Effect of First Line Gastric Cancer Chemotherapy Regime Treatment in the AGS Cell Line - MTT Assay Result

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Abstract

Background: Combination chemotherapy regimes are common treatments for cancer. The aim of this study was to evaluation the effect of individual chemotherapeutic agents in comparison with a first line chemotherapy regime treatment in the AGS gastric cancer cell line by MTT assay. Materials and Methods: In this experimental study, AGS cells were grown in RPMI-1640 supplemented with 10% fetal calf serum and 100 IU/ml penicillin, and 10 µg/ml streptomycin, under a humidified condition at 37°C with 5% CO2. All cells were washed with PBS and detached with trypsin, centrifuged and 8000 cells re-plated on to 96- well plates. LD50 doses of Epirubicin, Cisplatin and 5-fluorouracil were added to each well in mono or triple therapy. Anti-proliferative activities were determined by MTT assay after 24, 48 or 72 h. Results: Results of MTT assays showed that there were no significant differences among 3 drugs in monotherapy (p=0.088), but there was significant difference between combination therapy with epirubicin (P=0.031) and 5FU (p=0.013) on cell survival at 24 h. After 48 and 72 hours, cell viability showed significant differences between the 3 drugs (p=0.048 and P=0.000 for 48 and 72 h, respectively) and there was significant difference between combination therapy with epirubicin (P=0.035 and P=0.002 for 48 and 72 h, respectively). Conclusions: The results showed no significant differences between these chemotherapy drugs each given alone, but combination therapy with 3 drugs had significant effects on cell viability in comparison with epirubicin alone.

Keywords: Gastric cancer - chemotherapy - AGS cell line - MTT - monotherapy - combined therapy

Introduction

Gastric cancer is the third most common cause of death worldwide, that affecting both people with low socioeconomic status and developing countries (Arnold et al., 2014; Guedes et al., 2014). Gastric cancer is accounting for 8% of cancer incidence and 10% of cancer deaths (Lin et al., 2015). Several risk factors for gastric cancer have been identified, including *Helicobacter pylori* infection, salt-preserved foods, dietary nitrite, smoking, alcohol, obesity, radiation, and family history (Tsugane et al., 2004). The incidence is particularly high in East Asia, Eastern Europe, and parts of Central and South America, and it is about twice as high among men than among women. Prognosis is generally rather poor, with 5-year relative survival below 30% in most countries (Brenner et al., 2009). Researchers also found that the incidence rates of gastric cancer varied across different geographic regions and this variation may be associated with genetic, lifestyle or environmental factors, including diet (Armstrong and Doll, 1975). Treatment strategy in gastric cancer includes surgery, radiation therapy, chemotherapy, or targeted therapy (Choi et al., 2015). Chemotherapy remains the cornerstone of treatment for gastric cancer patients with locally advanced and metastatic disease. Many chemotherapy agents have activity including platinums, irinotecan, fluorouracil, taxanes and anthracyclines. Treatment with a combination of three agents has been shown to lead to modest improvements in survival compared to two agents, but at the expense of significant toxicity (Ajani et al., 2007). Combination chemotherapy may improve the length and quality of survival of some patients with advanced gastric cancer. First line chemotherapy regimens for gastric cancer include epirubicin, cisplatin and 5-fluorouracil (Kim, 2005; Benson, 2008).

There was some combination chemotherapy in chemotherapy regime treatment, so the aim of this study was to evaluation the effect of each individual chemotherapeutic agent in LD50 in comparisons with first line chemotherapy regime treatment in the AGS gastric cancer cell line by MTT assay.

Materials and Methods

In this experimental study, AGS cells (NCBI No.C131, Human gastric adenocarcinoma cell line) was purchased from Pasteur Institute of Iran (Pasteur Institute, Tehran, Iran) for correspondence: rafiei1710@gmail.com

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The cells were grown in RPMI-1640 supplemented with 10% fetal calf serum and 100 IU/ml penicillin, and 10 μg/ml streptomycin (PAA, Pasching, Austria) in flask (Nunc, Denmark). The cells were cultured in a humidified condition at 37°C, and 5% CO₂. When the cultures were 80-90% confluent, all cells were washed with PBS (pH=7.4), detached with 0.25% trypsin, centrifuged at 1200 rpm for 5 min and re-plated onto 96-well plates and returned to the incubator for 24 h to allow cell adherence to the plate. Cell counts were performed using a haemocytometer and 8000 cells were used for all experiments. Then, LD50 doses of three chemotherapeutic agents including Epirubicin, Cisplatin and 5-fluorouracil were added to each well in mono or triple therapy and the plate returned to the incubator for either 24, 48 or 72 h. Anti-proliferative activities of mono or triple therapy on the AGS cell line, was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay. After 24, 48 or 72 h of exposure to the chemotherapy agents, MTT (Sigma) was added and then incubated for a further 4 h at 37°C. 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 MTT solvent and shaken for 10 minutes. Then, spectrophotometric absorbance was measured at 570 nm and read by ELISA reader and Optical density (OD) for each well was determined.

There was a control sample which remained untreated and received the equal volume of medium. All different treatments carried out in triplicate.

Cell Viability was determined using the following formula:

\[
\text{Cell Viability (\%) in comparison to the controls} = \frac{\text{OD of cell in each well}}{\text{Mean OD of control cells}} \times 100
\]

Data were analyzed by SPSS software (version 15). All results were expressed as mean ± SD. The significance of difference was evaluated with ANOVA and Tukey test. Significant difference was set at P<0.05.

**Results**

![Figure 1. The effect of chemotherapy drug on viability of AGS by MTT assay at 24 h.](image1.png)

* Significant difference between combination therapy with epirubicin (P=0.031) and 5FU (P=0.013). E: Epirubicin, C: Cisplatin, F: 5-fluorouracil, ECF: Epirubicin + Cisplatin + 5-fluorouracil

![Figure 2. The effect of chemotherapy drug on viability of AGS by MTT assay at 48 h.](image2.png)

* Significant difference between combination therapy with epirubicin (P=0.035). E: Epirubicin, C: Cisplatin, F: 5-fluorouracil, ECF: Epirubicin + Cisplatin + 5-fluorouracil

![Figure 3. The Effect of Chemotherapy Drug on Viability of AGS by MTT assay at 72 h.](image3.png)

* Significant difference between combination therapy with epirubicin (P=0.035) E: Epirubicin, C: Cisplatin, F: 5-fluorouracil, ECF: Epirubicin + Cisplatin + 5-fluorouracil

of living AGS cells compared to control showed significant difference between 3 drugs at concentrations of LD50 of each chemotherapy drugs (p=0.048). In this time, there was significant difference between combination therapy with epirubicin (P=0.035). Cell viability changes in third day were shown in figure 3. Percentage of surviving AGS and cells showed significant difference between 3 drugs (p=0.000). Also, there was significant difference between combination therapy with epirubicin (P=0.002)

**Discussion**

Chemotherapy for gastric cancer is varied, with most regimes including one of epirubicin, cisplatin or 5-FU (Lordick and Siewert, 2006) and the aim of this study was to evaluate the effect of each individual chemotherapeutic agent in LD50 in comparisons with first line chemotherapy regime treatment in the AGS gastric cancer cell line by MTT assay. Result of present study show that, after 48 h the cell viability was difference between epirubicin, cisplatin and 5-fluorouracil in mono therapy and cell viability was lowest in cisplatin group. In comparison between monotherapy and combinational regime, there was significant difference between combination therapy with epirubicin and cell viability combination therapy.
and monotherapy by cisplatin or 5-fluorouracil was not significant difference in 48 and 72 h, especially. Similar to our result, in Weinreich et al. study reported that replacing 5-FU in ECF (epirubicin + cisplatin + 5-fluorouracil) with a novel duplex drug resulted in greater growth inhibition of cancer cells than of the non-malignant cell lines and the inversion of the chemosensitivity of MKN-45 and 23132/87 cells (Weinreich et al., 2015). But, in Suttie et al study, among three these chemotherapeutic agents, epirubicin having the greatest effectin AGS cells, followed by 5-fluorouracil then cisplatin (Suttrie et al., 2007). Previous studies show that exposure to epirubicin and cisplatin induced G1 and G2 cell cycle arrest (Zoli et al., 2004; Suttie et al., 2007). Epirubicin, an anthracycline derivative of doxorubicin effects as a DNA intercalating agent and as an inhibitor of topoisomerase II (Zoli et al., 2004). Cisplatin as an alkylating agent binds to DNA within G2, forming cisplatin-DNA adducts which causes an alteration in the conformation of DNA leading to cell cycle arrest and apoptosis (Suttie et al., 2007). It is reported that action of 5-FU is related to its conversion to 5-fluoro-2'-deoxy-5'-monophosphate leading to the inhibition of thymidylate synthase and hence DNA synthesis (Pinedo and Peters, 1988). Studies have described an increase S-phase fraction in tumour cells, caused by 5-fluorouracil (Yamane et al., 1999; Park et al., 2004).

One of the limitations of our study is that we didn’t use normal cell line for comparison but we have negative control as control group for chemotherapy drug on well-perfused cells. In conclusion result of present study show that there was no significant difference between these chemotherapy drugs, but combination therapy with 3 drugs had significant effect on cell viability in comparison with epirubicin.

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