RESEARCH ARTICLE

Comparison of in Vitro Cytotoxicity and Apoptogenic Activity of Magnesium Chloride and Cisplatin as Conventional Chemotherapeutic Agents in the MCF-7 Cell Line

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Abstract

Breast cancer is the most common malignancy and also the second leading cause of cancer death among women and also in women that have a high mortality. Previous studies showed that magnesium (Mg) has cytotoxic effects on malignant cell lines. However, the anti-cancer effects of Mg on MCF-7 breast cancer cells are uncertain. This study was aimed at the comparison of the cytotoxic effect of Mg salt (MgCl2) and cisplatin on MCF-7 cells and fibroblasts (as normal cells). After treatment with various concentrations of MgCl2, and cisplatin as a positive control for 24 and 48 hours (h), cytotoxicity activity was measured by MTT assay. In addition, apoptosis was determined by annexin V/propidium iide assay. Both cisplatin and the MgCl2 exhibited dose-dependent cytotoxic effects in the MCF-7 cell line, although the LD50 of the Mg was significantly higher when compared to cispaltin (40 µg/ml vs. 20 µg/ml). Regarding annexin V/propidium results, treatment of MCF-7 cells with LD50 concentrations of cisplatin and Mg showed 59% and 44% apoptosis at 24h, respectively. Finally, the results indicated that Mg has cytotoxic effects on MCF-7 cells, but less than cisplatin as a conventional chemotherapeutic agent. However, regarding the side effects of chemotherapy drugs, it seems that Mg can be considered as a supplement for the treatment of breast cancer.

Keywords: Breast cancer - MCF-7 cells - cisplatin - magnesium - apoptosis

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Introduction

Breast cancer is considered a global public health problem due to its increasing incidence and associated socioeconomic implications (Dieterich et al., 2014). It is the most frequent cancer among women in the world (Isfoss et al., 2013). The median age of breast cancer patients at diagnosis was 62 and 58 years among White females and Black females, respectively (Howlader et al., 2013). While the mean age of cancer in Iran is 5 to 10 years less than other countries (Movahedi et al., 2012; Ren et al., 2014). Breast cancer is a complex multifactorial disease in which genetic and environmental factors are involved (Torre et al., 2015). Moreover, being a woman is the main risk factor for developing breast cancer (González-Jiménez et al., 2015). About 5 to 10 percent of breast cancer cases are congenital background (Maskarinec et al., 2015; Takahashi and Suzuki 1993; Niknafs, 2011).

Although surgery, chemotherapy, radiation therapy and hormone therapy as the most common methods used to treat breast cancer, these therapies in turn lead to short and long-term complications in these patients (Liedtke et al., 2008). Most anticancer drugs used in chemotherapy are unable to selectively destroy cancer cells and unfortunately, it also causes serious damage to the surrounding healthy tissues. They have many complications on the patient's level of physical, mental, and social performance and cause cessation of the treatment period (Partridge and Winer, 2004).

Cis-Diamminedichloroplatinum (Cisplatin) and its derivatives as active anti-tumor agents are widely used

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in human chemotherapy. The major target of cisplatin is DNA. Cisplatin becomes active by forming DNA adducts. Formation of Cisplatin DNA adducts interferes with cell cycle checkpoints, cell replication and transcription or regulation of signal transduction pathways. The outcome of these alterations is DNA damage and then occurrence of cell death (Goel et al., 2010).

Due to the increased morbidity and mortality from cancer because of the failure of chemotherapy in advanced forms of cancer, finding new methods and materials of treating cancer is necessary (Roy et al., 2011).

Magnesium (Mg) is the second most abundant intracellular element in the body, involved with over 300 biological activities (Seri and French 1984). Mg plays an essential role in DNA repair, cell differentiation, proliferation, apoptosis and angiogenesis (Wolf et al., 2007). There are evidences that suggest Mg have apoptosis effects on cancer cells (Tanaka et al., 1994). Dai et al found that low blood Mg levels were associated with high-grade prostate cancer (Dai et al. 2011). In addition, Demir et al. demonstrated levels of Mg to be lowered in patients with leukemia (Demir et al., 2011).

Searching for a safe and effective method of delivering bioactive molecules and drugs, especially anticancer drugs, to specific cells is an intriguing area of research in modern pharmaceutics (Suberu et al., 2014).

Hence, this study was aimed at the comparison of the cytotoxic effect of Mg salts (MgCl2) and Cisplatine on the MCF-7 human breast cancer cell line.

Materials and Methods

Cell Line and Materials

MCF-7 cells (a human breast cancer cell line, NCBI No. C135) were purchased from Pasteur Institute of Iran (Pasteur Institute, Tehran, Iran). Cisplatine and 3-[4,5-Dimethyl-2-thiazolyl] -2,5-diphenyl-2-tetrazolium bromide (MTT) assay Kit were obtained from Sigma Aldrich (USA). The cell culture plastic ware was obtained from Nunc (Denmark). Dulbecco's Modified Eagle's Medium (DMEM), fetal bovine serum (FBS), penicillin, streptomycin, phosphate-buffered saline (PBS) and trypsin EDTA were obtained from Gibco (USA). Annexin V-FITC apoptosis detection kit and MgCl2 were purchased from Roche (Basel, Switzerland) and Merck (Germany), respectively.

Cell Culture

MCF-7 cell was cultured in high glucose DMEM supplemented with 10% heated-inactivate FBS, containing penicillin (100 U/mL) and streptomycin (100µg/mL) at 37 °C in a humidified atmosphere of 95% air and 5% CO2, and the medium was changed every other day. When the cultures were 80-90% confluent, all cells were washed with PBS (pH 7.4), detached with 0.25% trypsin, centrifuged (1200 rpm, 5 min) and re-plated onto 96-or 24-well plates at an appropriate density according to each experimental scale. All experiments were carried out 24–48 h after the cells were plated.

Cells were seeded overnight, and then incubated

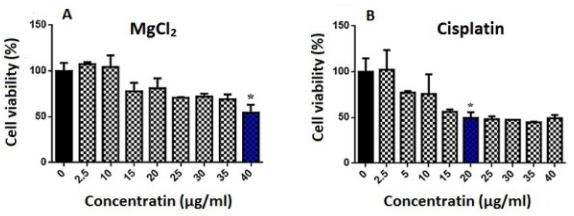

Figure 1. MTT Assay Results for Cisplatin and MgCl2 in 24 h on MCF-7 Cell Line. Treatment of MCF-7 Cells with 40 (µg/ml) Concentration of MgCl2 Showed 50% Cell Death (=LD50 or IC50) (A, Blue Column), Whereas LD50 of Cisplatin in This Time Calculated to be 20 (µg/ml) (B, blue column) (P<0.05).

with various concentrations of MgCl2 for 24 and 48 h. For MTT assay, cells were seeded at 8000/well into 96-well culture plates. For assay of apoptosis, cells were seeded at 100,000/well into a 24-well plate. For each concentration and time course study, there was a control sample which remained untreated and received the equal volume of medium. All different treatments were carried out in triplicate.

Cell Viability by MTT Assay

To evaluate the cytotoxicity effect of MgCl2 on the MCF-7 cell line, the MTT colorimetric assay was applied (Takahashi and Suzuki 1993). Briefly, cells (8,000/well) in 200ml DMEM containing 10% FBS were seeded on 96-well plates and incubated overnight. These cells subsequently treated with various concentrations of MgCl2, and Cisplatine as a positive control for 24 and 48 h. Afterwards, 20µl of MTT solution (5 mg/ml in PBS) was added to each well and incubated for an additional 4 h followed by adding 200µl of dimethyl sulfoxide. Cell viability was measured at 570nm by an ELISA reader (Biotek ELX800 microplate reader). IC50, the concentration reducing the absorbance of treated cells by 50% with respect to untreated cells, were determined by the standard curve method.

Detection of Apoptosis by Flow Cytometry

Apoptotic cells were determined by Annexin V/ Propidium Iodide assay according to the manufacturer's recommendation. Briefly, MCF-7 cells were cultured overnight in a 24-well plate (100,000/well) and treated with MgCl2 or Cisplatin for 24 h. Floating and adherent cells were then harvested and incubated overnight at 4°C

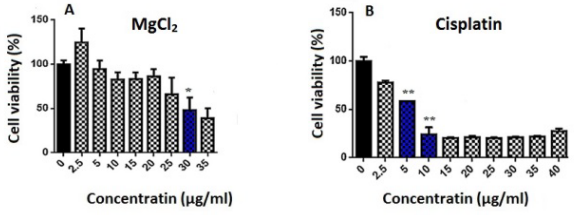

Figure 2. MTT Assay Results for Cisplatin and MgCl2 in 48 h on MCF-7 Cell Line. The LD50 of Treatment of MgCl2 on the MCF-7 Cells Calculated to be 30 (µg/ml) (Left Diagram, Blue Column) in 48 h, Whereas LD50 of Cisplatin in This Time Calculated to be 7.5 (µg/ml) (Right Diagram, Blue Column) (P<0.05).

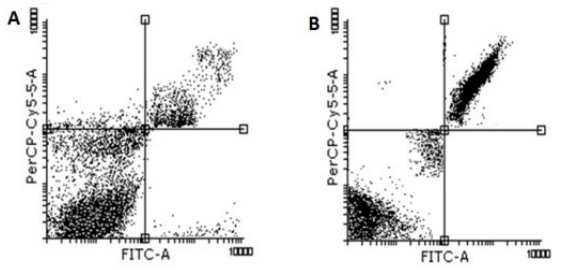

Figure 3. Evaluating Apoptosis Percentage for LD50 Dose of Cisplatin and MgCl2 in 24 h on MCF-7 Cells. Treating Cells with 20 (µg/ml) Concentration of Cisplatin (LD50 Dose of Cisplatin in 24h) Showed 59% Apoptosis (B, Top Right Quadrant); Flow Cytometery Results for Treatment the Cells with 40 (µg/ml) Concentration of MgCl2 (LD50 Dose of MgCl2 in 24h) Showed 44% Apoptosis (A, Top Right Quadrant).

in the dark with 750 µl of a hypotonic buffer (50 µg/ml PI in 0.1% sodium citrate plus 0.1% Triton X-100) before flow cytometric analysis using a FACScan flow cytometer (BD FacsCanto II, USA).

Statistical Analysis

All results were expressed as mean ± SD. The significance of difference was evaluated with ANOVA and Bonfrroni's test. Data were analyzed by SPSS software (version 6.01). Significant difference was set at P=0.05.

Results

MTT assay

The cell viability MTT assay was performed to determine the cytotoxicity effect of MgCl2 on the MCF-7. As it is shown in Figure 1, lethal dose 50 (LD50) in 24 h for MgCl2 was evaluated to be 40 (µg/ml); and that of Cisplatin was 20 (µg/ml). Treatments of fibroblasts (as a normal cell line) with Cisplatin have no significant effect on the cell survival in 24 h, in concentrations up to 30 (µg/ml) (P=0.07). MgCl2 have no significant effect on fibroblast cells' survival at 24 h, in concentrations up to 40 (µg/ml) (P=0.56).

We also performed MTT assay in 48 h to investigate LD50 of MgCl2 and Cisplatin on MCF7. As it is shown in Figure-2, the LD50 of Cisplatin in this time was evaluated to be 10 (µg/ml) (more precisely 7.5 (µg/ml)) and that of MgCl2 in the same time was calculated to be 20 (µg/ml). Similar to MTT assay results in 24 h, Cisplatin has no significant effect on the fibroblast survival in 48 h (P=0.06), in concentrations up to 30 (µg/ml). In addition, MgCl2 have no significant effect on fibroblast cells' survival in 48 h (P=0.57), in concentrations up to 40 (µg/ml).

MTT assay results for Cisplatin and MgCl2 in 24 and 48 h on MCF-7 cell line show that LD50 of MgCl2 at these times was higher than Cisplatin and these differences were significant (P=0.03)

Flow cytometery

Flow cytometery results for treatment of MCF-7 cells with LD50 concentration of Cisplatin and MgCl2 showed 59% and 44% apoptosis respectively in 24h (Figure 3).

Discussion

Resistance in responsive tumors is a common problem and a cause for failure in the curative therapy for malignancy. Therefore, there is a vital need to develop new anti-cancer drug (Song et al., 2011). Apoptosis as an underlying mechanism of MgCl2 cytoxocity was shown by flowcytometery in the present study. Flowcytometery results show that the rate of apoptosis in treatment of cells with 40 (µg/ml) concentration of MgCl2 (44%) was lower than this rate in treatment of cells with 20 (µg/ml) of cisplatin (59%). Mg is involved in a wide range of biochemical reactions and is an important mineral that affects carcinogenesis by promoting genomic stability, DNA synthesis and repair, glucose metabolism, the regulation of cell proliferation and apoptosis, and defense against oxidative stress (Wolf et al., 2007; Hartwig 2001; Anghileri 2009). In a meta-analysis by Ko et al (2014) showed that higher dietary magnesium intake seems to have a protective effect for cancer. In similar to our result, Zhang et al (2015) study showed that the viability and proliferation of AGS gastric adenocarcinoma cell were inhibited by MgSO4 at concentrations of 25-50 mM. In another study, by Kabadere et al (2004) the effects of MgSO4 and lazaroid on glutamate toxicity on glial cells were investigated. Compared to the L-glu-treated group, MgSO4 at the dose of 0.01 mM induced C6 and human glioma cell growth by 17%, 15% and 5%, respectively in MTT test after 24 hours. In the Park et al (2000) study, the effect of extracellular cations such as calcium and magnesium was investigated on the therapeutic efficacy of drug uptake/excretion and the chemosensitivity of the human breast cancer cell lines, MCF-7 and MCF-7/ADR. Both calcium and magnesium ions decreased the membrane permeability of cancer cells. These divalent ions also lowered the drug uptake and the cytoplasmic levels of Rhodamine 123 and Adriamycin, and their results indicate that these extracellular cations might play an important role in the therapeutic activities of anticancer drugs in cancer patients.

In conclusion, the results of the present study show that on MCF-7 cell line, the LD50 of MgCl2 was higher than Cisplatin. In addition, the rate of apoptosis in treatment of cells with MgCl2 was lower than Cisplatin and it seems that Mg has a cytotoxic effect against breast cancer cells. Although the Cisplatine is more cytotoxic than Mg on MCF-7, but regarding the side effects of chemotherapy drugs, Mg can be considered as a supplement agent in the treatment of breast cancer patients. Hence, further studies are still recommended on the various aspects and side effects of this agent.

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Tina Parsa, Seyed Alireza Salimi-Tabatabaee, Mohammad Jafari and Reza Alizadeh-Navaei. All authors participated in drafting of the manuscript. Critical revision of the manuscript for important intellectual content was done by Tina Parsa, Hossein Ghasemzadeh Kolagar and Saeed Khazaei Jalil. All authors have read and approved the final version of the manuscript.The authors declare no conflict of interests.This article does not contain any studies with human participants or animals performed by any of the authors.

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