Unexpected Hepatotoxicities in Patients with Non-Hodgkin's Lymphoma Treated with Irinotecan (CPT-11) and Etoposide

Tomoko Ohtsu¹, Yasutsuna Sasaki¹, Tadahiko Igarashi¹, Tohru Murayama², Yukio Kobayashi² and Kensei Tobinai²

¹Division of Hematology/Oncology, Department of Medicine, National Cancer Center Hospital East, Kashiwa, Chiba and ²Department of Medical Oncology, National Cancer Center Hospital, Tokyo, Japan

Background: Irinotecan (CPT-11) is a topoisomerase I inhibitor that has been confirmed to be active against a broad spectrum of neoplasms including non-Hodgkin's lymphoma (NHL). Because the combination of topoisomerase I and II inhibitors seemed to be an attractive therapeutic strategy owing to their complementary functions, we conducted a combination phase I study of CPT-11 and etoposide, a topoisomerase II inhibitor, in relapsed or refractory non-Hodgkin's lymphoma (NHL).

Methods: The starting doses of CPT-11 and etoposide were 30 mg/m²/day (days 1-3 and 8-10) and 40 mg/m² (days 1-3), respectively.

Results: All three patients who received the starting dose developed dose-limiting toxicities including one case of grade 4 neutropenia lasting for >7 days, one of grade 3 serum transaminase elevation and one of grade 3 hyperbilirubinemia. All three patients presented hepatotoxicity >grade 2. The starting dose level was judged to be the maximum tolerated dose (MTD) and further dose escalation of this combination was halted. The patient who developed grade 3 hyperbilirubinemia presented a second peak of plasma SN-38, an active metabolite of CPT-11, on the concentration–time curve for day 3, suggesting the possibility of the enterohepatic circulation of SN-38 and of a drug-to-drug interaction. No durable objective response was observed in the three patients treated at the starting dose.

Conclusions: We conclude that etoposide is not recommended for combination with CPT-11 in NHL patients because of unexpected frequent hepatotoxicities.

Key words: CPT-11 – etoposide – non-Hodgkin’s lymphoma – hepatotoxicity

INTRODUCTION

Irinotecan (CPT-11) (7-ethyl-10-[4-(1-piperidino)-1-piperidinol] carbonyloxy camptothecin) is a topoisomerase I inhibitor that has shown anti-tumor activity against a broad spectrum of neoplasms including non-Hodgkin’s lymphoma (NHL) (1–5). Early and late phase II studies of CPT-11 obtained a high response rate of 38% (21/56) by relapsed or refractory NHL patients on a schedule of daily infusions of 40 mg/m²/day for three or five consecutive days, in contrast to no response to a single infusion of 200 mg/m² every 3–4 weeks (4,5). In the treatment of NHL, combination chemotherapy rather than mono-chemotherapy has usually been performed and has shown higher efficacy in most cases. We previously conducted a combination phase I-II study of CPT-11 and carboplatin (6). Six of eight patients who received the starting doses of CPT-11 (20 mg/m², days 1–3 and 8–10) and carboplatin (300 mg/m², day 1) experienced critical toxicities and the starting doses were judged to be the maximum tolerated dose (MTD). Because the response rate (25%, 2/8) to the combination was not superior to that of CPT-11 alone, it was concluded that the combination was not recommended and that other suitable agents for a combination with CPT-11 should be sought.

The combination of topoisomerase I and II inhibitors seems to be an attractive therapeutic strategy because of the complementary functions of these agents. Several investigators have reported a lack of cross-resistance between the camptothecins and topoisomerase II inhibitors and that CPT-11 enhances the antitumor activity of topoisomerase II inhibitors (7–10). Etoposide, a topoisomerase II inhibitor, has been shown to be effective...
for malignant lymphoma. A phase II study of intravenous etoposide for NHL showed a response rate of 48% (19/40) (11). Hence a combination of CPT-11 and etoposide could be an effective salvage therapy for relapsed and refractory NHL patients.

The aims of the present study were to determine the MTD of CPT-11 and etoposide in combination for the treatment of relapsed and refractory NHL, to observe the therapeutic efficacy of this regimen and to analyze the pharmacokinetics of this combination.

PATIENTS AND METHODS

Patients

Relapsed or refractory lymphoma patients who had received standard chemotherapeutic regimens were eligible if they met the following criteria: (a) they had histologically and/or cytologically confirmed NHL; (b) they had NHL refractory to standard chemotherapy or had relapsed after attaining complete remission (CR) or showed disease progression after attaining partial remission (PR); (c) the presence of measurable disease; (d) no chemotherapy or irradiation within 2 weeks; (e) life expectancy of at least 2 months; (f) 15 years of age or older and younger than 75 years; (g) performance status (PS) two or better on the Eastern Cooperative Oncology Group Scale; (h) adequate bone marrow function (leukocyte count ≥3000/µl, neutrophil count ≥1200/µl, hemoglobin ≥8.0 g/dl, platelet count ≥100 000/µl), adequate hepatic function (bilirubin ≤2.0 mg/dl, transaminases ≤2.5 times the upper limits of normal), adequate renal function (creatinine ≤1.5 mg/dl, creatinine clearance ≥50 ml/min) and adequate pulmonary function (PaO2 ≥70 mmHg); (i) no severe complications; (j) no active double cancer; (k) serum negativity for anti-hepatitis B virus, hepatitis C virus and human immunodeficiency virus antibodies; (l) they provided written informed consent. The study was approved by the Institutional Review Board of the National Cancer Center, Japan.

Administration and Evaluation

CPT-11 was provided by Yakult Honsha and Daiichi Pharmaceutical Co., Tokyo, Japan. CPT-11 was dissolved in 250 ml of 5% glucose and administered as a 90 min intravenous infusion daily on days 1–3 and 8–10. Etoposide was dissolved in 250 ml of normal saline or 5% glucose and administered as a 60 min intravenous infusion daily on days 1–3 immediately after the end of CPT-11 infusion. The starting doses of CPT-11 and etoposide were 30 mg/m²/day and 40 mg/m² day, respectively. The dose escalation scheme of the combination is shown in Table 1. The treatment was planned to be repeated every 28 days, provided that patients did not develop progressive disease or critical toxicities. The dose-limiting toxicities (DLTs) defined in this study were any of the following: (a) grade 4 leucopenia and/or neutropenia lasting for more than 4 days, (b) grade 4 leucopenia and/or neutropenia complicated with fever (≥38°C), (c) grade 4 thrombocytopenia, (d) non-hematological toxicity of grade 3 or more except for nausea and vomiting and (e) delay of CPT-11 administration at days 8–10 for more than 7 days, according to the toxicity grading criteria of the Japan Clinical Oncology Group (12), which is an expanded and modified version of the National Cancer Institute Common Toxicity Criteria. To avoid the occurrence of severe toxicities, the administration of CPT-11 in the second week (days 8–10) was delayed until recovery if one of the following toxicities was noted on day 8: (a) leucopenia and/or neutropenia of grade 3 or more, (b) thrombocytopenia of grade 2 or more or (c) diarrhea of grade 2 or more. Granulocyte colony-stimulating factor (G-CSF) was given daily when the neutrophil count decreased to less than 1000/µl. Patients received 3 mg of granisetron intravenously before each administration of the anticancer drug. We planned to enter at least three patients at each dose level and the MTD was defined as the dose level at which the DLTs were observed during the first course in all three of patients or three or more of six patients. Tumor response was assessed according to the World Health Organization criteria (13).

PHARMACOKINETIC STUDY

Plasma for the pharmacokinetic evaluation of CPT-11, SN-38 (7-ethyl-10-hydroxycamptothecin) (an active metabolite of CPT-11), SN-38G (SN-38 glucuronide) and etoposide was collected from all patients in their first course. Heparinized blood samples were obtained before the administration of CPT-11 on days 1–3 and at the following times: at 0.5, 1, 1.5, 2, 2.5, 3, 5, 7 and 9 h from the start of the CPT-11 administration on day 1 and at 0.5, 1, 1.5, 2, 2.5, 3, 5, 7, 9, 24 and 48 h from the start of CPT-11 administration on day 3. Immediately separated plasma was frozen at −80°C until assayed. The plasma levels of CPT-11 and SN-38 were determined using the method reported by Kaneda et al. (14) with high-performance liquid chromatography. The SN-38G concentrations were determined using a modified assay method for CPT-11 (13). Pharmacokinetic parameters were obtained by a non-compartmental moment method. The maximum plasma concentration (Cmax) was the actually observed peak concentration. The area under the plasma concentration–time curve (AUC) and the mean residence time (MRT) were computed by trapezoidal integration with extrapolation to infinite time.

Table 1. Dose escalation scheme of the combination phase I study with CPT-11 and etoposide for relapsed or refractory NHL

<table>
<thead>
<tr>
<th>Level</th>
<th>CPT-11 (mg/m²/day)</th>
<th>Etoposide (mg/m²/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>30</td>
<td>40</td>
</tr>
<tr>
<td>2</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>3</td>
<td>40</td>
<td>60</td>
</tr>
<tr>
<td>4</td>
<td>40</td>
<td>80</td>
</tr>
<tr>
<td>5</td>
<td>40</td>
<td>100</td>
</tr>
</tbody>
</table>

The treatment was planned to be repeated every 4 weeks.
Hepatotoxicity of CPT-II and etoposide

Table 2. Characteristics of the NHL patients who were entered into the phase I study of CPT-II and etoposide

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age/gender</th>
<th>PS</th>
<th>Histology (immunophenotype)</th>
<th>Prior therapy (No. of regimens)</th>
<th>Disease status</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>64/M</td>
<td>0</td>
<td>Follicular large cell (B)</td>
<td>CHOP (1)</td>
<td>Relapsed after CR</td>
</tr>
<tr>
<td>2</td>
<td>60/M</td>
<td>1</td>
<td>Diffuse large cell (B)</td>
<td>CHOP, irradiation (1)</td>
<td>Progressed after PR</td>
</tr>
<tr>
<td>3</td>
<td>57/M</td>
<td>1</td>
<td>Follicular mixed cell (B)</td>
<td>COP, irradiation (1)</td>
<td>Relapsed after CR</td>
</tr>
</tbody>
</table>

PS, performance status; M, male; CHOP, cyclophosphamide (CPA) + doxorubicin (DOX) + vincristine (VCR) + prednisolone (PSL); COP, CPA + VCR + PSL; CR, complete remission; PR, partial remission.

Table 3. Toxicity grades and response of three NHL patients who received CPT-II (30 mg/m²/day, days 1-3 and 8-10) and etoposide (40 mg/m², days 1-3)

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Administered doses (mg/m²) at the 1st/2nd course</th>
<th>Grade of hematological toxicities (grade at the 1st/2nd course)</th>
<th>Non-hematological toxicities (grade at the 1st/2nd course)</th>
<th>Response</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CPT-II</td>
<td>Etoposide</td>
<td>WBC</td>
<td>Neu</td>
</tr>
<tr>
<td>1</td>
<td>30 x 6/30 x 6</td>
<td>40 x 3/40 x 3</td>
<td>3/4</td>
<td>4*7/4</td>
</tr>
<tr>
<td>2</td>
<td>30 x 3/30 x 3</td>
<td>40 x 3/20 x 3</td>
<td>3/3</td>
<td>3/3</td>
</tr>
<tr>
<td>3</td>
<td>30 x 3/-</td>
<td>40 x 3/-</td>
<td>3/-</td>
<td>3/-</td>
</tr>
</tbody>
</table>

WBC, white blood cell; Neu, neutrophil; Hb, hemoglobin; PIt, platelet; T-Bil, total bilirubin; Alp, alkaline phosphatase; Cr, creatinine; NC, no change; NE, not evaluable. *Dose-limiting toxicity. †Grade 4 neutropenia lasting for more than 7 days.

Table 4. Pharmacokinetic parameters of CPT-II, SN-38 and etoposide on days 1 and 3 in the three study patients

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Day</th>
<th>CPT-II</th>
<th>SN-38</th>
<th>Etoposide</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>$T_{\text{max}}$ ($h$)</td>
<td>$C_{\text{max}}$ (µg/ml)</td>
<td>AUC (µg h/ml)</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>1.00</td>
<td>0.35</td>
<td>3.13</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1.00</td>
<td>0.41</td>
<td>2.70</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>1.00</td>
<td>0.38</td>
<td>2.56</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1.00</td>
<td>0.53</td>
<td>2.92</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>0.50</td>
<td>0.39</td>
<td>2.78</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1.00</td>
<td>0.66</td>
<td>3.58</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>0.83</td>
<td>0.37</td>
<td>2.82</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1.00</td>
<td>0.53</td>
<td>3.07</td>
</tr>
</tbody>
</table>

$C_{\text{max}}$, maximum plasma concentration; $T_{\text{max}}$, time from the start of the drug administration to $C_{\text{max}}$; AUC, area under the concentration versus time curve; MRT, mean residence time.

RESULTS

PATIENTS AND TOXICITIES

Between May and July 1996, three men with relapsed NHL (ages 64, 60 and 57 years) were entered in the study of the starting dose level (CPT-II 30 mg/m²/day, days 1–3 and 8–10 and etoposide 40 mg/m², days 1–3). The characteristics of the patients are listed in Table 2. Their histological subtypes of NHL were follicular mixed-cell, follicular large-cell and diffuse large-cell type, each according to the Working Formulation (15). Two patients had relapsed after complete remission and one had progressed after partial remission. All three had good performance status (0 or 1).
and normal liver function and had received only one prior chemotherapy regimen such as COP (cyclophosphamide + vincristine + prednisolone) and CHOP (COP + doxorubicin). As shown in Table 3, all three patients developed DLTs, including grade 4 neutropenia lasting for more than 7 days under the G-CSF administration (case 1), grade 3 elevation of hepatic transaminase (case 2) and grade 3 hyperbilirubinemia with severe general fatigue, ameliorated with dexamethasone administration (case 3). Case 1 also presented grade 2 hyperbilirubinemia. Mild to moderate diarrhea was seen in all patients. Two (cases 2 and 3) did not receive CPT-II in the second week because of grade 3 hepatotoxicities. Two patients (cases 1 and 2) showing some tumor shrinkage by the first cycle of the treatment received a subsequent CPT-II for 3 or 5 consecutive days produced incidences of 10% for mild hyperbilirubinemia and 20% for mild hepatic transaminase elevation (4). Intravenous etoposide administration is reported to produce <20% mild hepatotoxicities (11,17). Several combinations of CPT-II with etoposide with different schedules have been reported, mainly for lung cancer. In two reports (21,22) where CPT-II and etoposide were administered on days 1–3 simultaneously, similarly to our protocol, only a few mild transaminase elevations (less than grade 3) (21) and 3/61 (4.9%) cases of liver toxicity (22) occurred. Most courses of those studies were given with prophylactic dexamethasone to reduce nausea and vomiting, suggesting a role of dexamethasone for the prevention of hepatic toxicity. In another study of a weekly administration of CPT-II combined with etoposide on days 1–3, grade 3 hepatotoxicity was observed in only 1/50 courses (2%) (23). The study reporting the highest frequency of hepatotoxicity was a phase I trial of a combination of CPT-II and etoposide given sequentially (24): CPT-II and etoposide were administered on days 1–3 or 4–6 consecutively by two different schedules, that is, one schedule was CPT-II followed by etoposide and the other vice versa. Grade 3 elevations of liver transaminases were not receive CPT-II in the second week because of grade 3 hepatotoxicities. Two patients (cases 1 and 2) showing some tumor shrinkage by the first cycle of the treatment received a subsequent CPT-II for 3 or 5 consecutive days produced incidences of 10% for mild hyperbilirubinemia and 20% for mild hepatic transaminase elevation (4). Intravenous etoposide administration is reported to produce <20% mild hepatotoxicities (11,17). Several combinations of CPT-II with etoposide with different schedules have been reported, mainly for lung cancer. In two reports (21,22) where CPT-II and etoposide were administered on days 1–3 simultaneously, similarly to our protocol, only a few mild transaminase elevations (less than grade 3) (21) and 3/61 (4.9%) cases of liver toxicity (22) occurred. Most courses of those studies were given with prophylactic dexamethasone to reduce nausea and vomiting, suggesting a role of dexamethasone for the prevention of hepatic toxicity. In another study of a weekly administration of CPT-II combined with etoposide on days 1–3, grade 3 hepatotoxicity was observed in only 1/50 courses (2%) (23). The study reporting the highest frequency of hepatotoxicity was a phase I trial of a combination of CPT-II and etoposide given sequentially (24): CPT-II and etoposide were administered on days 1–3 or 4–6 consecutively by two different schedules, that is, one schedule was CPT-II followed by etoposide and the other vice versa. Grade 3 elevations of liver transaminases were

**Pharmacokinetics**

The pharmacokinetic parameters of CPT-II, SN-38, SN-38G and etoposide on days 1 and 3 are shown in Table 4. With regard to CPT-II pharmacokinetics, the maximum plasma concentrations ($C_{\text{max}}$) of CPT-II, SN-38 and SN-38G were achieved just after, 2–4 h after and 3–3.5 h after the end of the CPT-II infusion on average, respectively. The mean parameters of CPT-II, SN-38 and etoposide on day 1 did not seem to be very different from the values of each drug used alone expected from the literature regarding the Japanese population (16,17). The mean AUCs of CPT-II, SN-38, SN-38G and etoposide were similar between days 1 and 3. Interestingly, case 3, who developed grade 3 hyperbilirubinemia, showed a second peak of SN-38 6 h after the end of the CPT-II infusion on day 3 (Fig. 1), suggesting the possibility of the enterohepatic circulation of SN-38 and of a potential drug-to-drug interaction in this patient. The biliary index ($BI$), which is considered to be correlated with the grade of diarrhea, was calculated according to the method of Gupta et al. (18) as follows: $BI = \frac{\text{AUC}_{\text{CPT-II}} \times \text{AUC}_{\text{SN-38G}}}{\text{AUC}_{\text{SN-38}}}$. The BIs of our patients were relatively low on both days 1 and 3, showing that the BI may not be associated with hepatotoxicity in combinations including etoposide. The grades of diarrhea in our patients were all less than grade 3 and the present data are compatible with the BIs in patients with lower grades of diarrhea reported previously (18).

**Discussion**

Hepatotoxicity by CPT-II has not been a subject of extensive discussion. According to Slichenmyer et al.’s review (19) regarding phase I and phase II studies of CPT-II, hepatotoxicity was referred as a non-dose-limiting adverse effect only when CPT-II was administered as a 5-day continuous infusion, which showed 1/36 (2.8%) grade 3 and 4/36 (11.8%) grade 2 hepatotoxicity (20). A schedule of daily 60 min infusions of CPT-II for 3 or 5 consecutive days produced incidences of 10% for mild hyperbilirubinemia and 20% for mild hepatic transaminase elevation (4). Intravenous etoposide administration is reported to produce <20% mild hepatotoxicities (11,17). Several combinations of CPT-II with etoposide with different schedules have been reported, mainly for lung cancer. In two reports (21,22) where CPT-II and etoposide were administered on days 1–3 simultaneously, similarly to our protocol, only a few mild transaminase elevations (less than grade 3) (21) and 3/61 (4.9%) cases of liver toxicity (22) occurred. Most courses of those studies were given with prophylactic dexamethasone to reduce nausea and vomiting, suggesting a role of dexamethasone for the prevention of hepatic toxicity. In another study of a weekly administration of CPT-II combined with etoposide on days 1–3, grade 3 hepatotoxicity was observed in only 1/50 courses (2%) (23). The study reporting the highest frequency of hepatotoxicity was a phase I trial of a combination of CPT-II and etoposide given sequentially (24): CPT-II and etoposide were administered on days 1–3 or 4–6 consecutively by two different schedules, that is, one schedule was CPT-II followed by etoposide and the other vice versa. Grade 3 elevations of liver transaminases were

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**Figure 1.** Plasma concentration-time curves for (A) CPT-II, (B) SN-38 and (C) SN-38G after the infusion of CPT-II in case 3, who presented grade 3 hyperbilirubinemia.
observed in 4/20 patients (20%). As reviewed here, hepatic toxicity seems to be frequently induced by 3–5 consecutive administrations of CPT-II alone or combination with etoposide. In the present study, no objective response was observed. The response rates to CPT-II in combination with etoposide for non-small cell lung cancer have been reported to be no greater than that to CPT-II alone (22–24). It might be possible that the low response rate is partly due to the decreased dose intensity of CPT-II when combined with etoposide.

In summary, the concurrent administration of CPT-II and etoposide is not recommended for the therapy of NHL because of unexpected hepatotoxicities. The possibility of pharmacodynamic interaction between these two agents should be investigated.

Acknowledgments

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