Phase I Trial of Oral 2′-Deoxy-2′-methylidenectydine: On a Daily × 14-day Schedule

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ABSTRACT

2′-deoxy-2′-methylidenectydine (DMDC) is a potent deoxycytidine analogue. Preclinical studies of DMDC demonstrated activity against a variety of murine and human tumors in cell cultures and murine models and indicate enhanced antitumor activity of DMDC when it was administered in a manner that provided prolonged systemic exposure. In view of this observation, this study was designed to determine the toxicities, maximum-tolerated dose, and pharmacokinetic profile of DMDC. DMDC was given p.o. under fasting conditions for 14 consecutive days every 4 weeks in patients with advanced solid tumors. The starting dose was 12 mg/m²/day. Pharmacokinetic studies were carried out on days 1 and 14 of the first cycle. Forty patients received 22 courses of DMDC. The dose-limiting toxicities were anorexia, leukopenia, thrombocytopenia, and anemia. General fatigue was the common nonhematological toxicity. The maximum-tolerated dose was 18 mg/m²/day, at which two of six patients developed grade 3 toxicities. This dose level could also be considered for Phase II testing with this schedule. At the 18-mg/m²/day dose level, the mean terminal half-life, maximum plasma concentration (Cmax), the area under the plasma drug concentration-time curve (AUC0-∞) on day 1 were 1.7496 h, 112.9 ng/ml, and 399.8 ng/h/ml, respectively. Forty to 50% of the administered dose was recovered in the urine, indicating a good bioavailability and resulting significant systemic exposure to the drug, which may enable chronic oral treatment.

INTRODUCTION

DMDC, synthesized by Dr. K. Takenuki et al. (1), is a novel deoxycytidine analogue with structural similarity to ara-C and gemcitabine (Fig. 1). DMDC is activated by deoxycytidine kinase to the diphosphate and triphosphate metabolites. DMDC triphosphate inhibits human DNA polymerases with resultant inhibition of additional elongation of DNA (2, 3). DMDC diphosphate inhibits ribonucleotide reductase, which is a key enzyme involved in DNA synthesis and, therefore, a potential target for cancer chemotherapy (2, 4).

IC50s toward cell lines are in the range of 0.025–60.5 µg/ml (1). Preclinical antitumor activity was identified in murine P388 leukemia, colon 26 carcinoma, and M5076 reticulum cell sarcoma, in the xenografts of SK-Mel-28 human melanoma, and in LX-1 human lung cancer in nude mice (5). Like ara-C and gemcitabine, DMDC exhibited schedule dependency with increased therapeutic efficiency, obtained by daily injection for 5 days compared with a single injection on day 1 (5).

In contrast to gemcitabine and ara-C, which are susceptible to cytidine deaminase, DMDC is highly resistant, so that it is minimally converted to an inactive metabolite, DMDU (1, 6). In tumors with high cytidine deaminase activity, the enzyme converts 2′-deoxycytidine to 2′-deoxyuridine and lowers intracellular 2′-deoxycytidine concentrations. Because 2′-deoxycytidine competitively inhibits the activation of DMDC by deoxycytidine kinase, DMDC is phosphorylated more effectively because of the decreased level of intracellular 2′-deoxycytidine. DMDC was highly effective in human cancer xenograft models with high levels of cytidine deaminase activity, whereas gemcitabine was less effective in such tumors (7, 8). These features distinguish DMDC from other deoxycytidine analogues such as ara-C and gemcitabine. Therefore, non-small cell lung cancer, colon cancer, esophageal cancer, pancreas cancer, and cervical cancer, which show high cytidine deaminase activity, seem to be rational targets for therapy with DMDC.

Preclinical features of DMDC were high potency, a broad spectrum of antitumor activity against murine tumors as well as...
the xenografts of human tumors in nude mice, and increased activity with prolonged exposure (5, 7). The availability of a p.o. active drug would provide significant advantages for administration of chronic dosing regimens and the opportunity for cost-effective outpatient treatment. These data led to the selection of an oral daily schedule for 14 consecutive days for the clinical trial.

The objectives of this Phase I study were (a) to determine the maximum tolerated dose of DMDC on this schedule; (b) to describe and quantify the clinical toxicities; (c) to determine the pharmacokinetics of DMDC and DMDU and to evaluate whether there is a relationship between pharmacokinetic parameters and clinical toxicities; and (d) to obtain preliminary evidence of therapeutic activity in patients with advanced solid tumors.

PATIENTS AND METHODS

Patient Selection. Patients were enrolled in this study if they met the following criteria: histological or cytological evidence of a malignant solid tumor that was no longer amenable to established forms of treatment or for which no such standard therapy exists; no therapy within 4 weeks before entry (within 6 weeks for nitrosourea or mitomycin C); no whole blood, blood constituent transfusions, or administration of hematopoietic factors, including recombinant human granulocyte colony-stimulating factor within 2 weeks before enrollment; life expectancy of at least 12 weeks; age of 20–74 years; performance status of 0–2 by the Eastern Cooperative Oncology Group scale; adequate bone marrow function (leukocyte count, $4,000–120,000/\text{µl}$; granulocyte count, $\geq 2,000/\text{µl}$; platelet count, $\geq 100,000/\text{µl}$; and hemoglobin concentration, $\geq 10.0 \text{ g/dl}$), normal hepatic function (bilirubin, $\leq 1.5 \text{ mg/dl}$; transaminases, $\leq 2.5 \times$ upper limit of normal), and renal function (creatinine, $\leq 1.5 \text{ mg/dl}$; serum uric acid, $\leq 1.25 \times$ upper limit of normal; and serum calcium, $\leq 11.5 \text{ mg/dl}$); free of any concurrent active malignancy; no medical problem sufficiently severe to prevent compliance with the study requirements or that exposes the patients to excessive risk; and informed consent of the patient. Patients were ineligible if they had a history of congestive heart failure (cases rated as New York Heart Association Functional Classification of grade III or IV) or acute myocardial infarction within the previous 6 months; had a history of drug allergy; had a diagnosis of serious obstructive pulmonary disease; were lactating or pregnant women or those willing to be pregnant; had a history of gastrointestinal disorders or kidney diseases assumed to influence the pharmacokinetics of the investigational product; had a history of hepatocellular carcinoma or hepatitis B (hepatitis B antigen-positive) or hepatitis C (hepatitis C virus antibody-positive); not willing to use contraception; had a history of organ alogravts (except autologous bone marrow transplant); were treated with other investigational drugs within 6 weeks before the initiation of this study; had symptomatic brain metastases; had massive pleural effusion or ascitic fluid; and had serious infectious diseases including HIV infection. Patients receiving systemic corticosteroid therapy were also ineligible. The study was approved in advance by the Institutional Review Board and by the Hospital Ethics Committee.

Drug Formulation and Administration. DMDC was supplied by Nippon Roche Co., Ltd. (Tokyo, Japan) as light-red, film-coated tablets containing 5 and 20 mg of product. Patients received once daily oral DMDC for 14 consecutive days 1 h before breakfast, followed by a 14-day rest period. Because the smallest DMDC tablet size available was 5 mg, it was necessary to make some approximations in the calculated daily dose. For example, if the patient, whose body surface area was from 1.25 to 1.66 m², should be treated at 12 mg/m²/day, the drug was administered at 15 mg/day for 14 days.

Study Design. A starting dose of 12 mg/m²/day, corresponding to one-third of the oral dose at which there was no observable adverse event in monkeys in the 4-week toxicity study (9), was used. Then the dose was planned to be increased in increments of 6 mg/m² for successive patient cohorts until the MTD was reached, and at least three patients were to be included at each dose level (see Table 2). The MTD was defined as the dose causing grade 3–4 adverse reactions (grade 4 in case of granulocytopenia) in two or more of six patients (or two or more of three patients). No intrapatient dose escalation was allowed in this trial.

Evaluation. Tumor staging was done on the basis of a complete medical history and physical examination; routine chest radiography; whole-lung tomography; bone scintiscanning; computed tomography of the head, chest, and abdomen; and fiberoptic bronchoscopy. Staging was performed according to the tumor-node-metastasis system (10). Before the first course of treatment, a complete blood count (including a differential white cell count and platelet count), biochemistry tests (renal and hepatic function and electrolytes), urinalysis, and a urine pregnancy test in women of childbearing potential were performed. The complete blood count, biochemistry tests, and urinalysis were repeated at least once a week after this initial evaluation, and chest radiography and other investigations were also repeated every 4 weeks to evaluate marker lesions. Tumor response was classified according to WHO criteria (11). NCI common toxicity criteria were used to grade organ damage.

Pharmacokinetics. Pharmacokinetic studies were performed during the first cycle. Heparinized blood samples (3 ml) for the pharmacokinetic study were obtained before and at 30 and 60 min as well as 2, 3, 4, 5, 6, 8, 12, and 24 h after oral administration on days 1 and 14. The blood was centrifuged immediately, and the plasma thus obtained was stored at $-20^\circ \text{C}$ until analysis.

Urine samples were collected at intervals of 0–4, 4–8,
Dose-limiting toxicities of DMDC at each dose level during the first cycle

<table>
<thead>
<tr>
<th>Dose of DMDC (mg/m²/day)</th>
<th>Number of patients</th>
<th>Number of patients with WHO grade 3/4 toxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>12 mg/m²</td>
<td>6</td>
<td>0/0</td>
</tr>
<tr>
<td>18 mg/m²</td>
<td>6</td>
<td>1/0</td>
</tr>
</tbody>
</table>

Includes one patient simultaneously.

RESULTS

Between November 1997 and September 1998, 14 patients participated in this four-center trial and received a total of 22 courses of therapy. Five patients were women and nine were men, and the median age was 61 years (range, 44–66 years; Table 1). Most patients received prior chemotherapy. The number of DMDC cycles administered per patient ranged from 1 to 4 (one cycle in 8 patients, two in 5 patients, and four in 1 patient).

Toxicity. Two patients were not fully assessable for toxicity. One patient at dose level 1 was nonevaluable because of protocol violation. The second patient at dose level 2 was not assessable because of an error in drug administration. The remaining 12 patients were assessable for toxicity. The most common grade 3/4 toxicities were neutropenia (1/0), leukopenia (0/0), anorexia (0/0), and thrombocytopenia (0/0). There were no grade 4 toxicities. One patient at dose level 1 was nonevaluable because of a protocol violation.

Statistical Analysis. The significance of difference of dose-independent pharmacokinetic parameters between 12 and 18 mg/m² was determined using one-way ANOVA.
starting dose level of 12 mg/m²/day experienced grade 3 anorexia, three more fully assessable patients were accrued (Table 2). No other toxicities were seen, and escalation continued. One patient at dose level 2 had grade 3 leukopenia and neutropenia, and, therefore, three more patients were entered.

**Hematological Toxicity.** At 12 mg/m²/day, no patients experienced grade 3 or worse myelosuppression (Table 2). A patient treated at 18 mg/m²/day had grade 3 leukopenia, grade 3 neutropenia, and grade 3 anemia. Another patient experienced grade 3 thrombocytopenia. The onset of neutropenia and thrombocytopenia were relatively late, with neutrophil and platelet nadirs observed on days 22–25 and 15–22, respectively. Because two of six fully evaluable patients of the 18-mg/m²/day dose level had DLTs, defining the MTD as 18 mg/m²/day for 14 cycles. There was no evidence of hepatic or renal toxicity or alopecia in any of the patients. One patient at dose level 2 died on day 15 during the second cycle from pulmonary artery thrombosis, which was unlikely to be related to the study drug.

**Pharmacokinetics.** The pharmacokinetic study was carried out in 7 patients at dose level 1 and 7 at dose level 2. $C_{\text{max}}$ was achieved about 1.5 h after oral dosing. DMDC declined monophasically in patients after $C_{\text{max}}$ (see Fig. 3). The dose levels at 0, 12, and 24 h were below the detection level. The pharmacokinetic parameters are listed in Table 4. The mean (+SD) $C_{\text{max}}$ and $AUC_{(0-\infty)}$ for DMDC on day 1 were $0.1129 \pm 0.0293 \mu$g/ml and 0.3998 \pm 0.0753 \mu$g/ml at 12 mg/m²/day and 0.1457 \pm 0.0583 \mu$g/ml and 0.6033 \pm 0.2527 \mu$g/ml at 18 mg/m²/day, respectively. Dose-adjusted $AUC_{(0-\infty)}$, dose-adjusted $C_{\text{max}}$, and $T_{\text{max}}$ of DMDC at 12 mg/m²/day were similar to those at 18 mg/m²/day. On the other hand, $t_{1/2}$ for 18 mg/m²/day was longer than that for 12 mg/m²/day: the difference was statistically significant [$P < 0.01$ (two-tailed) on day 1 and $P = 0.047$ (two-tailed) on day 14]. There was no significant accumulation in $C_{\text{max}}$ (day 1 vs day 14; Table 4). At dose of 18 mg/m²/day, the simulation curves for 12 and 18 mg/m²/day were well fitted with the observed data after multiple dosing (Figs. 2 and 3; Table 5).

The 24-h urinary excretion values of DMDC + DMDU on day 1 were $53.27 \pm 12.44\%$ at 12 mg/m²/day and $38.78 \pm 9.49\%$ at 18 mg/m²/day. Those on day 14 were $44.02 \pm 12.19\%$ at 12 mg/m²/day and $42.44 \pm 14.53\%$ at 18 mg/m²/day (Table 6). The proportion of inactive metabolite, DMDU, recovered in the urine was higher than the parent compound DMDC.

**Response.** All patients were evaluable for response. A 61-year-old man treated at 18 mg/m²/day showed complete resolution of 14 pulmonary nodules ranging from 3 to 5 mm in diameter seen previously on chest computed tomography. This resolution of the lesions observed after four cycles of treatment and lasted for 11+ weeks. The patient was considered to achieve a complete response although the size of the pulmonary metastases was small. Three patients had stable disease, and 10 had disease progression.
DISCUSSION

This article describes a Phase I trial of the novel deoxycytidine analogue, DMDC, administered p.o. once a day for 14 days and repeated at 4-week intervals. DMDC given on this schedule was generally well tolerated. The DLTs of DMDC were anorexia and myelosuppression (anemia and thrombocytopenia). Because the MTD was defined as the dose at which one-third or more of the patients experienced DLT during course 1 therapy, it was reached at the 18-mg/m²/day level, with DLTs observed in two of six patients (Table 2). This dose (18 mg/m²/day) could also be considered appropriate for Phase II testing of DMDC administered for 14 days p.o., because these DLTs were short-lasting, predictable, and easily manageable.

The observed toxicity pattern was quite different from that in preclinical studies of monkeys. With prolongation of exposure time, dermatitis characterized by erythema, pain, bullae, and desquamation prevailed, and there were only mild hematological toxicities in monkeys (9). Gemcitabine, another deoxycytidine analogue in a well-advanced stage of clinical development, frequently induces flu-like symptoms (12–17). However, in this study flu-like syndrome and fever were not frequent nonhematological toxicities. Other toxicities were relatively mild, and no episodes of toxicities grade 3 or worse were noted (Table 3).

In comparison, in a single i.v. administration Phase I trial, the dose was escalated from 200 to 450 mg/m². The MTD on this schedule was >400 mg/m², and no antitumor activity was noted on this schedule (18). On a daily i.v. injection for 5-day schedule, the MTD was 40 mg/m²/day. Although no major responses were observed in this trial, a sign of the potential antitumor activity was observed in one patient with lung cancer who experienced some shrinkage of liver metastasis. The DLTs for both trials were leukopenia and neutropenia (18). A Phase I trial with the drug given p.o. once a day for 10 consecutive days every 4 weeks revealed that the MTD was 40 mg/m²/day with the dose-limiting toxicities being neutropenia and thrombocytopenia (19).

The pharmacokinetic study showed that after oral administration of DMDC, the drug was readily detected in plasma with a short lag time of 0.42–1.29 min and a peak at about 1.2–1.6 h, and rapid absorption [absorption rate constant (k12) = 0.74–0.93 h⁻¹]. The pharmacokinetic data, obtained on days 1 and 14 of the cycle 1, suggest that the pharmacokinetic parameters of DMDC were not changed by multiple dosing. Because the t₁/₂ of

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**Fig. 2** Schematic presentation of the compartmental model simultaneously fitted to the mean values of DMDC and DMDU.

**Fig. 3** Observed and simulated mean plasma concentrations after oral administrations of DMDC in patients at a dose of 18 mg/m²/day. The simulated plasma concentrations were calculated based on compartmental model.
DMDC was short relative to the dosing interval of 24 h. DMDC did not accumulate on repeated dosing (Fig. 3). Furthermore, there was a trend toward an apparent time-dependent reduction in systemic exposure in some patients.

After oral administration of DMDC, 40–50% of the administered dose was recovered in the urine as DMDC and DMDU combined (Table 6). In contrast, in another deoxycytidine analogue, (E)-2'-deoxy-2'-((fluoromethylene)cytidine) only 3.2–4.8% of the total dose was found in the first 24-h urine (20).

Although DMDC was about 700-fold more resistant to metabolic degradation by cytidine deaminase than gemcitabine (7), the proportion of metabolite DMDU to parent compound DMDC was significant (Table 6), indicating that the extensive metabolism of DMDC by cytidine deaminase occurred in vivo.

Although no major responses were observed in this trial, signs of the potential antitumor activity were observed in one patient with advanced non-small cell lung cancer who was heavily pretreated with cisplatin plus vindesine and thoracic radiotherapy.

In conclusion, this trial demonstrated that the MTD of DMDC and the recommended dose for an additional Phase II trial on this schedule were the same dose of 18 mg/m²/day. The DLTs were anorexia, leukopenia, anemia, and thrombocytopenia. General fatigue was the most frequent nonhematological toxic effect. Because there were signs of the antitumor activity, additional development of this drug is recommended.

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**REFERENCES**


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