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High-dose toremifene as a cisplatin modulator in metastatic non-small cell lung cancer: targeted plasma levels are achievable clinically

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Abstract *Purpose*: The triphenylethylenes tamoxifen and toremifene have been reported to enhance the cytotoxicity of cisplatin by inhibition of protein kinase C (PKC) signal transduction pathways. However, the concentrations of tamoxifen and toremifene required for chemosensitization in preclinical models are generally ≥5 μM, at least tenfold higher than plasma levels observed in patients receiving these agents as antiestrogenic therapy. As part of a translational phase II trial investigating the efficacy and potential molecular mechanism of high-dose toremifene as a cisplatin modulator in metastatic non-small-cell lung cancer, plasma concentrations of toremifene and its active metabolite N-desmethyltoremifene were measured to determine whether targeted levels could be achieved clinically. *Methods*: Treatment consisted of toremifene, 600 mg orally on days 1–7, and cisplatin, 50 mg/m^2 intravenously on days 4 and 11, repeated every 28 days. Toremifene and N-desmethyltoremifene were measured by reverse-phase HPLC assay on days 4 and 11 prior to cisplatin infusion. *Results*: In the initial 14 patients, the mean total plasma concentrations of toremifene plus its N-desmethyl metabolite on days 4 and 11 were 14.04 (±8.6) μ*M* and 9.8 (±4.4) μ*M*, respectively. Variability in concentrations achieved did not correlate with renal or hepatic function, gender, or body surface area. Levels of N-desmethyltoremifene were higher on day 11 relative to toremifene concentrations. *Conclusions*: We conclude that plasma levels achieved compare favorably with the levels required for cisplatin chemosensitization and PKC modulation in vitro. Targeted toremifene levels can be achieved clinically with 600 mg orally daily in combination with cisplatin and are well tolerated.

Key words Lung cancer · Toremifene · Cisplatin · Protein kinase C · Phase II trial

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Introduction

Cisplatin is one of the most active agents available in the treatment of non-small cell lung cancer (NSCLC). However, its usefulness is limited by the relatively rapid development of drug resistance. One proposed mechanism of cisplatin resistance involves protein kinase C (PKC) signal transduction pathways [2, 10], stimulating a search for therapeutic agents that can modulate this process.

The triphenylethylenes tamoxifen and toremifene have been reported to possess chemosensitizing properties for several classes of chemotherapy, including cisplatin [6, 11, 14]. Cisplatin potentiation by tripheny lethylenes has been associated with decreased levels of PKC [12]. In a study in which tamoxifen was synergistic with cisplatin in vitro, the dose-effect curves for PKC

inhibition closely resembled the dose-response curves for cisplatin's antiproliferative activities [6].

However, the concentrations of tamoxifen required to achieve anthracycline chemosensitization in vitro are approximately tenfold higher than those observed in patients receiving tamoxifen as antiestrogenic therapy, increasing the risk of known complications from this therapy [1, 3]. Similar tamoxifen levels are necessary to achieve cisplatin chemosensitization in vitro in human NSCLC cell lines [14]. 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 metastatic breast cancer patients [5]. When toremifene was combined with cisplatin in human NSCLC cell lines, chemosensitization was observed similar to that reported for reported for tamoxifen. Toremifene levels (> 5 μM) at least tenfold higher than those typically achieved at doses used for antiestrogenic effects are required to achieve cisplatin chemosensitization [13].

As part of a phase II trial to evaluate the efficacy of high-dose toremifene plus cisplatin in patients with metastatic NSCLC, we measured plasma concentrations of toremifene and its active N-desmethyl metabolite to determine whether levels required for cisplatin chemosensitization in vitro are achievable clinically.

Materials and methods

Drug supply

Toremifene was supplied by its maker, Orion-Farmos, Espoo, Finland as 60-mg tablets. Cis-dichlorodiamineplatinum(II) (Platinol for injection) was obtained from Bristol Laboratories (Syracuse, N.Y.). All other reagents and chemicals used were of analytical grade.

Eligibility criteria

Patients were required to have pathologically demonstrated metastatic (TNM stage IV, M1) NSCLC. Patients who have had any number of prior chemotherapy regimens, one of which must have been platinum-based, as well as chemotherapy-naive patients, were eligible. In those who had been previously treated, the maximum cumulative cisplatin dose of prior chemotherapy must have been ≤ 300 mg/m^2. Also required were: measurable disease with at least one bidimensional (perpendicular diameters) objectively measurable lesion; adequate performance status of 0–2 by Southwest Oncology Group criteria; adequate bone marrow reserve defined as a WBC count $\geq 3500/\mu L$ and platelet count $\geq 100\,000/\mu L$; adequate renal and hepatic function defined by a pretreatment creatinine clearance of ≥ 60 ml/min, a serum creatinine of ≤ 1.5 mg/ml, and serum bilirubin and SGOT not more than twice the institutional upper limit of normal. Prior radiation therapy was allowed provided 3 weeks had elapsed since the completion of radiation. Prior surgical therapy of the primary tumor was also allowed. Pregnant or nursing women were excluded. Patients with a prior malignancy were ineligible except for those with adequately treated basal cell carcinoma of the skin, in situ cervical cancer, or other cancer of which the patient had been free 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 ineligible.

Treatment schedule

Written informed consent was obtained from all patients on a protocol approved by the Institutional Review Boards of participating institutions. 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 in normal saline administered intravenously over 1 h on days 4 and 11, repeated every 28 days. 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 per h for a minimum of 2 h. Potassium chloride (20 mEq) and 1 g of 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. Patients were encouraged to drink at least 3 liters of fluid on the day after cisplatin treatment.

Assessment of toremifene levels

Plasma samples for analysis of toremifene and its major metabolite N-desmethyltoremifene were collected from enrolled patients on days 4 and 11 of the first cycle of treatment, placed immediately into prechilled sodium-heparinized tubes, and centrifuged. Plasma was collected and frozen at −20 °C. Plasma ultrafiltrate was prepared by placing plasma specimens in Amicon (Danvers, Mass.) CF-10 filters (MW cutoff 10 000 Da), followed by centrifugation at 5000 *g*, at 4 °C for 20 min. Toremifene and N-desmethyltoremifene were quantitated in plasma samples using photoactivation and a high-performance liquid chromatographic (HPLC) assay as previously described [7, 16]. Total plasma and ultrafiltrate samples were thawed at room temperature and spiked with an internal standard (nafoxidine). Each sample was extracted with 2% *n*-butanol in hexane and irradiated with high intensity ultraviolet light (254 nm). Samples were analyzed by HPLC using a C-18 reverse-phase column and eluted isocratically with a mobile phase of water and triethylamine in methanol. Fluorescence of all compounds was measured at a wavelength of 266 nm. The limits of detection of this assay were 8.0 and 15.0 ng/ml for toremifene and *N*-desmethyltoremifene, respectively. Standard curves were linear over a concentration range of 25 to 750 ng/ml for both compounds with a correlation coefficient of greater than 0.98.

Toxicity evaluation

All subjects enrolled were subject to toxicity assessment, graded according to the National Cancer Institute common toxicity criteria.

Statistical analysis

Results were entered into a database utilizing MS Access for Windows 95 from Microsoft Corporation (Redmond, Wash.). Summary statistics, correlation coefficients, and regression analysis were performed using MS Excel from Microsoft Corporation.

Results

Patient characteristics are summarized in Table 1. The mean age was 59 years (median 58, range 46–84 years) with ten males and four females. Plasma concentrations of toremifene and N-desmethyltoremifene are reported in Table 2. Wide variability was observed in drug concentrations achieved. Total concentrations were higher on day 4 (14.04 ± 8.6 μM) and had a wider range of values (1.5–31.9 μM) compared with day 11. The ratios

Table 1 Patient characteristics

Patient no.	Age (years)	Gender	Race	Histology	Creatinine clearance (ml/min)	Total billrubin (mg/dl)	Body surface area (m^2)
1	55	F	Hispanic	Adenocarcinoma	120	0.5	1.86
2	72	M	Caucasian	Bronchoalveolar	63	0.8	2.02
3	59	M	Caucasian	Adenocarcinoma	98	0.4	2.10
4	68	F	Caucasian	Squamous	79	0.3	1.98
5	58	F	Black	Unspecified	107	0	1.79
6	56	M	Caucasian	Squamous	146	0.7	1.86
7	46	M	Caucasian	Adenocarcinoma	99	0.9	1.97
8	53	M	Caucasian	Adenocarcinoma	76	0.9	1.82
9	84	M	Asian	Adenocarcinoma	59	0.6	1.86
10	47	M	Pacific lander	Adenocarcinoma	97	0.5	1.72
11	61	F	Caucasian	Unspecified	79	0.4	1.66
12	48	M	Asian	Unspecified	62	0.5	1.5
13	63	M	Asian	Large cell undifferentiated	53	0.4	1.74
14	55	M	Asian	Adenocarcinoma	83	0.3	1.6
Means	59				87	0.5	

Table 2 Plasma levels (μM) of toremifene (TOR) and N-desmethyltoremifene (N-des TOR)

	Day 4 ($n = 4$)			Day 11 ($n = 13$)[a]		
	TOR	N-des TOR	Total	TOR	N-des TOR	Total
Mean	8.83	5.22	14.04	2.94	6.9	9.84
SD	7.23	1.99	8.6	2.18	2.52	4.45
Median	6.11	5.19	11.22	2.28	7.67	9.79
Range	0.70–25.17	0.74–6.98	1.52–31.92	0.48–6.15	2.05–10.45	2.93–17.15

[a] Not performed in one patient because of noncompliance

Table 3 Ratios of N-desmethyltoremifine to toremifene on days 4 and 11

Patient no.	Day 4	Day 11
1	0.27	1.36
2	0.47	1.56
3	1.20	3.83
4	2.10	4.03
5	1.01	3.17
6	1.06	4.33
7	0.41	2.12
8	0.67	–
9	0.43	1.59
10	1.15	6.98
11	1.71	3.97
12	0.81	4.86
13	0.33	1.85
14	0.95	2.33

in individual patients of N-desmethyltoremifene to toremifene on days 4 and 11 are summarized in Table 3. There was an increase in the ratio of the metabolite to parent compound in all patients evaluated.

The toxicity of toremifene plus cisplatin during the first cycle of therapy is summarized in Table 4. Grade 3 or 4 toxicity consisted only of nausea and vomiting in two patients. No additional episodes of grade 3 or 4 toxicity were seen in succeeding cycles. No adverse effects specifically attributable to toremifene were observed.

Discussion

Reversal of intrinsic or acquired drug resistance continues to be a major theme of both basic and clinical cancer research. An important focus of such research activity has been the platinum compounds cisplatin and carboplatin. These agents have a broad spectrum of anticancer activity, and platinum-based therapy has become part of the standard chemotherapeutic approach in a number of different solid tumor types. 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 signal transduction pathways have all been implicated [2]. Specifically, the PKC system may play an important role in platinum resistance [2, 4, 9].

The triphenylethylenes tamoxifen and toremifene are reported to have broad chemosensitizing activity. Previous studies by our group and others have demonstrated that these agents are capable of reversing p-glycoprotein-mediated anthracycline resistance and of enhancing cisplatin cytotoxicity in vitro [1, 6, 13, 14]. Cisplatin chemosensitization has been associated with reduced levels of intracellular PKC [6, 11, 12, 13]. In some preclinical models, however, the toremifene concentrations required for chemosensitization are greater

Table 4 Toxicity. The table shows the incidence of adverse events in cycle 1 possibly/probably/definitely related to treatment ($n = 14$). No significant toxicities, i.e., grade III or higher, were noted in subsequent cycles

	Toxicity grade			
	I No. (%)	II No. (%)	III No. (%)	IV No. (%)
Nausea	3 (21)	3 (21)	1 (7)	0
Vomiting	2 (14)	2 (14)	0	1 (7)
Flu-like symptoms	5 (36)	1 (7)	0	0
Haematologic	4 (28)	0	0	0
Anorexia	2 (14)	0	0	0
Renal	2 (14)	0	0	0
Hepatic	1 (7)	0	0	0
Metabolic	0	1 (7)	0	0
Peripheral neuropathy	2 (14)	0	0	0
Miscellaneous	3 (21)	1 (1)	0	0

than 5 μM, at least tenfold higher than plasma levels observed in patients receiving these agents as antiestrogen therapy [3, 13]. Here, we present evidence that targeted levels of toremifene required for enhancing cisplatin cytotoxicity in vitro are clinically achievable in vivo in patients receiving the drug orally at 600 mg daily for seven days in combination with cisplatin. Similarly, targeted plasma levels of toremifene have been achieved in combination with doxorubicin in a recent phase I trial [17]. These findings support the clinical feasibility of testing the hypothesis that toremifene functions as a platinum chemosensitizer.

Other studies attempting to optimize the dose-schedule of toremifene in solid tumors have explored high-dose regimens, administered alone or in combination with standard chemotherapeutic agents. Gershanovich et al. treated 36 patients with renal cell carcinoma with single-agent toremifene at a dose of 300 mg/day [4]. Total plasma concentrations of approximately 4 μg/ml (approximately 10 μM) were obtained. A recent pharmacodynamic study by Liipo et al. [8] has provided insight into the potential use of high-dose toremifene as a chemosensitizer. In this study, toremifene was administered to 18 patients with operable lung cancer at 240, 480, or 600 mg/day for 7 days prior to surgical resection. Toremifene levels were measured in serum, normal lung, and tumor tissue at all dose levels. Levels required to reverse multidrug resistance of cancer cells in vitro were achieved at 480 and 600 mg/day. Furthermore, the results suggest a preferential dose-dependent accumulation in tumor tissue as opposed to normal lung.

Toremifene has been evaluated as a chemosensitizer in 11 patients with recurrent, drug-resistant gynecologic cancer at a dose of 240 mg daily for 1 week prior to the identical chemotherapy to which drug resistance had been observed [9]. Three partial responders in eight evaluable patients were reported. Salomaa et al. [15] treated 16 chemotherapy-naive patients diagnosed with unresectable, locally advanced NSCLC with toremifene at 420 mg orally on days 1–4 and 240 mg on day 5 followed by ifosfamide at 5 g/m^2 on day 5, repeated every 3 weeks. The investigators reported no enhancement of ifosfamide's clinical efficacy when preceded by high-dose toremifene therapy. Furthermore, the study concluded that the levels achieved clinically were insufficient to enhance cytotoxicity.

The present trial was designed to deliver toremifene at a dose level sufficient to reach the theoretical plasma levels required to achieve PKC inhibition and cisplatin modulation based on previous preclinical models. In this study population, the plasma levels of toremifene achieved did not correlate with gender, body surface area, or renal or hepatic function (data not shown).

The long half-life of toremifene and its active metabolite N-desmethyltoremifene (5 to 6 days) permits maintenance of high plasma concentrations for several days after discontinuation of single daily oral dosing at 600 mg. However, one observation from our study is the finding of lower total drug concentrations on day 11 compared with day 4. As expected, levels of the N-desmethyl metabolite had risen relative to the parent compound toremifene by day 11 in all patients, as demonstrated by higher day 11 N-desmethyltoremifene to toremifene ratios (Table 3). Since the last toremifene dose was administered on day 7, total drug concentrations would be anticipated to be slightly lower by day 11 because of ongoing drug metabolism.

In conclusion, these results demonstrate that toremifene levels which achieve cisplatin chemosensitization and PKC modulation in vitro are clinically achievable with a daily oral dose of 600 mg in combination with cisplatin, and that this combination is well tolerated. Phase II trials designed to determine the clinical efficacy of high-dose toremifene in combination with a number of chemotherapeutic agents are currently underway.

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