

Prepared 10 different dilutions (10 µg/ml, 20 µg/ml, 30 µg/ml, 40 µg/ml, 50 µg/ml, 60 µg/ml, 70 µg/ml, 80 µg/ml, 90 µg/ml,100µg/ml ) of the stock solution. Standard curve was drawn out with help of UV-VIS Spectrophotometer at 261 nm for Genistein and at 275nm for Docetaxel.

Differential scanning calorimetry (DSC):

Interaction Studies between Genistein & Docetaxel was determined by DSC at Department of textile, IIT, Delhi.

DSC method

A differential scanning calorimeter was used for thermal analysis of the drug and mixtures of drug and perciipients. Excipients expected to be used in the development of immediate release drug at an appropriate ratio were selected for the study. Individual samples of the drug and excipients as well as physical mixtures of the drug and selected excipients were weighed directly into the DSC aluminum crucible and scanned in the temperature range of 50-300°C under a dry nitrogen atmosphere. The heating rate was 20°C min-1 and the thermo grams obtained were observed for interactions.

Procedure of cytotoxicity assay

1) Exponentially growing MCF-7 and MDAMB-231 cells were harvested from T-25 tissue culture flask and a stock cell suspension (1X105cell/ml) was prepared.

2) A sterile 96-well flat bottom tissue culture plate was seeded with 1x104 cells in 0.1 mL of MEM medium supplemented with 10% FBS and allowed to attach for 24 h.

3) Cells were treated with different conc. of test compound (12.5-100µM) in triplicates and incubated for 24 h. The control group cells were treated with only the medium containing 0.1% DMSO (DMSO control).

4) Drug containing media was removed and washed with 100µl of phosphate buffer saline (PBS) and 100µl of MTT reagent (1mg/ml) was added and incubated for 3 h at 37°C.

5) After 3 h of incubation, MTT was removed draining on tissue paper and the formazan crystals formed in each well were dissolved in 100µl of DMSO.

6) The absorbance was measured by an ELISA plate reader at 540nm, and the % cytotoxicity was calculated as follows.

\[
\text{% cytotoxicity} = \frac{(\text{Abs of Control} - \text{Abs of blank}) - (\text{Abs of test} - \text{Abs of blank})}{(\text{Abs of Control} - \text{Abs of blank})} \times 100
\]

Data analysis

Each experiment was repeated three times, and the results were analyzed by Student’s t test and one-way analysis of variance. A P value less than 0.05 was considered significant. Data are reported as mean ± SEM/SD. Statistical analysis was performed in Prism 4 (Graph Pad Software, Inc. San Diego, USA). Combination Index was calculated by using CompuSyn 1 (CompuSyn software ComboSyn, Inc. USA).

3. Result, Discussion and Conclusion

Extraction and isolation results suggested that glycine max seed contain 0.12 % of total isoflavone and 0.051% genistein was obtained from the total isoflavone of glycine max seed. So this extraction and isolation method can be used for Genistein, the amount obtained is a bit low but still it is substantial amount of Genistein.
Characterization of Genistein was done by NMR, FTIR and UV. data of isolated Genistein was compared with standard Genistein, all data suggested that the isolated material have the same peaks as standard Genistein.

The drug-excipient interaction study was carried out by FT-IR and DSC to ensure that there is no interaction between Genistein and Docetaxel.

Docetaxel is a clinically well-established anti-mitotic chemotherapy medication (that is, it interferes with cell division. It is used mainly for the treatment of breast, ovarian, prostate, and non-small cell lung cancer (18).

Aromatase inhibitors (AIs) are a class of drugs used in the treatment of breast cancer and ovarian cancer in postmenopausal women.

Use of aromatase inhibitors may prevent or inhibit many of the dose-limiting toxicities of anti-neoplastic agents. The results of most of the recent clinical studies have been suggested that combining the natural aromatase inhibitors with chemotherapies can lead to improve effects. So here we performed the experiment on the combination of both Genistein and Docetaxel because of their therapeutic applications.

In this work we are combining aromatase inhibitor (Genistein) with anti-cancer drug (Docetaxel) to observe the synergistic /preventive/therapeutic potential of Genistein against ER positive cell lines MCF-7 and ER negative cell lines MDAMB-231. The cell viability has been tested with the help of MTT Bioassay. The current study describes in detail the critical link between aromatase inhibitors and cancer and Optimization of the doses for anticancer combination has been done with the help of MTT bioassay (ED_{50}, ED_{75}, ED_{90}, ED_{95}).

MTT assay were performed to assess the cytotoxic potential of Genistein and Docetaxel combination against hormone dependent (ER positive cell lines) breast cancer cell lines MCF-7, hormone independent (ER negative cell lines) cell lines MDAMB-231. Results suggested that combination have the better cytotoxic potential than the either drugs alone on ER positive Cell Lines may be due to the inhibitory effect of Genistein on aromatase production which in turn is responsible for overproduction of estrogen in cancerous cells. To check this concept further we tested the Genistein, Docetaxel alone and Genistein in combination with docetaxel on hormone independent (ER negative cell lines) cell lines MDAMB-231 through MTT bioassay. We did not found synergistic effect on this cell lines.

4. Results

In vitro cytotoxicity of the drugs was performed. MTT assay for selected combination Docetaxel and Genistein against hormone dependent breast cancer cell lines (MCF-7) & hormone independent breast cancer lines (MDAMB-231). Results suggested that combination have synergistic cytotoxic potential against hormone dependent breast cancer cell lines (MCF-7) with ED_{50} of 0.880 but antagonistic effect against hormone independent breast cancer lines (MDAMB-231) with ED_{50} of 1.409. The difference was statistically significant.

References

[17] Xu et al., 2002