Introduction
Cancer is one of the leading causes of mortality worldwide having a death rate of 9 million in 2016. Among different types of cancer like colon, breast, pancreas, stomach, colorectal, lung, there is an increased surge of breast carcinoma that has been observed over several years [1]. Breast cancer is the second leading cause of cancer death in women with a mortality rate of 1 in 37 (about 2.7%) [2].

Chemotherapy is the best option in different types of treatments for advanced stages of tumors. Existing therapy includes the widespread use of chemotherapeutic drugs such as Doxorubicin, Paclitaxel, Cisplatin, Etoposide, 5-Flurouracil, Methoxetrate etc [3]. Molecular targeting by these drugs surely have had a substantial impact on cancer treatment, but patients either eventually develop resistance to these agents or get affected by their toxic side effects. Among different types of chemotherapeutic drugs we have chosen 5-FU as a targeting chemotherapeutic drug in this current study. The anticancer effects of 5-FU have already been studied on different cell lines like cardiomyocytes [4], colorectal cancer cells [5,6,7] and breast cancers [8] etc. 5-FU is a structural analogue of pyrimidine. 5-FU has some common side effects like nausea, vomiting, headache, itching, diarrhea etc and also some critical adverse effects like cardiotoxicity [9], vein pigmentation [10], GI ulceration and bleeding etc.

Reduction of toxic and adverse effects of chemotherapeutic drugs with minimum impact on efficacy has led to the concept of combination therapy. Combination therapy has a potential for long-term disease modification [11]. Combination of drugs with differing modes of action often attains a synergistic effect without the toxic side effects associated with a particular drug when used at its optimal dose. So in this study we want to develop a logical combination of 5-FU and MG to achieve synergistic killing of cancer cells while avoiding additive toxicity.

The anticancer role of methylglyoxal (MG) has been investigated and established in our laboratory. Methylglyoxal generally targets malignant cells by affecting glycolysis and mitochondrial respiration with minimum or no toxicity on normal cells [12-17]. Methylglyoxal is formed as a byproduct of several metabolic pathways. MG specifically decreases cellular ATP pool by blocking glycolysis and mitochondrial electron transport chain by inhibiting Glyceraldehyde-3-phosphate dehydrogenase and mitochondrial complex I, respectively, of malignant cells. This selectivity of MG towards malignant cells has been attributed to the differential molecular association of GAPDH (glyceraldehyde-3-phosphate dehydrogenase) in cancer cells compared to normal cells. It is well established that GAPDH from normal tissue sources is a homo-tetrameric protein with four identical subunits of 36 kDa each [18]. By contrast, GAPDH from Erlich Ascites carcinoma (EAC) cells and other cancer cells is a heterodimer having two non-identical subunits of ~33 and 55 kDa.

Abstract
Reduction of toxicity due to uses of conventional chemotherapeutics drugs is a major challenge in cancer treatment. With this aim we combined 5-FU, a widely used chemotherapeutic drug and methylglyoxal (MG), a non-toxic anticancer agent in breast cancer cell line, MCF-7. Treatment with 5-FU in combination with MG on MCF 7 cells exert synergistic effects in proliferation and destruction of those cells as determined by MTT, clonogenic assay and scratch wound healing assay. Results clearly showed that 5-FU is more effective at lower doses in presence of MG. Taken together, our preliminary results revealed that MG can be used in combination therapy to reduce the concentration of toxic 5-FU against breast cancer cell.

Keywords: Methylglyoxal; 5-Fluoro Uracil; Cancer; MCF-7

5FU Synergistically Inhibits MCF-7 in Combination with Methylglyoxal

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Materials

5-Fluorouracil, MG (40 % aqueous solution, w/v), MTT (3-(4,5-dimethylthiazol-2-yl)2,5-diphenyltetrazolium bromide), Crystal violet solution, 3.7% Formaldehyde, cell culture media and fetal bovine serum were purchased from Sigma Chemical Co., St. Louis, Mo, USA. MCF-7 human breast cancer cell lines were obtained from American Type Culture Collection (ATCC) Manassas, VA, USA). Other chemicals were of analytical grade and obtained from local manufacturers.

Methods

Cell culture

Human breast adenocarcinoma cell line (MCF-7) were maintained in Roswell Park Memorial Institute medium (RPMI), supplemented with 10 % fetal bovine serum, penicillin (100 μg/ mL), streptomycin (100 μg/mL), and gentamycin (100 μg/mL) and grown in a 37 °C humidified incubator flushed by an air mix containing 5 % CO₂.

Cell viability analysis by MTT assay on MCF-7 cells

The MTT assay [21] was performed to analyze the cell viability of MCF-7 cells (breast cancer cell line) with 5-FU supplemented and/or in combination with MG. Cells in their log phase were seeded overnight in colorless RPMI in 48 well tissue culture plates such that each well contained ~15,000 cells. The cells were then incubated with increasing concentrations of 5- FU (0.25, 0.5, 0.75, 1.0, 1.25, 1.5, and 2.0 μM) and different combinations of MG (1, 2 and 3 mM) for 24 h at 37 °C. The medium was then replaced with 200 μL MTT (0.5 mg/mL) per well and further incubated for 4 h at 37 °C. Then the insoluble formazan was dissolved in DMSO (dimethyl sulfoxide) (100 μL/ well) and absorbance was measured using a 96-well plate reader (ELX 800; Biotek, Winooski, VT, USA) at 490 nm. The percentage of cell viability was calculated compared with the untreated control (considered as 100 % viable).

Clonogenic assay on MCF-7 cells

Clonogenic assay [22] was performed to analyze the cell reproductive ability of MCF-7 cells. In 6 well plates around 150 MCF-7 cells were seeded in each of the wells and cells were allowed to attach to the plates for 2 h. Then the cells were treated with lower and higher concentration of 5- FU (.05μM, .25μM) and in combination with constant dose of MG (.05 mM). For the next 7 days media was changed on every 2nd day. After 7 days the cells were washed with 1X PBS and incubated with 3 mL of a mixture of 3.7% formaldehyde in 1X PBS and 0.5% crystal violet for 30 minutes. The formaldehyde crystal violet mixture was carefully removed by rinsing with tap water and plates were allowed to dry at room temperature. Finally, the image was captured using Gel Doc XR + (Bio-Rad) and colony numbers were counted.

Wound healing assay

Cells (MCF-7)) were grown to almost 90% confluency in RPMI media supplemented with FBS. Next, two separate linear scratches were made by a 200 μl micropipette tip. Wounded monolayers were washed thrice to remove cell debris, kept in 10% PBS medium and incubated with 5 FU (10 and 20μM) and MG (.1mM and .25mM) alone or their combination for 24 h. Images were taken using phase contrast microscopy.

Results

Effect of 5-FU supplemented with MG on cell viability

To evaluate the effect of 5-FU in combination with MG, varying doses of 5-FU and MG were tested on MCF-7 cells by MTT assay. 5-FU and MG in combination affected viability of MCF-7 cells in a dose dependent manner. 5-FU alone at a concentration of 20μM was
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able to reduce cell viability to almost 50% (Figure 1A) and MG at a concentration of 0.75 mM and 0.25 mM were able to reduce cell viability to 50% and 20% respectively. (Figure.1B). Treatment with combination of MG at a concentration of 0.25 mM with varying concentration of 5-FU has been performed. 50% cell viability was achieved at a concentration of only 1 µM of 5-FU in combination with 0.25 mM of MG (Figure.1C). In contrast the cell viability was reduced by approximately 8% by the treatment of 5FU alone. These results clearly demonstrate that 5-FU, if combined with MG significantly reduced MCF-7 cell viability compare to 5-FU alone. Phase contrast microscopic images were also shown morphological changes when treated with 5FU in combination with MG (Figure.1 D, E, F, and G). Only 5-FU and MG treated cells were rounded up and many floating cells in the culture plate were observed. These results clearly suggest a synergistic effect of 5FU when treated with MG on MCF-7 cells.

Treatment with 5-FU combined with MG significantly increased cell reproductive death

MG supplemented with 5-FU reduced reproductive ability was detected by clonogenic assay (colony formation). It was performed in MCF-7 cells using two different concentration of 5-FU (.05µM and .25 µM) (Figure. 2C and 2D) and a concentration of MG (.05 mM) alone (Figure. 2B) or in combination (Figure. 2E and 2F) to check the ability of a single cell to grow into a colony for 7 days. Counting the colonies significantly demonstrated that 5-FU decreased the colony numbers when it was treated with MG in MCF-7 cells. At a dose of 0.25µM of 5-FU and .05mM in combination with MG, very few colonies were observed than without combination (Figure. 2F).

Figure 2: Effects of 5Fu and MG on colony formation on MCF-7 cells A) Untreated, B) Treated with FU (.05 µM), C) Treated with FU (.25 µM), D) Treated with MG (.05mM), E) FU+MG (.05µM+.05mM), F) FU+MG (.25µM+.05mM), G) After counting the number of colonies the data is plotted in a bar diagram. Concentrations of all the mentioned compounds are indicated in the X axis of the figure. Each datum is the mean ± standard deviation of three experiments (n=3). P-value of <0.05 was considered to be significant in all cases (by one-way ANOVA followed by Dunnett’s multiple comparison test (to compare between control vs. all the treated groups).

Effect of 5-FU supplemented with MG on cell migration and growth

To investigate the effect of 5-FU in combination with MG on cell growth and migration ability, wound healing assay was performed. MCF-7 cells were treated with varying concentration of 5-FU (10 µM and 20 µM) (Figure. 3B and 3C) and MG (.1mM and .25mM) (Figure.3D and 3E) and in combination (MG .1+FU10 and MG.1+FU20) (Figure.3F and 3G) to check the ability of cells to migrate. Pictures were taken by Phase contrast microscopy after 24 hour of treatment.

Discussion

It is evident from the results presented here that MG augments the anti-cancer effect of 5-FU. This is supported by experiments performed in vitro. Previously from our laboratory it has been established that MG specifically targets malignant cells by different molecular association of GAPDH (glyceraldehyde-3-phosphate dehydrogenase) in cancer cells compared to normal cells. In this study the goal was to increase the efficacy of 5-FU in combination with MG. In this paper, the efficacy of this combination was studied in human breast carcinoma cell line MCF-7 cells which were established by cell viability assay by MTT assay. Result showed that when cells were treated in combination with a constant dose of MG with different doses of 5-FU, number of colonies had been decreased by 12% and 38%.When cells were treated with lower and higher dose of 5-FU along with MG, colony...
number was decreased by 30% and 95%. The wound healing assay showed additional decreased motility of MCF-7 cells in combination treatment as indicated by unchanged area of wound. Cells treated with 5FU and MG were migrated little compared to single treatments. These data assured that breast cancer cell motility is inhibited more when MG was used in combination with 5FU. Taken together, our findings suggested that MG can synergistically increase the efficacy of 5-FU against MCF-7 cells.

Acknowledgements

This research is funded by the Council of Scientific and Industrial Research (CSIR), Government of India; and Department of Science and Technology (DST) INSPIRE scheme, Government of India.

References


