An Early Phase II Study of Etoposide (VP-16) in Advanced Gastric Cancer

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An early phase II study was conducted to evaluate the anti-tumor effects and toxicity of etoposide in patients with unresectable or relapsed advanced gastric cancer. From April 1991 to December 1992, 13 patients were enrolled into this study; one was subsequently considered ineligible. Before enrollment, all the patients had been treated with chemotherapy which did not include etoposide. Etoposide (100 mg/m²/day) was administered as an intravenous infusion over 120 min for five consecutive days and was repeated every four weeks. Seven patients received one course of this therapy and the remaining five received two. No patient showed a complete or a partial response. No change and progressive disease were observed in three and nine patients, respectively. The clinical toxicities (grade 3-4; WHO) of leukocytopenia, anemia and alopecia occurred in 50, 42, and 42% of the patients, respectively. We conclude that this dose of etoposide administered according to the present schedule is ineffective in previously treated patients with advanced gastric cancer.

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Key words: Etoposide (VP-16)—Early phase II study—Gastric cancer—Chemotherapy—Pharmacokinetics

Introduction

Gastric cancer is one of the most common neoplasms worldwide. Despite remarkable progress in its early detection and surgical treatment, gastric cancer still has the highest mortality rate of all malignancies in Japan. Since metastatic or recurrent advanced gastric cancer is a systemic disease, systemic chemotherapy should offer the best therapeutic outcome. Currently, however, there are few agents effective against gastric cancer; 5-fluorouracil (5-FU), mitomycin-C (MMC), doxorubicin (ADM), cisplatinum (CDDP), nitrosoureas and others, have yielded response rates of only around 10-25% in previous phase II studies.⁴⁻⁷

Etoposide (VP-16) is a semisynthetic podophyllotoxin derivative, which is believed to have anti-tumor activity by virtue of its inhibition of DNA synthesis. Podophyllotoxin and its derivatives have been shown to be antimitotic. Typically, they arrest cells in the G2 or S phase of the cell cycle, then inhibit DNA synthesis. The cytotoxic mechanism of etoposide is inhibition topoisomerase II, which is essential for normal DNA synthesis.⁴ Etoposide has undergone extensive clinical trials and has demonstrated anti-tumor activity against small-cell lung cancer, leukemia, lymphoma and ovarian and testicular cancers.⁴⁻⁷

In 1987, Preusser et al. reported a significantly high response rate in gastric cancer with the combination of etoposide, ADM and CDDP (EAP).⁷ Subsequently, EAP and other etoposide-containing regimens were tested and high response rates were observed. However, little information regarding the use of etoposide alone against advanced gastric cancer is available. Only one prior phase II trial of etoposide in patients with upper gastrointestinal tract malignancies, which included cancer of the esophagus, gastroesophageal junction, stomach and duodenum, conducted by Kelsen et al., has been reported.⁸ In their trial, etoposide (120 mg/m²/day) was administered intravenously on days 1, 3 and 5 every three weeks. Partial responses were seen in three of 36 patients with evaluable disease (8%). Two of the responders had gastric cancer and one had duodenal adenocarcinoma. None of the three
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Patients and Methods

Patients

Patients were entered into this study if they fulfilled the following eligibility criteria: (1) unresectable or recurrent advanced gastric cancer; (2) previous treatment with chemotherapy which did not include etoposide prior to enrollment; (3) an interval of over four weeks between the prior chemotherapy and enrollment into this study; (4) histological confirmation of gastric cancer; (5) measurable or evaluable disease; (6) performance status of 0, 1, 2, or 3 on the Eastern Cooperative Oncology Group (ECOG) scale; (7) age <75 years; (8) adequate bone marrow WBC >4000/mm$^3$, platelets >100,000/mm$^3$ and hemoglobin >8.0 g/dl), liver (serum bilirubin <1.5 mg/dl, serum transaminase ≤three times normal), and renal (serum creatinine ≤1.5 mg/dl) function and (9) no serious coexistent disease. The protocol was approved by the institutional review board of the National Cancer Center Hospital and all the patients gave their written informed consent.

Treatment Schedule

Etoposide (100 mg/m$^2$/day) was administered by intravenous infusion over 120 min for five consecutive days and was repeated every four weeks. Neither premedication for nausea and vomiting nor prophylactic granulocyte colony-stimulating factor for leukocytopenia were administered in this study and no dose reduction was planned. Etoposide administration was discontinued if disease progression or prolonged grade 4 side effects were observed and if no objective response was observed after two courses of treatment. The sample size of this study was determined according to Simon’s two-stage design. 93 Etoposide was kindly supplied by Nippon Kayaku Co., Tokyo, and Bristol-Myers Squibb Co., Tokyo.

Pharmacokinetic Study

Pharmacokinetic studies of three patients during first course of treatment were performed. Blood samples (5 ml) were collected from a forearm vein -2 h, and immediately before and 0.5, 1, 2, 4, 8 and 22 h after etoposide infusion on days 1 to 5, put immediately into heparinized tubes, spun down at 3000 rpm for 10 min and the plasma samples were stored at -20°C until drug assay. Part of each 24-h urine sample obtained on days 1 to 7 was also stored at -20°C until analysis. Etoposide was quantitated by high-performance liquid chromatography (HPLC) using a HPLC Auto Sampler System (Nippon Bunko, Tokyo), which included a Nucleosil 5C18 φ 4.6 mm ×25 cm column. The mobile phase consisted of a mixture of 30% (v/v) acetonitrile and 70% (v/v) sodium acetate buffer 10% (v/v), at a flow rate of 1.2 ml/min. The eluate was monitored using an 875-UV spectrophotometer (Nippon Bunko, Tokyo), detected at 290 nm and the retention time was 8.0 min. The limits of detection for plasma and urine were 0.1 and 0.3 μg/ml, respectively. The linearity of the calibration curve between 0.1 and 20 μg/ml was very good. The intra- and inter-day coefficients of variation were less than 0.8 and 5.3%, respectively. Plasma concentration (c) and time (t) data were analyzed using a non-compartmental method. The areas under the concentration versus time (AUC) and moment [AUMC = (c X t) versus t] curves were calculated using logarithmic and linear trapezoidal methods, respectively. The mean residence time (MRT) was calculated by the following equation: MRT = AUMC/AUC-T/2, where T is the duration of the etoposide infusion.

Evaluation of Response

The responses of measurable lesions other than the primary lesion were evaluated according to the World Health Organization (WHO) criteria. 10 The eligibility and suitability of the subjects for assessment and their responses to treatment were reviewed extramurally. A complete response (CR) was defined as the disappearance of all evidence of cancer for at least four weeks. A partial response (PR) was defined as a 50% or greater reduction in the sum of the products of the perpendicular diameters of all lesions for at least four weeks with no evidence of development of new lesions or progression of any lesions. No change (NC) was defined as a less than 50% reduction or less than 25% increase in the sum of the products of the perpendicular diameters of all lesions with no evidence of new ones. Progressive disease (PD) was defined as an increase in the diameter of one or more lesions of over 25% or the appearance of
new ones. The lesions were measured by CT scanning, ultrasonography and plain chest x-rays every four weeks. The responses of the primary lesion were evaluated according to the response assessment criteria of chemotherapy for gastric carcinoma outlined by the Japanese Research Society of Gastric Cancer, which are based on the macroscopic appearance on the x-ray and/or endoscopic films. The WHO criteria for clinical toxicity were also used to evaluate toxicity in this study. A complete blood cell count, liver function tests, renal function tests and urinalysis were performed at regular intervals. Serum tumor markers, including CEA and CA19-9, were also examined and an ECG was recorded before and after each treatment course.

**Results**

Between April 1991 and December 1992, 13 patients with metastatic adenocarcinoma of the stomach, all of whom had measurable or evaluable disease, were entered into this study. One, however, was considered ineligible due to the short interval between prior chemotherapy and entry into our study. The characteristics of the 12 remaining patients are shown in Table I. Their median age was 52 years (range, 40 to 69) and 10 of them (83%) had good performance status (0 or 1). The histological types of the primary lesions, according to the Japanese Classification of Gastric Cancer, were tubular adenocarcinoma in four patients, poorly differentiated adenocarcinoma in five and papillary adenocarcinoma, signet-ring cell carcinoma and mucinous adenocarcinoma in one each.

Nine patients had received surgical treatment before enrollment into this study. The prior chemotherapy regimens of the 12 patients are also shown in Table I. Nine had been pretreated with 5-FU and CDDP (FP-therapy) combination chemotherapy and the others had been treated with 5-FU or its derivative, either alone or with MMC or methotrexate (MTX). One patient had also been treated with irinotecan (CPT-11). Of the 11 patients whose objective responses to prior chemotherapy were evaluable, four (36%) had achieved PR with FP-therapy. We could not evaluate the response to prior chemotherapy of one patient who had been pretreated in another hospital.

Seven patients received only one course of treatment because their disease progressed and five received two courses and treatment was then discontinued because no responses were observed. The median dose administered was 500 mg/m² (range; 500–1000) and the median treatment interval was 29 days (range; 28–38). The objective responses of the 12 patients are listed in Table II. No patient had a CR or PR. NC occurred in three and the remain-
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ing nine showed PD.

The major adverse reactions were hematological toxicity, gastrointestinal toxicity and alopecia (Table III). Leukocytopenia occurred in 11 patients (91%); in six of whom it was grade 3, and decreased neutrophil counts occurred in all the patients and was grade 4 in seven. The time from the beginning of treatment to the WBC and neutrophil count nadirs was 14 days and nine days were needed for recovery. The median nadir WBC and neutrophil counts were 2300/mm$^3$ (range: 1000–5100) and 372/mm$^3$ (range, 185–1924), respectively. Anemia occurred in nine patients (75%), one of whom required a blood transfusion for severe anemia and the incidence of mild thrombocytopenia (grade 1 or 2) was 16% (two of 12 patients).

Alopecia was observed in nine patients (75%) and was grade 3 in five (42%).

Nausea and vomiting were observed in nine patients (75%), including one with grade 3 toxicity. These side effects appeared most often on the fifth day of treatment and persisted for an average of nine days. Vomiting, even when severe, which was uncommon, could be managed with metoclopramide. Grade 2 or 3 diarrhea and grade 1 stomatitis each occurred in two patients (17%).

The pharmacokinetic data for the three subjects tested are listed in Table IV. The AUC was 105.1±37.1 (mean±SD) µg·h/ml. For each patient, no significant differences between the values obtained on each of the five consecutive days and no tendency for the drug to accumulate was observed. The mean plasma half-life and MRT were 6.9±2.2 and 8.9±2.6 h, respectively. The proportion of the etoposide dose excreted as its unchanged form in the urine in 24 h was 35.4±18.3% and urinary excretion of etoposide did not increase with repeated administration.

Discussion

This early phase II study was undertaken to investigate the anti-tumor activity and toxicity of etoposide alone in patients with gastric cancer. All the patients enrolled into the present study had been treated previously, mainly with 5-FU-based regimens, but had not yet received etoposide. As the mode of action of etoposide differs from those of other agents, such as 5-FU, MMC and CDDP, commonly used to treat gastric cancer, the selection

<table>
<thead>
<tr>
<th>WHO grade</th>
<th>Incidence of grade 3 or 4 (%)</th>
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<tbody>
<tr>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Leukocytopenia</td>
<td>1</td>
</tr>
<tr>
<td>Neutrocytopenia</td>
<td>0</td>
</tr>
<tr>
<td>Anemia</td>
<td>3</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>10</td>
</tr>
<tr>
<td>Alopecia</td>
<td>3</td>
</tr>
<tr>
<td>Nausea/vomiting</td>
<td>3</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>10</td>
</tr>
<tr>
<td>Stomatitis</td>
<td>10</td>
</tr>
<tr>
<td>Hepatotoxicity</td>
<td>10</td>
</tr>
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</table>

Table IV. Pharmacokinetic Parameters during Administration of Etoposide at 100 mg/m$^2$/day for Five Consecutive Days

<table>
<thead>
<tr>
<th>Day</th>
<th>AUC (µg·h/ml)</th>
<th>$t^{1/2}$ (h)</th>
<th>MRT (h)</th>
<th>Renal excretion (% of dose)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P-1</td>
<td>P-2</td>
<td>P-3</td>
<td>P-1</td>
</tr>
<tr>
<td>Day 1</td>
<td>79.3</td>
<td>107.0</td>
<td>80.7</td>
<td>4.9</td>
</tr>
<tr>
<td></td>
<td>(89.0±15.6)</td>
<td>(6.5±2.4)</td>
<td>(7.5±3.0)</td>
<td>(8.7±2.2)</td>
</tr>
<tr>
<td>Day 2</td>
<td>91.2</td>
<td>140.0</td>
<td>105.0</td>
<td>4.3</td>
</tr>
<tr>
<td></td>
<td>(112.1±25.2)</td>
<td>(5.9±2.4)</td>
<td>(7.5±3.0)</td>
<td>(7.5±3.0)</td>
</tr>
<tr>
<td>Day 3</td>
<td>102.0</td>
<td>154.0</td>
<td>87.6</td>
<td>11.4</td>
</tr>
<tr>
<td></td>
<td>(114.5±34.9)</td>
<td>(8.1±3.2)</td>
<td>(9.7±3.6)</td>
<td>(9.7±3.6)</td>
</tr>
<tr>
<td>Day 4</td>
<td>101.0</td>
<td>129.0</td>
<td>94.6</td>
<td>8.6</td>
</tr>
<tr>
<td></td>
<td>(108.2±18.3)</td>
<td>(7.2±2.5)</td>
<td>(9.2±3.2)</td>
<td>(9.2±3.2)</td>
</tr>
<tr>
<td>Day 5</td>
<td>120.0</td>
<td>73.4</td>
<td>111.0</td>
<td>8.7</td>
</tr>
<tr>
<td></td>
<td>(101.5±24.7)</td>
<td>(7.1±1.7)</td>
<td>(9.5±2.5)</td>
<td>(9.5±2.5)</td>
</tr>
<tr>
<td>Day 6</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Day 7</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>3.7</td>
</tr>
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P, patient number; ND, no data; ( ), mean±SD.
criteria for patients in the present study seemed reasonable. The dose and schedule used were based on the results of phase I studies performed in Japan, in which the recommended dose of etoposide for a phase II study was 80–100 mg/m²/day for five consecutive days, and we adopted the maximum dose of 100 mg/m²/day.

In the present study, no objective responses were observed. Therefore, the etoposide dosage regimen used was considered ineffective, at least in previously treated but etoposide-naïve patients with gastric cancer. The anti-tumor activity of etoposide in previously untreated patients with gastric cancer remains uncertain. When interpreting the present results, it should be borne in mind that the activity of a drug may be underestimated when patients who have previously undergone chemotherapy are studied. However, in view of the modest activity observed in Kelsen’s study and the negative result in ours, the activity of etoposide against gastric cancer, if any, appears to be quite low. As with other drugs, the mechanism of resistance to etoposide is considered likely to be multifactorial. A possible explanation for etoposide resistance in vitro involves the expression of P-glycoprotein encoded by the MDR1 gene. P-glycoprotein is a membrane glycoprotein, which acts as an energy-dependent drug-efflux pump for various anti-tumor agents in multi-drug-resistant cells. Recent data have suggested that the intrinsic insensitivity of gastric cancer to chemotherapy can be explained, at least in part, by P-glycoprotein expression. The resistance to etoposide may also be the result of reduced topoisomerase II concentrations or the presence of an altered form of topoisomerase II in the tumor cells. Further studies are needed to elucidate the mechanism of the clinical resistance of gastric cancer to etoposide.

Significant toxicity, particularly myelosuppression, was observed in the present study. Half of the patients experienced grade 3 leukocytopenia, and 58% showed grade 4 neutrocytopenia. Furthermore, alopecia also occurred frequently. Although several etoposide-containing combination regimens have been reported to yield high response rates, they have also been associated with significant toxicity. Preusser et al. first reported a high response rate of 64% with the EAP regimen. However, later follow-up studies yielded disappointing results, with lower efficacy and higher toxicity, including treatment-related death. Therefore, the need for etoposide in combination chemotherapy for gastric cancer appears questionable, in view of its low activity and high toxicity as a single agent administered by standard intravenous infusion. The use of etoposide in the treatment of gastric cancer should be considered investigational at present.

The efficacy of etoposide in the treatment of small-cell lung cancer is schedule-dependent and this may also be true with other sensitive neoplasms, such as lymphoma and germ cell tumor. Recent data have suggested that a longer administration schedule (14 to 21 days) may be more effective than the standard three- to five-day schedule. It is unknown whether these observations also hold true for use of etoposide against advanced gastric cancer. Recently, however, Ajani et al. described the preliminary results of their ongoing phase II study, which suggested that prolonged administration of oral etoposide was effective against previously untreated gastric carcinoma. Creaven and Allen carried out the initial pharmacokinetic studies of intravenous etoposide using radio-labeled etoposide. In more recent studies, HPLC was used to assay etoposide and its metabolites, resulting in greatly improved sensitivity and specificity. Our pharmacokinetic data were comparable to those of prior studies using HPLC, but due to the small number of patients analyzed in the present study, correlations between the pharmacokinetic parameters and clinical response and toxicity could not be determined. However, Miller et al. found a close correlation between the AUC of etoposide and its hematological toxicity. In addition, Stewart et al. reported that the AUC of free etoposide was correlated much more strongly with hematological toxicity than was the AUC of the total drug. These findings suggest that drug monitoring may be useful to optimize the etoposide dose in future.

The results of the present early phase II study show that monotherapy with etoposide using the present dosage regimen is ineffective for previously treated patients with advanced gastric cancer. If etoposide is to be used in future to treat advanced gastric cancer, further studies to identify a more effective and less toxic dose and schedule must be conducted.

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