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

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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.


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.1) CBDCA is a platinum agent, and inhibits cell growth by forming intrastrand DNA cross-links.2) PTX is an anti-microtubule agent, and disturbs tubulin depolymerization.2) 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.4–5) 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.1–3) 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.4–9) Recombinant human TPO increases the circulating platelet counts, and reduces the need for platelet transfusion in cancer patients with CBDCA-induced thrombocytopenia.7–10) 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.10

Patients and methods

Patient selection

Patients with histologically or cytologically documented lung cancer were candidates for this study.
Patients with non-small-cell lung cancer were consecu-
tively 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 diffi-
culties, patients could not be allocated to the treat-
ment with CBDCA alone or the combination with an-
other drug. Since our phase II study has recently
revealed that CBDCA/CPT-11 is promising for the
treatment of small-cell lung cancer, 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/μL, platelet counts≥100,000/μL, and he-
matocrit levels≥9.0g/dL; and normal hepatic and
renal function. Specific exclusion criteria included
bone marrow metastasis, uncontrolled brain meta-
tasis, 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, the same target AUC
of 5 mg·min/mL was selected in both the CBDCA/
PTX and CBDCA/CPT-11 regimens. The actual dose
of CBDCA was determined by multiplying 5 mg·min/
ML by CBDCA clearance predicted by the Chatelut
formula.

In the CBDCA/PTX regimen, 210 mg/m² 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 mg/m² when com-
bined with CBDCA in the USA and European coun-
tries, the maximum dose of PTX approved by the
Japanese Government was 210 mg/m². Patients re-
ceived 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 admin-
istered on day 1, and 50 mg/m² of CPT-11 on days 1,
8 and 15. The dose of CPT-11 was determined based
on our phase I study. 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 co-
unts<3,000/μL or platelet counts<50,000/μL were
observed, or if any grade of diarrhea or fever > 38°C
developed. Each treatment cycle was 4 weeks. When the
circulating neutrophil counts were less than 500 / μL,
recombinant human granulocyte colony stimulating fac-
tor (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°C until
use. The plasma TPO levels were measured by en-
zyme-linked immunosorbent assay. Platelet counts,
total leukocyte counts, and differential cell counts
were determined with an automated hematology ana-
lyzer (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 pa-
tients were assessable for plasma TPO levels and cir-
culating platelet counts. There was no difference of
sex-, age-distribution, performance status, circulating
neutrophil counts, and platelet counts before the treat-
ment. The CBDCA/CPT-11 group included more pa-
tients 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

<table>
<thead>
<tr>
<th></th>
<th>CBDCA/PTX</th>
<th>CBDCA/CPT-11</th>
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<tr>
<td>No. of patients</td>
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<td>5</td>
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<tr>
<td>Sex</td>
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<td>4/1</td>
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<tr>
<td>Median age (range)</td>
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<td>65 (52-70)</td>
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<tr>
<td>Performance status</td>
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<td>3/2/0/0</td>
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<tr>
<td>Stage</td>
<td>III/IV</td>
<td>4/1/1/4</td>
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<tr>
<td>Pretreatment counts</td>
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<td>Neutrophil counts (μL)</td>
<td>4,352 ± 1,451</td>
<td>3,554 ± 1,390</td>
</tr>
<tr>
<td>Platelet counts (x10^3/μL)</td>
<td>289 ± 98</td>
<td>258 ± 94</td>
</tr>
</tbody>
</table>

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.
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 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.\(^4_{-5}\) A mathematical model shows that patients receiving CBDCA/PTX experience...
less severe thrombocytopenia, compared to historical controls receiving CBDCA alone. In another study, thrombocytopenia was significantly less in patients treated with intraperitoneal CBDCA and intravenous PTX infusion, compared to those with intraperitoneal CBDCA alone. 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. Patients treated with CBDCA/PTX are reported to show an elevation of serum TPO levels but not stem-cell factor. 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. Although the majority of TPO is produced in the liver and enters into the circulation, 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. The megakaryocytic progenitors derived from normal bone marrow are likely to be resistant to CBDCA/PTX, compared to the erythroid or myeloid progenitors. 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. 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.

### References