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ORIGINAL ARTICLE

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Activity of high-dose toremifene plus cisplatin in platinum-treated non-small-cell lung cancer: a phase II California Cancer Consortium Trial

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Abstract *Purpose*: Although cisplatin is an important agent in non-small-cell lung cancer (NSCLC), de novo resistance is common and acquired resistance emerges rapidly during therapy. Proposed mediators of platinum resistance include the protein kinase C (PKC) signal transduction pathway and associated *c-FOS* overexpression. While estrogen administration has been reported to upregulate PKC and *c-FOS* expression, the triphenylethylenes tamoxifen and toremifene potentiate platinum cytotoxicity by inhibition of PKC. Downregulation of *c-FOS* expression has been reported to result from PKC inhibition. In view of these findings, we hypothesized that toremifene would reverse platinum resistance and that this interaction would be influenced by tumor estrogen receptor (ER) status. *Materials and methods*: A phase II trial of high-dose toremifene (600 mg orally daily on days 1–7) plus cisplatin (50 mg/m^2 intravenously on days 4 and 11) every 28 days in NSCLC patients was conducted. A group of 30 patients with metastatic NSCLC who had been previously treated with platinum-based therapy were enrolled. *Results*: All of the 30 patients were assessable for toxicity and 28 for tumor response. Therapy was well tolerated with minimal hematologic and non-hematologic toxicity. Common toxicity criteria grade 3 hematologic toxicity was seen in only three patients. Five patients achieved a partial response for an overall response rate of 18% (95% CI 6–37). Median overall survival was 8.1 months (95% CI 5.4–17). To assess PKC, ER, and c-Fos expression by immunohistochemistry, 12 informative pretreatment patient tumor specimens were obtained. Four patient tumor specimens were positive for one or both PKC isoforms (α and ϵ) while c-Fos was overexpressed in three. None of the responding patient tumors exhibited *c-FOS* or PKC-ϵ overexpression. ER expression was found to be infrequent (8%), contrasting with previous reports in this tumor type. *Conclusion*: While this phase II study indicates that high-dose toremifene plus cisplatin is feasible, active, and well tolerated in NSCLC patients previously treated with platinum compounds, the mechanism of action remains unclear. Further study of this regimen is warranted.

Keywords Lung cancer · Chemotherapy · Cisplatin · Toremifene

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Introduction

Cisplatin is generally considered the most important chemotherapeutic agent in advanced non-small-cell lung cancer (NSCLC). However, cisplatin resistance often develops rapidly and ultimately leads to treatment

failure and disease progression. The identification and development of novel compounds that modulate platinum resistance is therefore of interest. Several mechanisms responsible for the development of platinum resistance have been identified, including enhanced activation of cellular detoxification systems, decreased drug accumulation, and altered DNA repair [37]. Other proposed mediators of cisplatin resistance include activation of the protein kinase C (PKC) signal transduction pathway [23], resulting in overexpression of the *c-FOS* oncogene [8, 19]. Of interest, an antisense ribozyme (catalytic RNA) to *c-FOS* has been reported to reverse cisplatin resistance in an in vitro model [36].

The triphenylethylenes tamoxifen and toremifene possess chemosensitizing properties for several classes of chemotherapy, including anthracyclines, vinca alkaloids, and cisplatin [14]. Potentiation of cisplatin cytotoxicity by triphenylethylenes has been correlated with decreased levels of PKC [28]. In a study demonstrating synergy of tamoxifen with cisplatin, the dose-response curves for the antiproliferative activity of cisplatin paralleled the dose-effect curves for PKC inhibition [14]. However, concentrations of tamoxifen required to achieve chemosensitization in vitro are approximately tenfold higher than those observed in patients receiving tamoxifen as antiestrogenic therapy [5, 32]. Initial clinical trials attempting to administer high-dose tamoxifen in combination with cisplatin have shown severe tamoxifen-related toxicities in some patients, reducing enthusiasm for this approach [26, 41].

Toremifene is a newer triphenylethylene that was initially developed to improve the therapeutic index of antiestrogens [12]. The activity of toremifene in metastatic, hormone-responsive breast cancer appears to be similar to that of tamoxifen. In human NSCLC cell lines, toremifene also demonstrates platinum chemosensitization [30]. We have previously reported that toremifene levels required for cisplatin chemosensitization and PKC modulation in vitro are achievable clinically with little associated toxicity [21]. Here, we report the results of a phase II trial of high-dose toremifene as a cisplatin modulator in NSCLC patients previously treated with platinum-based chemotherapy. Molecular correlative studies to investigate the potential roles of tumor estrogen receptor (ER), PKC, and *c-FOS* expression in patient response and outcome were also performed.

Materials and methods

Patients

Patients with histologically confirmed metastatic NSCLC were eligible. Patients were required to be at least 18 years of age and must have been previously treated with platinum-based chemotherapy. The maximum cumulative cisplatin dose prior to chemotherapy was required to be ≤ 300 mg/m^2 to avoid excessive neurotoxicity. Any number of prior chemotherapy regimens was allowed. Patients were required to have measurable disease with at least one bidimensional (perpendicular diameters) objectively measurable lesion; adequate performance status of 0–2 according to the Southwest Oncology Group (SWOG) criteria; adequate bone marrow reserve defined as a WBC count $\geq$3500/μl and a platelet count of $\geq$100,000/μl; adequate renal and hepatic function defined by a pretreatment creatinine clearance of $\geq$60 ml/min, a serum creatinine of ≤ 1.5 mg/ml, and serum bilirubin and SGOT of not more than twice the institutional upper limit of normal. Prior radiation therapy was allowed provided 3 weeks had elapsed since its completion. Pregnant and nursing women were excluded because of undefined risks to the fetus or infant. Patients with a prior malignancy were ineligible except for those with adequately treated basal cell carcinoma of the skin, in situ cervix cancer, or any other cancer in which the patient had been free of disease for 5 years. Patients with congestive heart failure, cardiomyopathy, or severe chronic obstructive pulmonary disease, which would all preclude the use of vigorous hydration, and those with documented brain metastases were excluded.

Ethics

Written informed consent was obtained from all patients on a protocol approved by the Institutional Review Boards of the participating institutions.

Treatment plan

Treatment cycles consisted of toremifene at a dose of 600 mg orally daily on days 1 through 7 plus cisplatin at 50 mg/m^2 administered intravenously over 1 h on days 4 and 11, repeated every 28 days. This cisplatin schedule was selected because it coincides with steady-state levels of toremifene which are achieved on day 4 and maintained through day 11. This represents a modification of a split dose (days 1 and 8) cisplatin schedule with which our group has had prior experience in the Southwest Oncology Group. Cisplatin was preceded by the administration of appropriate antiemetics (including ondansetron or granisetron and dexamethasone) and intravenous hydration with normal saline at 250–400 ml/h for a minimum of 2 h. Potassium chloride (20 mEq) and 1 g magnesium sulfate were added to each liter of normal saline unless contraindicated by hyperkalemia. Following completion of cisplatin infusion, hydration was continued at 250–400 ml/h for a minimum of 2 h. A maximum of six treatment cycles was planned.

Response evaluation

Prior to treatment, patients underwent a medical history, physical examination, laboratory tests (including a complete blood count, differential, electrolytes, liver and renal function tests, and a urine pregnancy test for women with child bearing potential), and a baseline chest computed tomography (CT) scan. Other imaging procedures such as bone scans were performed if clinically indicated or if they were positive pre-study. Creatinine clearance was calculated prior to each treatment course. Tumor measurements were performed by chest radiography or computed tomography after every two cycles. Tumor response was assessed according to standard World Health Organization criteria [44].

Toxicity assessment

All patients were examined and graded each treatment day for subjective and objective evidence of toxicities using the National Cancer Institute Common Toxicity Criteria version 1.0. Chemotherapy dose modification and delay was based on day 1 complete blood count, days 1 and 11 calculated creatinine clearance, and other interim toxicities. All dose reductions were permanent.

Criteria for removal from protocol treatment

Patients were removed from the study if any one of the following criteria were met: (1) progression of disease; (2) unacceptable toxicity (as determined by the treating physician or patient);

(3) toxicity requiring discontinuation of treatment despite dosage modification; (4) completion of six cycles of treatment; and (5) patient withdrawal for any reason.

Statistical analysis

A two-stage design was used with response rate as the primary efficacy endpoint. Secondary endpoints included overall survival, progression-free survival, and toxicity. We assumed that further study of this regimen would be warranted for a true response rate ≥20%, and would not be warranted for a true response rate of ≤ 5%. In the first stage of accrual, 14 evaluable patients would be enrolled and assessed. If no response was observed, then accrual would stop, with the conclusion that the regimen was not promising for further study in these patients. If one or more responses were seen in the first 14 patients, an additional 16 patients would be accrued in the second stage. Four or more responses out of 30 was considered evidence that the regimen warranted further study, provided that other factors such as toxicity and survival also appeared favorable. Using this design, the probability of correctly declaring that an agent with a true response rate of 20% warrants further study is 0.88 (power). The probability of declaring that an agent with only a 5% true response rate warrants further study is 0.06 (alpha). With 30 patients, the true response probabilities can be estimated with a maximum standard of error of 0.09. Any toxicity occurring with at least a 5% probability was likely to be seen at least once (79% chance). Duration of response, time to progression, and survival were estimated using the methods of Kaplan and Meier [18].

Molecular correlative studies

Pretreatment lung cancer tissues from 12 patients with NSCLC were obtained. Specimens were evaluated by a board-certified reference pathologist (R.G.E.) blinded to the clinical data. Tumor was confirmed on histologic sections and each immunostained slide scored for reactivity by light microscopy. We utilized commercial monoclonal antibodies to evaluate the expression of ER (DAKO, Carpinteria, Calif.), *c-FOS* protein (Oncogene Science, Cambridge, Mass.), and protein kinase pathway proteins PKC-α, and PKC-ϵ (Santa Cruz Biotechnology, Santa Cruz, Calif.). Immunohistochemistry was performed on 4-μm formalin-fixed, paraffin-embedded tissue sections from archival blocks using standardized avidin-biotin techniques. Briefly, sections were deparaffinized and cleared. Endogenous peroxidase activity was blocked by a 3% solution of hydrogen peroxide and methanol. Sections were then hydrated in graded alcohol to distilled water. Microwave heat-induced (100°C) antigen retrieval was performed in citrated buffer solution for a total of 4 min (two 2-min cycles). Slides were then cooled to room temperature and placed in a humidified chamber. A solution of 10% normal horse serum (Vector Laboratories, Burlingame, Calif.) was applied for 20 min.

After blotting, the primary antibodies were applied and the slides were incubated overnight at 4°C. After PBS rinsing, a horse anti-mouse biotinylated antibody (Vector Laboratories) was applied at a dilution of 1:800 followed by incubation for 60 min at room temperature. Two PBS rinses were followed by a 30-min incubation with avidin-biotin complex (Vector Laboratories). Color development was achieved by application of the chromogen DAB (Vector Laboratories) and careful monitoring of staining by direct light microscopy visualization. This generally was 2–3 min per section. The slides were then rinsed thoroughly in running tap-water, counterstained in Mayers hematoxylin, dehydrated, cleared and coverslipped. Duplicate sections subjected to all phases of staining except the primary antibody served as negative controls. Positive controls included paraffin sections of breast carcinoma for ER and *c-FOS* and Jurkat cells for the PKCs. Expression of the PKC-α and -ϵ and *c-FOS* were determined by cytoplasmic staining. ER expression was counted only when strong nuclear staining was observed. For all three markers, overexpression was defined as staining in more than 10% of the neoplastic cells in the specimen.

Results

Between September 1995 and December 1998, 30 patients with histologically proven, progressive metastatic NSCLC were enrolled by the three California Cancer Consortium institutions. Patient characteristics are shown in Table 1.

Toxicity

All patients were assessable for toxicity. A median of two treatment cycles were administered (range 0–6). Treatment was generally well tolerated, with no grade 4 hematologic toxicities or treatment-related deaths observed. Grade 3 hematologic toxicity occurred in only three patients, and consisted principally of anemia. Significant non-hematologic toxicities (grade 3 or 4) included the following: one patient with grade 3 nausea, one patient with grade 4 vomiting, four patients with grade 3 hyperglycemia, and two patients with 'flu-like symptoms. No severe toxicities attributable to toremifene were observed. No significant neuropathy was seen.

Response

Of the 30 patients, 28 were assessable for response. In five patients, a partial response was observed, for an overall response rate of 18% (95% CI 6–37). Nine patients (32%) had stable disease while 14 patients (50%) progressed. Three responders (60%) had plati-

Table 1 Patient characteristics

Gender (male/female)	20/10
Age (years)	
Median	58
Range	38–77
Performance status	
0	9
1	18
2	2
Ethnicity	
Caucasian	15
Asian/Oriental	7
Hispanic	3
African American	2
Native American	1
Unspecified	1
Histology	
Adenocarcinoma	16
Squamous	6
Large cell	1
Unspecified	6
Number of prior chemotherapy regimens	
1	15
2	10
3 or more	5
Platinum response status	
Potentially sensitive	8
Refractory	22

num-refractory disease, defined prospectively as progression within 6 months of platinum-based therapy.

Survival

Survival data are plotted in Fig. 1. Median progression-free survival was 3.4 months (95% CI 2.0–5.6) while median survival time was 8.1 months (95% CI 5.4–17). Females had a median survival of 12.7 months while males had a median survival of 7.2 months (not significant).

Molecular correlative studies

Informative pretreatment tumor specimens were obtained from 12 patients. Three of these patients had a partial response, three had stable disease, five progressed, and one was not assessable for response. Results of IHC staining for ER, PKC-α and -ϵ, and *c-FOS*, and correlation with patient response are shown in Table 2. In summary, four patients were positive for one or both PKC isoforms. None of the three responders overexpressed *c-FOS* or PKC-ϵ. ER status was found to be positive in only one female patient (8%).

Discussion

Reversal of intrinsic or acquired resistance to anticancer agents continues to be a major theme in both basic and clinical cancer research. An important focus of such research has been the platinum compounds cisplatin and carboplatin. The broad spectrum of anticancer activity of these platinum agents is reflected by their current role as the backbone of chemotherapeutic regimens in a variety of solid tumors, including NSCLC. Although the exact mechanisms of platinum resistance continue to be clarified, altered drug uptake, increased intracellular glutathione, increased metallothionein levels, enhanced DNA repair, and overexpression of the PKC signal transduction pathway have all been implicated [4].

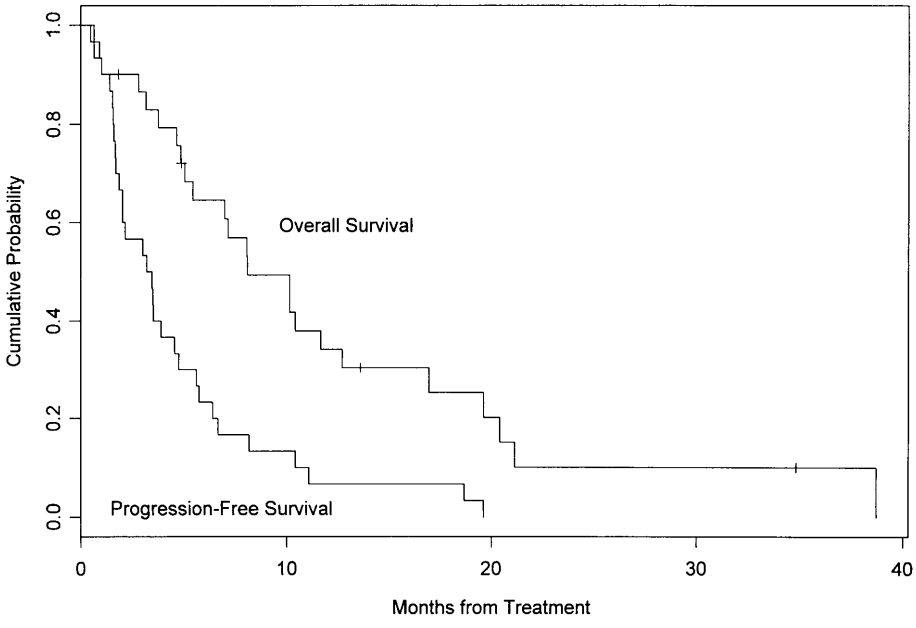

Fig. 1 Kaplan-Meier progression-free and overall survival curves

Table 2 Molecular-clinical correlation

No.	Gender	PKC-α	PKC-ϵ	cFOS	ER	Best response
1	M	–	–		–	Partial response
2	M	+	–	–	–	Partial response
3	M	–	–	–	–	Partial response
4	F			+		Stable
5	M	+	+	–	–	Stable
6	F	–	–	+	+	Stable
7	F				–	Progression
8	M	–	–	–	–	Progression
9	F	–	–	+		Progression
10	M	–	–	–	–	Progression
11	F	+	+		–	Progression
12	M	+	+		–	Inevaluable

Preclinical studies have demonstrated that triphenylethylene compounds are effective in modulating cisplatin cytotoxicity. In an in vitro study of tamoxifen's synergism with cisplatin, the dose-effect curve for inhibition of PKC was closely correlated with cisplatin's antiproliferative activity. In bladder cancer cell lines, tamoxifen has been found to be an effective dose-dependent chemosensitizing agent in combination with methotrexate, vinblastine, doxorubicin and cisplatin [34]. In that study, as in many others, the levels of tamoxifen required to achieve optimal chemosensitization were 5–15 μM [23, 24, 31]. When used as an antiestrogen in the prevention and treatment of breast cancer, steady-state plasma levels of tamoxifen at a dose of 20 mg daily are commonly 0.1–0.5 μM [5, 6]. Although high-dose tamoxifen has generally been well tolerated [25, 33, 45], complications such as cataracts, hepatic necrosis, thrombosis, ataxia, seizures, and peripheral neuropathy have been described [26, 41]. Therefore, tamoxifen may not be the optimal triphenylethylene for use as a cisplatin chemosensitizer.

Toremifene, a newer triphenylethylene, was initially developed to improve the therapeutic-to-toxic ratio of antiestrogens, and has been demonstrated to possess clinical efficacy similar to that of tamoxifen in patients with metastatic breast cancer [12]. Furthermore, toremifene has been reported to possess cisplatin-chemosensitizing properties in a preclinical model, but at levels tenfold that expected for its antiestrogenic indication, similar to that observed for tamoxifen [30]. In a study comparing toremifene at a relatively high dose (200 or 240 mg/day) with standard doses of tamoxifen (20 or 40 mg/day) in patients with metastatic breast cancer, toxicity did not differ significantly between the treatments [10]. The present trial was designed to deliver toremifene at a dose level sufficient to reach plasma levels (> 5 μM) required to achieve PKC inhibition and cisplatin modulation based on previous preclinical models and pharmacokinetic studies [15]. We have previously reported that these levels are clinically achievable in this patient cohort [21].

This study demonstrated that high-dose toremifene in combination with cisplatin is active and tolerable in NSCLC patients previously treated with platinum-based chemotherapy. The observed overall response rate of 18% and median survival of 8 months compare favorably with similar NSCLC patient populations following treatment with the most effective second-line therapy. For example, the response rate to docetaxel in NSCLC patients who had received first-line cisplatin-based chemotherapy ranges from 6–22% [7]. This toremifene-cisplatin regimen was also well tolerated. The toxicities observed were those typically seen with cisplatin treatment alone or dexamethasone premedication. There was no apparent increase in toxicity with a toremifene dose ten times higher than that used for its antiestrogenic indication.

Although the clinical results of the current trial are supportive of the hypothesis that toremifene modulates cisplatin resistance, the relationship to the PKC signal transduction pathway and *c-FOS* remains unclear. *C-FOS* belongs to a group of immediate-early genes activated in response to a variety of stimuli, including growth factors and chemotherapy. *C-FOS* has been suggested to play an important role in the development of platinum resistance, as supported by the observation that cisplatin-resistant human carcinoma cells contain elevated levels of *c-FOS* mRNA [35]. Furthermore, clinical data demonstrate *c-FOS* gene amplification in cell samples obtained from patients failing platinum-based therapy [13]. The postulated mechanisms of *c-FOS* action in cisplatin resistance may be in DNA synthesis and repair processes, including modulation of dTMP synthase and DNA polymerase [16]. Of interest, it has been shown that platinum resistance can be reversed in vitro following treatment with an antisense ribozyme directed against *c-FOS* [36].

The *c-FOS* oncogene has also been implicated in estrogen-ER interaction and the PKC signal transduction pathway. Estrogen administration has been shown to upregulate PKC and increase *c-FOS* expression in human breast cancer cells [38, 42]. Furthermore, activation of the PKC pathway directly increases *c-FOS* expression [11, 20, 27]. Specifically, the PKC isoforms α and ϵ have been shown to enhance the activities of at least three signaling pathways that converge on the serum response element in the *c-FOS* promoter necessary for transcription of *c-FOS* as induced by serum, growth factors, and phorbol esters [39]. Conversely, inhibition of the PKC pathway can lead to downregulation of *c-FOS* expression [9]. Informative patient tumor specimens were available in only a subset of 12 patients on this study, primarily because of limited samples obtained by fine needle aspiration. None of the responding patients overexpressed PKC-ϵ, the PKC isoform associated with a cisplatin-resistant phenotype [1]. Likewise, there were no responders that overexpressed *c-FOS*.

It has been observed that stage-for-stage, females with NSCLC may have a survival advantage over males [29]. In our study, the median survival for females was almost 13 months compared to 7.2 months for males. One proposed explanation for this gender-specific observation is that increased expression of ER in female patients influences response to chemotherapy. In one retrospective analysis, actuarial survival at 3 years in ER-positive NSCLC significantly favored women (94% vs 75%, $P < 0.05$) [2]. Surprisingly, ER expression in our cohort was found to be only 8%, as measured by the DAKO IHC kit. This is in contrast to previous reports of frequent ER overexpression in NSCLC [17, 40]. In one study, up to 97% of lung cancer tissue specimens were positive for ER by IHC [2]. The findings of our study are in accordance with more recent reports of infrequent ER expression in this tumor type [3, 43]. However, our analysis was limited by the sole evaluation of the ER-α isoform. A recently identified ER isoform (ER-β), which is likewise involved in signal transduction pathways, was not assessed in this study [22].

In summary, this combination of high-dose toremifene and cisplatin is feasible, well tolerated, and active in the treatment of NSCLC patients who have had prior platinum-based chemotherapy. The mechanism of toremifene in this combination is unclear. Further studies of this approach are warranted.

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