The Pharmacologic Effects of Recombinant, Human Colony-Stimulating Factors and Their Modulation by Theophylline


Study Objectives. To investigate the effect of colony-stimulating factors (CSFs) on drug metabolism using theophylline as a substrate (phase I), and to evaluate the influence of theophylline on endogenous serum cytokine concentrations (phase II).

Design. Open-label, prospective study.

Setting. The bone marrow transplant unit of a tertiary university teaching hospital.

Patients. Thirty-seven women with breast cancer (28 phase I, 9 phase II).

Interventions. Patients received aminophylline 0.58 mg/kg either as a 30-minute intravenous infusion before receiving recombinant CSFs or after several days of CSF therapy, just before high-dose chemotherapy (phase I) or as a continuous intravenous infusion after bone marrow transplantation (phase II).

Measurements and Main Results. Clearance of theophylline was significantly higher after CSF administration (0.76 vs 0.99 ml/min/kg, p=0.019). Continuous infusion of aminophylline resulted in elevations of serum macrophage-CSF and interleukin-6.

Conclusions. Administration of CSFs before autologous bone marrow transplantation for priming progenitor cells may alter drug metabolism. Studies should be conducted to evaluate the potential effects of CSFs on the disposition of chemotherapeutic agents.

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Recombinant colony-stimulating factor (CSF) use has dramatically reduced the hematologic toxicity associated with high-dose chemotherapy and autologous bone marrow transplantation (ABMT). The addition of CSF-induced peripheral blood progenitor cells (PBPCs) into supportive care regimens has also attenuated some nonhematologic toxicities. The mechanism behind the nonhematologic effects of PBPC support is not well understood and may include alteration of chemotherapy metabolism in addition to correlation of accelerated hematopoiesis.

Administration of some recombinant CSFs, such as granulocyte-macrophage (GM)-CSF, affects the serum concentrations of endogenous cytokines including tumor necrosis factor (TNF). Tumor necrosis factor is a potent
inhibitor of hepatic P-450 enzymes in animal models. The potential effect of administered or secondarily generated cytokines on hepatic enzyme systems is extremely important, since stimulation and collection of autologous PBPCs typically occurs 1–2 weeks before administration of high-dose chemotherapy. If use of CSF in this manner modulates drug metabolism by direct or indirect effects, it could potentially alter systemic exposure to cancer chemotherapy.

Elevations in endogenous cytokines are also associated with organ toxicities after ABMT, and therefore are a target for pharmacologic blockade. Several methylxanthine derivatives can impede TNF production in white blood cells. Among these, pentoxifylline was under clinical evaluation at the beginning of the current trial. We anticipated that theophylline might be a better selection, since the plasma concentrations achieved with typical dosages are closer to the amount required for in vitro inhibition of TNF.

This study was designed around two objectives. First was to investigate the effect of PBPC induction with CSF on drug metabolism using theophylline as a substrate. The second was to perform a pilot evaluation on the ability of theophylline to alter endogenous cytokine disposition after ABMT.

Materials and Methods

This was a prospective, open-label, non-randomized evaluation of the effects and metabolism of theophylline in women with metastatic breast cancer who were receiving high-dose chemotherapy with autologous cellular support. Approval for the study was obtained from the institutional review board before initiation, and all subjects provided written informed consent.

Patients with stage IV breast cancer who were receiving recombinant granulocyte (G)-CSF (filgrastim) or GM-CSF (sargramostim) for facilitation of PBPC collection were eligible (Table 1). All patients received exactly the same prior therapy for their malignancy and were fully recovered from the last cycle of chemotherapy at the time of enrollment. Details of the CSF regimen and leukapheresis procedures are described elsewhere. Patients were eligible to participate in only one of the two phases of the study. Exclusion criteria were a history of smoking within the previous 8 months, and exposure to any known inducers or inhibitors of hepatic metabolism within 3 weeks of CSF initiation for PBPC induction.

Phase I

Drug Administration

Aminophylline was given after informed consent as a 5.8-mg/kg (ideal body weight) intravenous infusion over 30 minutes by a volumetric infusion device before beginning growth factor, or after 6 days of CSF use (immediately preceding high-dose chemotherapy; Figure 1). Plasma was obtained to determine theophylline concentrations just before the infusion and at 0.5, 0.75, 1, 2, 3, 4, 6, 8, 10, and 12 hours after its initiation.

Analytic Methods

Plasma was analyzed for theophylline concentration by a high-performance liquid chromatographic method. Samples were tested in duplicate and compared with a standard curve that was linear over the concentration range of 0.5–25.0 µg/ml. Results were considered acceptable if both high- and low-spiked controls

Day from Marrow Infusion

<table>
<thead>
<tr>
<th>Phase I</th>
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<tbody>
<tr>
<td>0.03 mg/kg IV</td>
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<tr>
<td>Phase II</td>
</tr>
</tbody>
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Figure 1. Study treatment schema including ancillary drugs. All patients received exactly the same high-dose chemotherapy regimen. Solid lines indicate drugs given as continuous infusions, and dots and boxes represent intermittent infusions. CGF = filgrastim or sargramostim; PBPC = peripheral blood progenitor cells.
Table 2. Median [quartiles] Pharmacokinetic Variables After 30-Minute Infusions of Aminophylline (phase I)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Before CSF (n=22)</th>
<th>Before ABMT (n=16)</th>
<th>p Value*</th>
</tr>
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<tbody>
<tr>
<td>Systemic clearance</td>
<td>0.764 [0.533-0.822]</td>
<td>0.994 [0.774-1.112]</td>
<td>0.019</td>
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<tr>
<td>(ml/min/kg)</td>
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<tr>
<td>AUC (μg/ml*min)</td>
<td>6072 [5648-8712]</td>
<td>4669 [4175-5995]</td>
<td>0.019</td>
</tr>
<tr>
<td>C_max (μg/ml)</td>
<td>13.8 [10.3-18.5]</td>
<td>12.7 [12.3-13.5]</td>
<td>0.584</td>
</tr>
<tr>
<td>Half-life (hrs)</td>
<td>4.3 [2.6-6.3]</td>
<td>3.4 [2.1-4.7]</td>
<td>0.315</td>
</tr>
<tr>
<td>Vdss (L/kg)</td>
<td>0.612 [0.514-0.685]</td>
<td>0.589 [0.492-0.626]</td>
<td>0.089</td>
</tr>
</tbody>
</table>

*Mann-Whitney U test.

were within 10% of expected values and the sample concentrations were within the standard curve range. Interassay and intraassay coefficients of variation were less than 5%.

Pharmacokinetic Modeling

The appropriate pharmacokinetic model and initial parameter estimations for each patient were selected by curve stripping (RSTIRIP V.4.03, MicroMath, Salt Lake City, UT). Subsequent evaluations of individual data sets were conducted using a two-compartment model with zero-order input and a first-order elimination process (PCNONLIN V.4.2, ClinTrials, Apex, NC). All pharmacokinetic variables were calculated from the modeled output.

Number of Patients

The sample size was selected based on an 80% power to detect a difference in clearance before versus after growth factor administration. An assumption was made that the interpatient standard deviation of the clearance difference was not more than twice the mean change.

Phase II

Drug Administration

The high-dose chemotherapy regimen consisted of cyclophosphamide 5625 mg/m², carmustine 600 mg/m², and cisplatin 165 mg/m² administered over 4 days (day -6 to day -3). All patients received prophylactic antibiotics consisting of oral ciprofloxacin and rifampin commencing on day -2. They were started on a continuous intravenous infusion of aminophylline 0.58 mg/kg/hour on transplant day +1 (day of marrow reinfusion). A plasma sample for theophylline determination was sent to the clinical laboratory 48 hours into the continuous infusion to adjust the rate, if necessary, to maintain a target concentration of 12 μg/ml ± 20% (acceptable range 9.6-14.4 μg/ml). Follow-up samples were drawn every 4 days, or as required, for adjustment or clinical evaluation. Infusions were intended to continue until the absolute neutrophil count was above 500 cells/mm³ on two repeated measurements.

Apparent systemic drug clearance was determined using noncompartmental methods as described by the equation: clearance = infusion rate/serum concentration. A blood sample was also drawn every morning during the infusion to evaluate endogenous cytokine concentrations.

Analytic Methods

Fluorescence polarization immunoassay was performed by the clinical laboratory to quantify theophylline concentrations during continuous infusions. Serum concentrations of endogenous cytokines (TNF-α, interleukin-6, M-CSF) were determined by enzyme-linked immunosorbent assay.

Statistical Analysis

The Mann-Whitney U test was used for comparisons of pharmacokinetic values in the treatment groups in phase I, as well as the day +8 endogenous M-CSF concentrations in controls and patients in phase II. The criterion for statistical significance was defined as probability of 0.05. Mean log concentration-time curves for endogenous M-CSF were obtained by regression analysis using the equation: log y = A + Bx + Cx².
where \( y \) = concentration and \( x \) = day of therapy after bone marrow reinfusion.

Results

Phase I

Thirty women with stage IV breast cancer were enrolled in this portion of the study. Two were excluded due to problems with intravenous access that hindered collection of adequate numbers of samples. Thirty-eight aminophylline disposition studies were conducted in these 28 patients. Ten patients received aminophylline at both phase I administration periods. Twenty-two studies were performed before administration of CSFs and 16 just before the high-dose regimen (after PBPC induction). Of these 16 women, 15 received G-CSF as the inducing agent, and the remaining patient received GM-CSF.

No adverse effects attributable to the aminophylline infusions were observed. The median systemic theophylline clearance and systemic exposure (area under the curve [AUC]) were significantly higher after CSF administration for inducing PBPCs, and the AUC was lower (Table 2). No other pharmacokinetic parameters were different.

Phase II

Continuous infusions of aminophylline were administered to nine patients with stage IV breast cancer. Four of them were concurrently receiving GM-CSF and five G-CSF to aid hematopoietic recovery. The apparent systemic clearance of theophylline was higher than expected during the infusion (median 1.23 ml/min/kg, interquartile range 0.965–1.40 ml/min/kg); therefore, the initial dosing regimen required modification in most patients to achieve the desired target. We were able to maintain concentrations above 9.6 \( \mu \)g/ml on an average of 86% of surveyed treatment days after day +3.

Two women were discontinued from the study before neutrophil reconstitution (both on day +8) due to diarrhea or urine retention that was thought possibly attributable to aminophylline. Only one patient experienced hepatic venoocclusive disease. She also had the only documented fungal infection during this trial. Her cytokine disposition and theophylline pharmacokinetic data are shown in Figure 2. It is of interest that infusion of the study drug did not prevent the appearance of circulating TNF-\( \alpha \) or M-CSF in this woman.

Descriptions of the M-CSF kinetic patterns in study patients and controls (matched for type of CSF therapy and cellular support as described below) are shown in Figure 3. A quadratic model was fit to all available concentration-time points for each group and cytokine treatment subgroup. Aminophylline seemed to be associated with an alteration in the pattern of M-CSF disposition over time. The concentrations increased in a linear fashion in those receiving treatment, whereas the pattern of disposition in controls was a function of both day of therapy and day of therapy squared.

Serum concentrations of interleukin (IL)-6 and M-CSF were analyzed on day +8 (final day on

![Figure 2. Cytokine and theophylline kinetics in a woman who received GM-CSF-primed PBPCs after ABMT. The arrow indicates the day (+9) on which a fungemia was first detected. Apparent theophylline clearance is expressed in ml/hr/kg.](image)

![Figure 3. Regression of serum M-CSF concentrations from days 2 to 10 in patients treated with sargramostim (upper two lines) or filgrastim (lower lines). Solid lines depict data for women who received aminophylline during phase II of the protocol, and broken lines represent control patients.](image)
which all patients were still receiving aminophylline) and compared statistically with the group of previously reported control patients, matching for type of CSF and cellular support prescribed in addition to day after ABMT. The median (intraquartile range) M-CSF concentration was 3.05 ng/ml (2.05–3.88 ng/ml) in the nine women receiving aminophylline versus 1.43 ng/ml (0.98–1.75 ng/ml) in the 18 controls (p=0.008). Concentrations of IL-6 were also higher in the study patients than in controls [25.0 (13.5–258) vs 14.7 (4.2–33.8) pg/ml, respectively, p=0.095].

Discussion

The typical peripheral leukocyte concentration after a 6-day cycle of G-CSF as given in this study for inducing PBPCs reaches approximately 40,000 cells/mm³ by the end of the last day. Since many cytokines are made by white blood cells, this leukocytosis could potentially increase the circulating concentrations of these molecules. Endogenous concentrations of some cytokines influence liver metabolism in animal models, and thus altered metabolism of subsequently administered chemotherapy is a possibility. Other evidence pointing toward a potential interaction between CSFs and hepatic function comes from investigations of patients with cytokine-producing tumors showing that liver function is altered. In addition, patients given recombinant CSFs with or without chemotherapy occasionally have elevations in liver function tests.

Theophylline was chosen as a nonspecific marker substrate for hepatic drug metabolism due to its relatively innocuous adverse effect profile at the dosages and duration prescribed here, and its ease of measurement and extensive metabolism. Theophylline systemic clearance was significantly faster (1.3-fold) after the administration of CSFs for priming of PBPCs. This suggests that hepatic metabolism of subsequent high-dose chemotherapy might also be altered. These data may be of particular importance for drugs such as cyclophosphamide, which require metabolic activation for efficacy. This work highlights the potential for all interventions, both during and before administration of high-dose chemotherapy, to affect the ablative regimen. Further evaluations should be conducted to determine if the metabolism of specific chemotherapeutic agents is altered by CSF induction of PBPCs.

Dosage escalation studies of some CSFs noted significant toxicities including severe hypotension, renal dysfunction, and a capillary leak syndrome. One potential mechanism of these effects is thought attributable to the ability of a recombinant cytokine to stimulate secretion of endogenous molecules such as TNF. Secretion of TNF in pathologic processes (e.g., septic shock) is correlated to the severity of symptoms. Elevated concentrations of TNF and several other cytokines were also detected in patients experiencing complications after bone marrow transplantation. These data have led investigators to pursue strategies that pharmacologically block cytokine synthesis or action.

Animal studies with the methylxanthine derivative pentoxifylline demonstrated its ability to modulate endotoxin-induced TNF production and prolong survival. The cellular mechanism of this interaction is suspected to be blockade of TNF mRNA accumulation. Randomized, placebo-controlled, clinical studies of pentoxifylline for the prevention of transplant-related complications failed to confirm the promising results noted in a phase I-II trial; however, some methods and concomitant drug concerns may confound these data. A 50% suppression of TNF production occurred in cell culture at pentoxifylline concentrations of approximately 7 µg/ml or theophylline concentrations of 19 µg/ml. Unfortunately, the usual maximum plasma concentration of pentoxifylline after a 400-mg oral dose is less than 1 µg/ml, and the parent drug and metabolites are rapidly excreted. Taking these in vitro and pharmacokinetic differences into account, we postulated that theophylline may be a more clinically useful inhibitor of TNF secretion.

Endogenous cytokine data available to us before beginning this study were obtained in patients who did not receive PBPCs. A majority of patients in that setting had measurable TNF-α concentrations after ABMT. The frequency of detection dropped precipitously with use of PBPCs. Thus we were not able to address the impact of theophylline on serum TNF concentrations in this study. We did note that TNF was not totally blocked.

Concentrations of other endogenous cytokines (M-CSF and IL-6) were higher in women who received continuous infusion of aminophylline than in controls. It must be emphasized that these patients, but not the controls, were also receiving ciprofloxacin, another known modulator
of cytokines.27 The results are not totally surprising, given the fact that administration of pentoxifylline selectively inhibits TNF but not IL-6.28 The clinical implications of a pharmacologic elevation of endogenous IL-6 are unknown; however, one could postulate an augmentation of thrombosis or an increase in adverse effects such as fever.29 Elevations in M-CSF seem to be a normal response to foreign invasion by organisms such as fungi.30 In addition, M-CSF is increased in patients with organ dysfunction4 and may negatively affect thrombopoiesis.31

The theophylline clearance during continuous infusion after ABMT (1.23 ml/min/kg) was substantially higher than anticipated for a group of nonsmoking adults (typical population mean 0.72 ml/min/kg).32 A possible explanation for this finding may lie in the ability of high-dose cyclophosphamide to induce hepatic enzymes.33 We are unaware of any previous reports suggesting this specific drug interaction. Rifampin also may have contributed to the hypermetabolic state since studies in healthy subjects showed that 1–2 weeks of therapy can increase theophylline clearance by 38–45%.34,35 The women in the present study were also simultaneously receiving a known inhibitor of theophylline metabolism (ciprofloxacin).36 A firm conclusion as to the etiology of elevations in theophylline clearance after ABMT is not discernable, but we believe that the evidence is highly suggestive of a role for cyclophosphamide in the metabolic induction of theophylline.

The data from this pilot study do not substantiate a therapeutically meaningful role for use of aminophylline as a toxicity-modulating agent after high-dose chemotherapy. More work has to be done to discern the potential influence of PBPC induction by CSFs on the metabolic fate of chemotherapeutic agents.

References