Research Article

Gemcitabine and brucine inhibit MDA MB-436 human breast cancer cells
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Abstract: Combinations of anticancer agents are likely to be more effective in breast cancer therapy. Hence, in the present study we evaluated the in vitro anticancer activity of brucine when combined with gemcitabine in MDA MB-436 human breast cancer cells. The cells were cultured in vitro and treated with brucine and gemcitabine combinations. Cell viability was assessed by trypan blue exclusion assay and cell migration was determined by in vitro scratch assay. Brucine and gemcitabine treatment showed inhibition of cell proliferation in a dose dependent manner. Combination treatment resulted in additive inhibitory effects. MDA MB-436 cells treated with both drugs showed additive inhibition of cell migration in the in vitro cell migration assay.

Keywords: MDA MB-436, Trypan blue assay, In vitro scratch assay

INTRODUCTION

Breast cancer is the most common cancer in women [1] and the second most cause of death [2]. Surgery, radiation and chemotherapeutic approaches are often not enough to improve the survival rate in breast cancer [3]. Thus, alternative interventions such as substances from natural herbal sources can be tested to combine with the existing anticancer agents. Toxicity and drug resistance are the major drawbacks of anticancer chemotherapeutic therapies. The goal of developing newer agents is to overcome such problems and side effects could be less [4]. In this regard, testing the newer agents and their combinations is anticipated to benefit breast cancer drug discovery approaches.

In the current study we investigated whether adding brucine to the gemcitabine will have any additive effects in inhibiting MDA MB-436 cells. Brucine has been isolated from Strychnos nux-vomica, a medicinal plant present in many Asian countries. It has been widely used in Chinese folk medicine [5] for the treatment of nervous diseases, analgesic, anti-inflammatory [6] and anti-tumour [7]. Brucine is a plant alkaloid and isolated from the seeds of Strychnos nux-vomica. It has anti-proliferative and cytotoxic effects have been reported in HepG2 [7, 8] SMMC-7721 [9] hepatoma cells, and multiple myeloma RPMI 8226 cell lines [10].

Gemcitabine (2’,2’-difluorodeoxycytidine, dFdC) is a dCyd analog, which is active against variety of cancers including non-small cell lung cancer [11], pancreatic carcinoma [12, 13] and breast cancer [14]. Gemcitabine requires phosphorylation by deoxycytidine kinase (dCK) in order to be active, and can be inactivated by deamination by deoxycytidine deaminase (dCDA) to 2’,2’-difluorodeoxyuridine (dFdU) [15, 16]. After conversion by kinases to its active form, 2’,2’-difluorodeoxycytidine triphosphate (dFdCTP), it can be incorporated into DNA and is a potent inhibitor of DNA-synthesis by inhibition of DNA polymerase [17]. Moreover, gemcitabine, as dFdCTP, is also incorporated into RNA [18].

Thus, we set out to determine if the combination of brucine and gemcitabine is more potent than either agent alone. Our findings suggest that the combination of brucine and gemcitabine are effective in inhibiting the proliferation and migration of MDA-MB 436 cells.

MATERIALS AND METHODS

Cell culture and Reagents

The human breast cancer cell lines (MDA MB-436) was purchased from American Type Culture Collection (Manassas, VA) and cultured in DMEM (Gibco-BRL, Grand Island, NY) pH 7.4 containing penicillin (100 U/ml), streptomycin (100 μg/ml), and gentamicin (60 μg/ml) and supplemented with 10% Fetal Bovine Serum (FBS- Gibco-BRL, Grand Island, NY) and humidified 5% CO₂ incubator at 37°C. 50 mM brucine and 10 mM gemcitabine (purchased from...
Sigma, St. Louis, MO, USA) stocks were prepared in dimethyl sulfoxide (DMSO; Merck, Darmstadt, Germany) and phosphate buffer saline pH 7.2 (PBS) respectively and stored at -20°C until use. All other chemicals of analytical grade were purchased from Sigma, USA.

**Trypan blue dye exclusion method**

To analyse the cytotoxic activity of brucine and/or gemcitabine by trypan blue dye exclusion method adapted to [19]. MDA MB-436 (2×10^5) cells were seeded in 6 well plates and allowed to adhere for overnight at 37°C. Then the cells were exposed to different concentrations of the brucine (100, 200 and 300 µM) or gemcitabine (10, 50, 100 and 150 µM) or in combination of both drugs. The control well received only maintenance of medium. The plates were incubated at 37°C in a humidified incubator with 5% CO_2 for 48 h. After drug treatment cells were washed with phosphate buffer saline (PBS), trypsinized and cells were centrifuged at 1200 rpm for 4 min and re-suspended in complete media. Each sample was mixed with an equal volume of 0.4% trypan blue and counted using a hemocytometer (Hausser Scientific, Horsham, PA, USA) under an inverted microscope (Olympus, Germany). At the end of 48 h incubation, cell viability was determined.

**In vitro scratch assay**

MDA MB-436 (5×10^4) cells were seeded in 60 mm culture plates for monolayer formation up to 80% confluence and the cells were subjected to serum starvation for 6 h and treated for 48 h either with brucine (50 and 100 µM) or gemcitabine (25 and 50 µM) and their combination. After incubation the drug containing medium was removed, scratch was created by sterile p200 tip after washed with PBS (X2) to remove floating cells and 2% media was added. Photographic images were taken at 0, 24 and 48 h using inverted phase microscope (Zeiss, Axiovert 25, Germany). Cell migration was expressed as the percentage of the gap relative to the total area of the cell-free region using Image-J software (National Institutes of Health, Bethesda, MD, USA) [20].

**RESULTS**

**Viability staining by trypan blue assay**

To determine the cell viability of MDA MB-436 cells treated with brucine and/or gemcitabine by trypan blue dye exclusion assay (Figure 1). Individually brucine and gemcitabine exhibited a suppressive effect on cell viability in a dose dependent manner.

Cell viability was significantly decreased to 58.4% at 100 µM brucine (p≤0.001, Figure 1A). Similarly, cell viability was significantly decreased to 60% at 50 µM gemcitabine (p≤0.001, Figure 1B). The IC50 values were 100 and 30 µM for brucine and gemcitabine respectively.

In contrast cells treated with both the drugs at same time showed highest inhibition (Figure 2) of cell proliferation compared to either brucine or gemcitabine (Figures 1A and B). Combination treatment with both brucine (100 µM) and gemcitabine (50 µM) showed significant (p≤0.001) decrease in cell proliferation (84%) compared to untreated cells.

**Brucine and gemcitabine inhibits the MDA MB-436 cell migration**

To investigate the inhibitory effect of brucine and/or gemcitabine on MDA MB-436 cell migration, in vitro scratch assay was performed. The % of cells that had migrated into the scratch after 0, 24 and 48 h was calculated and shown in figure 3. There was complete gap closure in 24 to 48 hours in the untreated cells. There was no significant change in cell migration which was treated with brucine (50µM) and gemcitabine (25µM) compared with control cells. Cell migration was reduced by 25% in cells treated with 100 µM brucine and 36% in cells treated with 50 µM gemcitabine. However, the cell migration was significantly inhibited (83%) when the cells were treated with both brucine (100 µM) and gemcitabine (50 µM). These results suggest that in combination treatment shown more additive effect in inhibiting the migration of MDA MB-436 cells compared to individual drugs treated alone.
Figure 2: Effect of brucine or gemcitabine treatment on cell viability of MDA MB-436 cells. Cells were treated with brucine (50 or 100 μM) in combination with gemcitabine (25 or 50 or 100 μM). After 48 h treatment, to evaluate the cell viability using trypan blue assay. Data represented as Mean ± SD.*p ≤ 0.001, treated cells were compared with untreated cells.

Figure 3: Effect of brucine and gemcitabine on cell migration. Cells were plated to obtain 80% confluence in 60 mm culture plate. A scratch was created and treated the cells for 48 h with either brucine or gemcitabine or both. After drug treatment the migration of cells to the gap region created was monitored and photographed at 0, 24 and 48 h and the cell migration was analyzed and represented as Mean ± SD obtained from three independent experiments.*p ≤ 0.001, when treated samples are compared with respective untreated cells (Bru-Brucine; Gem-Gemcitabine).

DISCUSSION

Current cancer treatment strategies favour combination therapies which would offer low toxicities to the cancer patients [4]. Natural products have proven to be the most reliable source of new and effective anticancer agents [21, 22]. The most important rationale is the realization that individual chemotherapeutic agents for the majority of tumours have not increased cure rates in the treatment of cancer [14]. Hence, the present study focussed on combinational therapy of brucine and gemcitabine in breast cancer cells. Gemcitabine an analogue of deoxycytidine is active against several types of solid tumors, including breast cancer [14] and pancreatic carcinoma [12]. In addition, brucine has been used as an anticancer agent in various types of cancers including lung cancer [23] and hepatoma cancer [24]. Previous reports have investigated the mechanisms of brucine in HepG2 cells [7]. In the present study, brucine as well as gemcitabine exhibited a suppressive effect on MDA MB-436 cell viability in a dose dependent manner (Figure 1). Such an inhibitory activity of cell viability was enhanced significantly when the MDA MB-436 cells were exposed to combination with brucine and gemcitabine (Figure 2). Recent studies showed that combination of gemcitabine and curcumin is effective in pancreatic [25] and bladder cancers [26]. Results of the present study revealed that combination exposure of both drugs at same time point exhibited highest inhibition in cell viability than either brucine or gemcitabine alone.
These observations may be of value while carefully considering the combination therapies in a clinical setting. In addition, cell migration was significantly inhibited (47%) with 100 μM brucine and 50 μM gemcitabine combination (Figure 3). The combined results may conclude that the combinational therapy with brucine and gemcitabine would be more effective and beneficial in breast cancer therapy. Our results suggest that concurrent treatment of brucine and gemcitabine show additive effect in human breast cancer (MDA MB-436) cells and these drugs may have future clinical utility for treating breast cancer.

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