Original Article

The in vitro and in vivo effects of cisplatin on growth of oral cancer cells regulated by miR-633 and miR-210

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Abstract: The objective of this study was to explore the effects of cisplatin on growth of human oral cancer cells, and the differential expression of two micro RNAs (miRs), miR-633 and miR-210 in vitro and in vivo. The growth-inhibiting effects of cisplatin on the human oral cancer cell line SCC25 were assessed by MTT assay, while cell apoptosis was examined using DAPI staining. In addition, the effects of cisplatin on the growth of oral cancer in a murine model were tested and the expressions of miR-633 and miR-210 were quantified using real-time PCR. We observed that, cisplatin significantly decreased the growth of human oral cancer cells in vitro (P < 0.05 vs. control cells) at both 24 and 48 hours of incubation, with effects being more pronounced at the latter time point. Furthermore, Microscopic studies demonstrated apoptotic signs in cispatin-treated cells with a clear dose-response pattern. In the murine tumor model, tumor volumes were smaller in cisplatin-treated animals, tumor weight were reduced in cisplatin-treated animals. Subsequently, the miR-633 was down-regulated, while miR-210 was up-regulated by cisplatin. Thus, cisplatin significantly inhibits proliferation of oral cancer cells, and the effects are associated with down-regulation of miR-633 expression and up-regulation of miR-210 expression.

Keywords: Cisplatin, oral cancer, miR-633, miR-210

Introduction

Oral cancer is a malignant cancer and the largest group of the cancers that categorize into the head and neck cancers which is associated with is associated with high mortality [1-4]. Currently, chemotherapy is the main therapy for oral cancer [5, 6]. Oral cancer is revealed in most patients at an advanced stage, making the surgical treatment impossible due to advanced tumor size, and/or intrahepatic or metastases [7, 8]. Thus, non-surgical treatment, and especially, chemotherapy, remains the principal therapeutic method [9, 10].

Cisplatin is a chemotherapeutic drug that principal mechanism of action is DNA damage. Cisplatin is widely used to treat solid tumors [11, 12]. In this study, we examined the effects of cisplatin on growth of oral cancer cells, and also verified the expression of miR-633 and miR-210 in vitro and in vivo.

Materials and methods

Cells and reagents

The SCC25 oral cancer cell line was obtained from the Third Military Medical University. Cisplatin was provided by Yunnan Gejiu Biological Pharmaceutical Co., Ltd. The clean grade male healthy Kunming mice, weighing 18 g to 22 g were purchased from Kunming Institute of Zoology. The 25- and 50-ml culture bottles, 96- and 6-well plates were from purchased from Coster Company. Automatic microplate reader Elx-800 was from Omega Bio-Tek Inc., CO₂ constant temperature incubator was from Thermo Fisher Scientific Inc., centrifuge was from Changsha Xiangyi Centrifuge Co., Ltd, and Olympus optical microscope was purchased from Olympus Corporation. DMEM culture medium, trypsin, and antibiotics were provided by Thermo Fisher Scientific Inc., while fetal calf serum, MTT, and DMSO were from Sigma-
Aldrich. The hematoxylin-eosin staining solution was from Beijing ZhongshanJinqiao Biotechnology Co., Ltd. Primers for miR-633, miR-210, and control U6 were obtained from Beijing Keyingmei Technology Co., Ltd.

Effects of cisplatin on SCC25 oral cancer cell line

SCC25 cells sub-cultured for 48 hours with a uniform growth were digested with trypsin, counted, and 6×10^3/well cells were seeded onto 96-well culture plates. After 24 hours, culture medium was removed and replaced with culture medium containing cisplatin. Cisplatin was administered at drug concentrations (0 µg/ml = basal, and 5, 10, 20, 40, and 80 µg/ml) in the final volume of 200 µl. All experimental conditions were done in triplicate. Afterwards, the cells were cultured for 24 or 48 hours. Then, culture medium was discarded and 50 µl/well MTT in 150 ml of culture medium were added in each well. The plates were incubated for 4 hours, following which culture supernatants were discarded, and cells were lysed by 150 µl of DMSO for 12 min under mild shaking. Optical densities were registered at a wavelength of 490 nm. The growth inhibition rate (%) was calculated as follows: (OD of control well - OD of cisplatin-treated well)/OD of control well.

Apoptosis of SCC25 cells on cisplatin

The cells were seeded onto sterile glass coverslips placed in the bottom of a 6-well plate. The cells were incubated overnight and then exposed to 10 or 20 µg/ml of cisplatin at a final volume of 2 ml. After incubation for 48 hours, cells were washed three times with cold PBS, and 4% triformol was added to cells to fix for 30 min. Then, 2 ml of a 1 µg/mL DAPI staining solution was added to cells to stain the nuclei for 15 min. Cover slips were placed over the slides and dried. Cell fluorescence was observed under the microscope, with the excitation wavelength of 350 nm and emission wavelength of 460 nm.

Effects of cisplatin on growth of SCC25 oral cancer cells in mice

Mice were inoculated with SCC25 oral cancer cells except control mice. Then, Ascitic fluid from the abdominal cavity of these animals was collected, and PBS was added to prepare the cell suspension at a concentration of 5×10^6/ml. Then, 0.2 ml of SCC25 cell suspension was inoculated in the subcutaneous armpit of left forelegs. Inoculation was completed within 90 min to achieve a solid tumor model. Tumor size was observed and measured each day following the inoculation. When the volume reached 100 mm^3, mice were divided into 5 groups, with 6 animals per group: (i) control group (no injection of cancer cells) received a gavage of 0.2 ml/10 g 0.5% CMC-Na every day, (ii) untreated group: mice received a gavage of 0.2 ml/10 g 0.5% CMC-Na every day, (iii) low-dose cisplatin group (gavage with 5 mg/kg cisplatin), (iv) medium-dose cisplatin group (gavage with 10 mg/kg cisplatin), and (v) high-dose cisplatin group (gavage with 20 mg/kg cisplatin). All mice were euthanized on day 10.

Effect of cisplatin on tumor size in mice

After cisplatin treatment, the smallest and the biggest diameter of the tumor were measured to calculate the tumor volume. The tumor volume (mm^3) = (a × b × a) / 2 (“a” = the longest diameter in mm, b = the shortest diameter in mm). Six mice from each group were euthanized to obtain solid tumors. The tumors were washed with normal saline. The tumor inhibition rate (%) was calculated as: 1 - tumor weight in cisplatin-treated group/tumor weight in untreated group × 100%.

Hematoxylin-eosin staining

Tumors were washed with cold normal saline and fixed with 4% triformol. After fixation, tumors were washed and kept overnight, and then immersed sequentially in 70%, 80%, 90% and 100% ethanol for three hours. Afterwards, tumors were immersed in xylene I and II (30 min each), and then in paraffin I, II, and III (30 min each). Finally, tumors were embedded in paraffin and sliced into 5 um paraffin sections. The following procedures were dewaxing, staining with hematoxylin, washing with running water, staining with eosin, repeated washing, and sealing. Afterwards, the slides were observed under the microscope.

Expressions of miR-633 and miR-210

Logarithmical growing SCC25 cells obtained after 48 hours subculture was digested with trypsin, counted, and 3×10^5/well cells were seeded onto 6-well culture plates. After 24
The anti-cancer effects of cisplatin on oral cancer

Table 1. The growth-inhibiting rate of cisplatin on SCC25 oral cancer cells

<table>
<thead>
<tr>
<th>Group</th>
<th>Inhibit rate after 24 hours (%)</th>
<th>Inhibit rate after 48 hours (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated cells</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5 µg/ml cisplatin</td>
<td>13.27±0.87*</td>
<td>19.07±1.05*#</td>
</tr>
<tr>
<td>10 µg/ml cisplatin</td>
<td>25.37±1.25*</td>
<td>30.81±2.53*#</td>
</tr>
<tr>
<td>20 µg/ml cisplatin</td>
<td>37.81±2.89*</td>
<td>46.27±2.05*#</td>
</tr>
<tr>
<td>40 µg/ml cisplatin</td>
<td>44.61±2.48*</td>
<td>53.61±1.87*#</td>
</tr>
<tr>
<td>80 µg/ml cisplatin</td>
<td>51.26±2.07*</td>
<td>62.48±2.36*#</td>
</tr>
</tbody>
</table>

Footnote: Cell growth was assessed by MTT test. Data are presented as mean ± SD. *P < 0.05 vs. untreated cells; #P < 0.05 vs. untreated cells.

Figure 1. The growth-inhibiting rate of cisplatin on SCC25 oral cancer cell. Cell growth was assessed using MTT test.

Cisplatin significantly increased the inhibitory rate of cisplatin on SCC25 oral cancer cells after 24 and 48 hours of treatment (P < 0.05 vs. untreated group) (Table 1 and Figure 1). The inhibitory effects were more pronounced at 48 hours of treatment (50% inhibitory concentration of 57.94 at 24 hours vs. 32.93 at 48 hours) (Table 1 and Figure 1).

DAPI staining

After cells were treated with cisplatin, the cells exhibited growth inhibition and shrinkage of cell volume (Figure 2). In addition, DAPI staining revealed a marked obvious karyopyknosis and breakage. Furthermore, cell apoptosis became more prominent with increasing drug concentrations.

Inhibitory effects of cisplatin in in vivo mouse cancer model

The inhibitory rates in low, medium and high dose cisplatin groups were significantly higher than the model group (P < 0.05), which was statistically significant (P < 0.05); however, there was no statistically significant difference observed among low, medium and high dose cisplatin treated groups (Table 2).
The anti-cancer effects of cisplatin on oral cancer

The untreated group which showed highest tumor volume also displayed obvious tissue morphology, with bigger cytoplasm and more karyokinesis (Table 3). In contrast, tissues from cisplatin-treated mice showed more necrotic areas of tumor tissue with smaller cells, while chromatin was darker and karyokinesis was less pronounced, as compared with untreated animals (Figure 3).

Expression of miR-633 and miR-210

The relative mRNA expression level of miR-633 was down-regulated, while miR-210 was up-regulated (Tables 4 and 5).

Discussion

Cisplatin is the first generation platinum drug and belongs to alkylating anti-tumor drugs [13, 14]. Cisplatin is inactive in high chlorine environment [15]. It is believed that cisplatin enters the cells using the copper transport protein. It has been reported that cisplatin concentration is correlated with copper and that absorption and transportation of cisplatin is defined by pharmacokinetic of copper [16, 17]. A transport protein, CTR1 has high affinity to platinum and copper. Deficiency and mutation of the gene that encodes this transport protein can increase cancer resistance to cisplatin [18-20]. Cisplatin causes cross-linking of nucleoprotein and DNA strands by adding bivalent platinum by adding bivalent platinum [21-24]. Cisplatin was shown to inhibit proliferation of SCC25 human oral cancer cells in vitro [25, 26]. Our study also showed similar findings. Cisplatin is a cytotoxic drug and can inhibit lots of tumor growth. In this study, the inhibition of growth of SCC25 oral cancer cells may associate with toxic effect. We also observed that cisplatin caused karyopyknosis and breakage with pronounced cell apoptosis. We tested the effects of cisplatin in a murine model of oral cancer and observed inhibition of tumor growth.
miR-633 was up-regulated by cisplatin treatment. miR-210 inhibits cell proliferation and promotes cell apoptosis in cancer cells. In contrast, miR-633 was down-regulated by cisplatin. It is possible that miR-633 acts as an oncogene in SCC25 cancer cells. Therefore, cisplatin may exert its cancer inhibiting effects by down-regulating miR-633.

In conclusion, cisplatin exerts a significant inhibitory effect on SCC25 human oral cancer cells, and this effect is both time- and concentration-dependent. In a murine tumor model, cisplatin significantly inhibits tumor growth and promotes cell apoptosis, possibly, via up-regulating miR-210 and down-regulating miR-633 expressions.

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Disclosure of conflict of interest

None.

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References


Figure 3. Hematoxylin-eosing staining of tumor tissue (×100). A. Untreated group; B. Low-dose cisplatin group; C. Medium-dose cisplatin group; D. High-dose cisplatin group.

Table 4. The relative mRNA expression level of miR-633

<table>
<thead>
<tr>
<th>Sample</th>
<th>Relative mRNA expression level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>1.00±0.03</td>
</tr>
<tr>
<td>Medicine group</td>
<td>0.86±0.01*</td>
</tr>
</tbody>
</table>

Footnote: Data are presented as mean ± SD, *P < 0.05 (P = 0.001) vs. Control group.

Table 5. The relative mRNA expression level of miR-210

<table>
<thead>
<tr>
<th>Sample</th>
<th>Relative mRNA expression level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>1.01±0.01</td>
</tr>
<tr>
<td>Medicine group</td>
<td>1.20±0.01*</td>
</tr>
</tbody>
</table>

Footnote: Data are presented as mean ± SD, *P < 0.05 (P = 0.000) vs. Control group.
The anti-cancer effects of cisplatin on oral cancer


