RESEARCH ARTICLE

Intervention Effects of Nedaplatin and Cisplatin on Proliferation and Apoptosis of Human Tumour Cells in Vitro

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Abstract

Objective: To study synergistic effects of nedaplatin and cisplatin on three human carcinoma cell lines (esophageal carcinoma cell line Eca-109, ovarian carcinoma Skov-3 and cervical carcinoma Hela). Methods: Inhibition effects were evaluated by MTT assay and cell apoptosis was detected by flow cytometry. In addition, changes of Ki-67, Bax and Bcl-2 at mRNA and protein levels were quantified by RT-PCR and Western blotting. Results: Growth inhibition in each cell lines was dose-dependent after exposure to nedaplatin or cisplatin alone. The interaction of the two drugs was synergistic at higher concentrations according to the median-effect principle. The inhibition rates with nedaplatin, cisplatin and combined treatment were 41.9±4.1%, 47.4±2.9%, 52.5±0.9% (Eca-109), 39.0±1.26%, 45.0±1.45% , 56.2±1.44% (Skov-3) and 44.8±2.11%, 46.9±0.99%, 56.6±1.83% (Hela) respectively, with increase in apoptosis. Compared with the nedaplatin or cisplatin alone treatment group, the combinative treatment group's Ki-67 and bcl-2 mRNA (protein) expression was decreased while that of Bax mRNA (protein) was increased. Conclusion: Compared to the effects of nedaplatin or cisplatin alone at high concentrations, combination of nedaplatin and cisplatin at low concentrations proved to be much more effective for inhibition of proliferation and the induction of apoptosis in the Eca-109, Skov-3 and Hela cell lines.

Keywords: Nedaplatin/cispaltin combined treatment - carcinoma - esophagus - ovaries - cervix - median-effect principle

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Introduction

Cisplatin, a first-generation platinum complex, has been successfully used for treatment of several types of cancer, including ovarian, cervical and esophagea carcinoma (Nial et al., 2010). Cisplatin-based combinations were well reported to show high response rates, varying from 15 to 76%. Unfortunately, most patients who were treated with cisplatin developped dose-limiting neurotoxicity, hearing loss, and nephrotoxicity. Because the toxicities reported with cisplatin-based regimens are substantial, there has been a keen interest in newer platinum analogues.

Nedaplatin is a cytotoxic drug with the same mechanism but different spectrum of toxic effects as cisplatin (Takeki et al., 2011). It has definite antitumour effect on varied tumours no matter single or combined with other antitumour drugs. The main dose-limiting toxicity is thrombocytopenia caused by marrow suppression. The recommended dose of nedaplatin is (80~100) mg/m^2, however chemotherapy delay or even discontinuance always happen because of drug toxicity in this dosage (Taguchi et al., 1992). The only way to solve this problem is to decrease the dosage of nedaplatin within the chemotherapy cycles. This will certainly affect the therapeutic effect because maintaining certain concentration in blood is the assurance of effect of platinum drugs in which there is an obvious dose-effect relationship.

Cisplatin is the parent compound of platinum drugs with the same mechanism as nedaplatin. The spectrum of antitumour effects of both drugs is also similar, but the spectrum of toxity effects is not completely overlapped (Koshiyama et al., 2005; Uehara et al., 2005). Combination of the two drugs ensure sufficient concentration of platinum in tumour tissue, so as to achieve the best effect but without an increased toxicity. Preliminary clinical investigations of the nedaplatin/cisplatin combination have been initiated. Although there may be some rationale for the administration of platinum analogues in combination, in vitro interactions between these two drugs have not previously been studied (Nishida et al., 1999; Adachi et al., 2001; Li et al., 2008). The present study investigated the intervention effects of nedaplatin and cisplatin on proliferation and apoptosis of human carcinoma cell lines (human esophageal carcinoma cell line Eca-109, human ovarian carcinoma cell line Skov-3 and human cervical carcinoma cell line Hela) and its mechanism in vitro.

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Materials and Methods

Cell lines and culture

The human esophageal carcinoma cell line Eca-109 (squamous cell) , human ovarian carcinoma cell line Skov-3 and cervical carcinoma cell line Hela were obtained from Shanghai Institute of Cell Biology (Shanghai, China). The cell lines maintained in RPMI1640 (GIBCO, Grand Ireland, NY, USA) , and supplemented with 10% heat-inactivated fetal calf serum (GIBCO, Grand Ireland, NY, USA) in an incubator at 37 °C and 100% humidity with 5% CO_2 and air. No antibiotics were added to the medium. The cells were trypsinized and passed twice a week. Nedaplatin and cisplatin were prepared extemporaneously in complete culture medium immediately prior to use in vitro.

Cytotoxicity assay

Eca-109, Skov-3 and Hela cells were seeded onto 96-well microplate at a density of 5×10^4 cells per well and incubated overnight in 10% FCS medium respectively. The cells were then treated with different concentrations of nedaplatin or cisplatin in serum-free conditions. Cells incubated in serum-free medium were as control. After incubation for 48 h at 37 °C, 20μl of MTT (Sigma, St. Louis, MO, USA) solution (5 mg/ml in phosphate buffered saline [PBS]) was added to each well and continued to incubate for another 4 h at 37 °C. Then, 100μl of dimethylsulfoxide (DMSO) was added into each well at 37 °C for 2 h. The optical density (OD) was determined by spectrophotometer (Bio-Rad, Hercules, CA, USA) at 570 nm. Growth inhibition was calculated as a percentage of the untreated controls, which were not exposed to drugs. Each assay was performed triplicate.

Analysis of combination effects

On the basis of growth inhibition curve for each single drug, we analyzed the effects of the drug combinations using the method described by Chou and Talalay (Median-effect principle) and the Calcusyn software program for automated analysis (Biosoft, Cambridge, United Kingdom). The effect of combining the two drugs was evaluated by comparing the results of the sequential assays with those of the assays involving nedaplatin or cisplatin exposure alone. The combination effect was evaluated under median-effect principle, calculated as follows: CI=Cnedaplatin/Cxnedaplatin+Ccisplatin/Cxcisplatin+(Dnedaplatin*Dcisplatin)/(Dxnedaplatin*Dxcisplatin). Cxnedaplatin and Cxcisplatin are the concentrations of nedaplatin and cisplatin alone needed to achieve a given effect (Fractional cell growth inhibition, fa), respectively. Cnedaplatin and Ccisplatin are the concentrations of nedaplatin and cisplatin needed for the fa when the drugs are combined. These concentrations were calculated for each experiment and for each combination experiment at a fixed concentration ratio. The combination was considered as positive (synergistic) when the combination index was <1 and negative (antagonistic) when it was >1, and values of 1 were considered to indicate additivity(Chou, 2007). Apoptosis Assays. IC_{50} (50% concentration of inhibition) of nedaplatin and cisplatin were determined by median-effect principle generated from each experiment. Cells were plated at 1×10^6 in 6-well dishes (Grier) and were allowed to attach for 24 h. After treated with nedaplatin (24 h), cisplatin (24 h) along at their IC_{50} levels and their combinations at their half of IC_{50} levels followed by a 24 h washout, cells were harvested with trypsin and washed twice with phosphate-buffered saline (PBS). Follow to the manufacturer's instruction, cells were stained with Annexin V-FITC and PI. Samples were then processed by flow cytometry. Untreated cells Eca-109 were used as a control. Data acquisition and analysis were done on a BD (Becton Dickinson) FACSCaliber using CellQuest software (BD Biosciences).

RT-PCR analysis

The expressions of Bcl-2, Bax and Ki-67 mRNAs were assessed by RT-PCR analysis. Total RNA was extracted from cells treated as described above in "Apoptosis Assays" using the Trizol (Invitrogen Co., Carlsbad, CA, USA) according to the manufacturer's recommendations. First-strand cDNA synthesis was performed on 1μg of total RNA. PCR was performed using 2μl cDNA as template, 10 pM of each primer, 10 mM deoxynucleoside triphosphates (dNTPs), 1.25U TaqDNA polymerase (Tiangen Biotech), 1× reaction buffer containing 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM MgCl2 and 0.01% gelatin in 25μl reaction volume. The primer sequences and PCR product sizes were as follows: (a) Bcl-2 sense (5'- CTTCGCCGAGATGTCCAGC-3') and Bcl-2 antisense (5'- CCAGGAGAAATCAAACAGAGGC-3'), 310 bp; (b) Bax sense (5'-TTTGCTTCAGGGTTTCATCC-3') and Bax antisense (5'- CAGTTGAAGTTGCCGTCAGA-3'), 246 bp; (c) Ki-67sense (5'- GTGCTCTGGGTTACCTGGTC-3') and Ki-67 antisense (5'- CAGGTGGAGTGTGCATTACC-3'), 215 bp; and (d) β-actin sense (5'-ACACTGTGCCCATCTACGAGG-3') and Ki-67 antisense (5'- AGGGGCCGGACTCGTCATACT-3'), 661 bp. The PCR products were separated by 1.5% agarose gel electrophoresis and stained with ethidium bromide (EB). Negative controls without DNA template were included for all PCR reaction mixtures. The mRNA bands were then visualized by UV light and quantified by BIO-RAD Image computer program. The relative expression of Bcl-2, Bax and Ki-67 mRNAs was normalized to that of β-actin.

Western blot analysis

Treated Eca-109, Skov-3 and Hela cells were rinsed twice with icecold PBS, then were extracted and the protein concentration was determined by Lowry method. Forty micrograms of protein lysates from each sample were subjected to SDS-PAGE on 10% acrylamide gel and the separated proteins transferred to a PVDF membrane. Blots were incubated for 1 hour with 5% nonfat dry milk to block nonspecific binding sites and then incubated with rabbit polyclonal antibody against Bax, Bcl-2, Ki-67 (Santa Cruz Biotechnology) at 4 °C overnight. The specific protein was detected using a Super Signal protein detection kit (Pierce, USA). After washed with buffer, the PVDF membrane was rehybridized with a primary antibody for β-actin, followed by the same procedures as described above. The relative quantification was determined as the

Table 1. Effects of NDP on Cell Proliferation of Eca-109, Skov-3 and Hela Cells

Group	Inhibition Rate of Eca-109 cells (%)			Inhibition Rate of Skov-3 cells (%)			Inhibition Rate of Hela cells (%)		
	24h	48h	72h	24h	48h	72h	24h	48h	72h
Control group	0	0	0	0	0	0	0	0	0
NDP 4 mg/L	4.3±0.6	16.2±4.3	43.9±4.1	8.03±1.01	21.53±0.54	42.03±1.60	5.49±0.80	14.56±1.79	35.33±1.13
NDP 8 mg/L	9.8±2.0	24.2±5.1	60.3±1.8	14.45±1.14	28.42±1.90	52.87±0.51	10.86±0.99	24.87±2.00	50.43±1.44
NDP 16 mg/L	11.8±2.7	26.1±0.6	69.2±1.4	22.72±0.98	37.38±1.59	57.60±0.65	19.21±1.78	38.86±1.22	62.87±0.87
NDP 32 mg/L	15.5±1.1	47.4±2.9	81.3±7.6	32.97±1.26	41.10±2.27	66.10±1.23	38.86±1.22	62.87±0.87	38.86±1.22
NDP 64 mg/L	32.2±3.0	69.2±9.0	84.8±5.5	48.08±1.85	67.29±0.78	80.86±0.98	38.86±1.22	62.87±0.87	38.86±1.22

Table 2. Effects of DDP on Cell Proliferation of Eca-109, Skov-3 and Hela Cells

Group	Inhibition Rate of Eca-109 cells (%)			Inhibition Rate of Skov-3 cells (%)			Inhibition Rate of Hela cells (%)		
	24h	48h	72h	24h	48h	72h	24h	48h	72h
Control group	0	0	0	0	0	0	0	0	0
DDP 5 mg/L	12.3±1.1	21.3±0.4	38.6±1.8	5.28±0.57	13.86±1.46	34.59±2.39	6.69±0.50	14.78±1.39	38.68±1.52
DDP 10 mg/L	17.6±0.9	25.4±0.3	50.5±1.6	10.33±2.21	19.03±2.82	49.18±0.82	11.75±1.46	23.54±2.17	53.86±1.30
DDP 20 mg/L	36.0±1.2	41.9±4.1	69.4±0.5	24.27±1.67	36.23±3.01	62.63±2.12	28.80±0.82	46.32±2.17	71.53±1.36
DDP 40 mg/L	58.4±2.5	63.2±1.1	84.4±3.1	51.53±2.55	63.67±3.11	83.68±1.59	42.48±o.83	67.12±1.14	86.50±1.02
DDP 80 mg/L	80.9±3.2	97.8±0.9	99.4±0.6	73.03±1.59	83.50±1.71	99.28±0.48	54.87±1.23	88.17±0.78	99.51±0.36

Table 3. Inhibition Rates of NDP Combined with DDP to Eca-109, Skov-3 and Hela Cells

Drugs	C(mg/L)	fa(%)		
		Eca-109	Skov-3	Hela
NDP+DDP	4+5	34.2±1.0	35.67±0.68	26.45±2.42
NDP+DDP	8+10	47.2±1.3	54.33±2.15	54.39±2.20
NDP+DDP	16+20	84.5±5.0	68.30±1.35	71.77±1.86
NDP+DDP	32+40	87.9±0.1	92.58±0.79	89.18±2.00
NDP+DDP	64+80	95.3±5.1	97.18±0.26	94.26±0.84

density of Bax, Bcl-2, Ki-67 divided by the density of β-actin.

Statistical analysis

Datas were shown as means ±standard deviation and then analyzed by one-way analysis of variance (ANOVA) using SPSS software (version 11.0; Chicago, USA). P values < 0.05 were considered to be significant.

Results

Effects of single nedaplatin or cisplatin on tumour cell proliferation. The inhibition rates of cell proliferation of Eca-109, Skov-3 and Hela cell lines intervened by different concentrations of single nedaplatin or cisplatin at 24h, 48h and 72h were showed in Table 1 and 2. Significant statistic differences were found in each time groups and each concentration groups (P<0.05), which indicated that nedaplatin and cisplatin had a dose-dependent and time-dependent antiproliferative effects on Eca-109, Skov-3 and Hela cells.

Effects of nedaplatin combined with cisplatin on tumour cell proliferation. The inhibition rates of cell proliferation of Eca-109, Skov-3 and Hela cell lines intervened by different concentrations and different concentration ratio of combination of nedaplatin and cisplatin were showed in Table 3.

We analysed different changes of IC_{50} concentrations about three cell lines. In Eca-109 cells, the IC_{50} concentration of single nedaplatin was 33.16mg/l while

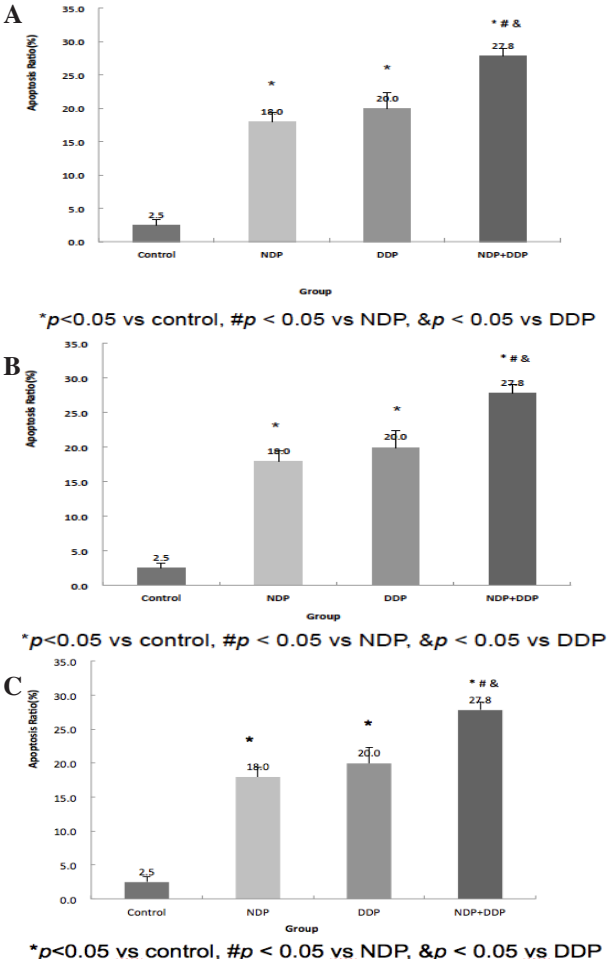

Figure 1. Early Apoptosis Rate of (A) Eca-109 Cells (B) Skov-3 Cells (C) Hela Cells Induced by NDP and DDP

6.70mg/l when nedaplatin combined with cisplatin, the former is latter 4.949 multiple. The IC_{50} concentration of single cisplatin was 16.52mg/l while 8.38 mg/l when cisplatin combined with nedaplatin, the former is latter 1.972 multiple. The relationship between effects of two drugs and combination index can be concluded as follows: 1. antagonistic effect appears when effective fa<0.14 (the required concentrations of two drugs are smaller) and

Table 4. mRNA Expressions of Bax, Bcl-2 and Ki-67 in Skov-3 and Hela Cells Treated with Different Interventional Factors after 24h

	Eca-109 cells			Skov-3 cells			Hela cells		
Group	Bax/β-actin	Bcl-2/β-actin	Ki-67/β-actin	Bax/β-actin	Bcl-2/β-actin	Ki-67/β-actin	Bax/β-actin	Bcl-2/β-actin	Ki-67/β-actin
Control	37.2±1.1	81.2±1.9	81.8±3.6	0.29±0.02	0.73±0.03	0.78±0.02	0.25±0.01	0.78±0.02	0.74±0.02
NDP	40.5±1.3	71.8±1.7	61.4±0.8	0.37±0.01	0.51±0.02	0.54±0.02	0.33±0.01	0.46±0.03	0.54±0.01
DDP	40.4±0.8	60.2±1.2	58.3±2.0	0.41±0.01	0.43±0.03	0.48±0.02	0.37±0.03	0.41±0.01	0.52±0.03
NDP+DDP	43.1±0.8	39.2±0.8	36.8±3.3	0.53±0.02	0.26±0.03	0.28±0.02	0.58±0.02	0.22±0.02	0.26±0.02*#

*P<0.01 vs NDP; #P<0.01 vs DDP

Table 5. Protein Expressions of Bax, Bcl-2 and Ki-67 in Eca-109 Cells Treated with Different Interventional Factors

	Eca-109 cells			Skov-3 cells			Hela cells		
Group	Bax/β-actin	Bcl-2/β-actin	Ki-67/β-actin	Bax/β-actin	Bcl-2/β-actin	Ki-67/β-actin	Bax/β-actin	Bcl-2/β-actin	Ki-67/β-actin
Control	0.26±0.04	0.83±0.06	0.89±0.07	0.46±0.05	0.91±0.02	0.92±0.03	0.42±0.02	0.93±0.05	0.90±0.04
NDP	0.51±0.04	0.57±0.05	0.67±0.03	0.67±0.02	0.77±0.03	0.80±0.03	0.69±0.03	0.72±0.03	0.77±0.03
DDP	0.64±0.07	0.60±0.01	0.72±0.04	0.81±0.03	0.66±0.03	0.79±0.04	0.74±0.03	0.68±0.01	0.75±0.05
NDP+DDP	0.92±0.06	0.49±0.06	0.50±0.07	0.96±0.02	0.50±0.05	0.60±0.03	0.92±0.02	0.44±0.02	0.55±0.01*#

*P<0.01 vs NDP; #P<0.01 vs DDP

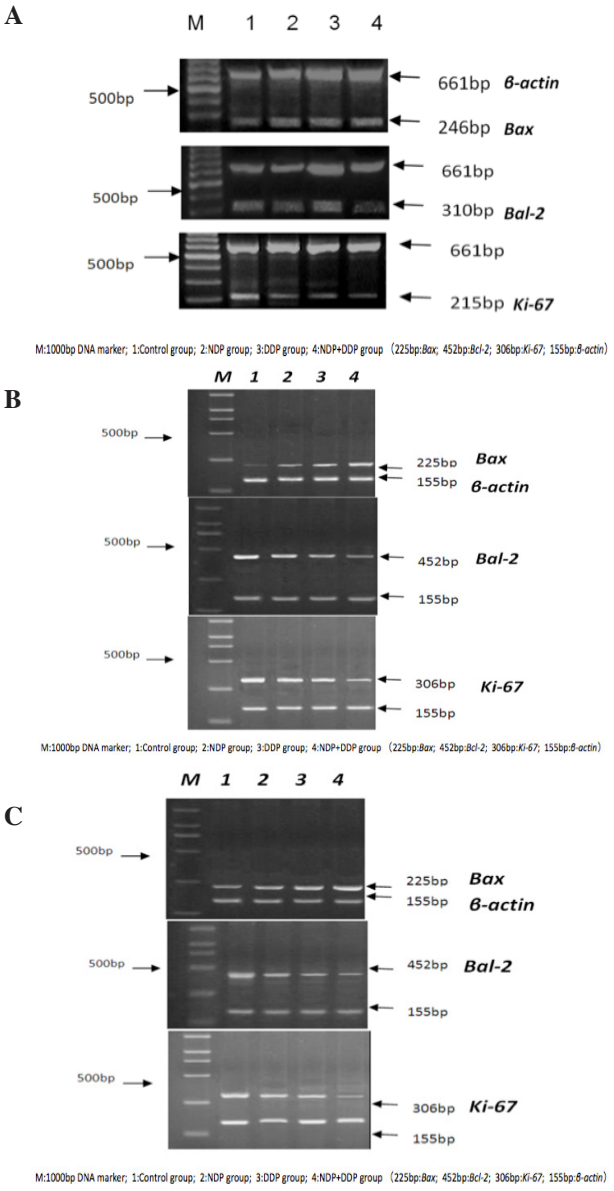

Figure 2. mRNA Expressions of Bax, Bcl-2 and Ki-67 in (A) Eca-109 Cells (B) Skov-3 Cells (C) Hela Cells Treated with Different Interventional Factors after 24h

two-drug combination index(CI)>1; 2.additional effect appears when fa=0.14 and two-drug CI=1; 3. synergistic effect appears when fa>0.14 (the required concentrations of two drugs increase gradually) and two-drug CI<1. moreover we found that synergistic effect would increase with the increasing concentration of the two drugs when 0.14<fa<0.6, however synergistic effect would decrease with the increasing concentration of the two drugs when fa>0.6.

In Skov-3 cells, the IC_{50} concentration of single nedaplatin was 32.74 mg/l while 6.92 mg/l when nedaplatin combined with cisplatin, the former is latter 4.73 multiple. The IC_{50} concentration of single cisplatin was 25.89 mg/l while 8.65 mg/l when cisplatin combined with nedaplatin, the former is latter 2.99 multiple. The relationship between effects of two drugs and combination index can be concluded as follows: 1. antagonistic effect appears when effective fa<0.20 (the required concentrations of two drugs are smaller) and two-drug CI>1; 2.additional effect appears when fa=0.20 and two-drug CI=1; 3. synergistic effect appears when fa>0.20 (the required concentrations of two drugs increase gradually) and two-drug CI<1. In Hela cells, the IC_{50} concentration of single nedaplatin was 26.03 mg/l while 7.76 mg/l when nedaplatin combined with cisplatin, the former is latter 3.36 multiple. The IC_{50} concentration of single cisplatin was 21.07 mg/l while 9.7 mg/l when cisplatin combined with nedaplatin, the former is latter 2.16 multiple. The relationship between effects of two drugs and combination index can be concluded as follows: 1. antagonistic effect appears when effective fa<0.25 (the required concentrations of two drugs are smaller) and two-drug CI>1; 2.additional effect appears when fa=0.25 and two-drug CI=1; 3. synergistic effect appears when fa>0.25 (the required concentrations of two drugs increase gradually) and two-drug CI<1.

Effects of nedaplatin combined with cisplatin on tumour cell apoptosis. Early cell apoptosis can be seen after dealing with different factors, and the early apoptosis rate induced by half of IC_{50} concentrations of nedaplatin combined with cisplatin is higher than single agent group and control group, which difference has statistically

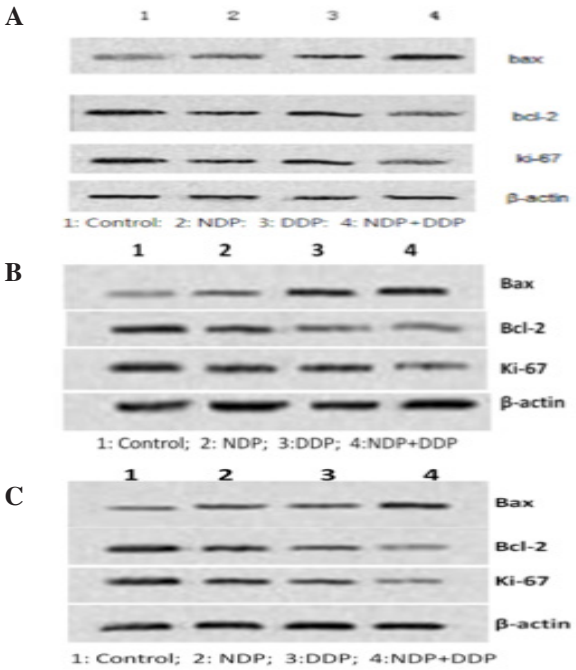

Figure 3. Protein Expression of Bax, Bcl-2 and Ki-67 in (A) Eca-109 Cells (B) Skov-3 Cells (C) Hela Cells Treated with Different Interventional Factors

significance (P<0.05, Figure 1).

The mRNA expressions of Bax, Bcl-2 and Ki-67 in Eca-109, Skov-3 and Hela cells dealing with different factors are shown in Table 4 and Figure 2. The strip grayscale value ratio of Bax, Bcl-2, Ki-67/β-actin were analysed by T test and Variance analysis. The mRNA expression of Bax increased in both combination group with half IC_{50} concentrations of nedaplatin and cisplatin and single agent group, while the expressions of Bcl-2 and Ki-67 decreased, which had statistic significant differences compared with control group (p<0.01). There were also statistic significant differences of the expressions of Bax, Bcl-2, Ki-67 in combination group with half IC_{50} concentrations of nedaplatin and cisplatin compared with that in single agent group (p<0.01).

Protein expressions of Bax, Bcl-2 and Ki-67. The protein expressions of Bax, Bcl-2 and Ki-67 in Eca-109, Skov-3 and Hela cells dealing with different factors were showed in Tab5 and Figure 3. The strip grayscale value ratio of Bax, Bcl-2, Ki-67/β-actin were analysed by T test and Variance analysis. The protein expressions of Bax increased in both combination group with half IC_{50} concentrations of nedaplatin and cisplatin and single agent group, while the expression of Bcl-2 and Ki-67 decreased, which had statistic significant differences compared with control group (p<0.01). There were also statistic significant differences of the expressions of Bax, Bcl-2, Ki-67 in combination group with half IC_{50} concentrations of nedaplatin and cisplatin compared with that in single agent group (p<0.01).

Discussion

Rosenberg reported that cisplatin had strong antitumour activity since 1960s. Platinum mainly reacts with nucleoside which generating platinum-nucleoside compounds, then the compounds combine with DNA so as to suppress the copy of DNA (Pasetto et al., 2006). The broad-spectrum antitumour activity and effectiveness of platinum attracted researcher dense interest and widely study. Over the half-century development platinum has become a group of widely used antitumour drugs for patients with solid tumours, and the function characteristic of platinum is in a dose-dependent manner (Yao et al., 2007). Nedaplatin is cis-glycolic diamine platinum, which molecular formula is C2H8N2O3Pt, and developed by Japanese pharmaceutical company Shionogi and launched in Japanese in 1995. The mechanism of antitumour activity of nedaplatin is identical with cisplatin, however the dissolution rate of nedaplatin is about ten times that of cisplatin (Tanaka et al., 2005). Nedaplatin is a broad-spectrum antitumour drug which is effective to esophageal carcinoma, ovarian cancer, cervical cancer and so on. Nedaplatin has no complete cross-resistant to cisplatin, and has excellent therapeutic effect when combined with other antitumour drugs (Brabec et al., 2006). The toxicity spectrum of nedaplatin is different with cisplatin, and the main dose-limiting toxicity is thrombocytopenia caused by marrow suppression, and the incidences of kidney toxicity and gastrointestinal reactions are low. Delay or interruption of chemotherapy always appear because of adverse reactions in current clinical recommended dosage, and reducing the treatment cycle dose of nedaplatin is the only solution. However simple lower dose will inevitably affect curative effect because maintaining a certain plasma concentration of platinum guarantee clinical curative effect. As the side-effects of the two drugs do not overlap, the combination of cisplatin and nedaplatin in clinical practice is used for patients with cervical cancer (Nishida et al., 1999) and esophageal cancer (Li et al., 2008), producing a favorable response and tolerance. However, the properties of this combination in vitro have not been reported.

In the present experiments, interactions between platinum analogues were variable in different cell lines. We assessed the inhibitory rates of proliferation by MTT assay in human esophageal carcinoma cell line Eca-109, human ovarian carcinoma cell line Skov-3 and human cervical carcinoma cell line Hela in vitro. Combination therapy is the basic method of cancer chemotherapy. The effects of synergy, addition or antagonism among drug ingredients can be quantitatively analysed by Chou-Talalay share index method (median effect) currently. The research method on dose-effect analysis of drugs based on median effect analysis was established by Chou, and the analysis can be done even the drug interaction is mutually exclusive or unknown non-exclusive nature (Kunihiko et al., 1995; Risa et al., 2005). We discovered that the combined effects of both drugs given concurrently on cells were synergistic in a certain concentration range, and the IC_{50} concentration of nedaplatin or cisplatin when combined together was lower than that when using single agent individually, which indicated that the effect was enhanced when reducing the dosage of each drug in combination therapy. The study also found that the inhibition rate when nedaplatin combined cisplatin in both half IC_{50} concentration together was significant higher than that when using single agent in IC_{50} concentration

individuall, which means the susceptibility of cells to nedaplatin or cisplatin increased when both drugs combined together.

We investigated Bcl-2 family members involved in the mitochondrial (intrinsic) pathway to determine whether nedaplatin and cisplatin were functioning in upstream initiator signaling for apoptosis. Involvement of the Bcl-2 and Bax proteins in cisplatin-mediated apoptosis was reported previously for human gastric cancer cells (Kim et al., 2001). Ki67 protein is a cellular marker for proliferation and is strictly associated with cell proliferation (Takeuchi et al., 2003; Liu et al., 2012). It often correlates with the clinical course of cancer and predicts the prognosis, recurrence and metastasis for the carcinomas of the prostate and the breast. Our results revealed that the combination of nedaplatin and cisplatin can up-regulate the mRNA or protein expression of Bax, and down-regulate the mRNA or protein expression of Bcl-2 and Ki67 in three tumour cell lines. Moreover, compared with nedaplatin or cisplatin group alone, the expressions of Bax, Bcl-2 and Ki67 were significantly increased in the combined group in three tumor cell lines.

In brief, our study showed the combination of nedaplatin and cisplatin had the synergistic phenomenon in the inhibition of the proliferation and the induction of the apoptosis of Eca-109, Skov-3 and Hela cell lines. So the synergistic effects of nedaplatin combined with cisplatin were achieved by enhancing the induction of apoptosis and inhibition of cell proliferation of the three tumour cell lines. However, cell culture in vitro cannot mimic complicated drug metabolism and activity progress in vivo, so cannot accurately reflect the complex relationship among drug-body-tumour, so extended animal experiments and further signal pathway system analysis are needed in the future study.

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