Effects of Combination Therapy with Cisplatin and Grape Seed Proanthocyanidins on Human Cervical Cancer Cells C33a

Jing Wang, Hong Yu

Abstract: To investigated the inhibitory effect of cisplatin in combination with Grape Seed Proanthocyanidins against human cervical cancer cell lines in vitro. Cervical cancer C33A cells were cultured and divided into four groups: cisplatin group, Grape Seed Proanthocyanidins group, cisplatin with Grape Seed Proanthocyanidins group and the blank control group. Morphologic changes of C33A cells were observed under invert microscope after C33A cells were treated 48, and 72 h. The proliferation of C33A cells was determined by CCK8 assay. Apoptosis were evaluated by PE Annexin V staining. Both of these two agents induced apoptosis and inhibited proliferation in C33A cells in a dose-dependent manner. In combination, cisplatin and Grape Seed Proanthocyanidins amplified these regulatory effects compared to each agent alone. The inhibition of proliferation can be observed in cisplatin group and in Grape Seed Proanthocyanidins group with different concentrations, while the notable effect was showed in Grape Seed Proanthocyanidins group. Combination treatment of cisplatin and Grape Seed Proanthocyanidins produces synergistic influence on C33A cells.

Keywords: cisplatin; Grape Seed Proanthocyanidins; human cervical cancer cells C33A

Introduction
Cervical cancer is one of the most common cancers, with an estimated global incidence of 470,000 new cases and over 200,000 deaths per year[1], making it the second cause of cancer deaths that kills women worldwide. Traditional treatments of cervical cancer include surgery, radiation therapy and chemotherapy. Among these treatments, chemotherapy combined with operation is the effective one for cervical cancer[2]. Docetaxe and cisplatin are the most common clinical chemotherapeutic drugs. Cisplatin (DDP) is a platinum Rn metal complex, its center atoms can interfering with DNA replication by binding DNA strands in tumor cells[3]. Cisplatinum is the common clinical platinum antineoplastic, but it has serious cytotoxicity to kidney and bone marrow, this feature restricts its application in cancer treatment[4]. Grape seed procyanidins (GSP), as a kind of polyphenols, exits widely in different kinds of seeds and grape skins. GSP is a kind of free radical scavenger and has strong antioxidation capacity[5]. GSP was proved to regulate cells growth and apoptosis on many kinds of tumor cells cultured in vitro[6-7]. Therefore, Cisplatin combined with Grape seed proanthocyanidin may enhance their anticancer property and reduce side affect brought by Cisplatin.

1. Materials and Methods
1.1 Materials
The human cervical cancer cells C33A was obtained from authors’ laboratory. DMEM medium was purchased from Hyclone, USA. Fetal bovine serum was from Sijiqing co., LTD. Cisplatin was from sigma.
Grape seed procyanidin was from melonepharma. CCK8 kit was from Bridgen. XDS-1B invert microscope bought from OLYMPUS Company. Enzyme micro-plate reader bought from Thermo.

1.2 Methods

1.2.1 Cell proliferation assay

C33A cells were cultured until in logarithmic phase, made into cell suspension and seeded in 96-well plates at 2×10^4 cells/well. After incubated for 24h at 37°C, the cells were divided into blank control group, cisplatin group, Grape seed procyanidins group and cisplatin plus Grape seed procyanidins group. DDP group was treated with different concentrations of cisplatin (9, 18, 37 μM). GSP group was treated with different concentrations of GSP (170, 340, 680 μM). DDP&GSP group was treated with 340 μM procyanidins first and then added with different concentrations of cisplatin (9, 18, 37 μM) respectively. Two parallel groups were set up in each concentration with 6 well per group. Cell proliferation was evaluated using the CCK8 after 48h and 72h. Briefly, 10 μl of CCK8 solution was added to the culture medium, and incubated for additional 2 h. The absorbance was determined at 450 nm wavelength using the microplate reader. Cell proliferation rate of different groups can be calculated by a formula.

Inhibition of cell proliferation rate /% = (control group A value - the value of the experimental group A) / A value of blank group × 100%. The value of every experimental group in this formula is the average of absorbance from the 6 wells in each group.

1.2.2 Cell apoptosis assay

C33A cells were cultured until in logarithmic phase, made into cell suspension and seeded in 6-well plates at 55 × 10^4 cells/well. After incubated for 24h at 37°C, the cells were divided into blank control group, DDP group and DDP&GSP group. DDP group was treated with different concentrations of DDP (9, 18, 37 μM). DDP&GSP group was treated with 340 μM GSP first and then added with different concentrations of DDP (9, 18, 37 μM) respectively. Two parallel well were set up in each group. After treatment, adherent cells were collected. Cells in suspension were digested using trypsin and centrifuged at 1000g for 5 min. The final cell concentration was adjusted to 2 ~ 5 × 10^5/mL. The single cell suspension added 100μL Guava Nexin Reagent (containing Annexin V-PE and 7-ADD buffer). After incubation in the dark at room temperature for 20 min, cells were analyzed by flow cytometry.

1.2.3 Statistical Evaluation

SPSS ver.17.0 software was used for the statistical analysis, and single-factor analysis of variance was used to analyze statistical differences. P < 0.05 was accepted as significant. The data were expressed as the mean ± standard deviation.

2 Result

2.1 normal morphology of C33A cells

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Fig. 1 unmedicated C33A cells cultured 48h, 72h (100×)
2.2 Effect of Cisplatin with Different Concentrations for 48h

Fig. 2 Effect of Cisplatin with Different Concentrations for 48h (100×)

A: Cisplatin 9 μM, 48h, cells morphology of C33A; B: Cisplatin 18 μM, 48h, cells morphology of C33A; C: Cisplatin 37 μM, 48h, cells morphology of C33A.

2.3 Effect of Grape Seed Procyanidins with Different Concentrations for 48h

Fig. 3 Effect of Grape Seed Procyanidins with Different Concentrations for 48h (100×)

A: GSP170 μM, 48h, cells morphology of C33A; B: GSP340 μM, 48h, cells morphology of C33A; C: GSP680 μM, 48h, cells morphology of C33A.

2.4 Effect of Cisplatin Plus Grape Seed Procyanidins with Different Concentrations for 48h

Fig. 4 Effect of Cisplatin Plus Grape Seed Procyanidins with Different Concentrations for 48h (×100)

A: GSP of 340 μM combined with DDP of 9 μM for 48h; B: GSP of 340 μM combined with DDP of 18 μM for 48h; C: GSP of 340 μM combined with DDP of 37 μM for 48h.
2.5 detect cell proliferation inhibition rate with cck8

Table 1: Cell proliferation inhibition rate of different concentrations of DDP incubate C33A for 48h

<table>
<thead>
<tr>
<th>group</th>
<th>absorbancy (x±s)</th>
<th>cell proliferation inhibition rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>control group</td>
<td>0.809±0.007</td>
<td></td>
</tr>
<tr>
<td>DDP group (9μM)</td>
<td>0.696±0.031</td>
<td>13.9</td>
</tr>
<tr>
<td>DDP group (18μM)</td>
<td>0.650±0.039</td>
<td>19.6</td>
</tr>
<tr>
<td>DDP group (37μM)</td>
<td>0.582±0.035</td>
<td>28.0</td>
</tr>
</tbody>
</table>

From Table 1 we conclude that the proliferation inhibition rate of DDP on C33A cells for 48h was different from control group. The differences had statistical significance (P < 0.05).

Table 2: Cell proliferation inhibition rate of different concentrations of GSP incubate C33A for 48h

<table>
<thead>
<tr>
<th>group</th>
<th>absorbancy (x±s)</th>
<th>cell proliferation inhibition rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>control group</td>
<td>0.752±0.043</td>
<td></td>
</tr>
<tr>
<td>GSP group (170μM)</td>
<td>0.265±0.002</td>
<td>64.7</td>
</tr>
<tr>
<td>GSP group (340μM)</td>
<td>0.231±0.009</td>
<td>69.2</td>
</tr>
<tr>
<td>GSP group (680μM)</td>
<td>0.228±0.006</td>
<td>69.6</td>
</tr>
</tbody>
</table>

From Table 2 we conclude that the proliferation inhibition rate GSP on C33A cells for 48h was higher than that in control group. The differences had statistical significance (P < 0.05).

Table 3: Cell proliferation inhibition rate of different concentrations of DDP&GSP incubate C33A for 48h

<table>
<thead>
<tr>
<th>group</th>
<th>absorbancy (x±s)</th>
<th>cell proliferation inhibition rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>control group</td>
<td>0.779±0.074</td>
<td></td>
</tr>
<tr>
<td>DDP&amp;GSP group (9+340μM)</td>
<td>0.235±0.008</td>
<td>69.8</td>
</tr>
<tr>
<td>DDP&amp;GSP group (18+340μM)</td>
<td>0.221±0.009</td>
<td>71.6</td>
</tr>
<tr>
<td>DDP&amp;GSP group (37+340μM)</td>
<td>0.210±0.006</td>
<td>73.0</td>
</tr>
</tbody>
</table>

From Table 3 we conclude that the proliferation inhibition rate DDP&GSP on C33A cells for 48h was higher than that in control group. The differences had statistical significance (P < 0.05).
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2.6 flow cytometry (FCM) detect cell apoptosis rate of C33A

Pic 5 Annexin V-PE Double staining to detect cell apoptosis

A: control group, the cell apoptosis rate is 0.1%; B: DDP 9μM, the C33A cell apoptosis rate is 0.8%; C: DDP 18μM, the C33A cell apoptosis rate is 2.5%; D: DDP 37μM, the C33A cell apoptosis rate is 1%; E: DDP & GSP group (μM) 9 + 340μM, the C33A cell apoptosis rate is 89.7%; F: DDP & GSP group (μM) 18 + 340μM, the C33A cell apoptosis rate is 89.7%; G: DDP & GSP group (μM) 37 + 340μM, the C33A cell apoptosis rate is 92.3%.

The figure 5 shows that the control group of cell apoptosis rate was 0.1%; the cell apoptosis rate of DDP group which is only add cisplatin for 24h was higher than the control group; the cell apoptosis rate of DDP & GSP group was 92.3%.

3 Discussion

Cervical cancer is one of the most common gynecological malignancy and 80% of this cases happen in developing countries; there are 529,000 new cases occur each year, and more than half of the patients died. Because of the high cytotoxicity of
radiotherapy, chemotherapy drugs, only a patients can survive over five-year survival after being treated[8]. DDP is a chemotherapeutic agent commonly used in the treatment of cervical cancer[9], and as a cell cycle non-specific drugs, DDP can inhibit the replication of cervical cancer’s DNA and has a strong inhibitory effect on kinds of cancers. Research discovered that cervical cancer cells showed lower sensitivity to DDP and produced drug resistance after a period of radiotherapy, increasing the treatment difficulty of cervical cancer.

DDP’s toxicity to renal is an important factor in limiting their extensive use. DDP’s toxicity to the kidneys is a important factor to limit its extensive use. The study found that DDP rise renal small tube net stress related proteins GRP78 and P – ERK and endoplasmic reticulum stress response leads to kidney function decline. Grape seed GSP extracted from grape seeds bio-active ingredients and it can increase apoptosis through the mitochondrial pathway protein Bak-1 and reduce apoptotic anti-apoptotic protein Bcl-2-induced cervical cancer cells[10]. GSP in the chemical structure of a phenolic hydroxyl group are easily oxidized quinone structure, which can consume oxygen in the atmosphere, on free radical it has a strong ability to capture and eliminate and the ability to eliminate free radicals in the body is very strong antioxidant. GSP is a good oxygen free radical scavenger. The study found that the appropriate dosage of GSP has certain protective effect on the toxicity of DDP cells and can improve cell survival rate[11].

In this study, different concentrations of DDP&GSP act on cancer cells C33A showed that apoptosis rate of DDP&GSP group is higher. apoptosis rate is up to 92.3% when 37 + 340μM. DDP&GSP plays role in C33A cells. Cell proliferation inhibition rate of DDP&GSP group was significantly higher than DDP group. cell proliferation inhibition rate of C33A which is just affected by GSP group is higher than 50%. This provides theoretical basis to improve the effect of chemotherapy on combination therapy and Provides a guidance for clinical treatment of patients with cervical cancer.

References