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SHORT COMMUNICATION

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Docetaxel and paclitaxel inhibit DNA-adduct formation and intracellular accumulation of cisplatin in human leukocytes

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Abstract The purpose of this study was to determine the mechanism of the pharmacodynamic interaction between docetaxel/paclitaxel and cisplatin. Cisplatin-induced DNA-adducts and cisplatin accumulation were quantitated in peripheral blood leukocytes (WBC). The WBC were obtained from patients treated with docetaxel or paclitaxel in phase I/II studies and were incubated in vitro with cisplatin. In addition, blank whole-blood samples were obtained from patients and healthy subjects and incubated in vitro with cisplatin or docetaxel/paclitaxel and cisplatin. The cisplatin-induced DNA-adduct levels measured in WBC after treatment with docetaxel or paclitaxel were significantly lower than those determined in non-pretreated WBC. Docetaxel and paclitaxel reduced the intracellular accumulation of cisplatin in WBC by 46–47%. If the pharmacodynamic interaction between docetaxel/paclitaxel and cisplatin also occurs in other normal tissues such as bone marrow, it may well contribute to the sequence dependent toxicity that has been observed in clinical studies.

Key words Cisplatin · Docetaxel · Paclitaxel

Introduction

Docetaxel and paclitaxel are novel antimicrotubule agents inducing tubulin polymerization and inhibiting microtubule depolymerization. Clinical studies have revealed high response rates, particularly in breast and ovarian cancer [8, 11]. In vitro, paclitaxel has shown synergism with cisplatin when given before cisplatin [3,4], whereas in vivo in humans administration of cisplatin before paclitaxel has induced more neutropenia than has the alternate sequence. This has been attributed to a reduced clearance of paclitaxel [10].

We have previously reported on a clinical and pharmacology study in patients with solid tumors, applying the sequence docetaxel/cisplatin in one group of patients and that of cisplatin/docetaxel in another group. It was shown that the levels of cisplatin-DNA adducts in WBC were markedly lower when docetaxel preceded cisplatin [13]. Indeed, the DNA adduct level measured at 1 h after cisplatin infusion (dose of 75 mg/m^2) was 0.54 ± 0.4 pg Pt/μg DNA for the sequence docetaxel/cisplatin and 1.21 ± 0.34 pg Pt/μg DNA for the reverse sequence ($P < 0.0001$) [13]. In addition, significantly less granulopenia ($P < 0.001$) and mucositis ($P < 0.01$) were observed for the sequence docetaxel/cisplatin than for the reverse sequence. There was no significant mutual pharmacokinetic interaction between docetaxel and cisplatin. Here we report on additional in vitro experiments that contribute to a better understanding of the mechanism of interaction.

Materials and methods

Patients were treated either with docetaxel (55–100 mg/m^2) and cisplatin (50–100 mg/m^2) in a phase I/II study ($n = 22$) [12] or with single agent docetaxel (100 mg/m^2) ($n = 6$) or paclitaxel (135 mg/m^2; $n = 3$) in phase I/II studies. All patients gave written informed consent and had a WHO performance status of ≤ 1 and adequate renal and liver function, i.e., a serum creatinine level of < 120 μmol/l (1.4 mg/dl) and a total bilirubin value of < 25 μmol/l (1.5 mg/dl), as well as a WBC of $> 4.0 \times 10^9$/l and a platelet count of $> 100 \times 10^9$/l.

For in vitro incubation with cisplatin, heparinized whole-blood samples (20 ml) were drawn prior to docetaxel or paclitaxel infusion and at 3 h postinfusion (i.e., prior to cisplatin administration in the docetaxel/cisplatin group). In the six patients treated with single-agent docetaxel and in one of the three patients treated with single-agent paclitaxel, a third sample was drawn at 24 h postinfusion.

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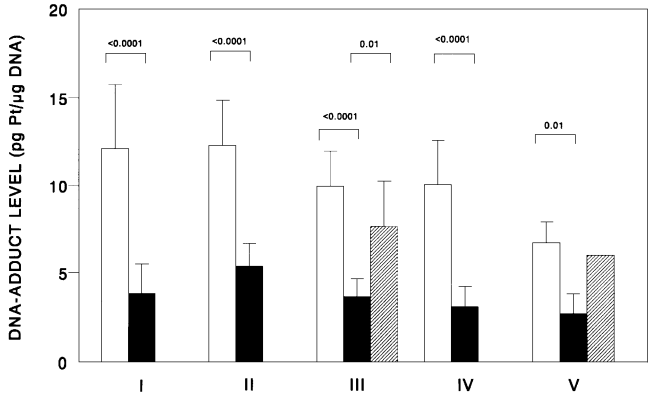

Fig. 1 Cisplatin-DNA adduct levels determined in vitro in WBC in blank blood and blood samples obtained after docetaxel or paclitaxel administration [*I* Blood collected prior to (*white bar*) and at 3 h after docetaxel infusion (*black bar*) and incubated with cisplatin in vitro ($n = 22$), *II* Patients' blank blood incubated in vitro with cisplatin alone (*white bar*)or with docetaxel + cisplatin (*black bar*; $n = 8$), *III* Blood collected prior to (*white bar*) and at 3 (*black bar*) and 24 h (*shaded bar*) after docetaxel infusion and then incubated with cisplatin in vitro ($n = 6$), *IV* Patients' blank blood incubated in vitro with cisplatin alone (*white bar*) or with paclitaxel + cisplatin (*black bar*; $n = 6$), *V* Blood collected prior to (*white bar*) and at 3 (*black bar*) and 24 h (*shaded bar*, $n = 1$) after paclitaxel infusion and then incubated with cisplatin in vitro ($n = 3$)]

From eight patients, additional blank blood samples were collected, i.e., prior to any drug treatment. The samples were incubated in vitro with docetaxel (1 µg/ml), immediately followed by cisplatin (10 µg/ml), for 2 h at 37° C in a humidified atmosphere of 5% CO_2 in air. The level of cisplatin-DNA adducts in WBC and the concentrations of total and unbound cisplatin in the incubation tubes were measured by atomic spectroscopy (AAS) [6, 7, 9].

The intracellular cisplatin concentration in WBC was measured in blank whole-blood samples obtained from five patients and two healthy volunteers. Three 10-ml portions were incubated for 2 h with cisplatin (10 µg/ml). Prior to cisplatin, docetaxel (1 µg/ml) was added to one portion; paclitaxel (1 µg/ml), to the second; and the third served as a control. WBC were collected after the incubation. The remaining red blood cells were lysed by the addition of 30 ml buffer [0.83% NH_4Cl, 0.1% $KHCO_3$, 1 m*M* ethylenediaminetetra- acetic acid (EDTA)]. The WBC were washed twice with ice-cold phosphate-buffered saline and lysed in 1 ml deionized water. The protein was quantitated by the Lowry method [5]. After evaporation at 70°C, the sample was digested by nitric acid (65%) at 75° C for 2 h. The platinum (Pt) concentration was measured by AAS. Student's paired and unpaired two-sided *t*-tests were used for data analysis.

Results and discussion

The DNA-adduct levels determined in vitro after treatment of blank blood with cisplatin in the 22 patients receiving the combination were 12.1 ± 3.7 and 3.8 ± 1.6 pg Pt/µg DNA before and after docetaxel infusion, respectively ($p < 0.0001$; Fig. 1, Table 1). In the six patients treated with single-agent docetaxel, DNA-adduct levels measured pretreatment were 10.0 ± 2.0 pg Pt/µg DNA, those determined at 3 h 3.7 ± 1.0 pg Pt/µg DNA ($P < 0.0001$ as compared with preinfusion levels) and those measured at 24 h post infusion were 7.7 ± 2.6 pg Pt/µg DNA ($P = 0.01$ as compared with 3 h post infusion). In the three patients treated with paclitaxel, the DNA-adduct level was 6.8 ± 1.2 pg Pt/µg DNA before treatment and 2.8 ± 1.1 pg Pt/µg DNA at 3 h after the infusion ($P < 0.0001$). In one of these three patients from whom a blood sample was also collected at 24 h after the infusion the DNA-adduct level was 6.6 pg Pt/µg DNA before treatment and 3.4 and 6.1 pg Pt/µg DNA at 3 and 24 h postinfusion, respectively. These results indicate a reversible interaction. The 4- to 14-h plasma elimination half-lives of docetaxel and paclitaxel [1, 2, 12] indicate that the concentrations determined at 24 h after infusion were already substantially reduced, which suggests that the interaction is reversed at that time.

Incubation of WBC in vitro with docetaxel ($n = 8$) or paclitaxel ($n = 6$) and cisplatin resulted in the same magnitude of inhibition of DNA-adduct formation (Fig. 1). The intracellular concentrations of platinum in WBC were reduced by 46% (docetaxel) and 47% (paclitaxel), respectively, as compared with the control values ($P < 0.0001$).

If the pharmacodynamic interaction between docetaxel/paclitaxel and cisplatin also occurs in other

Table 1 Cisplatin-induced DNA adducts determined in WBC of patients treated with docetaxel in the sequence docetaxel/cisplatin or with single-agent docetaxel or paclitaxel. All blood samples were incubated in vitro with cisplatin (10 µg/ml). Blood samples were collected prior to (time 0) and at 3 h after docetaxel administration from the 22 patients receiving docetaxel/cisplatin and prior to (time 0) and at 3 and 24 h after the infusion of docetaxel and paclitaxel given as single agents

	Cisplatin-DNA adducts in WBC (pg Pt/µg DNA)			
	n	0	3 h	24 h
Docetaxel + cisplatin	22	12.1 ± 3.7	3.8 ± 1.6**	–
Docetaxel, single-agent	6	10.0 ± 2.0	3.7 ± 1.0**	7.7 ± 2.6*
Paclitaxel, single-agent	3	6.8 ± 1.2	2.8 ± 1.1**	6.1[a]

*$P = 0.01$ as compared with 3 h after infusion; **$P < 0.0001$ as compared with time 0

[a]Single observation (in this patient the level at 0 hours was 6.6 and at 3 hours 3.4 pg Pt/g DNA)

normal tissues such as bone marrow, it may well contribute to the sequence-dependent toxicity. This suggests that the drug schedule is an important determinant not only of activity but also of toxicity.

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References

1. Brown T, Havlin K, Weiss G, Cagnola J, Koeller J, Kuhn J, Rizzo J, Craig J, Phillips J, Von Hof D (1991) A phase I trial of taxol given by a 6-hour intravenous infusion. J Clin Oncol 9:1261
2. Extra JM, Rousseau F, Bruno R, Clavel M, Le Bail N, Marty M (1993) Phase I and pharmacokinetic study of taxotere (RP 56976; NSC 628503) given as a short intravenous infusion. Cancer Res 53:1037
3. Jekunen A, Christen R, Shalinsky D, Howell SB (1994) Synergistic interaction between cisplatin and taxol in human ovarian carcinoma cells in vitro. Br J Cancer 69:299
4. Lee KB, Parker RJ, Dabholkar M, Bostick-Bruton F, Reed E (1993) Taxol effect on cisplatin sensitivity and cisplatin cellular accumulation in human ovarian cancer cells. Proc Ann Assoc Cancer Res 34:2114
5. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the folin phenol reagent. J Biol Chem 193:265
6. Ma J, Verweij J, Kolker HJ, Ingen HE van, Stoter G, Schellens JHM (1994) Pharmacokinetic-dynamic relationship of cisplatin in vitro: simulation of an i.v. bolus and 3 h and 20 h infusion. Br J Cancer 69:858
7. Ma J, Verweij J, Planting AST, Boer-Dennert M de, Ingen HE van, Burg MEL van der, Stoter G, Schellens JHM (1995) Current sample handling methods for measurement of platinum-DNA adducts in leucocytes in man lead to discrepant results in DNA adduct levels and DNA repair. Br J Cancer 71:512
8. Ravdin PM, Burris SM, Cooke G, Fields SM, Smith LS, Peacock N, Elledge R, Von Hoff (1994) Phase II evaluation of taxotere (RP56976) as chemotherapy for anthracycline refractory metastatic breast cancer. Ann Oncol 5 [Suppl 5]:512
9. Reed E, Sauerhoff S, Poirier MC (1988) Quantitation of platinum-DNA binding after therapeutic levels of drug exposure – a novel use of graphite furnace spectrometry. At Spectrosc 9:93
10. Rowinsky EK, Gilbert MR, McGuire WP, Noe DA, Grochow LB, Forastiere AA, Ettinger DS, Lubejko BG, Clark B, Sartorius SE, Cornblatt DR, Hendricks (CB), Donehower RC (1991) Sequences of taxol and cisplatin: a phase I and pharmacologic study. J Clin Oncol 9:1692
11. Rowinsky EK, Onetto N, Canetta M, Arbuck G (1992) Taxol: the first of the taxanes, an important new class of antitumor agents. Semin Oncol 19:646
12. Rowinsky EK, Donehower RC (1993) The clinical pharmacology of paclitaxel (taxol). Semin Oncol 20:16
13. Schellens JHM, Ma J, Bruno R, Paccaly D, Vergniol JC, Planting AST, Beor-Dennert M de, Burg MEL van der, Stoter G, Verweij J (1994) Pharmacokinetics of cisplatin and taxotere (docetaxel) and WBC DNA-adduct formation of cisplatin in the sequence taxotere/cisplatin and cisplatin/taxotere in a phase I/II study in solid tumor patients. Proc Am Soc Clin Oncol 13:132