

## Plasma Thrombopoietin Levels are Unlikely to Account for the Platelet-sparing Effect of Paclitaxel in Lung Cancer Patients

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**Purpose:** The present study was designed to determine whether the combination of carboplatin (CBDCA) with paclitaxel (PTX) spared CBDCA-induced thrombocytopenia by increased plasma thrombopoietin (TPO) levels.

**Methods:** Patients with non-small-cell and small-cell lung cancer were consecutively assigned to CBDCA with PTX regimen (CBDCA/PTX) and CBDCA with irinotecan (CPT-11) regimen (CBDCA/CPT-11), respectively.

**Results:** Ten patients were entered into either CBDCA/PTX (n=5) or CBDCA/CPT-11 (n=5). CBDCA/PTX showed a lesser reduction of platelet counts than CBDCA/CPT-11 ( $p < 0.05$ ), although more severe neutropenia was observed in CBDCA/PTX ( $p < 0.01$ ). The plasma TPO levels were inversely correlated with circulating platelet counts in CBDCA/PTX and CBDCA/CPT-11. However, the increased rate of plasma TPO levels in CBDCA/PTX was not significantly different from that in CBDCA/CPT-11.

**Conclusions:** These findings suggest that the increased plasma TPO levels in CBDCA/PTX result secondarily from thrombocytopenia, and that circulating TPO is probably not involved in the platelet-sparing effect of PTX.

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**Key Words:** *thrombopoietin, carboplatin, paclitaxel, thrombocytopenia*

### Introduction

The combination chemotherapy of carboplatin (CBDCA) with paclitaxel (PTX) is one of the common regimens for advanced non-small cell lung cancer.<sup>1)</sup> CBDCA is a platinum agent, and inhibits cell growth by forming intrastrand DNA cross-links.<sup>2)</sup> PTX is an

anti-microtubule agent, and disturbs tubulin depolymerization.<sup>3)</sup> The dose-limiting toxicity of CBDCA is thrombocytopenia, and that of PTX is neutropenia accompanied by moderate thrombocytopenia. The combination of CBDCA with other myelosuppressive drugs usually enhances the severity of thrombocytopenia. However, the combination with PTX is reported to rather decrease the degree of CBDCA-induced thrombocytopenia.<sup>4–5)</sup> The precise mechanism of the platelet-sparing effect of PTX remains undetermined.

Thrombopoietin (TPO) is one of the main regulators of megakaryocytopoiesis, and TPO is constitutively produced in the liver and enters into the circulation.<sup>6–7)</sup> The plasma TPO levels are inversely related to circulating platelet counts in cancer patients receiving chemotherapy, suggesting that circulating platelets regulate the plasma TPO levels.<sup>8–9)</sup> Recombinant human TPO increases the circulating platelet counts, and reduces the need for platelet transfusion in cancer patients with CBDCA-induced thrombocytopenia.<sup>7,10)</sup> Thus, the present study was designed to determine whether the combination with PTX further increased the plasma TPO levels and spared CBDCA-induced thrombocytopenia. In order to distinguish PTX-induced TPO from TPO up-regulated by thrombocytopenia, we examined the relationship of plasma TPO levels and circulating platelet counts in lung cancer patients receiving CBDCA and PTX, compared to those receiving CBDCA and irinotecan (CPT-11). CPT-11 is a topoisomerase-I inhibitor, and the major toxicity also includes neutropenia accompanied by moderate thrombocytopenia.<sup>11)</sup>

### Patients and methods

#### *Patient selection*

Patients with histologically or cytologically documented lung cancer were candidates for this study.

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Patients with non-small-cell lung cancer were consecutively assigned to the combination therapy of CBDCA with PTX (CBDCA/PTX), and those with small-cell lung cancer to the combination therapy of CBDCA with CPT-11 (CBDCA/CPT-11). Due to the ethical difficulties, patients could not be allocated to the treatment with CBDCA alone or the combination with another drug. Since our phase II study has recently revealed that CBDCA/CPT-11 is promising for the treatment of small-cell lung cancer,<sup>12)</sup> CBDCA/CPT-11 was selected as the control.

Eligibility criteria were as follows: Stage IIIB or IV disease without the indication of radiation therapy; age below 75 years; performance status of 2 or better; no prior chemotherapy or radiation therapy within 4 weeks; adequate hematopoietic function with neutrophil counts  $>2,000/\mu\text{L}$ , platelet counts  $>100,000/\mu\text{L}$ , and hemoglobin levels  $>9.0\text{g/dL}$ ; and normal hepatic and renal function. Specific exclusion criteria included bone marrow metastasis, uncontrolled brain metastasis, and massive pleural or pericardial effusion. All participants gave their written informed consent prior to the study.

#### *Treatment schedule*

Since the degree of CBDCA-induced thrombocytopenia is related to the area under the concentration time curve (AUC) of plasma CBDCA,<sup>9)</sup> the same target AUC of  $5\text{ mg}\cdot\text{min/mL}$  was selected in both the CBDCA/PTX and CBDCA/CPT-11 regimens. The actual dose of CBDCA was determined by multiplying  $5\text{ mg}\cdot\text{min/mL}$  by CBDCA clearance predicted by the Chatelut formula.<sup>13)</sup>

In the CBDCA/PTX regimen,  $210\text{ mg/m}^2$  of PTX was administered for 3 hours, followed by a 2-hour rest, and then CBDCA was given for 1 hour on day 1. Although the dose of PTX was  $225\text{ mg/m}^2$  when combined with CBDCA in the USA and European countries, the maximum dose of PTX approved by the Japanese Government was  $210\text{ mg/m}^2$ .<sup>14)</sup> Patients received premedication with 20 mg of intravenous dexamethasone 14 and 6 hours before PTX infusion. Additional premedication included 50 mg of oral diphenhydramine and 50 mg of intravenous ranitidine before PTX infusion. Each treatment cycle was 4 weeks.

In the CBDCA/CPT-11 regimen, CBDCA was administered on day 1, and  $50\text{ mg/m}^2$  of CPT-11 on days 1, 8 and 15. The dose of CPT-11 was determined based on our phase I study.<sup>15)</sup> CPT-11 was infused for 90 minutes, followed by a 2-hour rest, and then CBDCA was given for 1 hour on day 1. The administration of

CPT-11 on day 8 or 15 was canceled if leukocyte counts  $<3,000/\mu\text{L}$  or platelet counts  $<50,000/\mu\text{L}$  were observed, or if any grade of diarrhea or fever  $>38^\circ\text{C}$  developed. Each treatment cycle was 4 weeks. When the circulating neutrophil counts were less than  $500/\mu\text{L}$ , recombinant human granulocyte colony stimulating factor (rhG-CSF) was administered subcutaneously in both the CBDCA/PTX and CBDCA/CPT-11 regimens.

#### *Sample collection*

Blood samples for measuring the plasma TPO levels and platelet counts were collected in each EDTA-containing tube on days 1, 4, 8, 15, and 22 in the first cycle of chemotherapy. For the plasma TPO levels, plasma was immediately separated by centrifugation at 1,500 rpm for 10 minutes, and frozen at  $-20^\circ\text{C}$  until use. The plasma TPO levels were measured by enzyme-linked immunosorbent assay.<sup>16)</sup> Platelet counts, total leukocyte counts, and differential cell counts were determined with an automated hematology analyzer (NE-8000, Sysmex Corporation, Kobe, Japan).

#### *Statistical analysis*

The plasma TPO levels were log-transformed for normalization. The difference of neutrophil counts, platelet counts, and plasma TPO levels between the two groups was evaluated by the Student's *t*-test. The relationship of platelet counts and plasma TPO levels was evaluated by regression analysis. The difference of the regression lines between the two groups was determined by analysis of covariance. Two-tailed  $p < 0.05$  was considered significant. Data were analyzed with the StatView software program (version 5.0; SAS Institute Inc., Cary, NC).

## **Results**

Ten patients were entered into either CBDCA/PTX ( $n=5$ ) or CBDCA/CPT-11 ( $n=5$ ) (Table 1). All ten patients were assessable for plasma TPO levels and circulating platelet counts. There was no difference of sex-, age-distribution, performance status, circulating neutrophil counts, and platelet counts before the treatment. The CBDCA/CPT-11 group included more patients with stage IV disease because of small-cell lung cancer.

CBDCA/PTX showed lower nadir neutrophil counts than CBDCA/CPT-11 (Figure 1A,  $p < 0.01$ ). Contrary to the myelosuppression, however, CBDCA/PTX showed a lesser reduction of platelet counts than CBDCA/

**Table 1.** Patient characteristics

	CBDCA/PTX	CBDCA/CPT-11
No. of patients	5	5
Sex		
male/female	5/0	4/1
Median age (range)	61 (49-70)	65 (52-70)
Performance status		
1/2/3/4	4/1/0/0	3/2/0/0
Stage		
IIIB/IV	4/1	1/4
Pretreatment counts		
Neutrophil counts ( $\mu\text{L}$ )	4,352 $\pm$ 1,451	3,554 $\pm$ 1,390
Platelet counts ( $\times 10^3/\mu\text{L}$ )	289 $\pm$ 98	258 $\pm$ 94

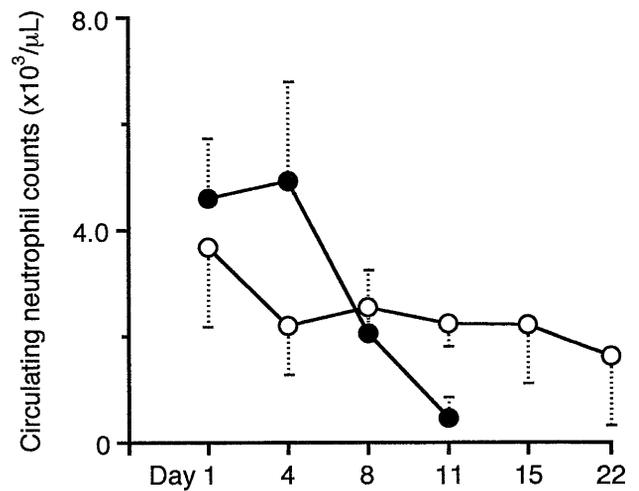
CBDCA, carboplatin; PTX, paclitaxel; CPT-11, irinotecan

CPT-11 (Figure 1B,  $p < 0.05$ ). There was no documented infection or platelet transfusion in the two groups.

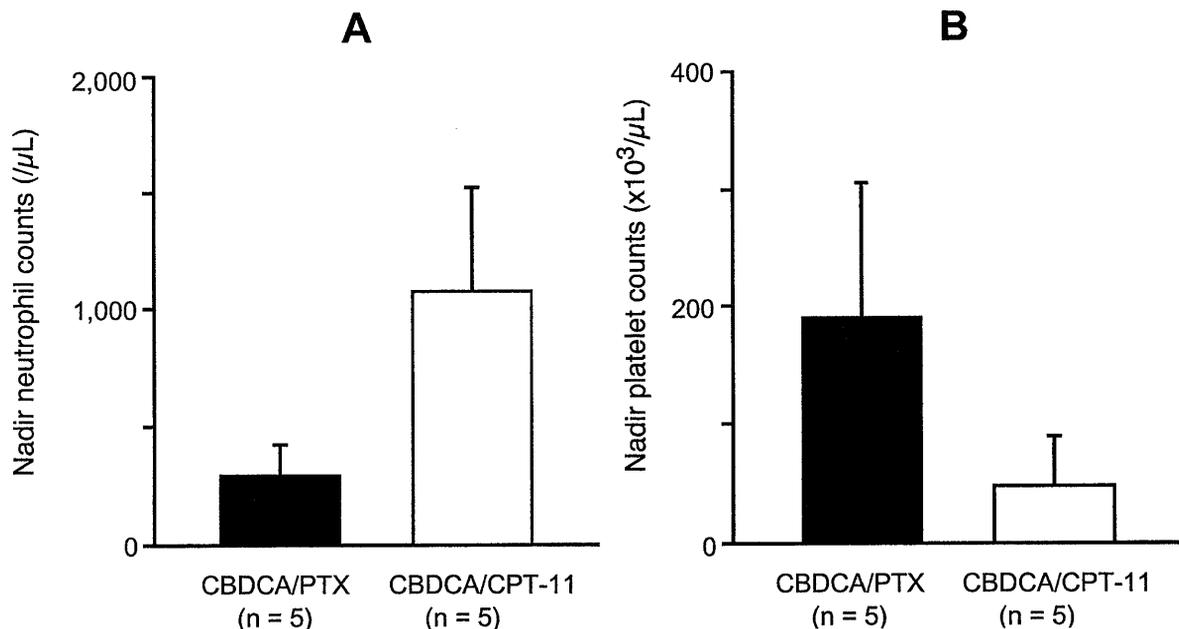
Serial changes in the neutrophil counts are shown in Figure 2. The neutrophil counts were decreased from day 4 in CBDCA/CPT-11, and were decreased from day 8 in CBDCA/PTX. The nadir of neutropenia occurred from day 19 to day 22 (median, day 21) in CBDCA/CPT-11, and that occurred from day 11 to day 15 (median, day 11) in CBDCA/PTX. Two of five patients were treated with G-CSF in CBDCA/CPT-11. In contrast, all of five patients were treated with G-CSF in CBDCA/PTX.

Serial changes in the platelet counts and plasma TPO levels were further examined. Between days 1

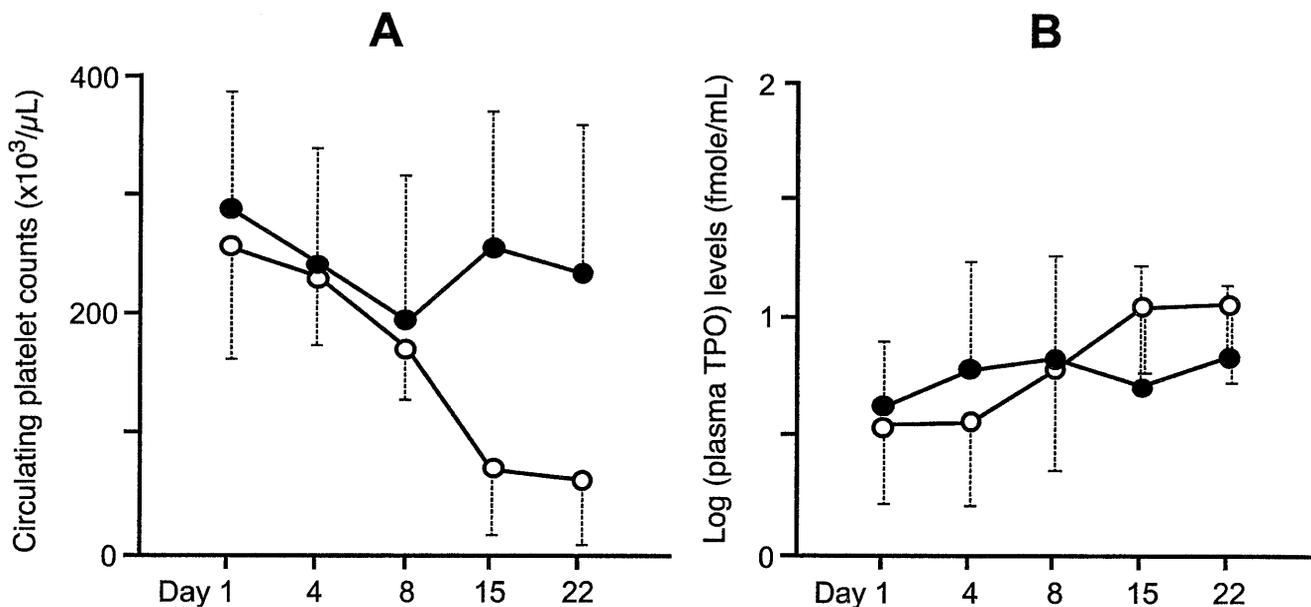
and 8, the platelet counts were similarly decreased in both CBDCA/PTX and CBDCA/CPT-11 (Figure 3A). On day 15, the platelet counts remained constant in CBDCA/PTX, and were higher than in CBDCA/CPT-11 ( $p < 0.01$ ). In contrast, the plasma TPO levels were increased after the chemotherapy, but the increase was not different between the two groups (Figure 3B).



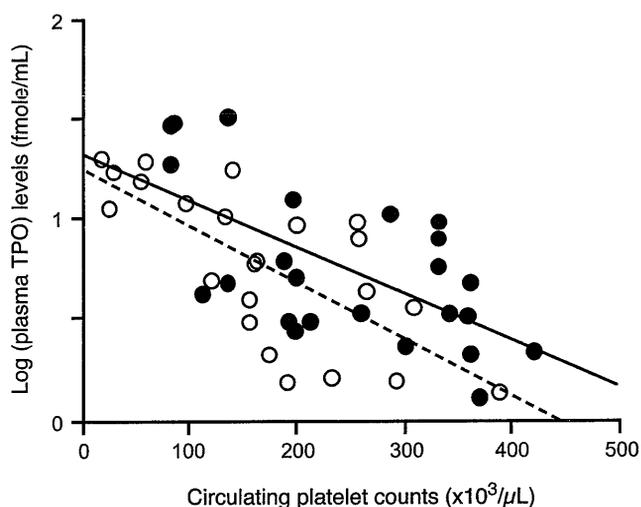
**Figure 2.** Serial changes in the circulating neutrophil counts after the chemotherapy. The closed circles represent the data for the combination of carboplatin with paclitaxel, and the open circles for the combination of carboplatin with irinotecan. The vertical lines show a standard deviation.



**Figure 1.** The nadir counts of circulating neutrophils (A) and platelets (B) after the chemotherapy. The closed bars represent the data for the combination of carboplatin with paclitaxel (CBDCA/PTX), and the open bars for the combination of carboplatin with irinotecan (CBDCA/CPT-11). The vertical lines showed a standard deviation. CBDCA/PTX showed more intense neutropenia ( $p < 0.05$ ) but less severe thrombocytopenia ( $p < 0.01$ ), compared to CBDCA/CPT-11.



**Figure 3.** Serial changes in the circulating platelet counts (A) and plasma thrombopoietin (TPO) levels (B) after the chemotherapy. The closed circles represent the data for the combination of carboplatin with paclitaxel, and the open circles for the combination of carboplatin with irinotecan. The vertical lines show a standard deviation.



**Figure 4.** The relationship between circulating platelet counts and plasma thrombopoietin (TPO) levels. The closed circles and solid line represent the data and the regression line for the combination of carboplatin with paclitaxel. The open circles and broken line show the data and the regression line for the combination of carboplatin with irinotecan. The plasma TPO levels were inversely correlated with the circulating platelet counts in each treatment. There was no significant difference between the two regression lines.

The relationship of platelet counts and plasma TPO levels is shown in Figure 4. The plasma TPO levels were inversely correlated with the circulating platelet counts in patients treated with CBDCA/PTX ( $r=-0.629$ ,  $p<0.001$ ) and those receiving CBDCA/CPT-11 ( $r=-0.714$ ,  $p<0.001$ ). There was no significant difference of the regression lines between the two groups.

**Discussion**

The present study demonstrated that the combination with PTX reduced the severity of CBDCA-induced thrombocytopenia, although this combination resulted in more intense neutropenia. Following CBDCA/PTX therapy, the plasma TPO levels were increased inversely proportional to the circulating platelet counts. As shown in Figure 3, however, the increased rate of plasma TPO levels was not different from that of CBDCA/CPT-11 therapy with more intense thrombocytopenia. This finding suggests that the increased plasma TPO results from the up-regulation response to thrombocytopenia, and that circulating TPO is probably not involved in the platelet-sparing effect of PTX.

Several studies have revealed that the combination with PTX provides a protective effect against CBDCA-induced thrombocytopenia.<sup>4-5)</sup> A mathematical model shows that patients receiving CBDCA/PTX experience

less severe thrombocytopenia, compared to historical controls receiving CBDCA alone.<sup>4)</sup> In another study, thrombocytopenia was significantly less in patients treated with intraperitoneal CBDCA and intravenous PTX infusion, compared to those with intraperitoneal CBDCA alone.<sup>5)</sup> In these studies, PTX does not affect the pharmacokinetics of CBDCA. A possible explanation for the platelet-sparing effect of PTX includes the induced production of hematopoietic factors and the enhanced survival of platelet precursors.

Gene-targeting studies have established that TPO and stem-cell factor play an important role in megakaryocyte production.<sup>7)</sup> Patients treated with CBDCA/PTX are reported to show an elevation of serum TPO levels but not stem-cell factor.<sup>17)</sup> Our study suggests that the increase in plasma TPO levels is secondarily due to thrombocytopenia, and that circulating TPO is probably not involved in the platelet-sparing effect of PTX. However, PTX is reported to induce the release of TPO from normal marrowstromal cells *in vitro*.<sup>17)</sup> Although the majority of TPO is produced in the liver and enters into the circulation,<sup>6)</sup> our results cannot exclude the possible roles of local hematopoietic factors in the bone marrow microenvironment.

Recent studies have showed that megakaryocytic cells are resistant to the cytotoxicity of CBDCA/PTX *in vitro*.<sup>17-18)</sup> The megakaryocytic progenitors derived from normal bone marrow are likely to be resistant to CBDCA/PTX, compared to the erythroid or myeloid progenitors.<sup>17)</sup> Furthermore, CBDCA and PTX show an antagonistic effect on survival in megakaryoblastic leukemia cells, and the intracellular CBDCA levels are not different in the presence or absence of PTX.<sup>18)</sup> The platelet-sparing effect is probably mediated by alternative mechanisms rather than circulating TPO, and further investigations are needed to clarify the cytoprotection of platelet progenitors.

## Conclusion

Our study demonstrated that PTX reduced the severity of CBDCA-induced thrombocytopenia in contrast to CPT-11, and that the platelet-sparing effect did not result from the increased plasma TPO levels.

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