A phase I investigation of the sequential use of methotrexate and paclitaxel with and without G-CSF for the treatment of solid tumors*

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Summary

Background: Paclitaxel is a novel agent with significant activity in several solid tumors. Preclinical data suggested that methotrexate prior to paclitaxel would be synergistic. To determine the qualitative and quantitative toxicity of this regimen we performed a phase I study in patients with solid tumors.

Patients and methods: Patients with solid tumors previously treated with no more than two prior chemotherapy regimens were given methotrexate intravenously on day 1, followed by paclitaxel, as a 24-hour infusion on day 2. The starting dose (level '0') was 40 mg/m² for methotrexate and 135 mg/m² for paclitaxel.

Results: After achieving a maximum tolerated dose, additional patients were enrolled with the addition of G-CSF 5 μg/kg/d on days 4–13. At the starting dose level, dose-limiting toxicity consisting of neutropenic fever occurred in 3 of 4 patients. At dose level -1, methotrexate 30 mg/m² and paclitaxel 110 mg/m², neutropenic fever occurred in 7 of 10 patients during the first course. At dose level -2, methotrexate 23 mg/m² and paclitaxel 85 mg/m², neutropenic fever occurred in 1 of 7 patients. To abrogate the neutropenia we explored the same combination with the addition of G-CSF. Neutropenic fever remained the only dose-limiting toxicity. At dose level '0' with G-CSF, 1 of 7 patients developed dose-limiting toxicity. At dose level 1 plus G-CSF, methotrexate 40 mg/m² and paclitaxel 170 mg/m², dose-limiting neutropenic fever occurred in 4 of 6 patients. Partial responses occurred in 4 of 41 patients entered on this study. Pharmacokinetic data suggested that methotrexate did not increase paclitaxel levels.

Conclusion: The combination of methotrexate and paclitaxel is feasible, but neutropenic fever, even with the addition of G-CSF prevents further escalations of paclitaxel beyond 135 mg/m² following methotrexate.

Key words: phase I, methotrexate, paclitaxel, solid tumors

Introduction

Taxol is a diterpene plant product derived from Taxus brevifolia which has been found to have activity in ovarian cancer [1], breast cancer [2], lung cancer [3, 4], and head and neck cancer [5]. Based on significant single-agent activity in phase II trials, current studies are aimed at optimizing the use of paclitaxel in combination with other agents [6]. Methotrexate has significant single-agent activity in a broad range of hematologic and solid tumors [7]. Furthermore, the combination of paclitaxel and methotrexate allows the combination of two agents with different mechanisms of action.

Methotrexate arrests cells in S-phase in vitro and in vivo [7]. Cells which are sensitive die after methotrexate exposure without reentry into the cell cycle, while surviving cells remain in S-phase until methotrexate levels decrease; then proceed through the cell cycle without cytotoxicity. The S-phase arrest, however, results in partial synchronization of the cells through completion of the cell cycle. Paclitaxel stabilizes microtubules interfering with the ability of cells to successfully pass through mitosis [8–12]. Therefore, administration of paclitaxel following the release of cells from S-phase blockage secondary to methotrexate should result in a population of cells entering mitosis during paclitaxel exposure which should optimize the cytotoxicity of paclitaxel. Chou et al. [13] showed that the administration of edatrexate, a methotrexate analogue, 24 hours prior to paclitaxel is more active than the administration of both agents simultaneously or paclitaxel administered prior to methotrexate supporting the proposed hypothesis. In the present study, we sought to define the maximum tolerated dose of administering methotrexate 24 hours prior to a 24-hour infusion of paclitaxel. Other paclitaxel infusion schedules are currently being investigated, but the 24-hour infusion was the only schedule extensively investigated in phase II trials at the time the present trial was initiated.

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In phase I studies the most frequent dose-limiting toxicity for paclitaxel was myelosuppression [6]; therefore, support with G-CSF has been given to maintain dose intensity. In a phase I trial in patients with refractory ovarian cancer, the maximum tolerated dose of paclitaxel was 300 mg/m² with G-CSF and the dose-limiting toxicity was peripheral neuropathy [14]. The recommended dose for phase II trials was paclitaxel 250 mg/m² over 24 hours followed by G-CSF [6]. Therefore, patients were given paclitaxel and methotrexate without G-CSF initially, and G-CSF was added for all subsequent patients once dose-limiting neutropenia was observed. As there is little data supporting dose intensification with methotrexate, and the primary goal was to synchronize tumor cells, paclitaxel was escalated to the maximum tolerated dose of this drug as a single-agent initially, then, if toxicity permitted, the methotrexate would be increased further.

Patients and methods

Patients were required to have a pathologic diagnosis of a solid tumor for which no higher priority treatment was available. Prior radiotherapy and as many as two prior chemotherapy regimens were permitted. Patients were required to have a performance status of at least 2 (Zubrod), a life expectancy of at least 12 weeks, and evaluable disease. Patients with a recent history (within the past six months) of congestive heart failure, acute myocardial infarction, second or third degree heart block, or current usage of digoxin, calcium channel blockers, or beta-blockers, were ineligible.

Study design

As seen in Table 1, patients were initially started at dose level '0', methotrexate 40 mg/m² and paclitaxel 135 mg/m². Methotrexate was given as an intravenous bolus 24 hours prior to the administration of paclitaxel as a 24-hour infusion. Once an MTD was established, prophylactic G-CSF 5 μg/kg was given subcutaneously on days 4–13 to patients enrolled on subsequent dose levels. Patients were given standard premedications consisting of dexamethasone 20 mg orally, 14 hours and 7 hours prior to paclitaxel, then, cimetidine 300 mg intravenously and diphenhydramine 50 mg intravenously were given one hour prior to paclitaxel. Cohorts of three patients were initially started at each dose level and if any patient developed dose-limiting toxicity, three additional patients were added. When two or more patients at any dose level developed dose-limiting toxicity, the immediately preceding dose was expanded to a total of six patients. The maximum tolerated dose (and the recommended phase II dose level) was defined as that dose immediately preceding the dose at which at least two out of six patients developed dose-limiting toxicity. If no hematologic toxicity greater than grade II and no nonhematologic toxicity greater than grade I occurred, patients were escalated one dose level for the subsequent course.

Dose-limiting toxicity

Toxicity was graded according to the standard National Cancer Institute criteria [15]. The development of grade III or IV nonhematological toxicity was considered dose-limiting. Dose-limiting hematological toxicity was defined as grade III or IV granulocytopenia or grade III or IV thrombocytopenia, which persisted for more than seven days or the development of a documented infection during granulocytopenia or bleeding during thrombocytopenia.

Pharmacokinetic methodology

Paclitaxel levels were determined using a modification of a sensitive liquid chromatography/mass spectrometry method as previously reported [16]. Briefly, the capillary HPLC equipment consisted of a fused 320 μm × 150 mm C18 capillary column and an Acurate flow splitter (LC Packings, Zurich, Switzerland), a Valco microinjector valve (Valco, Houston, TX), an electrospray interface (Analytica Inc., Branfort, CT) and a Nermag R-30-10 triple quadrupole mass spectrometer (Paris, France).

### Table 1. Graded toxicity during first course of methotrexate and paclitaxel.

<table>
<thead>
<tr>
<th>Dose level</th>
<th>Drug doses*</th>
<th>Pts.</th>
<th>Toxicity graded</th>
<th>Neutropenia</th>
<th>Thrombocytopenia</th>
<th>Nausea</th>
<th>Vomiting</th>
<th>Stomatitis</th>
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MTX = methotrexate; Pac = paclitaxel.

* Dose in mg/m².

+G-CSF: G-CSF dose: 5 μg/kg/day for days 4–13.
Echinomycin, used as an internal standard, was added to all plasma samples which were then extracted using Bondelut C2 mini-columns (Varian Assoc, Palo Alto, CA). Drug was eluted from the solid phase columns using 1 ml of 100% acetonitrile. Samples were dried under nitrogen and reconstituted in 20 μl methanol for analyses by capHPLC/ESPI/MS. This method has a lower limit of quantitation for paclitaxel of 100 pg/ml. The assay was validated with respect to both accuracy and reproducibility and was found to be useful over a broad drug detection range (0.1 ng/ml to 1 μg/ml).

Results

Patients

Forty-one patients were entered on this study. Median age was 56, and 85% had performance status of 1. Most patients had either head and neck cancer (46%) or non-small cell lung cancer (41%). Twenty-five patients had received prior chemotherapy and 27 had had radiotherapy; six patients had not undergone any prior cancer treatment.

Phase I without G-CSF

Toxicity data for the first course in all patients are shown in Table 1. All four patients treated at dose level ‘0’, methotrexate 40 mg/m² and paclitaxel 135 mg/m² developed grade 4 granulocytopenia, though it was not prolonged beyond seven days in any patient. However, three of the four patients treated at dose level ‘0’ developed infections during neutropenia requiring intravenous antibiotics and hospitalization. No other significant toxicity was noted except for grade 2 nausea and vomiting in one patient. At dose level —1, methotrexate 30 mg/m² and paclitaxel 110 mg/m², three of the first six patients developed fever during neutropenia, however, only 1 patient had a documented source of infection. Therefore, we sought to explore this dose level with four additional patients. All four of these patients developed neutropenic fever; therefore, among all ten patients entered at this dose level, eight developed grade 4 granulocytopenia and seven developed fever during grade 3 or 4 neutropenia. Other toxicity was limited to grade 2 mucositis in two patients and grade 2 diarrhea in one patient. At dose level —2, methotrexate 23 mg/m² and paclitaxel 85 mg/m², five of seven patients developed grade 4 granulocytopenia, but only one patient developed neutropenic fever. As seen in the previous dose levels no significant nonhematologic toxicity was noted; therefore, based on the dose-limiting toxicity of febrile neutropenia, dose level —2, methotrexate 23 mg/m² followed by paclitaxel 85 mg/m² over 24 hours, was the maximum tolerated dose recommended for phase II studies. The feasibility of the dose level was confirmed in an analysis of cumulative toxicity in that the majority of patients who required dose reductions from dose level —1 were able to tolerate dose level —2 for subsequent courses.

Phase I study with G-CSF

Based on the development of dose-limiting myelosuppression without any other significant toxicity we sought to determine if the administration of prophylactic G-CSF following the paclitaxel could abrogate the development of severe neutropenia and avoid infectious complications. The degree of myelosuppression for the first course at dose level —1 plus G-CSF, methotrexate 30 mg and paclitaxel 110 mg/m² was still profound as three of six patients developed grade 4 neutropenia and two of the remaining patients developed grade 3 neutropenia. The major difference between dose level —1 with G-CSF and dose level —1 without G-CSF was the absence of infectious complications as only one patient developed fever during grade 3 or 4 neutropenia. One patient did develop grade 3 thrombocytopenia which was of short duration, but no other severe or life-threatening toxicity was noted. Eight patients were treated at dose level ‘0’ with G-CSF, methotrexate 40 mg/m² and paclitaxel 135 mg/m². One patient was felt to be inevaluable for toxicity, as he died shortly after treatment at an outside hospital of a pulmonary embolus. A second patient had rapid progression and was placed in a hospice during his first course. Neither patient had evidence of infection, however hematological follow-up was not complete on either patient. Therefore, two additional patients were evaluated at this dose level. One patient at this dose level with small cell lung cancer, was hospitalized for respiratory distress and developed fever while hospitalized. He recovered following aggressive pulmonary treatment and antibiotic therapy. The same patient also had dose-limiting toxicity on the basis of duration, as his neutropenia persisted for 10 days. No other patient developed dose-limiting toxicity at this dose level. Among the six patients treated at dose level +1 with G-CSF, methotrexate 40 mg/m² and paclitaxel 170 mg/m², five of six patients developed grade 4 neutropenia and one developed grade 4 thrombocytopenia. Four patients developed dose limiting toxicity consisting of neutropenic fever. An additional patient who did not develop neutropenic fever was dose reduced on the basis of grade 3 fatigue. The feasibility of this dose level was confirmed in an analysis of cumulative toxicity, in that most patients requiring dose reductions from dose level +1 (with G-CSF) were able to tolerate treatment at the 0 dose level (with G-CSF). The MTD (and phase II dose level) was thus deemed to be paclitaxel 135 mg/m² plus methotrexate 40 mg/m² when administered with G-CSF.

Responses

As the primary goal of this study was to define the feasibility of this regimen, many patients had evaluable, but not measurable, disease making response difficult to quantitate. However, partial responses were noted in
patients with non-small cell lung cancer, and squamous cell carcinoma of the head and neck. Three responses occurred in patients who had not received prior chemotherapy. An additional patient with a mixed small cell and non-small cell carcinoma of the lung who progressed on cisplatin and etoposide also achieved a partial response. A patient with prostate cancer had decreased bone pain and a decrease in his prostate specific antigen from 109 to 89, which lasted for 3 cycles. When the analysis is restricted to the doses which have previously been used in phase II studies (≥135 mg/m^2), the response rate improves to 4 of 17 patients (24%).

**Clinical pharmacology**

Because of the extent of drug-mediated toxicity produced by the combination of methotrexate and paclitaxel in the present study, not all patients could be consistently followed for paclitaxel plasma levels. Drug levels were determined at 1, 4, 8, and 24 hours following the start of the 24-hour paclitaxel infusion. Paclitaxel clinical pharmacology was determined in a minimum of two patients and as many as four patients at each dose level. Presumed steady state paclitaxel plasma levels at 24 hours ranged from 0.13 μM to 0.62 μM over the dose range examined (85 to 170 mg/m^2). These data are consistent with those observed in several other 24 hour paclitaxel infusion regimens (lacking methotrexate) run at our institution within the past year (data not shown).

**Discussion**

The purpose of the current study was to define the feasibility of combining methotrexate with paclitaxel in patients with solid tumors. The initial portion of the study was designed to define the maximum tolerated dose of this combination without growth factor support. The maximum tolerated dose was methotrexate 23 mg/m^2 followed by paclitaxel 85 mg/m^2 and the dose-limiting toxicity was neutropenia complicated by infections. As nonhematologic toxicity was uncommon we evaluated the feasibility of escalating the dose of both agents with the addition of G-CSF. The addition of growth factor allowed us to increase the dose of both agents by approximately 50%, as the maximum tolerated dose was methotrexate 40 mg/m^2 and paclitaxel 135 mg/m^2; this is the recommended phase II dose level. The dose-limiting toxicity during this portion of the study remained febrile neutropenia though thrombocytopenia and nonhematologic toxicity became more prevalent. The majority of patients continued to have neutropenia despite G-CSF, but at equivalent doses of methotrexate and paclitaxel the incidence of infections was decreased. The activity of this combination was not as significant as would be hoped, despite it being a phase I study. The single agent activity of methotrexate in patients with lung cancer is limited, but responses may be seen in up to 30% of patients with head and neck cancer [17]. In the nine patients with head and neck cancer, only one had a major response. Paclitaxel single-agent response rates exceeded 30% in a recent ECOG trial in head and neck cancer [5, 6]. Therefore, the limited activity in this study is disappointing. However, most patients on this study had disease which had failed prior cisplatin therapy. Previous studies in non-small-cell lung cancer showed that the activity of paclitaxel in this setting is much less [18, 19]; therefore, the lack of activity in this study may be due to the prior treatment most patients had received. The occurrence of a partial response in a patient with a mixed small-cell and non-small-cell lung cancer who had failed cisplatin, is of note. Unfortunately, the duration of this response was short.

The results of the present study have shown that the combination of methotrexate and paclitaxel may have some promise of improved activity. However, the significant myelosuppression presents a considerable problem as the doses of paclitaxel that are feasible even with G-CSF are lower than would be hoped for phase II studies. Further studies, both preclinical and clinical will be necessary to define methods to overcome the profound neutropenia in order to further develop this regimen.

Combinations of agents must either be given at or near maximum single agent doses or have synergy in order to improve upon the efficacy of each agent individually. The degree of myelosuppression found in this trial strongly suggests that potentiation is indeed occurring especially with regard to bone marrow progenitor cells. The maximum tolerated dose of each agent in combination is well below the doses that could be given of either agent alone. An alternative explanation for this phenomenon was that the administration of methotrexate altered the clearance of paclitaxel thereby increasing paclitaxel serum levels. The pharmacokinetic data presented in this study suggest, however, that this is not the case. Recent studies [20, 21] have indicated that a complex sigmoid maximum response model is required to adequately explain the relationship of paclitaxel plasma concentrations and observed neutropenia. In addition, the major metabolite of paclitaxel, a 6-OH species, may serve as an indicator of the relative extent of paclitaxel metabolism within patients. Applications of these recent findings to studies such as ours will be important in future trials to more clearly understand the relationships of paclitaxel pharmacology especially when combined with other agents such as methotrexate in a sequence-dependent manner.

If the administration of methotrexate prior to paclitaxel results in synchronization of bone marrow progenitor cells, then schedule manipulations may be possible to develop an improved therapeutic index as cell cycle kinetics likely vary between bone marrow progenitors and solid tumors. Further preclinical work will
be necessary to define the potential benefit of variations in drug sequence and dose. Despite extensive experience, the cell cycle specific effects of methotrexate have been described in limited fashion in patients. The 24-hour interval for the current study was based on in vitro cell culture data and the actual kinetics in humans is likely to be substantially different; therefore, analysis of cell cycle specific effects of methotrexate prior to paclitaxel in humans will be necessary to determine the optimal interval.

Preclinical data suggests that a series of steps including the formation of microtubule bundles, arrest in mitosis, development of tetraploid multinucleated interphase cells, and entry into apoptosis may be necessary for cytotoxicity [6, 22]. Fine needle aspirates from tumors are being analyzed using antitubulin antibodies to define if paclitaxel is achieving intracellular levels adequate to induce the formation of microtubule bundles and mitotic arrest. Furthermore, BrdU pulse labeling and image analysis of feulgen stained specimens are being used to confirm the cell cycle specific effects of the combination of antifolates and paclitaxel in human tumors. Definition of these events may allow identification of a schedule which decreases myelosuppression and allow further exploration of this combination.

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References


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