# SHORT COMMUNICATION

**Jianguo Ma · Jaap Verweij · Andre S.T. Planting**
**Herman J. Kolker · Walter J. Loos**
**Maureen de Boer-Dennert · Maria E.L. van der Burg**
**Gerrit Stoter · Jan H.M. Schellens**

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

**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 $0.54 \pm 0.4$ pg Pt/µg DNA for the sequence docetaxel/cisplatin and $1.21 \pm 0.34$ 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.

J. Ma · J. Verweij · A.S.T. Planting · H.J. Kolker · W.J. Loos · M. de Boer-Dennert · M.E.L. van der Burg · G. Stoter

J.H.M. Schellens (✉)
Laboratory of Experimental Chemotherapy and Pharmacology, Department of Medical Oncology, Rotterdam Cancer Institute, Dr. Daniel den Hoed Kliniek, P.O. Box 5201, 3008 AE Rotterdam, The Netherlands
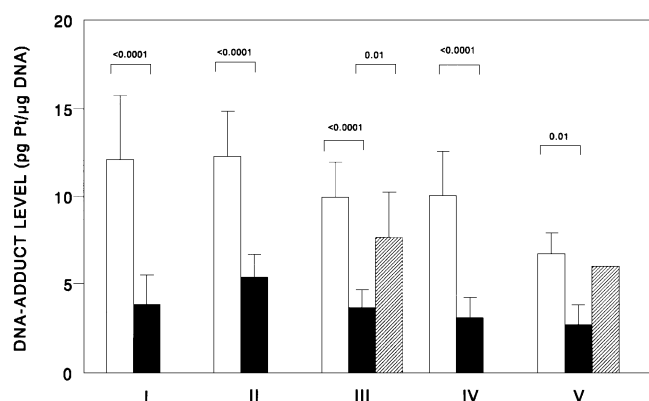
## Materials and methods

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 $\leq 1$ 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.

**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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