Cancer Chemother Pharmacol (2005) 56: 279–285
DOI 10.1007/s00280-004-0991-y

ORIGINAL ARTICLE

**Risa Tanaka · Yasushi Takii · Yoshihiro Shibata
Hiroshi Ariyama · Baoli Qin · Eishi Baba
Hitoshi Kusaba · Kenji Mitsugi · Mine Harada
Shuji Nakano**

In vitro sequence-dependent interaction between nedaplatin and paclitaxel in human cancer cell lines

Received: 6 September 2004 / Accepted: 19 November 2004 / Published online: 4 May 2005

Abstract *Purpose*: To define the most effective combination schedule of paclitaxel and nedaplatin, a new platinum derivative, we investigated the in vitro interaction between these drugs in AZ-521 and NUGC-4 gastric adenocarcinoma and KSE-1 esophageal squamous carcinoma cell lines. *Materials and methods*: Cytotoxic activity was determined by the WST-1 assay. Different treatment schedules of the two drugs were compared and evaluated for synergism, additivity, or antagonism using a quantitative method based on the median-effect principle of Chou and Talalay. Cell-cycle perturbation and apoptosis were evaluated by means of flow cytometry. *Results*: Upon 24-h sequential exposure, the sequence paclitaxel followed by nedaplatin induced greater than additive effects in all of the cell lines, with synergistic interactions in NUGC-4 and KSE-1 cells. By contrast, antagonistic effects were observed with the reverse sequence. Simultaneous treatment resulted in either a synergistic or antagonistic effect, depending on the cell line. Therefore, the sequence paclitaxel followed by nedaplatin appears most active, at least in these three cell lines. Flow cytometric analyses at IC_{50} indicated that paclitaxel induced G2/M arrest with subsequent induction of apoptosis (56%) in the sub-G1 phase. When paclitaxel preceded nedaplatin, apoptosis was most prominent (70%) with pronounced G2/M arrest. By contrast, the reverse sequence yielded only 28% induction of apoptotic cells, with almost identical cell-cycle distribution patterns to those observed with nedaplatin alone, indicating that the activity of paclitaxel is abolished by pretreatment with nedaplatin. *Conclusions*: Our findings suggest that the interaction of nedaplatin and paclitaxel is highly schedule dependent and that the sequential administration of paclitaxel followed by nedaplatin should be thus incorporated into the design of a clinical trial.

Keywords Nedaplatin · Paclitaxel · Sequence dependence · Drug interaction

R. Tanaka · Y. Takii · Y. Shibata · H. Ariyama · B. Qin
E. Baba · H. Kusaba · K. Mitsugi · M. Harada · S. Nakano (✉)
First Department of Internal Medicine and
Department of Biosystemic Science of Medicine,
Graduate School of Medicine, Kyushu University,
3-1-1 Maidashi, Fukuoka 812-8582, Japan
E-mail: sn@intmed1.med.kyushu-u.ac.jp
Tel.: +81-92-6425226
Fax: +81-92-6425247

Introduction

Cisplatin has played a major role in the chemotherapy of a variety of solid tumors over the past two decades. However, the clinical usefulness of cisplatin is limited due to its toxicity to many normal tissues, such as kidney. Nedaplatin is a new platinum derivative, selected from a series of platinum analogues based on its pronounced preclinical antitumor activity against various solid tumors with lower nephrotoxicity [10]. Preclinical studies indicate that nedaplatin has an antitumor activity comparable to cisplatin [2, 12] and has been shown experimentally to overcome cisplatin resistance in a cisplatin-resistant K562 cell line [12]. Clinically, single-agent nedaplatin has shown a wide spectrum of antitumor activity, producing the favorable response rates in head and neck [9], esophagus [24], non-small cell lung [7], and cervical cancers [18]. The activity of nedaplatin against gastric cancer, however, still remains unclear, despite the fact that nedaplatin has a spectrum of antitumor activity similar to that of cisplatin in phase-I and phase-II studies.

Paclitaxel has demonstrated broad clinical efficacy in a variety of malignancies including ovarian, non-small-cell lung [22], esophageal [1], head and neck [6], gastric [21] and cervical [16] cancers. Paclitaxel in combination with cisplatin is well known for its sequence-dependent synergy in vitro and in vivo [11, 17], and the sequence of paclitaxel followed by cisplatin has been recommended

for clinical studies. Although combination of paclitaxel and nedaplatin is expected to have a potent activity similar to that of paclitaxel and cisplatin combination, few preclinical data for the interaction between these drugs are currently available. Moreover, the efficacy of nedaplatin against gastric cancer cell lines has yet to be determined in vitro. In order to obtain the clinical rationale for the optimal administration schedule of this combination for the treatment of gastric and esophageal cancers, we investigated the interaction between nedaplatin and paclitaxel using an in vitro model of human cancer cell lines derived from esophagus and stomach, using a quantitative method that assesses the synergism or antagonism between these two agents.

Materials and methods

Cell lines and culture

The human AZ-521 and NUGC4 gastric adenocarcinoma cell lines were kindly provided by JCRB Cell Bank (Tokyo, Japan) and maintained in Dulbecco's minimum essential medium (DMEM) (Nissui, Tokyo, Japan) 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. The human KSE-1 esophageal squamous carcinoma cell line [15] was established in our laboratory and maintained under the same conditions as were AZ-521 cells.

Drugs

Nedaplatin was a gift from Shionogi (Osaka, Japan) and paclitaxel was a gift from Bristol-Myers (Tokyo, Japan). Stock solutions of nedaplatin were prepared in distilled water and those of paclitaxel were prepared in dimethylsulfoxide (DMSO). Both solutions were stored at −4°C prior to use. The final concentration of DMSO for all experiments and treatments was maintained at less than 0.02%. These conditions were found to be non-cytotoxic.

Cytotoxicity assay

Cytotoxic activity was measured by means of the WST-1 assay (Wako Chemicals, Osaka, Japan) using manufacturer's instructions [8]. The WST-1 assay is a colorimetric method in which the intensity of the dye is proportional to the number of the viable cells. Briefly, cells were plated into 96-well microtiter plates at a density of 5×10^3 cells/well, and incubated for 24 h for sufficient cell growth. Cells were then treated with graded concentrations of nedaplatin (0.3–1,000 μg/ml) or paclitaxel (0.3–1,000 ng/ml) alone for 24 h, and were incubated with drug-free medium for an additional 24 h. Cells were washed with PBS, and 100 μl medium and 10 μl WST-1 solution were added to each well; then the plates were incubated at 37°C for another 3 h. Absorbances at 450 nm and 620 nm were measured using a Delta Soft ELISA analysis program for Macintosh computer interfaced with a Bio-Tek microplate reader (Immuno-Mini NJ-2300). Wells containing only DMEM and WST-1 were used as controls. Each experiment was performed using six replicated wells for each drug concentration and carried out independently at least three times. The IC_{50} was defined as the concentration that reduced the absorbance in each test by 50%.

For the combination experiments, three different schemes were used to investigate the interaction of paclitaxel and oxaliplatin as shown in Fig. 1: (a) nedapaltin and paclitaxel were exposed simultaneously for 24 h and incubated for an additional 24 h with drug-free medium, (b) nedaplatin was administered for 24 h followed by paclitaxel for 24 h, or (c) paclitaxel was administered for 24 h followed by nedaplatin. Immediately after these treatments, the cytotoxic effects were evaluated by WST-1 assay.

Analysis of combination effects

On the basis of the growth inhibition curve for each single drug, we analyzed the effects of the drug combinations using the method described by Chou and Talalay and a Calcusyn software program for automated analysis (Biosoft, Cambridge, UK) [3, 4]. The influence on the combination of the two drugs was evaluated by comparing the sequential assays with assays involving nedaplatin or paclitaxel exposure alone. The combination effect was evaluated from isoeffect analysis (Cis), calculated as follows: $CI = C_{\text{nedaplatin}}/Cx_{\text{nedaplatin}} + C_{\text{paclitaxel}}/Cx_{\text{paclitaxel}}$ where $Cx_{\text{paclitaxel}}$ and $Cx_{\text{paclitaxel}}$ are

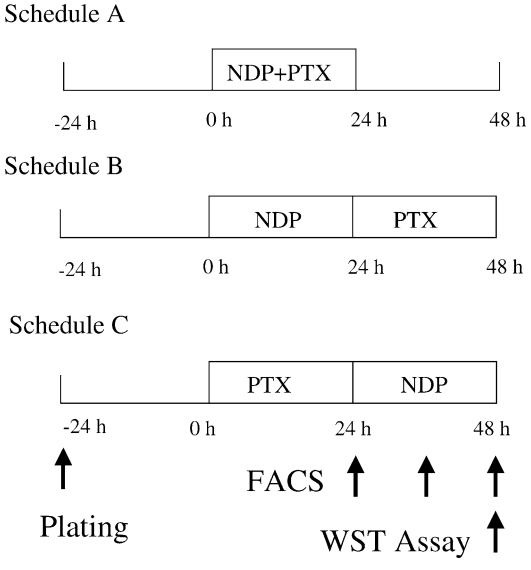

Fig. 1 Description of the three combination schedules. *NDP* nedaplatin, *PTX* paclitaxel

the concentrations of nedaplatin and paclitaxel alone, respectively, needed to achieve a given effect (x%) and $C_{\text{nedaplatin}}$ and $C_{\text{paclitaxel}}$ are the concentrations of nedaplatin and paclitaxel needed for the same effect (x%) when the drugs were 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.

Cell-cycle determination

Human gastric cancer cell line, AZ-521 cells were cultured at 1×10^5 cells per 60-mm dish. The same protocols as described in the growth inhibition assay were used. After treatment, the cells were harvested, washed twice in ice-cold PBS (pH 7.4), and then fixed in 100% ethanol and stored at 4°C for up to 3 days prior to cell-cycle analysis. After the removal of ethanol by centrifugation, cells were then washed with PBS and stained with a solution containing propidium iodide and RNase (Sigma-Aldrich, St. Louis, MO, USA) on ice for 30 min. Cell-cycle analysis was performed on a Becton Dickinson FACS/Calibur Flow Cytometer using the CELLQuest or ModFit 3.0 software packages (Becton Dickinson, San Jose, CA, USA), and the percentages of apoptotic populations were determined by measuring the sub-G1 phase using FACS analysis after collecting floating and trypsinized adherent cells at various times following drug exposure. Each experiment was performed in triplicate.

Results

Single-agent experiments

The cytotoxic activities of nedaplatin and paclitaxel were tested individually on the three tumor cell lines. Each drug was exposed to the cells for 24 h. The IC_{50} values ($\pm$SD) are summarized in Table 1. The IC_{50} value of nedaplatin for KSE-1 (3.4 μg/ml) was not significantly different from those for AZ-521 (3.8 μg/ml) and NUGC-4 (4.6 μg/ml) gastric cancer cells, indicating that nedaplatin appears to be equally effective against these esophageal and gastric cancer cell lines. By contrast, the IC_{50} of paclitaxel for these cell lines varied, depending on the cell type. AZ-521 gastric cancer cells were most sensitive to paclitaxel (14 ng/ml) among the three tumor cell lines, NUGC-4 gastric cancer cells being least sensitive (26 ng/ml).

Table 1 IC_{50} values of nedaplatin and paclitaxel in a panel of three cell lines. Cells were treated with various concentrations of nedaplatin or paclitaxel for 24 h. Results are expressed as the concentration that inhibits 50% of growth in comparison with controls (IC_{50}). The values are mean $\pm$ SD of three independent experiments

	AZ-521	NUGC-4	KSE-1
Nedaplatin (μg/ml)	3.8 ± 1.3	4.6 ± 1.3	3.4 ± 0.3
Paclitaxel (ng/ml)	14.0 ± 1.9	26.0 ± 2.6	18.9 ± 0.9

Median-effect analysis of paclitaxel and oxaliplatin combination in vitro

Nedaplatin and paclitaxel were tested in different combinations to define the most effective schedule. Three different schedules were tested (simultaneous exposure or sequential drug exposures) as shown in Fig. 1. When cells were treated with nedaplatin and paclitaxel simultaneously (Fig. 2), the CI values were below 1 at all levels of killed cell fraction in NUGC-4 and at higher levels of killed cell fraction in KSE-1 cells (Fig. 2b, c), indicating a marked synergistic effect, while moderately antagonistic effects ($CI > 1$) were seen in AZ-521 cells at the ranges corresponding to less than 70% killed cell fraction (Fig. 2a). When cells were treated with paclitaxel followed by nedaplatin, greater than additive effects were obtained in all of the cell lines, with synergistic interactions observed in NUGC-4 and KSE-1 cells at all ranges (Fig. 3). By contrast, largely antagonistic effects were seen in all of the cell lines when cells were treated with the reverse sequence (Fig. 4), although this sequence appeared to be synergistic in KSE-1 cells at the higher cytotoxic ranges (Fig. 4c). Therefore, the sequence paclitaxel followed by nedaplatin appears most active at least in these three cell lines.

Cell-cycle perturbation and apoptosis

In an attempt to explain the mechanisms underlying the different types of interaction, the effects of paclitaxel and nedaplatin on cell-cycle distribution and apoptosis were studied in AZ-521 cells (Table 2). The cells were treated with these drugs either alone or in combination, with different schedules, and cell-cycle distribution was analyzed 36 h and 48 h after the beginning of treatment, using flow cytometric analysis. Paclitaxel alone at a dose of 12.5 ng/ml induced the accumulation of cells in the G2/M phase. At a concentration of 5 μg/ml, nedaplatin alone caused an increase in the S population and a decrease in the G0/G1 population without affecting the population of G2/M, indicating that it inhibited both S to G2 and G2/M to G1 progression. The simultaneous exposure led to the accumulation of cells in the S and G2/M phase and a decrease in the population of G1, showing the combined activity of both drugs. The treatment with paclitaxel prior to nedaplatin led to the accumulation of cells more exclusively into the G2/M phase, showing similar distribution patterns to those observed in the cells treated with paclitaxel alone, although a significant increase in G2/M population was

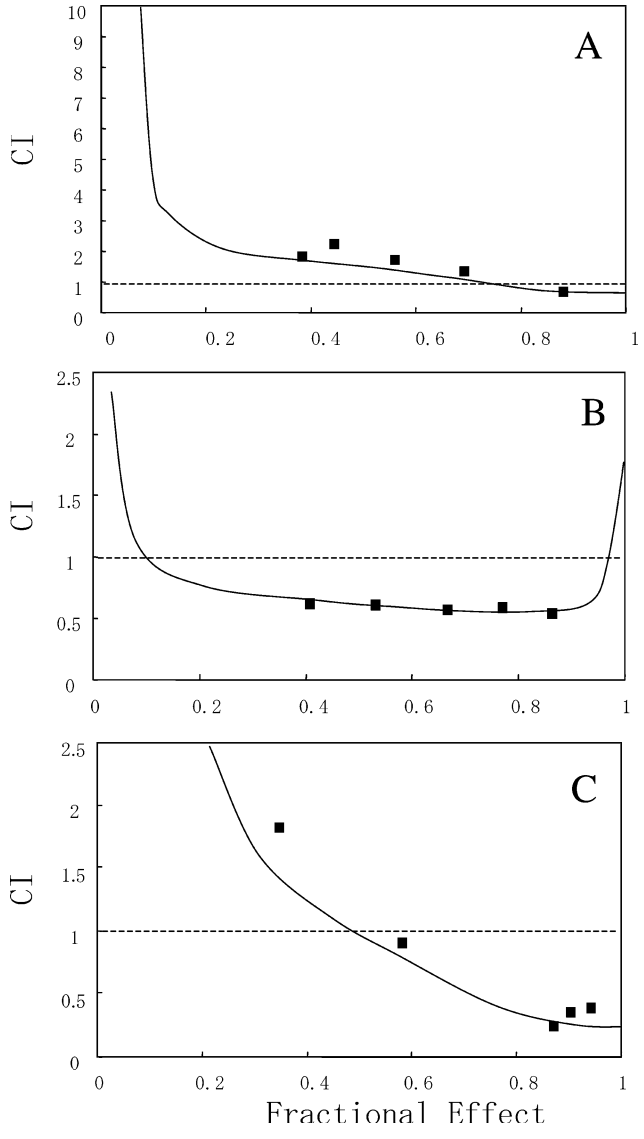

Fig. 2 Combination index (*CI*) plots obtained from three cancer cell lines exposed simultaneously to nedaplatin and paclitaxel for 24 h. **a** AZ-521; **b** NUGC-3; **c** KSE-1

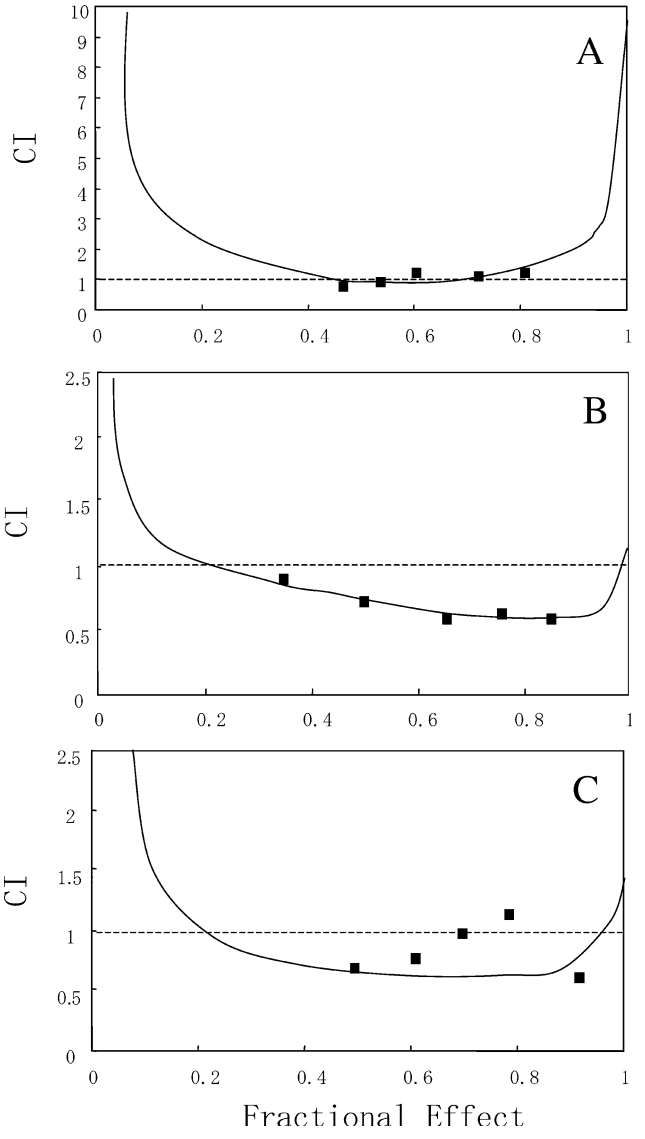

Fig. 3 Combination index (*CI*) plots obtained from three gastric cell lines exposed to paclitaxel for 24 h followed by nedaplatin for 24 h. **a** AZ-521, **b** NUGC-3, **c** KSE-1

observed when compared with treatment with paclitaxel alone. In contrast, nedaplatin prior to paclitaxel caused almost identical distribution patterns to those observed with nedaplatin alone. These data indicate that cell-cycle distribution patterns in sequential combination were mostly influenced by the initial drug administered.

To confirm the activities of sequential combination, the apoptotic activity was investigated after treatment of AZ-521 cells by measuring the population of sub-G1 phase using FACS analyses. The presence of hypodiploid DNA (sub-G1) is associated with cells undergoing apoptosis. As shown in Table 2, paclitaxel followed by nedaplatin induced the G2/M block, with substantial induction of apoptosis in the majority of the treated cells (70%). The induction rate of apoptosis by this sequential administration was greater than those of paclitaxel alone (56–66%) or nedaplatin alone (14–17%). By contrast, the reverse sequence caused S-phase block, and apoptotic population was 24–28%, being less than those induced by paclitaxel alone (56–66%), and slightly more than those induced by nedaplatin alone (14–17%). These data indicate that sequence nedaplatin followed by paclitaxel is antagonistic in inducing apoptosis.

Discussion

In this study, we examined the schedule-dependent interaction of nedaplatin and paclitaxel in a panel of three human cancer cell lines derived from stomach and esophagus in vitro. First, we compared the sensitivity of

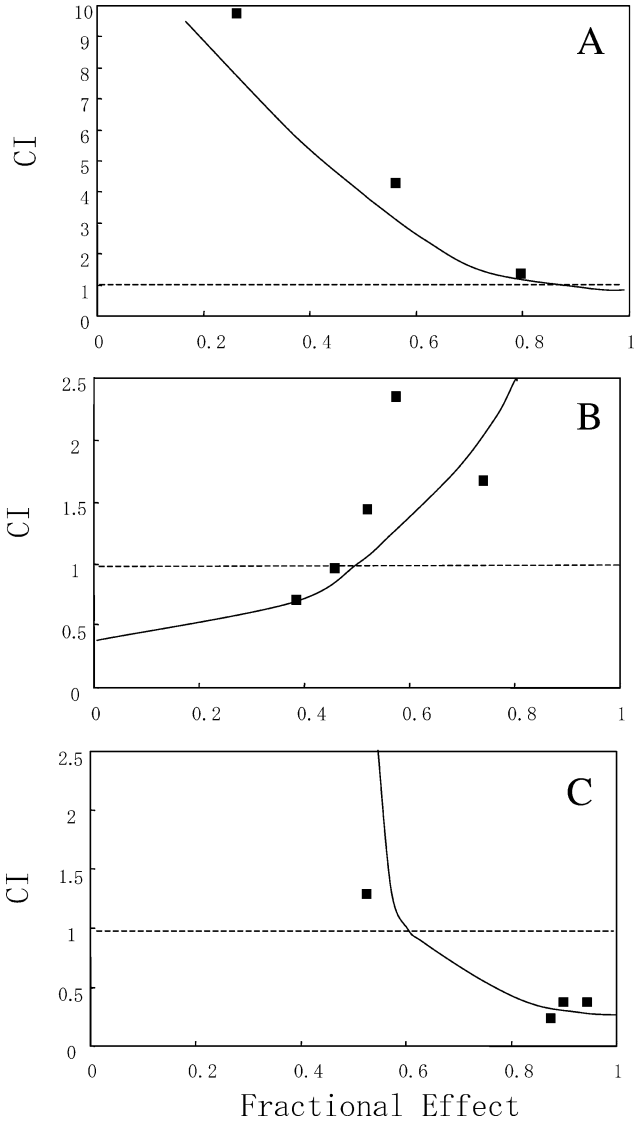

Fig. 4 Combination index (*CI*) plots obtained from three cell lines exposed to nedaplatin for 24 h followed by paclitaxel for 24 h. **a** AZ-521, **b** NUGC-3, **c** KSE-1

nedaplatin between these esophageal and gastric cancer cell lines, because nedaplatin has been clinically shown to be active against esophageal cancer [24], but its clinical efficacy against gastric cancer has not been determined. We have found that the IC_{50} of nedaplatin is not significantly different between these gastric and esophageal cancer cell lines, indicating that nedaplatin may be potentially effective against gastric cancer. Second, we found that the sequence paclitaxel followed by nedaplatin resulted in either synergism or additivity in all three cell lines. By contrast, a clear antagonism was observed in the reverse sequence in two of three cell lines. Simultaneous treatment with these two drugs resulted in either synergistic or antagonistic effects, depending on the cell line. Therefore, the sequence paclitaxel followed by nedaplatin appears to be the most active, at least in these three cell lines. A similar sequence-dependent antitumor activity of this combination was demonstrated in a preclinical in vivo mouse tumor model [26].

To explain the possible mechanism underlying the synergistic interaction of paclitaxel following nedaplatin sequence, we further analyzed the perturbations induced on cell cycle by flow cytometric analyses using the AZ-521 human gastric cancer cell line. We found that a 24-h treatment with paclitaxel markedly affected the cell-cycle distribution, producing a relevant accumulation in the G2/M phase with subsequent induction of apoptosis (56%) in the sub-G1 phase. Nedaplatin alone caused an increase in the S population and a decrease in the G0/G1 population without affecting the population of G2/M, suggesting that it inhibited both S to G2/M and G2/M to G1 progression. The treatment with paclitaxel prior to nedaplatin accumulated cells almost exclusively into the G2/M phase with prominent apoptosis (70%). By contrast, the reverse sequence yielded only 28% induction of apoptotic cells with almost identical cell-cycle distribution patterns to those observed with nedaplatin alone, indicating that the activity of paclitaxel is abolished by pretreatment of nedaplatin, accounting for an antagonistic interaction. The inhibition of paclitaxel-induced cytotoxicity by nedaplatin would probably be explained by the decrease in the number of G2 population targeted by paclitaxel, because pretreatment with

Table 2 Cell-cycle perturbation (%) and apoptosis induced by nedaplatin and paclitaxel in the AZ-521 cell line. Data are presented as mean percentage values from three independent experiments. *NDP* nedaplatin, *PTX* paclitaxel

Treatment	36-h				48-h			
	G0/G1(%)	S(%)	G2/M(%)	Apoptosis[a]	G0/G1(%)	S(%)	G2/M(%)	Apoptosis[a]
Control					58.34	19.91	21.76	3.52
NDP	40.85	31.89	27.26	14.52	37.81	39.91	22.28	17.05
PTX	16.33	24.65	58.42	66.61	19.45	18.02	62.53	56.47
NDP + PTX	12.85	41.48	45.66	49.53	16.98	38.93	44.09	54.76
PTX → NDP	10.91	20.28	68.81	66.10	11.70	13.88	74.42	70.14
NDP → PTX	37.31	44.97	17.72	24.05	36.99	40.40	22.60	28.46

[a] The percentages of apoptotic populations were assessed by measuring sub-G1 phase using FACS analyses after collecting floating and trypsinized adherent cells at various times following drug exposure

nedaplatin accumulates cells mostly in the phases before G2/M, thereby reducing the number of cells entering G2/M phase.

We have found that nedaplatin can arrest the cells mainly at both G1 and S phases, suggesting a distinct action from that of cisplatin or oxaliplatin, because cisplatin and oxaliplatin mainly accumulate cells into G2/M [14, 23] and G1 phases [25], respectively. However, sequence-dependent interactions between paclitaxel and these three platinum compounds did not differ; a synergistic or additive interaction was observed when paclitaxel precedes CDDP [13, 17, 20, 27] or oxaliplatin [25], whereas there were antagonistic interactions in the reverse sequence [13, 25, 27]. Several explanations for increased activity of the sequence paclitaxel followed by cisplatin are shown: cisplatin hastens the exit from mitosis in paclitaxel-treated cells [17]; paclitaxel induces an increase in intracellular uptake of cisplatin [5]; and paclitaxel inhibits repair of cisplatin-induced DNA damage [19]. Therefore, we hypothesize that the similar mechanisms, if not identical, to those as demonstrated in the interaction between CDDP and paclitaxel may also operate in the combination of nedaplatin and paclitaxel.

Clinically, single-agent nedaplatin produced promising response rates in phase-II trials for the treatment of head and neck, lung, esophagus and cervical cancers [7, 9, 18, 24]. In this study, we have shown that nedaplatin is effective against gastric cancer cells and exhibits a significant synergy with paclitaxel. Since these drugs have an overlapping spectrum of clinical efficacies, this combination is a promising chemotherapeutic regimen for the treatment of patients with these cancers. Although the biochemical basis for their interaction remains unknown, a clear sequence-dependent activity of nedaplatin and paclitaxel combination should be thus incorporated into the design of a clinical trial.

References

1. Ajani JA, Ilson DH, Daugherty K, Pazdur R, Lynch PM, Kelsen DP (1994) Activity of taxol in patients with squamous cell carcinoma and adenocarcinoma of the esophagus. J Natl Cancer Inst 86:1086–1091
2. Alberts DS, Fanta PT, Running KL, Adair LP Jr, Garcia DJ, Liu-Stevens R, Salmon SE (1997) In vitro phase II comparison of the cytotoxicity of a novel platinum analog, nedaplatin (254-S), with that of cisplatin and carboplatin against fresh, human ovarian cancers. Cancer Chemother Pharmacol 39:493–497
3. Chou TC, Talalay P (1984) Quantitative analysis of dose-effect relationships: the combined effects of multiple drugs or enzyme inhibitors. Adv Enzyme Regul 22:27–55
4. Chou TC, Motzer RJ, Tong V, Bosl GJ (1994) Computerized quantitation of synergism and antagonism of Taxol, topotecan, and cisplatin against human teratocarcinoma cell growth: a rational approach to clinical protocol design. J Natl Cancer Inst (Bethesda) 86:1517–1524
5. Christen RD, Jekunen AP, Jones JA, Thiebaut F, Shalinsky DR, Howell SB (1993) In vitro modulation of cisplatin accumulation in human ovarian carcinoma cells by pharmacologic alteration of microtubules. J Clin Invest 92:431–440
6. Forastiere AA, Shank D, Neuberg D, Taylor SG IV, DeConti RC, Adams G (1998) Final report of a phase II evaluation of paclitaxel in patients with advanced squamous cell carcinoma of the head and neck: an Eastern Cooperative Oncology Group trial (PA390). Cancer 82:2270–2274
7. Fukuda M, Shinkai T, Eguchi K, Sasaki Y, Tamura T, Ohe Y, Kojima A, Oshita F, Hara K, Saijo N (1990) Phase II study of (glycolate-O,O') diammineplatinum(II), a novel platinum complex, in the treatment of non-small-cell lung cancer. Cancer Chemother Pharmacol 26:393–396
8. Ishiyama M, Shiga M, Sasamoto K, Mizoguchi M, He P (1993) A new sulfonated tetrazolium salt that produces a highly water-soluble formazan dye. Chem Pharm Bull 41:1118
9. Inuyama Y, Miyake H, Horiuchi M, Hayasaki K, Komiyama S, Ota K (1992) A late phase II clinical study of cis-diammine glycolato platinum, 254-S, for head and neck cancers. Japanese Gan To Kagaku Ryoho 19:871–877
10. Kameyama Y, Okazaki N, Nakagawa M, Koshida H, Nakamura M, Gemba M. (1990) Nephrotoxicity of a new platinum compound, 254-S, evaluated with rat kidney cortical slices. Toxicol Lett 52:15–24
11. Kano Y, Akutsu M, Tsunoda S, Suzuki K, Yazawa Y (1996) In vitro schedule-dependent interaction between paclitaxel and cisplatin in human carcinoma cell lines. Cancer Chemother Pharmacol 37:525–530
12. Kobayashi H, Takemura Y, Miyachi H, Ogawa T (1991) Antitumor activities of new platinum compounds, DWA2114R, NK121 and 254-S, against human leukemia cells sensitive or resistant to cisplatin. Invest New Drugs 9:313–319
13. Liebmann JE, Fisher J, Teague D, Cook JA (1994) Sequence dependence of paclitaxel (Taxol) combined with cisplatin or alkylators in human cancer cells. Oncol Res 6:25–31
14. Ma J, Maliepaard M, Nooter K, Boersma AW, Verweij J, Stoter G, Schellens JH (1998) Synergistic cytotoxicity of cisplatin and topotecan or SN-38 in a panel of eight solid-tumor cell lines in vitro. Cancer Chemother Pharmacol 41:307–316
15. Matsuoka H, Sugimachi K, Ueo H, Kuwano H, Nakano S, Nakayama M (1987) Sex hormone response of a newly established squamous cell line derived from clinical esophageal carcinoma. Cancer Res 47:4134–4140
16. McGuire WP, Blessing JA, Moore D, Lentz SS, Photopulos G (1996) Paclitaxel has moderate activity in squamous cervix cancer. a gynecologic oncology group study. J Clin Oncol 14:792–795
17. Milross CG, Peters LJ, Hunter NR, Mason KA, Milas L (1995) Sequence-dependent antitumor activity of paclitaxel (taxol) and cisplatin in vivo. Int J Cancer 62:599–604
18. Noda K, Ikeda M, Yakushiji M, Nishimura H, Terashima Y, Sasaki H, Hata T, Kuramoto H, Tanaka K, Takahashi T et al (1992) A phase II clinical study of cis-diammine glycolato platinum, 254-S, for cervical cancer of the uterus. Japanese Gan To Kagaku Ryoho 19:885–892
19. Parker RJ, Lee KB, Dabholkar M, Bostick-Bruton F, Simmis M, Reed E (1993) Influence of taxol: cisplatin sequencing on cisplatin-DNA adduct repair in human ovarian cancer cells. Proc Am Assoc Cancer Res 34:356
20. Rowinsky EK, Citardi MJ, Noe DA, Donehower RC (1993) Sequence-dependent cytotoxic effects due to combinations of cisplatin and the antimicrotubule agents taxol and vincristine. J Cancer Res Clin Oncol 119:727–733
21. Sakamoto J, Morita S, Yumiba T, Narahara H, Kinoshita K, Nakane Y, Imamoto H, Shiozaki H (2003) Ascitic Gastric Cancer Study Group of the Japan South West Oncology Group. A phase II clinical trial to evaluate the effect of paclitaxel in patients with ascites caused by advanced or recurrent gastric carcinoma: a new concept of clinical benefit response for non-measurable type of gastric cancer. Jpn J Clin Oncol 33:238–240
22. Sekine I, Nishiwaki Y, Watanabe K, Yoneda S, Saijo N (1996) Phase II study of 3-hour infusion of paclitaxel in previously untreated non-small cell lung cancer. Clin Cancer Res 2:941–945

23. Sorenson CM, Eastman A (1988) Mechanism of cis-diamminedichloroplatinum (II)-induced cytotoxicity: role of G2 arrest and DNA double-strand breaks. Cancer Res 48:4484–4488
24. Taguchi T, Wakui A, Nabeya K, Kurihara M, Isono K, Kakegawa T, Ota K (1992) A phase II clinical study of cis-diammine glycolato platinum, 254-S, for gastrointestinal cancers. 254-S Gastrointestinal Cancer Study Group. Japanese Gan To Kagaku Ryoho 19:483–488
25. Tanaka R, Ariyama H, Qin B, Takii Y, Baba E, Mitsugi K, Harada M, Nakano S (2005) In vitro schedule-dependent interaction between Paclitaxel and Oxaliplatin in human cancer cell lines. Cancer Chemother Pharmacol (in press)
26. Yamada H, Uchida N, Maekawa R, Yoshioka T (2001) Sequence-dependent antitumor efficacy of combination chemotherapy with nedaplatin, a newly developed platinum, and paclitaxel. Cancer Lett 172:17–25
27. Vanhoefer U, Harstrick A, Wilke H, Schleucher N, Walles H, Schroder J, Seeber S (1995) Schedule-dependent antagonism of paclitaxel and cisplatin in human gastric and ovarian carcinoma cell lines in vitro. Eur J Cancer 31A:92–97