Investigations on curcumin’s ability to enhance anticancer drug sensitivity to cancer cells

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Introduction

Ovarian cancer is one of the most common cancers that affect women and it causes more deaths than other female reproductive cancers. In the early stage of this cancer, chemotherapeutic treatment is the standard approach employing combination of a platinum compound, such as cisplatin or carboplatin, and a taxane, such as paclitaxel or docetaxel. The chemotherapy course for ovarian cancer involves 3 to 6 cycles. Each treatment is followed by a rest period for 3- to 4 weeks so the host can recover from side effects. Unfortunately, cancer cells regrow if this period is too long. As a result of this strategy, most individuals will eventually develop chemoresistant disease, which is one of the major obstacles to successful ovarian cancer treatment. An alternative strategy: metronomic therapy, involving restriction of the treatment to lower dosages of the cytotoxic agent, combined with more frequently dosing, has been revealed to be more effective than using a standard maximum tolerated dose (MTD) regimen.¹

Curcumin is a hydrophobic polyphenol compound, derived from the rhizomes of the golden spice turmeric (*Curcuma longa* Linn.), which possesses multiple biological activities including anti-inflammatory,² antioxidant,³ and anti-oxidant⁴ properties. Consuming curcumin as a dietary phytochemical is one of the strategies to improve the therapeutic effectiveness and to reduce side effects of anticancer drugs. Additionally, curcumin acts synergistically when combined with conventional chemotherapeutic drugs to eradicate resistant cancer cell lines.⁵ This study is an attempt to develop combination of cisplatin and curcumin for improving cisplatin sensitivity to cancer cells. We have used cisplatin resistant ovarian cancer cells to test curcumin’s ability to improve efficacy.

Experimental methods

Cell lines and culture conditions

The cisplatin resistant ovarian cancer cell lines (A2780) were cultured in RMPI-1640 medium containing 10% fetal bovine serum (FBS; Life Technologies Inc., Gaithersburg, MD, USA), 1% antibiotics (100 units/ml penicillin, and 100 µg/ml streptomycin; Sigma Chemical Co.). Cells were incubated in a humidified atmosphere of 5% CO₂, 95% air at 37°C, and passaged after reaching 70-80% confluence.

Drug treatments and cell viability

Curcumin and cisplatin were dissolved in DMSO as stock solutions, and then diluted with culture media such that the final concentration of DMSO was not higher than 0.05%. Treatments were performed in triplicate. Cell viability was quantified using the 3-[4,5-dimethylthiazol-2-yl]2,5-diphenyltetrazolium bromide (MTT) assay. In summary, cells were seeded in a 96-well plate. After overnight growth, cells were then exposed to treatments, and incubation. Following treatment media was carefully removed, and 100 µl of MTT solution was added into each well, followed by a 3 h incubation period at 37 °C. MTT solution was then removed and 100 µl of DMSO
was added in each well to dissolve formazan crystals. The UV absorbance at 570 nm was subsequently measured by microtiter plate reader. The absorbance values obtained per treatment were transformed to percentages of cell viability and the results were expressed as the mean ± standard deviation (SD).

**Measurement of maximum tolerated dose of cisplatin or curcumin, or in combination, in cisplatin resistant ovarian cancer cells**

A2780 cells were adjusted to 1 x 10^4 cells/well in 200 µl of medium, plated into 96-well plates and cultured overnight. Cells were then exposed to cisplatin (0, 5, 10, 20, 30, 40, 50 µg/ml) for 24-, and 48 h to determine maximum tolerated dose (MTD). Cells were also treated with cisplatin 5- and 10 µg/ml plus or minus pretreatment with curcumin (2.5, 5 µM) for 48- and 72 h to investigate the activity of curcumin that could improve the effect of cisplatin to inhibit cell growth in cisplatin resistant cells. Cell viability was determined with MTT assay at wavelength 570 nm.

**Measurement of cytotoxicity of metronomic chemotherapy with cisplatin, and curcumin in cisplatin resistant ovarian cancer cells**

A2780 cells (2 x 10^3 cells/well) were seeded into 96-well plates and incubated overnight. Cells were then exposed to either low doses of cisplatin (0.25, 0.5 µg/ml) for daily treatment, or high doses of cisplatin (5, 10 µg/ml) for continuous treatment in the presence or absence of curcumin pretreatment (10 µM) for 6 days to determine the efficacy of treatment. To further investigate if cell viability was affected by cisplatin dose, different curcumin concentrations were assessed to define optimal concentration. Cells were treated daily with low cisplatin doses (0.25, 0.75 and 1 µg/ml) plus or minus curcumin pre-treatment (0.25, 0.5, 1, 2.5, 5 µM) for 6 days.

**Results**

**Effect of cisplatin or curcumin alone or in combination in cisplatin resistant ovarian cancer cells:**

To determine MTD of cisplatin, cell viability was analyzed using MTT assays. At cisplatin concentration of 5- and 10 µg/ml, cell viability was similar to control (around 90%) at both 24- and 48 h since ovarian cancer cells were exposed to 10 µg/ml of cisplatin to become cisplatin-resistant. Following treatment with increasing curcumin concentrations, cell viability was reduced in a concentration dependent manner up to 20%. Moreover, cell viability in combination (cisplatin-curcumin) treatment decreased as same as in curcumin alone (5 µM) (Fig. 1). This might be due to anticancer activity of curcumin.

**Effect of metronomic chemotherapy with cisplatin and curcumin in cisplatin resistant ovarian cancer cells**

Combination treatment with curcumin and either low- or high doses of cisplatin inhibited cell growth more than either drug alone. This was due to the effect of the combination treatment, since it appeared to be the effect of curcumin alone. Moreover, there was no significantly difference between each low dose cisplatin-treated cells after 6 days of incubation as shown in Fig. 2. However, curcumin inhibited cell growth in a dose dependent manner. Especially at curcumin concentration of 2.5- and 5 µM, cell viability of curcumin-treated cells...
was comparable to that of combination treated cells (approximately 50 and 30%, respectively). This revealed that the cell viability reduction might be due to the anticancer properties of curcumin.

**Figure 2.** Cell viability of A2780 cells pretreated with curcumin (CUR) for 1 h, followed by daily exposure with a low dose of cisplatin (CIS) for 6 days.

**Discussion**

Ovarian cancer is highly sensible to chemotherapy and often responds well initially to standard therapy, but most cases of ovarian cancer patients can be recurrent by development of clinical resistance to platinum-based therapy. An alternative strategy for improving current ovarian cancer therapy is to combine a chemo sensitizer with chemotherapy.

Curcumin has been shown anticancer activity and shown to be a potent chemo sensitizer for ovarian cancer and breast cancer. Yunos et al. determined the synergetic inhibitory effects from sequenced combinations of cisplatin with curcumin and epigallocatechin-3-gallate (EGCG) on human ovarian cell lines (cisplatin-sensitive; A2780, and cisplatin resistant; A2780 cisR). Curcumin was found to inhibit the growth of resistant cell lines more than the parent cell lines. Moreover, the authors reported that 4 h incubation of cisplatin before adding curcumin and EGCG resulted in the most synergistic action in both cell lines. In the current study, we have evaluated the combination of low dose cisplatin and curcumin in cisplatin resistant ovarian cancer cells. Our results show that an hour pre-treatment with curcumin effectively inhibited the growth of cisplatin resistant ovarian cancer cells while co-treatment with metronomic dose of cisplatin and curcumin did not show any effect on cell viability.

**Conclusion**

This study provides evidence that metronomic chemotherapy with cisplatin and curcumin could inhibit the growth of cisplatin resistant ovarian cancer cells: the inhibitory effect on cell viability appears to be a result of the anticancer activity of curcumin. In vitro investigations may not be appropriate for studying anticancer drug sensitivity, and in vivo cancer models might prove more beneficial for evaluation of the combination treatment.

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