Sequence-dependent Hematological Toxicity Associated with the 3-Hour Paclitaxel/Cyclophosphamide Doublet¹

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ABSTRACT
Paclitaxel is active in metastatic breast cancer. Combination studies have demonstrated complex interactions between paclitaxel and other cytotoxic agents, including sequence-dependent cytotoxic, toxicological, and pharmacological effects. The principal objectives of this study were to determine the maximum tolerated doses of paclitaxel (3-h infusion) and cyclophosphamide (1-h infusion) administered every 3 weeks with granulocyte colony-stimulating factor (Filgrastim) and to determine if the sequence-dependent toxicological effects that have previously been observed with this combination when paclitaxel was administered over 24 h were evident when paclitaxel was administered over 3 h. Fifteen women with metastatic breast cancer were treated. Starting doses were 200 mg/m² paclitaxel and 1600 mg/m² cyclophosphamide, with granulocyte colony-stimulating factor (5 µg/kg/day) given s.c. beginning 24 h after chemotherapy. Doses of both drugs were escalated in cohorts of at least four patients. The sequence of drug administration was alternated with each consecutive patient and with each subsequent course of therapy in each individual patient, enabling the evaluation of sequence-dependent toxicological and pharmacological effects. Severe myelosuppression was the principal dose-limiting toxicity for this regimen, precluding dose escalation above 200 mg/m² paclitaxel and 1600 mg/m² cyclophosphamide, the maximum tolerated dose for this combination on this schedule. As has been previously demonstrated with this combination, when paclitaxel is administered over 24 h, the hematopoietic toxicity was sequence dependent. Paired analysis of toxicity data using each patient as her own control indicated more severe hematological toxicity in courses in which paclitaxel was administered first. There was no evidence of sequence-dependent effects on the pharmacokinetics of these drugs that might account for this phenomenon. The impact of drug sequencing on toxicity should be considered in the further development of combination therapy containing alkylating agents and paclitaxel, when the latter is administered over 3 h.

INTRODUCTION
A series of Phase II studies has confirmed the clinical activity of single-agent paclitaxel in the treatment of women with both chemotherapy-naive and previously treated metastatic breast cancer (1–3). However, a variety of issues remain to be clarified concerning the optimal use of the drug in this disease. These include both the optimal dose and schedule of drug administration and the role of paclitaxel in the adjuvant treatment of high-risk patients with primary breast cancer. In addition, it is uncertain whether paclitaxel is best used as a single agent or in combination with other active cytotoxics (4).

A number of studies have demonstrated the activity of combination chemotherapy with paclitaxel and a variety of alkylating agents in women with metastatic breast cancer. Such regimens may be of major clinical utility in patients who have received anthracyclines. These authors observed a response rate of 55% when administered every 2 weeks to 27 women with metastatic breast cancer (5). Subsequent reports suggest that the activity of this combination is likely to be less, and response rates of between 23 and 60% have been reported (6, 7). Paclitaxel and cyclophosphamide combinations are also active, and substantial doses of both drugs can be administered before neutropenia is dose-limiting. Tolcher et al. (8) administered paclitaxel by 72-h infusion and cyclophosphamide by bolus on days 1, 2, and 3 every 3 weeks to 55 women with metastatic breast cancer, most of whom had been previously exposed to anthracyclines. These authors observed a response rate of 55%. Fennelly et al. (9) evaluated this combination for its ability to mobilize peripheral blood progenitor cells for subsequent apheresis to support sequential high-dose chemotherapy in women with ovarian cancer. In a Phase I trial with escalating doses of paclitaxel administered as a 24-h infusion, these authors reported 300 mg/m² paclitaxel could be administered every 2 weeks with 3 g/m² cyclophosphamide when supported with G-CSF without DLT.¹

¹ The abbreviations used are: G-CSF, granulocyte colony-stimulating factor; DLT, dose-limiting toxicity; MTD, maximum tolerated dose; AUC, area under the curve; CI, confidence interval; ANC, absolute neutrophil count; ECOG, Eastern Cooperative Oncology Group; USP, United States Pharmacopoeia.

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The combination of paclitaxel administered over 24 h with bolus cyclophosphamide and G-CSF support has previously been evaluated in women with heavily pretreated metastatic breast cancer (10). Neutropenia was dose-limiting. The MTD for this combination on this schedule was 200 mg/m² paclitaxel and 1250 mg/m² cyclophosphamide, and responses were noted in 29% of patients with anthracycline-resistant disease. Both sequences of drug administration (i.e. paclitaxel before cyclophosphamide and cyclophosphamide before paclitaxel) were evaluated in this study. Because as all patients were treated with both treatment sequences, each patient acted as her own control, and pairwise comparisons of toxicity with both sequences of drug administration could be performed. Platelet and neutrophil toxicities were more severe when paclitaxel was administered before cyclophosphamide, and the risk of neutropenia-associated fever was four times higher in courses in which paclitaxel was administered first. There seemed to be no pharmacokinetic explanation for this finding. Similar effects of drug sequencing on toxicity have been noted in studies of the paclitaxel/doxorubicin doublet. Holmes et al. (11) administered doxorubicin over 48 h and paclitaxel over 24 h in both sequences and noted that the clearance of doxorubicin was reduced by about 30%, and toxicity was greater in courses in which paclitaxel was administered first. However, in a recently reported study of paclitaxel administered over 3 h with doxorubicin infused over 15 min, Gianni et al. (12) noted no effect of sequence of drug administration on toxicity, although paclitaxel reduced the clearance of both doxorubicin and doxorubicinol in both treatment sequences (13). To determine whether sequence of drug administration has an impact on the toxicity of the paclitaxel/cyclophosphamide combination when thetaxane is administered over 3 h, we performed the study reported here. The goals of the study were to determine the MTD of paclitaxel (3-h infusion) and cyclophosphamide administered every 21 days, unless the ANC or platelet count was less than 1,500 or 100,000 cells/µL, respectively.

PATIENTS AND METHODS

Patient Eligibility. Women with histologically documented breast cancer that was metastatic who were 18 years of age or older were eligible to participate in the study. An ECOG performance status of 2 or better and a life expectancy of at least 3 months were required. Prior chemotherapy for metastatic disease, with the exception of high-dose therapy with autologous stem cell support or prior taxane treatment, was allowed. Adequate hematopoietic (WBC count ≥4,000 cells/µL with neutrophils (ANC) ≥1,500 cells/µL and platelets ≥100,000 cells/µL), hepatic (total bilirubin ≤1.5 mg/dL), and renal (creatinine ≤1.5 mg/dL) function was required. All patients gave written informed consent according to institutional and federal guidelines.

Dosage. In a prior study that evaluated the feasibility of administering the combination of paclitaxel given by 24-h infusion with cyclophosphamide over 1 h and G-CSF, the MTD was found to be 200 mg/m² paclitaxel and 1250 mg/m² cyclophosphamide. Because paclitaxel administered over 3 h results in less myelosuppression than paclitaxel administered over 24 h, the starting doses for the current study were 200 mg/m² paclitaxel given by 3-h infusion and 1600 mg/m² cyclophosphamide given over 1 h. All patients were treated with G-CSF (5 µg/kg/day) s.c. from day 3 until the ANC was ≥1500 cells/µL.

Doses of paclitaxel or cyclophosphamide were escalated sequentially in each successive cohort of new patients. Four new patients were treated at each dose level in which no DLTs were seen, and escalation to the next cohort was not attempted before at least three patients at the prior dose level had completed at least one course of treatment. Intra-individual dose escalation was not permitted, but dose reduction by one dose level was allowed for patients who received at least two courses of therapy and developed DLT or had one or more episodes of grade IV neutropenia (ANC <500 cells/µL) with fever, or grade IV thrombocytopenia (platelets <25,000 cells/µL).

DLT. Toxicity was graded according to the National Cancer Institute Common Toxicity Criteria. DLT was defined as grade 3/4 nonhematologic toxicity or the following hematologic toxicity: (a) ANC <500 cells/µL or platelets <25,000 cells/µL for more than 5 days; or (b) delay in the initiation of additional therapy for more than 7 days, due to unresolved hematologic toxicity. If DLT occurred in one of the first four patients treated at a specific dose level, then two additional patients were treated at that dose level. Accrual of additional new patients at the lower dose level was permitted if more than two patients had a DLT at the next highest dose level. The MTD was defined as the highest dose level at which less than two of six patients experienced a DLT.

Drug Administration. Paclitaxel (Taxol; Bristol Myers Squibb Pharmaceuticals, Princeton, NJ) was supplied by the Division of Cancer Treatment, National Cancer Institute (Bethesda, MD) as a concentrated sterile solution with 6 mg/ml in a 5-mI ampule in 50% polyoxyethylated castor oil (Cremophor EL) and 50% dehydrated alcohol, USP 50%. The total dose was administered over 3 h after dilution in 1.000 ml of 5% dextrose injection, USP. Glass infusion bottles and nitroglycerin tubing were used for administration. Cyclophosphamide (Neosar; Adriam Laboratories, Columbus, OH) was supplied in 100-, 200-, 500-, 1,000-, and 2,000-mg vials and dissolved in bacteriostatic water for injection, USP. The required dose was diluted in 500 mL of 5% dextrose water and administered over 1 h. The following premedications were administered to all patients: (a) dexamethasone (20 mg), i.v. or orally, 14 and 7 h before paclitaxel; and (b) diphenhydramine (50 mg) i.v. and famotidine (20 mg) i.v. 30 min before paclitaxel. Patients who received less than 1,500 mg/m² cyclophosphamide received hydration with 1,000 ml of 0.9% sodium chloride solution over 3 h following chemotherapy. Patients who received more than 1,500 mg/m² cyclophosphamide received 0.9% sodium chloride solution at 4 ml/kg/h for 6 h before and 12 h after treatment with cyclophosphamide. All patients were treated with recombinant human G-CSF (Filgrastim; Amgen, Thousand Oaks, CA; 5 µg/kg/day) s.c. beginning no sooner than 24 h after the end of chemotherapy. G-CSF was continued until the ANC exceeded 1,500 cells/µL on two consecutive determinations. Chemotherapy was administered every 21 days, unless the ANC or platelet count was less than 1,500 or 100,000 cells/µL, respectively.
Drug Sequence. The initial sequence of drug administration was alternated in each successive patient entered on study and with subsequent courses of therapy administered to each individual patient. The first patient received cyclophosphamide immediately before paclitaxel, and the second patient received paclitaxel immediately before cyclophosphamide during the first course. Thereafter, the sequence of drug administration for the first course alternated with each additional new patient entered on study. In each individual patient, the sequence of drug administration was also alternated with each successive course. Therefore, all patients were treated with both sequences.

The major statistical endpoint of the study was the ratio of pre- and posttreatment ANC and platelet count, which were assessed according to treatment sequence, i.e., cyclophosphamide and then paclitaxel or paclitaxel and then cyclophosphamide. The ANC and platelet ratios were regressed on treatment sequence, adjusted for patient identification number. Thus, the effects of sequence on hematological toxicity could be determined by performing a paired analysis, with each patient acting as her own control. Hospital admission for fever associated with severe neutropenia was also considered a statistical endpoint, and conditional logistic regression was used to determine whether treatment sequence was an independent determinant of this event.

Pretreatment and Follow-Up Evaluations. History and physical examination were performed, and routine laboratory studies were obtained on the first day of each course. Toxicity was evaluated weekly during therapy. Routine laboratory tests included complete blood count with WBC count differential, electrolytes, urea, creatinine, total protein, albumin, calcium, glucose, phosphate, uric acid, alkaline phosphatase, total and direct bilirubin, aspartate aminotransferase, alanine aminotransferase, amylase, prothrombin time, and urinalysis. All patients had an electrocardiogram before entry on study. Complete blood counts were obtained three times weekly until the ANC exceeded 1500/µl on two consecutive occasions. Assessment of tumor response was performed after every two courses of therapy, and treatment continued as long as there was no evidence of disease progression and toxicity permitted.

Pharmacokinetic Studies. An analysis of plasma concentrations of both paclitaxel and cyclophosphamide was performed with each sequence of drug administration in patients who received two or more courses of therapy. Blood samples were drawn for assay of paclitaxel concentrations, according to a limited sampling scheme: pre-infusion; 1 h into infusion; and 1 min, 3 h, and 24 h after infusion. Plasma paclitaxel concentrations were measured by high-performance liquid chromatography using the method described previously (10). Paclitaxel AUC was measured by the mixed linear-log trapezoidal rule. The 95% CI for the ratio of paired values was calculated to determine whether there was an effect of drug sequence on AUC.

RESULTS

Patient characteristics are outlined Table 1. Fifteen patients were entered on study, all of whom were assessable for toxicity. One patient treated at dose level 2 developed progressive disease during course 1; thus, 14 patients received at least 2 courses and are evaluable for assessments of sequence-dependent effects. Median age was 45 years (range, 35–68 years), and median ECOG performance status was 1. The median number of disease sites was 1, (range, 1–3). All patients had received prior anthracyclines, 11 in the adjuvant setting and 4 for metastatic disease. Patients were treated in the dose escalation scheme as outlined in Table 2. Fifteen patients received a total of 77 courses of treatment at 4 dose levels. The median number of courses per patient was 5 (range, 1–14).

The DLTs are summarized in Table 3. Whereas no DLTs were observed at dose level 1 (paclitaxel/cyclophosphamide, 200/1600 mg/m²), one of four patients did develop neutropenia-associated fevers on more than one occasion and required a dose reduction from dose level 3.
Table 3  DLTs at each dose level

<table>
<thead>
<tr>
<th>Level</th>
<th>Paclitaxel/cyclophosphamide (mg/m²)</th>
<th>New patients</th>
<th>New courses</th>
<th>DLT</th>
<th>Dose reduction due to recurrent NF a</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>200/1250</td>
<td>4</td>
<td>27</td>
<td>0</td>
<td>1/4</td>
</tr>
<tr>
<td>2</td>
<td>250/1600</td>
<td>7</td>
<td>25</td>
<td>2/7 b</td>
<td>0/7</td>
</tr>
<tr>
<td>3</td>
<td>250/2000</td>
<td>4</td>
<td>13</td>
<td>0</td>
<td>2/4</td>
</tr>
</tbody>
</table>

a NF, neutropenia-associated fever.
b One malaise, one syncope.

Table 4  Hematological toxicity

<table>
<thead>
<tr>
<th>Level</th>
<th>Paclitaxel/cyclophosphamide (mg/m²)</th>
<th>Total patients</th>
<th>Total courses</th>
<th>Grade 4 ANC a</th>
<th>Fever with neutropenia</th>
<th>Grade 3/4 platelets b</th>
<th>Dose reduction c</th>
</tr>
</thead>
<tbody>
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<td>1</td>
<td>200/1250</td>
<td>1</td>
<td>5</td>
<td>0/5 (0%)</td>
<td>0/5 (0%)</td>
<td>0/5 (0%)</td>
<td>0/1</td>
</tr>
<tr>
<td>2</td>
<td>200/1600</td>
<td>4</td>
<td>27</td>
<td>24/27 (88%)</td>
<td>4/27 (15%)</td>
<td>0/27 (0%)</td>
<td>1/4</td>
</tr>
<tr>
<td>3</td>
<td>250/1600</td>
<td>9</td>
<td>32</td>
<td>24/32 (47%)</td>
<td>3/32 (9%)</td>
<td>2/32 (6%)</td>
<td>0/9</td>
</tr>
<tr>
<td>4</td>
<td>250/2000</td>
<td>4</td>
<td>13</td>
<td>11/13 (85%)</td>
<td>6/13 (45%)</td>
<td>1/13 (8%)</td>
<td>2/4</td>
</tr>
</tbody>
</table>

a Neutrophil nadir count less than 500 cells/µl.
b Platelet nadir count less than 50,000 cells/µl.
c Dose reductions, per patient treated, due to repeated episodes of neutropenia-associated fever.

Reduction to dose level 1 (paclitaxel/cyclophosphamide, 200/1250 mg/m²). Treatment at this dose level was subsequently well tolerated for five courses. Only 1 of 24 courses of therapy administered to the other 3 patients treated at dose level 1 was complicated by a fever associated with severe neutropenia. One patient developed cumulative grade 2 malaise that contributed to the decision to discontinue therapy after six courses. Seven new patients were treated at dose level 2 (paclitaxel/cyclophosphamide, 250/1600 mg/m²). One patient developed progressive disease and was taken off study after one course of treatment. One patient developed syncpe with two courses of therapy at the end of the paclitaxel infusion and was taken off protocol because of this DLT. A second patient at dose level 2 developed dose-limiting malaise and was taken off protocol after two courses of therapy. At dose level 3 (paclitaxel/cyclophosphamide, 250/2000 mg/m²), two of four patients were hospitalized for treatment of fevers associated with severe neutropenia on more than one occasion and received further therapy after course 2 at dose level 2 (paclitaxel/cyclophosphamide, 250/1600 mg/m²). In retrospect, therefore, recurrent episodes of neutropenia-associated fevers were treated as DLTs, and dose modifications were made in patients who suffered these toxicities. Because DLTs occurred in two of seven patients treated at dose level 2 (paclitaxel/cyclophosphamide, 250/1600 mg/m²), and because two of four patients treated at dose level 3 (paclitaxel/cyclophosphamide, 250/2000 mg/m²) required dose reductions due to recurrent neutropenia-associated fevers, the MTD for the paclitaxel/cyclophosphamide combination on this schedule with G-CSF support is 200/1600 mg/m².

Hematological Toxicity and Sequence Dependence. Substantial hematological toxicity was observed at all dose levels (as outlined in Table 4). Fevers associated with severe neutropenia were uncommon at dose levels 1 and 2 (paclitaxel/cyclophosphamide, 200/1,600 mg/m² (15%) and 250/1,600 mg/m² (9%)) but occurred in 45% of courses at dose level 3 (paclitaxel/cyclophosphamide, 250/2,000 mg/m²). Recurrent neutropenia-associated fevers in each of the first two courses mandated dose reductions in two of four patients treated at this dose level. Severe platelet toxicity was uncommon, with platelet nadir counts of less than 50,000 cells/µl occurring in fewer than 10% of courses at all dose levels.

The sequence of drug administration had an impact on the severity of hematological toxicity observed (Table 5). There was no difference in mean ANC before therapy with either sequence (data not shown). As shown in Table 6, the posttreatment average ANC for paclitaxel-first courses (P/C) was 661 cells/µl and was not significantly different from the posttreatment average of 1,007 cells/µl for the cyclophosphamide-first (C/P) courses (P = 0.08). However, paired analysis of the ratio of nadir count to pretreatment ANC indicated a borderline significantly lower ratio for P/C courses (0.12) than for C/P courses (0.19; P = 0.05). Conditional logistic regression analysis demonstrated that the odds ratio for developing a neutropenia-associated fever on P/C courses was 1.7 times that for C/P courses. However, this was not statistically significantly increased for P/C courses as the 95% bound of the CI crossed 1 (95% CI, 0.5–5.7). An effect of sequence of drug administration was observed on platelet toxicity. Again, the mean pretreatment platelet count was similar for both sequences of therapy (data not shown). Platelet nadir were significantly lower in P/C courses (127,000 cells/µl) than in C/P courses (150,000 cells/µl; P = 0.01). Also, the ratio of paired nadir platelet count to pretreatment platelet count was also lower for P/C (0.43) than for C/P courses (0.51; P = 0.02). These effects were not clinically significant.

Nonhematological Toxicity. Neuropathy, myalgias, and malaise were the most common nonhematological toxicities. Grade 2 neuropathy was observed in three of four patients at...
Table 5  Impact of sequence of drug administration on hematological toxicity: average absolute neutrophil and platelet nadirs and the ratios of nadir counts to pretreatment values for each sequence

<table>
<thead>
<tr>
<th>Drug sequence</th>
<th>Course no.</th>
<th>Neutrophils</th>
<th>Platelets</th>
<th>Neutropenia-associated fevers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Nadir</td>
<td>Ratio$^a$</td>
<td>Nadir</td>
</tr>
<tr>
<td>C/P</td>
<td>38</td>
<td>1007</td>
<td>19%</td>
<td>150</td>
</tr>
<tr>
<td>P/C</td>
<td>39</td>
<td>661</td>
<td>12%</td>
<td>127</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td>0.08</td>
<td>0.05</td>
<td>0.01</td>
</tr>
</tbody>
</table>

$^a$ Paired analysis of the ratio of the nadir neutrophil count to the pretreatment count.
$^b$ Paired analysis of the ratio of the nadir platelet count to the pretreatment count.
$^c$ Cyclophosphamide followed by paclitaxel.
$^d$ Paclitaxel followed by cyclophosphamide.

Table 6  Impact of sequence of drug administration on hematological toxicity: comparison of nadir counts for each sequence of drug administration in the current study (3-h paclitaxel) and in the historical control population (24-h paclitaxel)

A. 3-h paclitaxel with cyclophosphamide

<table>
<thead>
<tr>
<th>Drug sequence</th>
<th>Course no.</th>
<th>Nadir ANC (mean)</th>
<th>Nadir platelets (mean)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C/P</td>
<td>38</td>
<td>1007</td>
<td>150</td>
</tr>
<tr>
<td>P/C</td>
<td>39</td>
<td>661</td>
<td>127</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td>0.08</td>
<td>0.01</td>
</tr>
</tbody>
</table>

B. 24-h paclitaxel with cyclophosphamide

<table>
<thead>
<tr>
<th>Drug sequence</th>
<th>Course no.</th>
<th>Nadir ANC (mean)</th>
<th>Nadir platelets (mean)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C/P</td>
<td>108</td>
<td>1283</td>
<td>112</td>
</tr>
<tr>
<td>P/C</td>
<td>98</td>
<td>546</td>
<td>96</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td>0.001</td>
<td>0.04</td>
</tr>
</tbody>
</table>

$^a$ Cyclophosphamide followed by paclitaxel.
$^b$ Paclitaxel followed by cyclophosphamide.

dose level 1, four of seven patients at dose level 2, and three of four patients at dose-level 3. One patient at dose level 1 who was treated with a total 14 courses of therapy received treatment on a 4-week schedule after course 11 because of cumulative neuropathy. In two other patients who received six and eight courses of therapy at dose level 1, cumulative sensory neuropathy contributed to the decision to discontinue therapy after maximal response had been achieved. Two patients (one each at dose levels 1 and 2) were treated with tricyclic antidepressants to ameliorate neurosensory neuropathy. Myalgias were commonly observed at all dose levels and treated with nonsteroidal anti-inflammatory drugs and narcotic analgesics as required. Myalgias were never dose-limiting. Severe malaise that required discontinuation of therapy was observed in one patient treated at dose level 2, and a second patient at that dose level discontinued therapy because of episodes of syncope at the end of her paclitaxel infusion. Gastrointestinal toxicities were uncommon. Grade 1 or 2 nausea, vomiting, mucositis, or diarrhea was observed on one or more occasions in three of four patients at dose level 1, four of seven patients at dose level 2, and three of four patients at dose level 3. Gastrointestinal toxicity was never dose-limiting.

Pharmacokinetics. Pharmacokinetic data are summarized in Table 7. Paired plasma samples were analyzed to evaluate the possibility of sequence-dependent pharmacological interactions between cyclophosphamide and paclitaxel in nine and four patients, respectively. The effect of drug administration sequence on the cyclophosphamide AUC was evaluated by calculating the 95% CI for the ratio of the paired values. The mean ratio $AUC_{CP}/AUC_{PCR}$ was 0.93 (95% CI: 0.79–1.08) for cyclophosphamide. The effect of drug sequencing on paclitaxel AUC was estimated calculating the 95% CI for the ratio of the paired values. The mean ratio of $AUC_{CP}/AUC_{PCR}$ was 1.09 (95% CI: 0.58–2.08) for paclitaxel.

Response to Therapy. Four of 15 patients had responses to therapy. Partial responses were observed in two patients with soft tissue disease and in one patient with liver metastases. One additional patient had a complete response in recurrent axillary nodal disease.

DISCUSSION

The activity of paclitaxel in the treatment of advanced chemotherapy-naive and -resistant breast cancer has been well documented, and this agent is currently undergoing evaluation in the adjuvant setting. Combination chemotherapy regimens, comprising paclitaxel and a variety of other active cytotoxics, notably doxorubicin, cisplatin, and cyclophosphamide, have previously been evaluated in Phase I and Phase II trials. Although these drug combinations have been shown to be active, it remains unclear whether there are advantages to combination...
over single-agent therapy in the treatment of women with advanced disease.

In vitro and in vivo evaluations have demonstrated that complex drug interactions occur when paclitaxel and certain other cytotoxics are combined. This was first observed in a Phase I study of paclitaxel (given over 24 h) and cisplatin (15). Patients were treated with both sequences of drug administration, i.e., C/P and P/C. The hematological toxicity of therapy was more severe in those courses in which cisplatin was administered first. Pharmacological analysis indicated that this was due to a 25% lower paclitaxel clearance rate in those courses. One possible hypothesis offered was that cisplatin may inhibit paclitaxel-metabolizing P450 mixed-function oxidases, thus increasing drug exposure and hematological toxicity when cisplatin is administered before paclitaxel (16). However, the sequence-dependent interaction of paclitaxel and alkylating agents is quite different in in vitro models. The cytotoxicity of the cisplatin/paclitaxel combination against L1210 leukemia cells was documented to be significantly greater when the cells were exposed to paclitaxel for 24 h before exposure to cisplatin when compared to the other sequence of administration (17). Additional similar in vitro studies by Cook et al. (18) have evaluated the sequence-specific effects of paclitaxel in combination with a variety of alkylating agents in A549 cells. In their studies, clonogenic cell survival assays have demonstrated subadditive toxicity for the combinations of paclitaxel with thiopeta, melphalan, or cisplatin when the alkylating agents were administered first. In contrast, toxicity was additive when the cells were exposed to paclitaxel first. From cell cycle analyses of these interactions in A549 cells, Cook et al. have suggested that the alkylator-first sequence is less toxic, because the initial exposure to alkylators induces G2 arrest, thereby inhibiting the progression of cells into mitosis, in which phase of the cell cycle cells may be most sensitive to the cytotoxic effects of paclitaxel. Therefore, less toxicity is observed with the alkylator-first sequence.

Pharmacological interactions have also had a profound impact on the toxicity of combination chemotherapy with paclitaxel and doxorubicin. Holmes et al. (11) evaluated paclitaxel by 24-h infusion and doxorubicin by 48-h infusion and found that doxorubicin clearance was reduced by 33% (from 51.6 to 34.3 liters/h/m²) when paclitaxel was administered before doxorubicin. The altered pharmacokinetics of doxorubicin resulted in more profound neutropenia in patients who received paclitaxel first. Further evaluation of the paclitaxel/doxorubicin doublet has been performed by Gianni et al. and by Gehl et al. in two separate studies (12, 19). Gianni et al. evaluated the combination of doxorubicin as a 5-min bolus and paclitaxel as a 3-h infusion in a Phase II study of 35 women with advanced breast cancer. The infusions were separated by 15 min. Gehl et al. treated 30 women with the paclitaxel/doxorubicin combination, also administering paclitaxel over 3 h. Although the response rates to therapy were high in both studies, cardiac toxicity complicated treatment to a significant extent. Gianni reported cardiac failure in 18% of patients after a median 480 mg/m² doxorubicin. Gehl et al. observed congestive cardiac failure in 20% of 30 patients. In 15 patients treated by Gehl, the left ventricular ejection fraction dropped below normal, and significant reductions were noted after only 360 mg/m² cumulative dose of doxorubicin. Sequences of drug administration were evaluated only in the study by Gianni, and no evidence of sequence-dependent toxicity was observed. However, Gianni et al. have recently reported on detailed pharmacological studies of the influence of sequence of drug administration, interval between drugs, drug dosing, and duration of doxorubicin infusion on the plasma disposition of doxorubicin and paclitaxel (administered over 3 h; ref. 13). These studies have in fact indicated that paclitaxel administered over 3 h before doxorubicin can increase the doxorubicin peak level and reduce both the peak level and AUC of the main metabolite of doxorubicin, doxorubicinol. Studies of the interval between drug infusions indicated a rebound in the concentration of both doxorubicin and the metabolite doxorubicinol as soon as paclitaxel infusions were begun. This rebound lasted about 6 h, and the effect was greater with higher doses of paclitaxel. Additional studies evaluating doxorubicin administered as a 3-h and 15-min infusion confirmed that these effects are also dependent on anthracycline concentration. These data clearly indicate a pharmacokinetic interaction between paclitaxel (by 3-h infusion) and doxorubicin that may be responsible for both the cardiac toxicity and substantial antitumor activity observed by Gianni for this combination administered on this schedule. Gianni has postulated that these interactions may result from competition between doxorubicin and paclitaxel, formulated in cremophor EL, for P-glycoprotein-mediated biliary excretion.

We have previously evaluated the combination of cyclophosphamide with paclitaxel administered as a 24-h infusion in women with advanced breast cancer (10). The MTD for this

<table>
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<tr>
<th>Table 7</th>
<th>Mean AUC for cyclophosphamide and paclitaxel in those patients for whom data from both sequences of drug administration was available</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose</td>
<td>C/P&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>---------</td>
<td>-----------------</td>
</tr>
<tr>
<td>Cyclophosphamide (mg/m²)</td>
<td></td>
</tr>
<tr>
<td>1600</td>
<td>2739</td>
</tr>
<tr>
<td>2000</td>
<td>2673</td>
</tr>
<tr>
<td>1600 and 2000 combined</td>
<td>250</td>
</tr>
<tr>
<td>Paclitaxel (mg/m²)</td>
<td></td>
</tr>
<tr>
<td>250</td>
<td>44.7</td>
</tr>
</tbody>
</table>

<sup>a</sup> C/P, cyclophosphamide followed by paclitaxel.

<sup>b</sup> P/C, paclitaxel followed by cyclophosphamide.
combination on this schedule was 200 mg/m² paclitaxel and 1250 mg/m² cyclophosphamide. Both sequences of drug administration were evaluated in all patients, and the hematological toxicity of therapy was more severe in courses in which paclitaxel was administered first. However, unlike the interaction between paclitaxel and doxorubicin noted by Holmes and by Gianni, in which paclitaxel reduces the clearance of doxorubicin, no effect of drug sequencing was noted on drug clearance for either cyclophosphamide or paclitaxel, although a full evaluation of the pharmacology of metabolites of both drugs was not attempted. Preclinical data, as reported by Cook et al. (see above) suggest that the interaction between paclitaxel and cyclophosphamide is due to intrinsic effects of the drugs on cell cycle progression rather than on pharmacokinetics. This is congruent with the observation that cyclophosphamide is not a target of P-glycoprotein-mediated biliary excretion. In the current study, the impact of drug sequencing on hematological toxicity was evaluated in patients treated with cyclophosphamide and paclitaxel administered as a 3-h infusion. The hematological toxicity of therapy was again observed to be sequence specific with this briefer infusion duration. Both neutrophil and platelet nadir counts, expressed as a proportion of pretreatment levels, were lower in courses in which paclitaxel was administered first. These effects are similar to those observed in the prior study of 24-h paclitaxel with cyclophosphamide. The pharmacokinetic analysis in the current report is very limited and has only focused on the parent compounds cyclophosphamide and paclitaxel. AUC of paclitaxel could only be analyzed in four patients with both sequences of drug administration, because in several patients, the 24-h sample was not drawn or was zero. Accordingly, because of the small numbers analyzed, the available data from this study do not rule out the possibility of a pharmacokinetic interaction between paclitaxel and cyclophosphamide.

Why is sequence-specific toxicity observed with both 3- and 24-h paclitaxel in combination with cyclophosphamide, but only with the 24-h paclitaxel in the paclitaxel/doxorubicin combination? The answer probably lies with the different causes of sequence-specific toxicity for these doublets. The available evidence suggests that for the paclitaxel/cyclophosphamide doublet, sequence-specific interactions are not due to alterations in drug disposition, but to effects on cell cycle progression of target tissues (18). Initial administration of cyclophosphamide delays cell cycle progression into G₂-M phase, thus reducing cytotoxicity due to paclitaxel and ameliorating hematological toxicity. This effect is due to the initial administration of cyclophosphamide and occurs independent of whether paclitaxel is subsequently administered over a 3- or 24-h infusion. However, the mechanism of sequence-specific toxicity is quite different when paclitaxel is administered with doxorubicin. For this combination, the evidence from the studies of Holmes and of Gianni suggests that paclitaxel reduces the clearance of doxorubicin, resulting in increased toxicity when paclitaxel as a 24-h infusion is administered first. Gianni et al. did not observe clinical evidence of sequence-dependent effects on toxicity in their study of 3-h paclitaxel and doxorubicin. Their pharmacological studies, however, do indicate that the initial administration of paclitaxel over 3 h can interfere with the disposition of subsequently administered doxorubicin as well as its metabolites. Gianni et al. have proposed that sequence did not have an impact on toxicity in their initial study, because the pharmacokinetic differences did not lead to significantly different AUCs of either drug between the two sequences.

Many issues related to the use of taxanes in the treatment of metastatic breast cancer are still unclear. For patients with previously treated disease, the optimal duration of infusion, dose of paclitaxel, and relative merits of single-agent or combination therapy remain the focus of clinical investigation. For paclitaxel-containing combinations, it is clear, however, that complex interactions between the drugs can have a significant impact on toxicity, and that these effects can occur when paclitaxel is infused over 3 h. It remains uncertain whether these differences in toxicity will be reflected in differences in activity against breast cancer, and this possibility is currently being tested in clinical trials.

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REFERENCES


Sequence-dependent hematological toxicity associated with the 3-hour paclitaxel/cyclophosphamide doublet.

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