A Phase I Clinical Trial of Novobiocin, a Modulator of Alkylating Agent Cytotoxicity

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ABSTRACT

Antineoplastic drug resistance is a major obstacle to improved treatment of most adult cancers in humans. Novobiocin, an antibacterial agent which inhibits the eukaryotic topoisomerase II enzyme, increases the cytotoxicity of several alkylating agents in vitro by the formation of lethal DNA-DNA interstrand cross-links, perhaps by decreasing the repair of drug monoaducts. In murine tumors treated in vitro novobiocin markedly potentiates alkylating agent cytotoxicity without concomitant increases in host toxicity. With this background, a Phase I trial of novobiocin and cyclophosphamide was performed in refractory cancer patients. Novobiocin was given p.o. for 96 h; 750 mg/m² of i.v. cyclophosphamide was administered at 48 h. Thirty-four patients received 65 courses. The dose-limiting toxicity of novobiocin in this trial was vomiting. The maximum tolerated dose was 6 g/day. Six of 34 patients had Grade III or IV myelosuppression but no dose escalation effect was noted. Three patients developed allergic reactions which resolved completely. No other significant toxicity occurred. While no dose-dependent effect on serum novobiocin levels occurred, 18 of 19 patients treated at ≥4 g daily had serum levels ≥100 μg/ml at steady state, a level which corresponds to levels used in vitro and seen in vivo where the murine novobiocin half-life of 82 min is far less than that seen in humans (6.0 h). Two of 30 evaluable patients had partial responses. Four other patients had stable disease. Four of six had prior disease progression on cyclophosphamide combination therapy. Novobiocin is well tolerated in patients receiving cyclophosphamide and blood levels are in the drug-potentiating range. Phase II trials in cyclophosphamide refractory patients are anticipated.

INTRODUCTION

Factors that have been shown to improve the efficacy of chemotherapy include the use of antineoplastic agents in combination, each of which is individually active against a particular tumor type, at the maximum tolerated dose and the minimal dosing interval possible (1). The use of drugs with nonoverlapping dose-limiting toxicities facilitates this approach. Nevertheless, the vast majority of adult cancers are either refractory to drug therapy at the initiation of treatment or develop resistance after initial cytoreduction. Approaches which selectively enhance tumor cytotoxicity, either empirically or by selective targeting of known mechanisms of resistance, might overcome drug resistance or enhance efficacy.

Novobiocin, an inhibitor of topoisomerase II, produces enhanced tumor cytotoxicity of several alkylating agents (2). In cell culture, novobiocin results in marked synergy with cisplatin and carmustine in association with an increased number of DNA interstrand cross-links (2). In a human lymphoblastic lymphoma cell line that is resistant to the alkylating agent nitrogen mustard and exhibits decreased DNA interstrand cross-links and elevated topoisomerase II levels, novobiocin restores sensitivity to nitrogen mustard and is uniquely toxic to the resistant cells (3, 4). Novobiocin is a coumermycin antibiotic used to treat soft tissue and urinary tract infections (5). Novobiocin inhibits the bacterial DNA gyrase, a topoisomerase II (6). A high level of allergic reactions and the rapid development of bacterial resistance have caused a decrease in the current use of novobiocin (7). The use of combination novobiocin and rifampin shows promise as a treatment for methicillin-resistant staphylococci infections (8).

In vivo, novobiocin increases the tumor growth delay and decreases the clonogenic survival produced by cyclophosphamide, carmustine, and cisplatin in the F5AsI mouse sarcoma (5). In these studies, the F5AsI tumors grows s.c. and drugs are given i.p. In the tumor excision studies, concomitant bone marrow obtained at the time of animal sacrifice and cultured in vitro showed a significantly lower toxic effect than the tumor, producing an enhanced therapeutic ratio. A novobiocin dose of 100 mg/kg daily produced significant chemotherapy modulation. The in vitro and in vivo animal studies were sufficiently promising to be extended to a clinical trial in humans.

The serum level of novobiocin necessary to potentiate cyclophosphamide or another alkylating agent in humans is unknown. In tissue culture experiments, 100 μg/ml produced synergy with cisplatin and carmustine. Since the preclinical studies suggest a dose-response effect for novobiocin, the objectives of this study were to establish the maximum tolerated dose of cyclophosphamide and novobiocin and to establish that serum levels in patients equal or exceed the serum levels in mice where a modulating effect is seen. Current doses of cyclophosphamide used in combination chemotherapy regimens range between 500 and 1000 mg/m². Since most patients in the Phase I study described below would have received extensive prior chemotherapy and some enhancement of the bone marrow toxicity of cyclophosphamide by novobiocin was seen in preclinical studies, an intermediate cyclophosphamide dose (750 mg/m²) was chosen which would provide antitumor efficacy and some myelosuppression and still be less than maximal, so that enhanced toxicity could be assessed. Steady state blood levels of novobiocin were obtained in all patients to provide a correlation between dose and clinical effect.

MATERIALS AND METHODS

Patients with inoperable or metastatic cancer for whom no curative or established palliative chemotherapy existed were eligible. All had signed an informed consent, had a WBC count ≥3,000/μl, a platelet count ≥100,000 μl, a creatinine clearance ≥50ml/min, and serum bilirubin and SGOT ≤1.5 times normal. No restriction was placed on performance status.

Novobiocin (Albamycin; Upjohn Co., Kalamazoo, MI) was administered p.o. for 48 h, twice or 3 times daily, prior to 750 mg/m² of i.v. cyclophosphamide and for 48 h thereafter (Fig. 1). Serum novobiocin levels were obtained prior to therapy, at the time of cyclophosphamide treatment and 24 h later. Samples were frozen at −70°C after centrifugation and were assayed by a microbiological technique (6). Patient samples at the higher doses (≥4 g) were also assayed by high perform-

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[510]
could receive multiple courses of therapy as individual circumstances which prevented patients from receiving novobiocin following chemotherapy regimens and showed evidence of progressive disease at the time of treatment. The maximum tolerated dose of p.o. novobiocin was 6 g daily. The dose-limiting toxicity was nausea and vomiting during the 48 h before cyclophosphamide, which prevented patients from receiving novobiocin following the cyclophosphamide. While only Grade II by WHO toxicity criteria, this nevertheless precluded further dose escalation, since the only preparation of novobiocin was 250-mg capsules for p.o. intake.

Toxicity was generally mild to moderate (Table 2). Six of 34 had Grade 3 or 4 myelosuppression. Despite this myelosuppression, there were only 2 episodes of febrile neutropenia, both of which resolved. Patients retreated at the same or increased novobiocin dose with cyclophosphamide had no evidence of increased myelosuppression at the higher dose and no evidence of cumulative myelosuppression. Two patients had significant thrombocytopenia. One patient had no thrombocytopenia or neutropenia on her first cycle but had severe thrombocytopenia (<20,000/μl, requiring transfusion) after 2 days of novobiocin on her second cycle. She never received her second dose of cyclophosphamide. A drug-mediated autoimmune reaction was suspected and she was not rechallenged.

Three patients had significant increases in liver transaminase or bilirubin levels. No patient developed an elevation who did not have known prior liver metastases. Several patients had isolated elevations of indirect bilirubin while taking novobiocin which later resolved. (A metabolite of novobiocin is known to react with the Van den Bergh test (11).) One patient developed a fever, rash, and mild urticaria while taking novobiocin. One patient developed mild joint pains in the absence of any other symptoms which resolved after novobiocin was discontinued. Two patients died on study. One patient with colon cancer developed an overwhelming pneumonia with normal neutrophil count. A man with a squamous cell cancer of the tongue had erosion of tumor into his carotid artery and exsanguinated with a normal platelet count.

In the pharmacological aspect of this study, novobiocin serum levels were obtained on each patient for at least one treatment course. Since the pharmacology of novobiocin has already been established, only steady state levels were measured. The microbiological assay generally conforms very well to the HPLC results (12). Levels measured however, were substantially less than those previously reported for this dose of novobiocin. HPLC was then used at novobiocin doses of 4 g daily or higher. The results are shown in Figure 1. Serum levels are displayed on a mg/kg basis rather than by dose level. There appears to be no significant correlation with serum level and dose for this p.o. absorbed drug (r² = 0.02). Nevertheless, 95% (18 of 19) of patients who received novobiocin doses ≥50 mg/kg had serum levels >100 μg/ml at the time of cyclophosphamide therapy. The half-life (t½) of p.o. novobiocin in healthy human volunteers is 6 h (12).

To provide a therapeutic context for the serum levels of novobiocin achieved in patients, serum levels were obtained from 24 male C3H/FeJ mice (Fig. 2), in which an enhanced therapeutic effect was observed between novobiocin and cyclophosphamide. Peak levels varied widely despite the i.p. route of administration. However, the serum half-life was nearly identical, with a t½ of 80 min. Although the short half-life would produce a total serum exposure (area under the curve) well below that achieved in patients taking 2 to 3 doses daily, therapeutic enhancement was observed in the mice.

Two patients had partial responses in this Phase I trial (Table 3). A woman with breast cancer metastatic to lung, pleura, and chest wall had resolution of all disease except pleural thickening for 6 months. A man with metastatic colorectal cancer had a decrease in lung metastases and a decreased carcinoembryonic antigen level for 3 months. Four additional patients had stab-
Table 2 Toxicity ≥ grade II (WHO grade)

<table>
<thead>
<tr>
<th>Dose level</th>
<th>Patients</th>
<th>Neutrophils</th>
<th>Platelets</th>
<th>Liver</th>
<th>Renal</th>
<th>Allergic</th>
<th>Bleeding</th>
<th>Infection</th>
<th>Emesis(^a)</th>
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<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>2</td>
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</table>

\(^a\) During novobiocin therapy.
\(^b\) Numbers in parentheses, not related to chemotherapy.
\(^c\) Died.

Table 3 Response data (evaluable)

<table>
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<tr>
<th>Disease site</th>
<th>No. of patients</th>
<th>CR(^a)</th>
<th>PR</th>
<th>SD</th>
<th>PD</th>
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<td></td>
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<tr>
<td>Other</td>
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<td></td>
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<tr>
<td>Total</td>
<td>30(^b)</td>
<td>2</td>
<td>4</td>
<td>24</td>
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</table>

\(^a\) CR, complete response; PR, partial response; SD, stabilization of metastatic disease; PD, partial stabilization of metastatic disease; NSCLC, non-small cell lung cancer.

\(^b\) Three patients never received cyclophosphamide. Two died by day 12 after treatment.

The mechanisms underlying chemotherapy drug resistance are multiple and include decreased transport, enhanced efflux, decreased activation, increased metabolism, expanded nucleotide pools, increased thiol binding, target gene amplification, and increased DNA repair (reviewed in Ref. 13). Biochemical interference or modulation with these mechanisms has the potential to enhance cytotoxicity of clinically available cancer treatments. Despite extensive knowledge about the chemistry, metabolism, and intracellular target binding of antineoplastics (14), the precise cytotoxic mechanism of cancer cell lethality is uncertain. In particular, the cytotoxic mechanism by which the DNA adducts of alkylating agents results in cell death is not clear, thus making precise intervention empiric. In vitro studies have identified multiple mechanisms of resistance to alkylating agents and empiric trials using agents directed against these pathways might enhance the cytotoxicity of alkylating agents in sensitive and resistant cells (15).

Novobiocin increases the cytotoxicity of several alkylating agents in association with an increase in the formation of DNA-DNA cross-links (2). That this effect is related to inhibition of topoisomerase II by novobiocin is supported by the fact that alkylating agent (nitrogen mustard) resistance is associated with elevated topoisomerase II levels (3) and is reversed by novobiocin. Preclinical trials of novobiocin and either carmustine, cisplatin, or cyclophosphamide showed significant enhancement of chemotherapeutic cytotoxicity with an increase in therapeutic index (5).

Novobiocin added very little, if any, toxicity to the cyclophosphamide. Despite a 6-fold increase in the usual dose of novobiocin, only 6 of 34 patients had major myelosuppression. Three of those patients were treated after investigational high dose chemotherapy regimens, including 1 following myeloablative chemotherapy and bone marrow autotransplant. One other patient in this group had bone marrow involvement by tumor. Grade III hepatotoxicity occurred in a single patient with progressive metastatic breast cancer to the liver and was likely due, in whole or part, to progressive cancer. Two patients developed clear allergic reactions which resolved after drug discontinuation. This 6% incidence of allergy is similar to the previous experience with novobiocin as an antibacterial (11). One other patient developed an autoimmune thrombocytopenia that was presumed to be due to novobiocin. This toxicity perhaps belongs more appropriately to the allergic category, underscoring the negligible nonallergic toxicity of this drug but also emphasizing the high incidence of these side effects, albeit usually mild.

Two of 30 patients (6.7%) had partial responses to chemotherapy. Six other patients had evidence of stabilization of previously progressive disease and were able to be treated for up to 8 cycles of novobiocin and cyclophosphamide. This degree of activity could have been due to cyclophosphamide alone. One of the 2 partial responders and 3 of 6 patients with disease stabilization had previously had disease progression on cyclophosphamide, and 5 of 6 had progressed on their most recent regimen.

At daily doses of 4 g or greater, plasma levels of novobiocin were consistently in excess of 100 µg/ml, the in vitro concentration required to observe synergy with cisplatin or carmustine. In mice receiving novobiocin plus cyclophosphamide under conditions that resulted in synergistic antitumor effects, the t\textsubscript{1/2}...
for novobiocin was 80 min. One can infer that the area under the curve in humans, considering the dosing interval and plasma levels of drug, is adequate to provide, in the plasma at least, concentrations of novobiocin that resulted in synergistic cytotoxicity in vitro and in mice.

Novobiocin represents a novel approach to the biochemical modulation of alkylating agent therapy. It is well tolerated and does not potentiate cyclophosphamide toxicity, as predicted by preclinical observations. A phase II trial is necessary to determine whether it has the capacity to enhance the efficacy of chemotherapy.

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