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Cyclophosphamide and Dimethylsulfoxide in the Treatment of Squamous Carcinoma of the Lung

Therapeutic Efficacy, Toxicity, and Pharmacokinetics

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Summary. *To determine whether dimethylsulfoxide (DMSO) can potentiate antitumor activity of cyclophosphamide (CYC) in patients with squamous cell carcinoma of the lung, 14 patients were treated with 5 l of a 5% or 6% DMSO solution PO over 3 days and 1,500 mg CYC/m² IV as a 60-min infusion on the third day of treatment. Serial blood, CSF, and urine samples were collected to assess the pharmacokinetics of CYC. Courses were repeated every 3–4 weeks. No antitumor responses were observed. Toxicity was mainly hematologic and similar to that of CYC alone. There was one death from infection during granulocytopenia. Nonhematologic toxicity was moderate to severe and included nausea (14 patients) and vomiting (five patients). The plasma pharmacokinetics of CYC in this study are similar to previously reported results for CYC alone, but the 24-h urinary excretion of CYC in our study is much lower than previously reported. Further studies in tumors more responsive to CYC may be warranted.*

Introduction

Therapy for advanced squamous cell carcinoma of the lung (SCCL) has been very disappointing. Single-agent chemotherapy has produced results with response rates of 10%–15% or less [21]. Various combination chemotherapy regimens have been studied, with reported response rates of 33%–86% [15, 16, 23]. The response rates obtained with these regimens are not always reproducible [2, 17] and the effects on survival cannot be clearly attributable to the chemotherapy independent of the prognostic factors [22]. Thus new treatment modalities are clearly indicated.

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The low order of antitumor activity of chemotherapeutic agents against SCCL might be due to the inability of these agents to permeate keratinized tumor. DMSO is known to enhance various drug penetration across cellular and vascular membranes [9, 10, 14, 20], and preliminary studies have suggested enhancement of the antineoplastic activity of CYC by DMSO [5, 24]. The present clinical trial was undertaken to determine whether DMSO can enhance CYC antitumor activity in patients with advanced SCCL. An additional purpose of this study was to determine the effect of DMSO on CYC pharmacokinetics.

Materials and Methods

Fourteen patients with histologically documented metastatic SCCL were entered in this study. Eligibility criteria included: a measurable lesion, an estimated survival in excess of 2 months, an interval of at least 4 weeks since any prior chemotherapy, and adequate renal and liver function tests (serum creatinine $\leq$ 1.5 mg/dl, bilirubin $\leq$ 1.5 mg/dl, and SGOT $\leq$ 50/dl). Pretreatment bone scan, liver scan, whole-lung tomography, or chest CT scan was obtained. Bone marrow aspirate or biopsy was performed as clinically indicated.

Five liters of a 5% (ten patients) or 6% (four patients) solution of DMSO in water was given PO over 3 days to be consumed from glass containers. On the third day of treatment CYC (1,500 mg/m²) was administered IV as a 60-min infusion. The drug was diluted to a volume of 1,000 cm³ in 5% dextrose and water. Therapy was repeated every 3–4 weeks, depending on hematologic recovery, and if the patient had improvement or stable disease, was continued until there was evidence of disease progression. Blood cell counts and serum chemistries were performed at least weekly. CYC dosage modifications were based on the nadir blood cell counts of each course according to the following criteria: If the WBC nadir was > 1,500/μl and the platelet nadir was > 100,000/μl, the same dose was repeated in the subsequent treatment; if the WBC nadir was < 1,500/μl and/or the platelet nadir count was < 100,000/μl the next dose of CYC was 50% of the previous course.

Blood samples were collected in heparin for determination of the concentration of unchanged CYC and alkylating activity and

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the plasma supernatant was obtained by centrifugation and then frozen. Urine was collected on ice from the beginning of CYC infusions. Cerebrospinal fluid (CSF) was obtained from three patients at various time following CYC infusion and immediately frozen.

All samples were stored at −20° C until assayed. Unchanged CYC was quantified by gas chromatography following extraction and derivatization by a previously described method [12]. Alkylating activity in plasma, urine, and CSF was assessed by the nitrobenzylpyridine technique described by Friedman and Boger [7]. Plasma terminal half-life of CYC and alkylating activity were calculated by a commercially available computerized linear regression program (TYMSHARE Corp, Houston, Texas, USA).

Patient Population. All 14 patients studied were evaluable for toxicity and response. Four females and ten males were treated. The median age of the patients was 58 (range 43–72). Five patients had involvement of more than one metastatic site. Two patients had received prior therapy with maytansine; two had received radiotherapy to a local lesion and then maytansine, and an additional two patients had received localized irradiation treatment only. One patient had undergone pneumonectomy 2 years prior to DMSO-CYC therapy. All previously treated patients had failed to respond to or relapsed from the prior treatment. Prior to the DMSO-CYC therapy, eight patients had a CALGB performance status of one and three patients each had performance status of two and three.

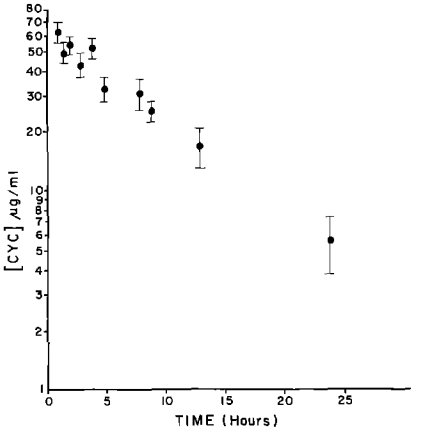

Fig. 1. Concentrations of cyclophosphamide in plasma of patients treated with DMSO. Each *point* represents the mean from 12 courses in eight patients. *Bars* indicate SEM

Table 1. Plasma and urine cyclophosphamide (CYC) levels and alkylating activity

Peak plasma CYC	62.5 ± 7.3 µg/ml[a]
Plasma CYC, terminal half-life	6.2 ± 9.0 h
Peak plasma alkylating activity	21.6 ± 9.0 nmol/ml
24-h urinary excretion of CYC (% administered dose)	3.3 ± 0.7
24-h urinary excretion of alkylating activity (% administered dose)	16.6 ± 1.5

[a] Mean ± SEM

Results

Response

No patient showed any regression of any measurable lesion after treatment with DMSO-CYC. Among 14 patients evaluable for response, in five the disease progressed after the first course of chemotherapy, seven patients received a second course of treatment, and another two patients received a third course. If calculated from the first day of treatment, seven patients initially had tumor stabilization for 6–9 weeks followed by progression; and two had stabilization for 11 and 13 weeks.

Toxicity

All 25 courses in the 14 patients were evaluable for toxicity. The major toxic effect of DMSO-CYC therapy was leukopenia. In two of the 25 evaluable courses the WBC nadir was < 100/µl, in two < 500/µl, in six < 1,000/µl, and in another two < 2,000/µl. In only two of the fourteen patients was the WBC nadir > 2,000/µl. The median WBC count was 800/µl (range 2,500–2,800/µl) for all evaluable courses with a median day to nadir of 12 (range 7–16) and a median number of days to recovery of 18 (range 13–26). One drug-related death occurred from bacteremia during granulocytopenia on day 14 of the first chemotherapy course. A platelet count of < 100,000/µl was observed in two patients. In one of these patients a platelet count nadir of 16,000/µl was observed, but this severe thrombocytopenia was due to disseminated intravascular coagulation secondary to infection. The other patient had a mild thrombocytopenia of 83,000/µl.

Nonhematologic toxicity was moderate to severe and consisted of nausea (14 patients) and vomiting (five patients). One patient had a syncopal episode on day 2 of the second chemotherapy course and was monitored with serial cardiac enzymes for 72 h. This patient had experienced vasovagal syncopal episodes on three occasions before DMSO-CYC therapy was initiated, and her two further DMSO-CYC courses were unassociated with similar toxicity.

There was no evidence of renal or hepatic toxicity. No patient experienced a conjunctival irritation and there were no alterations of the ophthalmoscopic findings or visual fields in the posttreatment examinations. No hemorrhagic cystitis was observed in any of the patients.

Pharmacology

After the 1-h CYC infusion, plasma CYC concentrations decayed monoexponentially with a mean $T^1/_2$

of 6.2 h (Fig. 1 and Table 1). Peak plasma CYC concentrations at the end of the CYC infusions ranged from 24.5–124.3 μg/ml (Fig. 1). These values agree closely with those recorded in previous reports of CYC given without DMSO [1, 4, 6, 11, 13, 19]. Plasma alkylating activity increased for several hours after cessation of CYC administration and then declined monoexponentially with a $T^1/_2$ of approximately 11 h (Fig. 2). Thus, the pharmacokinetics of plasma alkylating activity was similar to that in earlier studies of CYC alone [1, 3, 13]. The urinary excretion of alkylating activity in the first 24 h after CYC infusion was 16.6% ± 5.1% of the administered dose, and as such agrees closely with the previous studies employing CYC alone [1, 4, 11, 13]. In sharp contrast, patients who received DMSO plus CYC excreted approximately 80% less of the CYC dose as unmetabolized CYC than has been reported for patients receiving CYC alone (Table 1) [1, 4, 11, 13].

The results of cerebrospinal (CSF) studies performed in three patients are shown in Table 2. These CSF samples were obtained during the first course of therapy. There was no evidence in any of the patients of pleocytosis, elevated protein, abnormal cytology, or infection at the time the spinal fluid was obtained. The CSF CYC concentrations were lower than concomitant plasma concentrations at all three times, and the very low alkylating activity measured in the CSF was always much lower than concomitant concentration measured in the plasma.

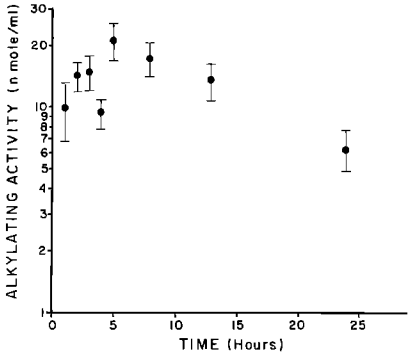

Fig. 2. Concentration of alkylating activity in plasma of patients treated with DMSO. Each *point* represents the mean from 12 courses in eight patients. *Bars* indicate SEM

Discussion

The combination of DMSO and CYC has been reported to enhance tumor response and to delay the onset of experimentally produced meningeal leukemia in Fisher rats inoculated intracerebrally with 1,000 NRL-1871 cells [24]. In addition, other workers have reported that DMSO potentiated the therapeutic efficacy of CYC in patients with advanced cancer [5]. This reported ability of DMSO to enhance the therapeutic efficacy of CYC led us to study the ability of DMSO to potentiate antitumor activity of CYC in patients with advanced squamous cell carcinoma of the lung. None experienced antitumor activity as determined by tumor regression, and this probably precludes a 20% level of activity for this combination. This is similar to a real appraisal of the single-agent data for CYC in squamous carcinoma of the lung.

Hematologic toxicity, particularly leukopenia, occurred with a median WBC nadir of 800/μl, and this is entirely comparable to the toxicity associated with a dosage of 1,500 mg CYC/m^2 used alone [18]. Thus, DMSO apparently did not enhance marrow toxicity.

The lack of antitumor activity and the lack of enhanced toxicity suggest that there was no untoward interaction between CYC and DMSO. The pharmacokinetic studies showed that the plasma terminal $T^1/_2$, the peak plasma concentrations of CYC and alkylating activity, and the urinary excretion of alkylating activity in patients receiving DMSO are similar to those in previously reported studies with CYC alone [8]. However, the 24-h excretion of the parent compound was much lower than has previously been reported for CYC alone [8]. There are several possible explanations for this decreased excretion. First, there may be increased tissue distribution of CYC or increased CYC conversion to nonalkylating metabolites such as 4-ketocyclophosphamide or alco-

Table 2. Cerebrospinal fluid levels of cyclophosphamide (CYC) and alkylating activity after initiation of CYC infusion

	4 h	5 h	24 h
CYC level in spinal fluid	29.4 μg/cm^3	36.6 μg/cm^3	0.6 μg/cm^3
% Concomitant CYC plasma level	43%	85%	56%
Alkylating activity in spinal fluid	0.1 nmol/cm^3	0.4 nmol/cm^3	2.3 nmol/cm^3
% Concomitant plasma alkylating activity	0.7%	2.6%	27.5%

phosphamide. Second, if there were increased conversion of CYC to alkylating metabolites and concomitant DMSO-enhanced tissue penetration of CYC, there would be a decreased urinary excretion of CYC with no increase in either plasma CYC or urinary alkylating activity.

Since we were unable to obtain tissue from these patients for analysis, our data cannot distinguish among these hypotheses. To elucidate the mechanism or prove whether increased amounts of active metabolites are entering the tissue, studies of normal and tumor tissue levels of CYC and specific metabolites are required. Clinically, it would be appropriate to test these hypotheses in a tumor potentially more sensitive to alkylating agents than squamous cancer of the lung.

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