Octreotide is the favorable alternative for cisplatin resistance reversal of ovarian cancer in vitro and in nude mice in vivo

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Summary

This study aimed to observe the effects of octreotide (OCT) on cisplatin resistance reversal of cancer cells in vitro and in nude mice in vivo. MTT method and flow cytometry were used to investigate the effect of cisplatin, OCT or the combination of these two compounds on the proliferation and apoptosis of SKOV3-DDP cells. The size and weight of xenograft tumors from the nude mice model were measured. Real-time PCR was used to detect the mRNA expression of SSTR2, MDR1, MRP2, GST-π and EGFR in SKOV3/DDP cells following the different treatment. At the concentration of 2.5-20 g/ml, OCT significantly reduced IC50 ($p < 0.05$) and promoted apoptosis ($p < 0.05$) of SKOV3-DDP cells' response to cisplatin. Unchanged expression was found in SSTR2 on the SKOV3/DDP cell in vitro after OCT treatment, but increased expression in vivo ($p < 0.05$). OCT increased GST-π expression ($p < 0.05$) and reduced MRP2 and EGFR expression ($p < 0.05$) in a dose-dependent manner. The similar results were obtained in mice in vivo experiment, except the reduced expression of GST-π. It is suggested that OCT could inhibit ovarian cancer proliferation and promote apoptosis, via the cell surface SSTR2, and reverse cisplatin resistance through inhibition of MRP2, EGFR, and even GST-π expressions.

Key words: Octreotide; Somatostatin; Epithelial ovarian cancer; Resistance reversal.

Introduction

Ovarian cancer is the most common cause of cancer death from gynecologic tumors in the world. Unfortunately, most cases are diagnosed in an advanced stage. Standard treatment involves aggressive debulking surgery followed by chemotherapy [1]. Platinum-based chemotherapy enhances the overall response rate, clinical remission rate, and median survival rate of ovarian cancer patients. However, it is also an obstacle to clinical treatment for primary and/or acquired multi-drug resistant (MDR) of tumor cells [2]. Therefore, there is a need to develop alternative new types of cytotoxic and non-cytotoxic drugs that can reverse chemotherapy resistance and enhance sensitivity to platinum-based chemotherapy drugs.

In a variety of non-cytotoxic agents, the somatostatin analogs (SSTA) have attached more attention in oncology community. It has been reported the somatostatin receptor (SSTR) is expressed in ovarian cancer cells [3,4], suggesting that SSTA could be involved in ovarian cancer. Recent studies have shown that SST and SSTA can enhance chemotherapeutic drug sensitivity in a variety of resistant tumor cells [5]. However, it is still unclear what the function of SST and SSTA are in the enhancement of cisplatin sensitivity to resistant ovarian cancer cells.

This study focuses on the effect of octreotide, one kind of octapeptide SSTA, *in vivo* and in nude mice *in vitro*, towards the cisplatin-resistant ovarian cancer cell SKOV3/DDP growth control and resistance reversal. The results provide a new understanding for the clinical treatment of ovarian cancer and drug resistance reversal.

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Materials and Methods

In vitro experiments

The effects of cisplatin, octreotide (OCT) and their combination on SKOV3/DDP cells proliferation

SKOV3/DDP cells (1×10^4, purchased from Chinese Academy of Medical Sciences Cell Bank) in the logarithmic phase were seeded in 96-well culture plates and cultured at 37°C under a 5% CO_2 atmosphere for 24 hours. After the cells were adhered to the wall, the cells were incubated in a 200 l media with OCT (0, 1.25, 2.5, 5, 10, 20 μg/ml), cisplatin (0,1, 2, 4, 8 μg/ml) or a combination of both OCT and cisplatin at each concentration listed previously. The blank control group was made with an equal volume of culture media without the drugs. Each group contained three parallel wells and the experiment was repeated three times for each group. For the OCT group, cells were cultured for predetermined times (24, 48, 72, 96 hours). Then, the cells were treated with 20 μl of the 3-(4,5-dimethylthiazol-2-yl)-2-5-diphenyl tetrazolium bromide (MTT) reagent (500 μg/ml) for 4 h, and lysed in 150 μl of dimethyl-sulfoxide (DMSO) solvent reagent for 10 min. Absorbance (A) was measured on an enzyme-linked immunosorbent assay plate reader. The inhibition rate was calculated using the following formula: cell proliferation inhibition rate = (average of value A from the control group - the average of value A from experimental group) / (average of value A from the control group - average of value A from blank controller) × 100%. All the experiments were repeated in triplicate and more than three wells were used for each treatment. According to the results of the octreotide, the same methods described above were used to detect the values of A for cisplatin and the two-drug combination groups. The time-concentration curve was made using the average value of three tests. The drug concentration of 50% inhibition rate (IC50) was calculated using the weighted linear regression method with Excel software. An IC50 curve of the effects of OCT on cisplatin was created.

The functions of cisplatin, OCT, and their combination in SKOV3/DDP cell apoptosis

According to the MTT results, the experiments were divided into four groups, including control, cisplatin (2.0 μg/ml), OCT (10.0 μg/ml), and two-drug combinations. Following the treatment for 36 h, the apoptosis test was done according to the Annexin V-FITC/PI staining kit's instructions, and the results were read using flow cytometry.

In nude mice in vivo *experiments:*

SKOV3/DDP nude model preparation and group

Forty female BALB/c-nu/nu nude mice, six to eight weeks of age, weighing 18-22 g, specific-pathogen-free (SPF) breeding conditions, were purchased from the Experimental Animal Center of Chinese Academy of Sciences. Human ovarian cancer cells SKOV3/DDP in the logarithmic phase were made of the density of 5×10^7/ml of single cell suspension to 5×10^6 cells/only (0.1 ml/only) inoculated into all nude mice subcutaneously near the right armpit, the daily observation of tumor growth and mice eating activity. Fifteen days after inoculation of tumor cells, the mice were randomly divided into four groups of 10 each: 1) octreotide group treated with octreotide 100 ug/kg, sc, qd, continuous four weeks, 2) cisplatin group processed with cisplatin of 4mg/kg, ip, qw, continuous four weeks, 3) combination group handled with the same dose of octreotide and cisplatin above at the same time, continuous four weeks, 4) control group dealt with the saline of 50 ml/kg, sc, qd, continuous four weeks. Twenty-four hours after the last treatment mice were sacrificed.

Evaluations of xenograft tumor status

Fifteen days after inoculation of tumor cells, the xenograft tumors were all at the similar volume in each group, of about 2 mm or so, which suggested the tumor formation of the same initial tumor volume, tumor growth, and good uniformity. After treatment of drugs in mice, the authors used a caliper to measure the size of tumor (a) as short-track and (b) long-track according to Steel formula tumor volume: $V = ab^2/2$ (cm^3). Meanwhile, after the xenograft tumors, removing blood, fat, and other non-tumor components, was stripped out from the killed mice, the tumor weight (gram) was measured and then the inhibitory rate of drug treatment was calculated. Inhibition rate = (V-V control group experimental group) / V control group × 100%.

Tumor cells extraction and preparation

Five of above fresh xenograft tumors in every group were randomly selected as part of polymerase chain reaction (PCR) experiments. The tumor was cut into small pieces, weighing about 80-100 mg, placed in liquid nitrogen, ground into powder, and every 100 mg of each tissue was added 1 ml of trizol reagent and homogenized with a homogenizer until it is particle-free homogenate and transparent. The cell lysates were transferred to a centrifuge tube at room temperature for 5 min, making the complete separation of nucleic acid protein complex. After centrifugation of 12,000 rpm at 4°C for 10 min, the supernatant was carefully draw into new tube.

Evaluation of SSTR2, epidermal growth factor receptor (EGFR), MDR1, and MRP2 mRNA expression in SKOV3/DDP cells in vitro and in vivo *in nude mice*

Total RNA was extracted from cells according to instructions of the RNeasy Mini Kit (Kaiji Company, KGA1203). The extracted RNA was dissolved in diethylpyrocarbonate (DEPC)-treated water. The absorption value at 260 nm and 280 nm were detected using a UV spectrophotometer. The RNA concentration was calculated using the following formula: RNA concentration = OD_{260} × dilution fold × 0.04 μg/ul. The quality was considered good when the OD260/280 value was in the range of 1.8 to 2.1. The OD260/280 value was also investigated using ultraviolet spectroscopy. A 2 μl sample of cDNA was added to the reaction mixture, and the cDNA was synthesized according to instructions of the RT-PCR Kit (Kaiji company, KGA1303). Primers were designed and synthesized by the Kaiji Company, showed as follows: SSTR2 (96bp) 5' TCAACCAACACCTCAAACCAGAC 3'/5' CCCAATGATGCAGACCACAAAAT 3', MDR1 (90bp) 5' TTGACAGCTACAGCACGGAAGG 3'/5' GTCGGGTGGGATAGTTGAATAC 3', EGFR (149bp) 5' TAACGGAATAGGTATTGGTGAAT 3'/5' GAGGAGGAGTATGTGTGAAGGAG 3', MRP2 (156bp) 5' CCATCATCCATAGCTTCATTCC 3'/5' GTGCGTTTCAAACTTGCTCACT 3', GST-π(128bp) 5' GATGCGTTCCCCCTGCTCTC 3'/5' CCCAACCCTCACTGTTTCCC 3', β-actin (136bp) 5' GCAGAAGGAGATCACTGCCCT 3'/5' GCTGATCCACATCTGCTGGAA 3'. Real-time PCR was performed in a Light Cycler (Roche Applied Science) with the following conditions: denaturation at 95ºC for 5 min with the addition of 15 seconds at 94ºC, 30 seconds annealing at 60ºC. Comparing the threshold method and the mathematical method, the amount of target gene = $2^{-\Delta\Delta ct}$. Ct is the number of cycles of fluorescence required for it to reach the threshold, $\Delta\Delta Ct = (Ct_{\text{objective gene}}\text{-}Ct_{\text{reference gene}})_{\text{experimental group}} - (Ct_{\text{objective gene}}\text{-}Ct_{\text{reference gene}})_{\text{control group}}$. Using this method, the authors could directly quantify the target gene relative to the reference gene (-actin) and compare the common logarithm of the relative value of the target gene and control gene.

Statistical analysis

Statistical analysis was performed using SPSS 16.0 software. The data was expressed as mean ± SD and was compared using Student's t-test and ANOVA. A p value of < 0.05 was considered significant.

Results

In vitro experiments

The effect of OCT on SKOV3/DDP cells proliferation

As the treatment time progressed, cell growth became slow, and there were more falling floating cells, necrotic and debris cells. Figure 1 shows the cell morphology following 48 h of treatment. OCT showed an inhibition effect on SKOV3/DDP cell proliferation beginning at the concentration of 1.25 μg/ml. An increase in the treatment concentration enhanced the inhibition effect. When the concentration of OCT was higher than 5 μg/ml, the inhibition rate on SKOV3/DDP cell proliferation sharply increased. There were significant differences between the control group and each of the OCT treatment groups. There were also significant differences among the different concentrations of OCT treatment groups ($p < 0.05$). Importantly, OCT (5, 10, 20 μg/ml) also inhibited proliferation in a time-dependent manner ($p < 0.05$, Table 1, Figure 2).

The functions of OCT and cisplatin combination on SKOV3/DDP growth inhibition rate and cisplatin IC50 value

Following treatment at the indicated concentrations of OCT and cisplatin, optical density (OD) was measured to calculate the cell growth inhibition rate. Compared to the control group, OCT decreased the IC50 value of cisplatin in a dose-dependent manner. A low dose OCT (1.25 μg/ml) had no significant effect on the IC50 of cisplatin ($p > 0.05$). However, a higher dose of OCT (2.5-20 μg/ml) significantly inhibited IC50 ($p < 0.05$, Figure 3). The combination drag index (CDI) was calculated and is shown in Table 2. The CDI is equal to the survival rate (A drug+B drug)/(survival rate(A drug)×survival rate(B drug)). A CDI < 1 indicates synergistic effects of cellular toxicity of A and B drugs in the combined treatment. It was shown that the CDI < 1, when the concentration of OCT and cisplatin were higher than 5 μg/ml and 2 μg/ml, respectively, suggesting that there was a synergistic effect with OCT and cisplatin.

The effect of cisplatin, OCT and their combination on SKOV3/DDP cell apoptosis

In contrast to the control group, apoptosis was induced in both the OCT (10 μg/ml) and cisplatin (2 μg/ml) groups ($p < 0.05$). This effect was much more powerful in the combination treatment group than in the individual treatment groups ($p < 0.05$). However, there was no significant difference between the OCT and cisplatin treatment groups ($p > 0.05$, Figure 4).

SSTR2, EGFR, MDR1, MRP2, GST-π mRNA expression in SKOV3/DDP cells

Figure 5 shows that, compared with the control group, OCT at different concentrations (2.5, 5, and 10 μg/ml) decreased EGFR, MRP2 mRNA expression in a dose-dependent manner following 48 h of treatment ($p < 0.05$). The basal mRNA level of MDR1 was low, but both the GST-π and SSTR2 mRNA expression were detectable. OCT significantly increased the GST-π mRNA expression ($p < 0.05$) but not MDR1 and SSTR2 ($p > 0.05$).

In vivo in nude mice experiments

Observation of tumor tissue samples in each group

Seven days after inoculation, indurations about the size of 1 mm can be promoted under the right armpit of nude mice. Also, two weeks after inoculation, visible pieces 1-2 mm of tumor growth can be seen, located subcutaneously near the right armpit and uplifted the skin surface with the characteristics of hard, good activity, skin color red. Luckily all mice were inoculated with tumor formation. Four weeks after, 40 mice were sacrificed for further observation and preparation. All the mice presented good growth of multi-section phyllodes solid tumor, coated with a layer of thin membrane, without local ulceration and other skin damage. It was clearly observed that the tumor surface had the clear boundary with the surrounding tissue, tumor gross specimen was pale yellow or white, and the cut surface was multiple cysts with thin yellow fluid and not smooth wall, shown in Figure 6. The tumor presented poorly-differentiated cancer, using hematoxylin and eosin (H&E) staining method, with the typical characteristics of density and flaky distribution cells, large stained and more mitotic nuclear, rare interstitial and large areas of necrosis, shown in Figure 7.

Table 1. — *Inhibition rate of OCT on SKOV3/DDP proliferation at the indicated concentration and time.*

Groups	Concentration	I (24 hours)	II (48 hours)	III (72 hours)	IV (96 hours)
1	0 g/ml	0	0	0	0
2	1.25 g/ml[1)]	5.35 ± 0.33	5.94 ± 0.20	7.02 ± 0.72[6)7)]	12.95 ± 0.23[6)7)8)]
3	2.5 g/ml[1)2)]	10.15 ± 0.40	12.23 ± 0.93[6)]	13.58 ± 1.21[6)]	16.91 ± 0.86[6)7)8)]
4	5.0 g/ml[1)2)3)]	13.42 ± 0.28	14.91 ± 0.15[6)]	17.18 ± 1.00[6)7)]	23.08 ± 0.67[6)7)8)]
5	10.0 g/ml[1)2)3)4)]	20.89 ± 0.88	26.23 ± 1.49[6)]	34.48 ± 1.99[6)7)]	47.73 ± 1.09[6)7)8)]
6	20.0 g/ml[1)2)3)4)5)]	32.94 ± 0.64	44.36 ± 2.08[6)]	48.20 ± 2.17[6)7)]	53.22 ± 1.31[6)7)8)]

1) $p < 0.05$ vs 1 2) $p < 0.05$ vs 2 3) $p < 0.05$ vs 3
4) $p < 0.05$ vs 4 5) $p < 0.05$ vs 5 6) $p < 0.05$ vs I
7) $p < 0.05$ vs II 8) $p < 0.05$ vs III

Table 2. — *Combination drag index of OCT and cisplatin.*

Cisplatin (g/ml)	OCT (μg/ml)			
	2.5	5	10	20
1	1.02	1.01	1.01	0.97
2	1.01	0.95	0.92	0.83
4	1.06	0.91	0.88	0.84
8	0.92	0.80	0.79	0.90

Table 3. — *Weight, size, and inhibition rates of xenograft tumor in all groups ($\bar{x} \pm s$, $n = 5$).*

Group	Weight (g)	Size (cm^3)	Inhibition rate (%)
Control	0.55 ± 0.02	0.51 ± 0.03	--
Cisplatin	0.37 ± 0.01[1)]	0.39 ± 0.02[1)]	24.62[1)]
Octreotide	0.22 ± 0.04[1)2)]	0.25 ± 0.03[1)2)]	52.25[1)2)]
Combination	0.07 ± 0.01[1)2)3)]	0.09 ± 0.01[1)2)3)]	82.24[1)2)3)]

1) $p < 0.05$ vs control group; 2) $p < 0.05$ vs cisplatin group; 3) $p < 0.05$ vs ctreotide group

Table 4. — *The expression of SSTR2, MDR1, MRP2, EGFR, and GST-π mRNA in xenograft tumor in all groups (n = 5).*

Group	SSTR2	MDR1	MRP2	EGFR	GST-π
Control	1	1	1	1	1
Cisplatin	0.982 ± 0.0137	0.965 ± 0.022	0.879 ± 0.035[1)]	0.944 ± 0.075	1.085 ± 0.041
Octreotide	7.513 ± 0.921[1)]	0.903 ± 0.214	0.364 ± 0.008[1)2)]	0.073 ± 0.011[1)2)]	0.715 ± 0.057[1)2)]
Combination	8.119 ± 1.261[1)]	0.931 ± 0.226	0.317 ± 0.022[1)2)]	0.014 ± 0.004[1)2)3)]	0.665 ± 0.109[1)2)]

1) $p < 0.05$ vs control group; 2) $p < 0.05$ vs cisplatin group; 3) $p < 0.05$ vs octreotide group.

Comparison of tumor weight and tumor volume between each group

Table 3 shows that, compared with control group, treatment groups, the average tumor volume were significantly reduced ($p < 0.05$), respectively, the average size from biggest to smallest: control group (0.51 ± 0.03 cm^3, Figure 6A) > cisplatin group (0.39 ± 0.02 cm^3, Figure 6B) > octreotide group (0.25 ± 0.03 cm^3, Figure 6C) > combination group (0.09 ± 0.01 cm^3, Figure 6D). Among them, the combination therapy group is smaller compare to the octreotide and cisplatin groups ($p < 0.05$), while the octreotide group is smaller than cisplatin ($p < 0.05$).

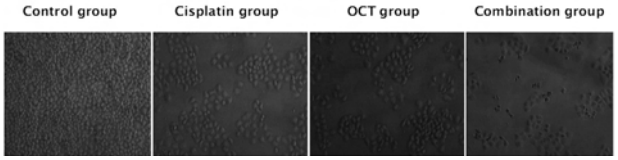

Figure 1. — The function of cisplatin, OCT, and the two-drug combination on SKOV3/DDP morphology.

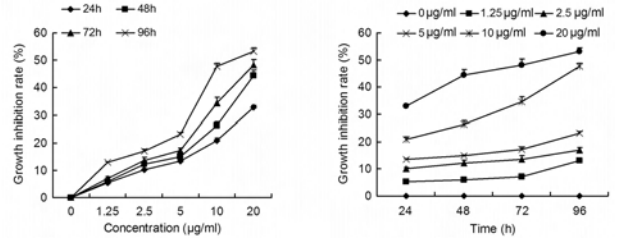

Figure 2. — The effect of OCT on SKOV3/DDP cell growth inhibition rate at the indicated concentration and time.

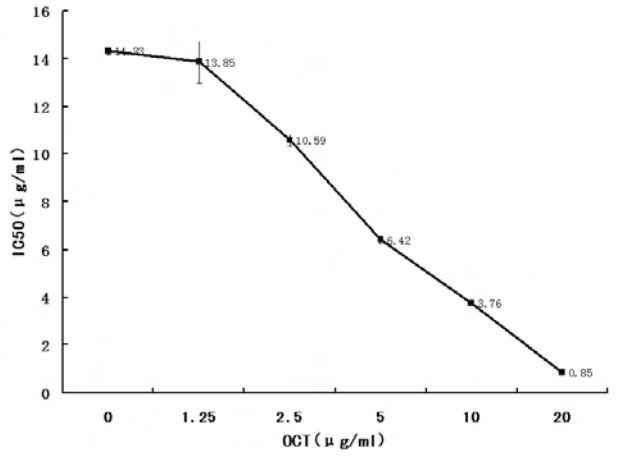

Figure 3. — Effect curve of different concentrations of OCT on cisplatin IC50.

Table 3 also indicates that, compared with the control group, tumor weight of each treatment group was significantly decreased ($p < 0.05$), the average tumor weight from heavier to lighter are: the control group (0.55 ± 0.02g, Figure 6A) > cisplatin group (0.37 ± 0.01g, Figure 6B) > octreotide group (0.22 ± 0.004 g, Figure 6C) > combination group (0.07 ± 0.01 g, Figure 6D). Among them, the combination therapy group the tumor weight was significantly reduced more than the octreotide group, cisplatin ($p < 0.05$), while the octreotide group tumor weight was smaller than the cisplatin group ($p < 0.05$).

Tumor inhibition rate in different group sorted in descending order in descending was as follows: combination group (82.24%) > octreotide group (52.25%) > cisplatin group (24.62%), group differences were statistically significant ($p < 0.05$) (Table 3).

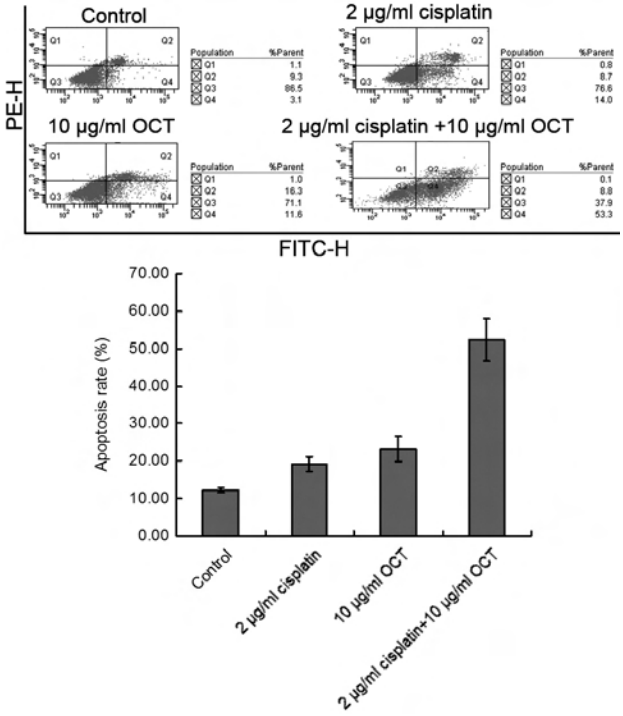

Figure 4. — Effects of cisplatin, OCT and the drug combination on SKOV3/DDP cell apoptosis.

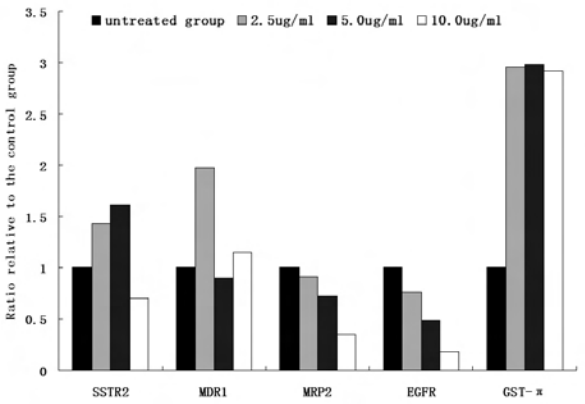

Figure 5. — Effect of OCT at different concentration on SSTR2, EGFR, MDR1, MRP2, GST-π mRNA expression in SKOV3/DDP cells.

The expression of SSTR2, MDR1, MRP2, EGFR, GST-π mRNA on xenograft tumors

According to the comparison threshold method, the authors calculated SSTR2, MDR1, MRP2, EGFR, GST-π mRNA expressions on tumor cells in every other intervention group compared with the control group, shown in Table 4, Figure 8.

After drug intervention, the SSTR2 mRNA expression in the octreotide group and the combination group was significantly higher, compared with the control group, the differences were statistically significant ($p < 0.05$), but

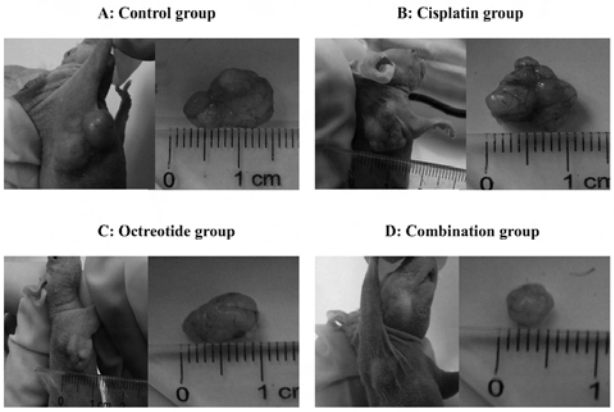

Figure 6. — The growth status of xenograft tumor in nude mice model.

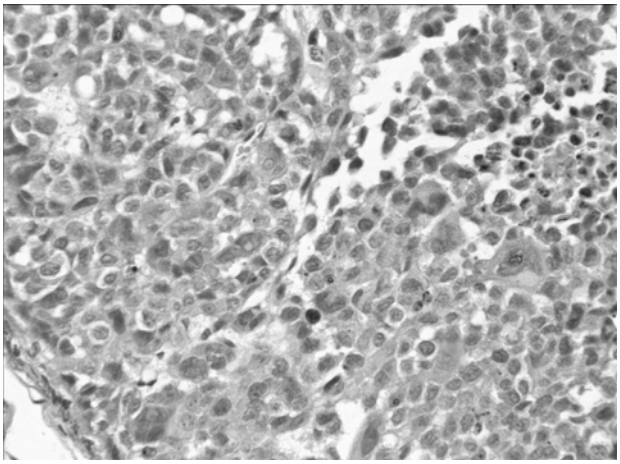

Figure 7. — HE staining of xenograft tumor specimen sections (×40).

there were no significant differences between the cisplatin and the control group ($p > 0.05$), even the octreotide group and the combination group ($p > 0.05$).

After drug intervention, there was no significant difference of MDR1 mRNA expression between each other group ($p > 0.05$). Compared with control group, the MRP2 mRNA expression was significantly reduced in every treated group ($p < 0.05$), and even decreased more significantly in the octreotide and combination groups than the cisplatin group ($p < 0.05$). Compared with the control or the cisplatin groups, the EGFR mRNA expression in the octreotide and the combination groups was significantly lower ($p < 0.05$), but the latter dropped more ($p > 0.05$). Compared with the control group, the GST-π mRNA expression in each group was all significantly reduced ($p < 0.05$), but the octreotide and the combination groups significantly lower the cisplatin group ($p < 0.05$).

Discussion

Platinum-based combination chemotherapy is the most widely used method in the treatment of ovarian cancer. However, due to resistance, it often fails to cure patients

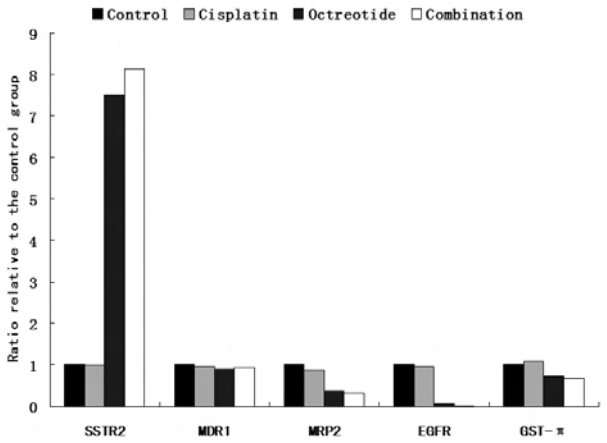

Figure 8. — The expression of SSTR2, EGFR, MDR1, MRP2, GST-π mRNA in xenograft tumor of all groups.

[2]. Therefore, how to reverse platinum resistance for ovarian cancer and to increased sensitivity to platinum-based chemotherapy is the main focus for cancer researchers and clinicians.

SST is a cyclic polypeptide hormone, which is located in most human organs and tissues. It performs the functions of inhibition of hormone secretion, regulation of neural transmission, and cell proliferation [6, 7]. Natural SST is limited in clinical applications because of its low selectivity, short half-life, and re-increases in hormone levels after drug treatment termination. However, SSTA is clinically widely used and has shown to have much more powerful effects and a longer half-life. OCT is the most widely used somatostatin analogue in clinical applications. Recently, the inhibition effects of SST and SSTA on cancer cells caught the attention of more people [8]. A body of studies reported that SST and SSTA can inhibit the growth of several non-neuroendocrine tumors [6, 9-10]. It is accepted that the functions of SST and SSTA are mediated by SSTR [11]. SSTR2, followed by SSTR1, 3 and 4, are widely-expressed in tumor tissues. The expression and functions of SSTR in ovarian cancer are still not very clear.

Previous studies showed that SSTR is expressed in ovarian cancer [3, 12]. Halmos *et al*. used RT-PCR to investigate all the subtypes SSTR mRNA expression in 17 cases of primary ovarian tumor tissue and found that 76% of the cases had advanced primary malignant ovarian tumors with a high SSTR expression. Of the cases found to have malignant ovarian tumors with high expression of SSTR, 65% of the patients highly-expressed SSTR1 and SSTR2A, followed by the SSTR3 and then SSTR5. These data suggest that SST and SSTA could be potential targets for ovarian cancer therapy. In this study, the authors found that SKOV3/DDP cells expressed SSTR2 and that OCT effectively inhibited the cisplatin-resistant ovarian cancer cell SKOV3/DDP proliferation in a dose-dependent manner and suppressed apoptosis. The present data confirmed that SST and SSTA, as the

endogenous hormone, can regulate ovarian cancer proliferation. One or several SSTRs, especially SSTR2, were expressed on the surface of most of the tumor cells, which could be inhibited by SST and SSTA [13-14]. OCT presented the highest binding affinity to SSTR2 and subsequently inhibited the activity of tyrosine phosphatase and the proliferation of SSTR2 expressed cells [15]. However, OCT did not change the SSTR2 expression level in vitro and in vivo, even unexpectedly up-regulated in vivo, which is not consistent with a previous study done by Hua *et al*. [16]. Their study showed that the short-term application of OCT induced SSTR2 desensitization and internalization, which partially inhibited the effect of OCT on liver cancer cells [17]. These contrary results may be due to different cell characteristics, expression of the receptor and their subtypes, and high OCT treatment concentration. Furthermore, mRNA could not reliably be used as the index to reflect the status of receptor, and the authors should therefore focus on the cell membrane localized receptor protein [16]. Also, due to this experimental data, it is considered that the long-term chronic stimulation of octreotide might induce upregulation of SSTR2, and the change could further strengthen the anti-tumor effect of octreotide, which suggest the long-term use of octreotide may not increase its resistance.

It has been shown that the synergistic effects of SSTA with chemotherapy drugs increased their clinical efficacy. These effects could enhance the sensitivity of several gastrointestinal cancers to chemotherapeutic drugs [18]. Cisplatin plays a pivotal role in the treatment of ovarian cancer. It has rarely been reported that OCT increases its clinical efficacy when SSTR expressed ovarian cancer patients undergo chemotherapy. In the current study, the authors found that OCT suppressed the inhibition effects of cisplatin on SKOV3/DDP cell IC50 in a dose-dependent manner, inhibited SKOV3/DDP cell proliferation, increased chemotherapeutic agents' sensitivity, and reversed chemotherapy resistance. In vivo results of the study also found the better inhibition effect of octreotide on the growth of SKOV3/DDP xenograft tumor in nude mice than cisplatin, and the combination of the two drugs enhanced anti-tumor effects, indicating that octreotide can inhibit the in vivo resistant ovarian cancer proliferation, and can play better than the inhibitory effect of cisplatin and a synergistic effect with cisplatin. These data suggest that OCT could reverse SKOV3/DDP cell resistance and increase the efficacy of chemotherapy of ovarian caner, but the detailed mechanism is still not clear. The authors investigated the expression of resistance-related genes MDR1, MRP2, GST-π, and EGFR using real-time PCR assays and compared the parameters before and after OCT treatment.

In this study, it was found that MRP2 and EGFR are expressed on the SKOV3/DDP cell surface. OCT treatment increased the cisplatin sensitivity, induced the synergistic cellular cytotoxic effects with cisplatin, and decreased MRP2 and EGFR expression in vitro and in vivo. These data demonstrate that OCT reverses ovarian cancer resistance and could be related to the down-regulated MRP2 and EGFR expression. MRP is an ATP-dependent membrane transport protein, with which MRP2 participates in cisplatin transport in combination with glutathione. Both animal experiments and clinical studies have shown that MRP2 could be associated with cisplatin resistance in ovarian cancer [19-20]. Based on these observations, the authors hypothesized that MRP2 down-regulation could increase intracellular cisplatin concentration to efficiently reverse drug resistance. The mechanism of OCT-reducing EGFR expression in ovarian cancer is not clear. However, studies have shown that EGFR over-expression in ovarian cancer cells indicates an increase in drug resistance. Suppressing EGFR expression in ovarian cancer cells increases cisplatin sensitivity [21-22]. Following OCT binding with SSTR, the activation of the tyrosine phosphatase and reversal of EGF-induced EGFR tyrosine kinase phosphorylation results in the reduction of EGFR, the termination of EGF signal transduction at the cell membrane and, eventually, the inhibition of cell proliferation [23]. EGFR is the producer of the oncogene ErbB1. It has the ability to connect the G-protein, cytokine receptors, integrins and other signals, and can affect many related gene-expressions [24]. Therefore, OCT may also, indirectly through EGFR, regulate the reversal of cisplatin resistance. These detailed mechanisms merit further investigations.

GST-π expression in ovarian cancer is not only related to primary cancer but also to acquired drug resistance [25]. In the current study, GST-π gene expression was increased but not decreased after treatment in vivo. Vanhoefer *et al*. reported that the drug has an outward flow caused by the non-specific binding of GST-π to the p-gp-induced drug pump at the early stage of resistance. Both GST-π and p-gp are involved in the modulation of the early-stage drug resistance development [26]. The synergistic effects of GST-π and p-gp may explain the phenomenon that GST-π and p-gp are not down-regulated in the OCT-induced reversal of cisplatin resistance. The resistance may be aggravated during the process of OCT-induced reversal of resistance for the increase of GST-π and p-gp. What is the meaning of this kind of change? Is it an accidental phenomenon or a necessity? Moreover, after octreotide intervention, in vivo, there has indeed been a reduction in GST-π. The result may be due to increased drug accumulation in the cells, and then depleted and lower-expressed GST-π after the long-term chronic effects of octreotide.

MDR1 can decrease the intracellular drug concentration by encoding cell surface transporter proteins p-gp, resulting in reduced or lost drug function, and induced resistance. It was shown that p-gp is expressed when ovarian cancer cells have high cisplatin resistance [27-28]. This study demonstrated that there is no MDR1 expression change in SKOV3/DDP cells following the combination treatment of OCT and cisplatin both in vitro and in vivo, suggesting that MDR1 is not involved in OCT inhibition of cell proliferation and that it reverses resistance.

However, the in vitro and in vivo results confirmed the

sensitization effect of OCT in ovarian cancer cisplatin resistance. This confirmation provided the new target for ovarian cancer therapy. Large-scale, randomized, double-blind, and controlled studies still need to be completed to confirm the OCT dose and timing issues of clinical treatment. In addition, it is suggested that the enhanced sensitivity to cisplatin maybe the mechanism through the changes in resistance gene expression following by action of the octreotide. However, the subsequent trial to explore the molecular mechanism of SSTR2 downstream signals transduction pathway in cisplatin resistance of ovarian cancer has been on-going. It will allow to acquire the theoretical basis for further studies.

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