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Synergistic effect of docetaxel combined with cisplatin on inhibiting human osteosarcoma in nude mice

Hai Tao [a], Xiaoyan Tang [b], Lin Jin [a], Yingchun Zhao [a], Yue Luo [a], Zheng Zhang [a], Lin Cai [c], Fenghua Tao [a, **], Weichun Guo [a, *]

[a] Department of Orthopaedics, Renmin Hospital of Wuhan University, Wuhan, 430060, Hubei province, PR China
[b] Department of Gastroenterology, Zhongnan Hospital of Wuhan University, Wuhan, 430071, Hubei province, PR China
[c] Department of Orthopaedics, Zhongnan Hospital of Wuhan University, Wuhan, 430071, Hubei province, PR China

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ABSTRACT

Cisplatin (CDDP) has been shown to be a promising anticancer drug that is effective against many types of cancer, which include osteosarcoma (OS). However, its therapeutic application is restricted by its toxicity in normal tissues, side effects caused in patients, and chemotherapy resistance. Thus, to further improve patients' treatment, the development of novel, more effective and well tolerated therapeutic approaches against OS in clinical is urgent and important. In the present study, nude mice were inoculated subcutaneously with injections of HOS8603 cells, CDDP and docetaxel (DTX) were administered intraperitoneally respectively. The inhibitive effects and the side effects were observed. Tumor weights and volumes were significantly lower and the tumor inhibition rate was significantly higher in the combination group than those of either drug alone or vehicle. The cell density in the tumor tissue was significantly decreased, apoptotic and necrotic cell death was significantly increased in the combination group, as compared with those of either drug alone or vehicle. In addition, there was no obvious side effect happening besides the appearance of erythema and papules in some mice. These results suggest that the combined effects of CDDP and DTX on the growth of human OS in vivo were superior to the single effects. CDDP combined with DTX had synergistic effects at lower concentrations and promoted apoptosis, but did not increase the side effects of chemotherapy.

1. Introduction

OS is the most frequent type of malignant primary bone tumor in childhood [1]. The prognosis for localized extremity OS treated with surgery alone is poor, with a 2-year survival rate of <20%, as the tumor exhibits a high propensity to metastasize to the lungs [2]. Although there have been developments in treatment with the administration of large doses of adjuvant/neoadjuvant chemotherapy, 30% of patients with localized disease and 80% of patients with metastatic disease at diagnosis suffer relapse [3]. In addition, the failure of standard multimodal therapy in OS is common, which is associated with a very poor prognosis. Therefore, new drugs or combination therapies are needed for patients with OS.

CDDP, one of the compounds of platinum, can integrate DNA base pair, cross link base pair, and inactivate DNA template, so as to disturb DNA duplication and induce the apoptosis of cancer cells by blanching catalysis effect of DNA polymerase. It is a cell cycle nonspecific agent.

DTX is a semisynthetic taxane analog of paclitaxel. This molecule induces cytotoxicity by stabilizing microtubules, preventing depolymerization, which results in cell cycle arrest and subsequent apoptosis.

CDDP and DTX are both broad-spectrum antineoplastic drugs, and they both are effective in treating OS when used alone [4]. As the two drugs have different mechanisms for treating OS, the combined use of them may have some synergistic effects.

Several studies have demonstrated that in gastric cancer, breast cancer and thyroid carcinoma, CDDP plus DTX combination treatment may prolong survival times in certain patients compared with treatment with CDDP or DTX alone [5–7]. There are clinical trials and case reports that have shown that CDDP and DTX can be

* Corresponding author.
** Corresponding author.
E-mail addresses: taohai2004@sina.com (H. Tao), 369482810@qq.com (F. Tao), taohai2004@126.com (W. Guo).

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successfully used for differentiated and anaplastic thyroid cancer. Since CDDP is one of the most active and widely used drugs in the treatment of high-grade OS, the CDDP plus DTX combination may have improved efficacy compared with CDDP alone.

In 2010, we [8] previously reported that the combined effects of CDDP and DTX on proliferation inhibition of OS cells were superior to the single effects, CDDP and DTX combination has been observed to exert additive and synergistic antitumor effects on OS cells. The results are interesting and encourage further research in the combined effects of CDDP and DTX in vivo.

The toxicity of these chemotherapy medicines to normal tissues has been one of the major obstacles to successful cancer chemotherapy. Therefore, the combined treatments with several chemotherapy regimens or even chemopreventive medicine are often used not only for the enhancement of the treatment effect, but also for the reduction of the toxicity of these drugs.

To investigate the efficacy and safety of the CDDP plus DTX combination with OS, the present study was conducted and the results obtained were compared with those of nude mice receiving the CDDP plus DTX combination.

2. Materials and methods

2.1. Animals and reagents

48 healthy male nude mice (BALB/c-nu/nu) at 5–6 weeks of age were selected (Laboratory Animal Central of Wuhan University,Wuhan, China) and weighed approximately 20 g at the time of experimentation. CDDP and DTX was obtained from Shandong Qilu Pharmaceutical Co. Ltd (China).

2.2. Cell line and cell culture

The OS cell line HOS8603 was obtained from the Chinese Academy of Medical Science (China). The cells were incubated in DMEM (Sigma, USA), 10% fetal bovine serum(FBS, GibcoBRL), 50 μg/ml streptomycin, 50 IU/ml penicillin, and 2 mM glutamine in a 5% humidified CO2 atmosphere at 37 °C.

2.3. Establishment of the model of human OS xenografts in nude mice

The Male nude mice (BALB/c-nu/nu) were inoculated subcutaneously with injections of 1×10^7 cells/mouse. When the tumors had reached a size of approximately 100 mg (10–15 days after transplantation), the mice were pair-matched into treatment and control groups. Mice were randomized into four groups(all 8 to 12 animals): vehicle, DTX, CDDP and DTX combined with CDDP respectively.

2.4. Drug treatment in nude mice

Mice as above were ear-tagged (Day 1) and followed individually throughout the experiment. Initial doses were given on Day 1 with saline control. The control and experimental groups were administered intraperitoneally (i.p.) at 4 mg/kg saline, 4 mg/kg CDDP, 4 mg/kg DTX, 2 mg/kg CDDP and 2 mg/kg DTX,respectively, twice a week for 4 weeks. The experiment was terminated on Day 28 when the control group tumor size had reached an average of 1 g. The mice were weighed twice weekly and observed daily for viability.

2.5. Evaluation of the synergistic effect in nude mice

The mice were weighed, sacrificed and their tumors xenografts were excised, weighed and measured. The mean tumor weights per group were calculated to determine tumor growth inhibition (TGI) for each group. Tumor volume (TV) was calculated using the following formula: TV (mm^3) = $d^2 \times D/2$, where d and D are the shortest and the longest diameters, respectively. This study was approved by the Institutional Review Board (IRB) of Renmin Hospital of Wuhan University.

2.6. Detection of pathological features

Tumor samples harvested from tumor-bearing nude mice under different treatments were fixed in formalin, sectioned (4 μm), and stained with hematoxylin and eosin (H&E).

2.7. Detection of apoptotic cell death by flow cytometry

Tumor tissues were isolated, washed by PBS, and cut into pieces. They were then immediately homogenized to make single-cell suspensions by ethylenediamine tetraacetic acid chelation, based on the published method. These single cells were fixed by 70% ethanol at −20 °C for 4 h. Cell suspensions were centrifuged and the supernatant discarded. Cells were resuspended in 3 mL of PBS and incubated with RNase at 37 °C for 30 min. Then, suspensions were centrifuged again to obtain cell pellets. Cells were stained with 1 mL of propidium iodide (PI) for 30 min and then detected on an Epics Altra II flow cytometer (Beckman, Fullerton, CA).

2.8. Evaluation of side effects

The livers and kidneys of different groups were fixed in 10% buffered formalin for 24 h at room temperature, dehydrated by grading ethanol and paraffin embedded, and the preserved tissues were cleaned in running water and processed for histological examination according to the conventional methods and stained with hematoxylin and eosin (H&E). Subsequently, the liver and kidney tissues were cut into 5 μm sections and stained with Hematoxylin-eosin (H&E) and Periodic Acid Schiff (PAS), respectively. The images were captured with an Olympus microscopy.

Blood was collected by cardiac puncture using heparin rinsed 1 ml syringes (20 gauge needles), and serum samples were collected after centrifugation at 1500 rpm for 10 min. BUN, Cr, ALT and AST levels were evaluated using a serum biochemical auto-analyzer Beckman 700 (Beckman, America).

Blood samples for blood cell counts were obtained from eye sockets of mice. Erythrocytes, leukocytes and platelets were counted by MEDONIC CA620 VET automated cell counter (Boule medical AB, Sweden). Blood marrow obtained from femur isolated from mice were made smears, and then observed by light microscope after trypan blue staining.

3. Statistic analysis

Biomedical variables obtained from this study were expressed as the mean ± standard error of the mean. The data were analyzed by using SPSSversion 20.0 software (SPSS, Inc., Chicago, IL). The significance association was determined by one-way analysis of variance. A value of $p < 0.05$ was considered statistically significant.

4. Results

4.1. Effect of CDDP and DTX on tumor development in nude mice

To determine a role of CDDP and DTX in progression of osteosarcoma, the antitumor effects of CDDP and DTX alone or in combination in human xenograft tumor models were evaluated.

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