Cancer Chemother Pharmacol (1997) 39: 212–216

ORIGINAL ARTICLE

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Toremifene concentration and multidrug resistance in lung tumors

Received: 26 July 1995/Accepted: 15 May 1996

Abstract In this pharmacokinetics study, concentrations of toremifene (TOR), a new antiestrogen, were measured after a 7-day oral treatment in serum, lung, and tumor tissue to determine the optimal dose of TOR for the modulation of clinical multidrug resistance in patients with lung cancer. Target levels of the antiestrogen were based on previous in vitro studies. Altogether, 18 patients with operable lung tumors were studied. TOR was given in an open, nonrandomized, phase I study at three different dose levels. The medication consisted of oral TOR given for 7 days at either 240, 480, or 600 mg/day before surgical removal of the tumor. At least five patients were scheduled to be included at each dose level, with all five receiving the full course of therapy before escalation of the dose. Blood samples for serum TOR concentration measurements were taken on days 0 and 7. Specimens of tumor and normal lung tissue of approximately 0.5 g were taken on day 7. The concentrations of TOR and its metabolites were determined in serum, lung, and tumor tissue at different dose levels. Altogether, 12 evaluable patients completed the scheduled treatment. The concentrations measured in serum, lung, and tumor tissue increased along with the dose used, such that the highest TOR values were achieved at 600 mg/day, with mean values being 4.9 μmol/l, 175.0 μmol/g, and 122.7 μmol/g, respectively. The concentrations of TOR and its metabolite *N*-demethyltoremifene were highest in lung tissue, but the values measured in tumor specimens were also well above the respective concentrations detected in serum samples. The TOR doses of 240 and 480 mg/day were well tolerated. One patient in the group treated at 600 mg/day had to discontinue the treatment because of headache and nausea. TOR given at doses ranging from 480 to 600 mg/day for 7 days will produce serum, lung, and tumor concentrations of the parent drug and its metabolites that have been shown to reverse multidrug resistance of cancer cells in vitro. As the 480-mg/day dose of TOR produced tumor concentrations high enough to reverse multidrug resistance without producing adverse drug reactions, the dose recommended for the foreseen clinical trials in the reversal of multidrug resistance would be 480 mg/day for 7 days.

Key words Multidrug resistance · Lung cancer · Antiestrogen · Toremifene · Pharmacokinetics study

Introduction

In recent years there has been substantial progress in our understanding of the mechanism by which tumor cells become resistant to anticancer drugs. The leading mechanism is considered to be multidrug resistance (MDR), whereby cells generate resistance to multiple drugs by increased expression of the *mdr* gene and its product, P-glycoprotein (PG-170). PG-170 acts as an efflux pump for toxic substances in a cell [5, 7, 9, 12, 14]. On the basis of the frequency of PG-170 expression in cancer patients and the links emerging with resistance to therapy, the ability to inhibit this efflux pump could be expected to produce a substantial benefit to patients if an inhibitor of efflux could be combined with conventional chemotherapy.

This work was supported by a grant from the Finnish Antituberculosis Association and the Southwestern Cancer Society of Finland

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Toremifene (TOR) and its metabolites *N*-demethyltoremifene (DMT) and 4-hydroxytoremifene (4-HT) sensitize drug-resistant cancer cell lines to doxorubicin and vinblastine in vitro [6, 10]. TOR displays a linear dose-response relationship in potentiation of doxorubicin toxicity at concentrations ranging from 2.5 to 10 μmol/l in resistant MCF-7 doxorubicin-resistant breast cancer cells [6]. Vinblastine resistance of mdr1 transfectant S1/1.1 cells may be completely reversed in vitro by TOR concentrations of 10 μmol/l [10]. Thus, the concentration sufficient to reverse MDR optimally in tumor tissue should be at least 10 μmol/l. The main metabolite of TOR, DMT has been shown to reverse MDR by increasing doxorubicin accumulation in MDA A-1 doxorubicin-resistant cells with about one-third of the capacity of the parent drug [18]. A similar effect of the metabolite on vinblastine cytotoxicity has been seen in resistant MCF-7 breast cancer cells [10]. Another significant metabolite of TOR, 4-HT, possesses MDR-reversing potential similar to that of DMT [10, 18]. As these metabolites, especially DMT, are present in tumor tissue, they may have an effect on MDR reversal in vivo.

A wide range of noncytotoxic drugs have shown activity in reversing MDR in vitro. These drugs include verapamil, quinidine, amiodarone, cyclosporine, and phenothiazines [16, 17, 20]. However, the achievement of concentrations of these drugs required to reverse MDR in vivo would cause substantial toxicity to the patients. TOR is a new triphenylethylene derivative, an antiestrogen, used clinically in the treatment of breast cancer. TOR has been given to these patients at high doses without producing significant toxicity [3]. TOR is extensively metabolized and is excreted mainly as metabolites in the feces. In addition to DMT and 4-HT, the main metabolites of TOR are *N*,*N*-didemethyltoremifene (DDMT) and (deaminohydroxy)-toremifene (DAHT) [1]. Like TOR, the metabolites have been shown to reverse MDR in vitro at concentrations achievable clinically in serum [6]. Antiestrogens are extensively bound to plasma proteins [15], which may have an effect on the tissue distribution of the drug [15, 19]. In a clinical study, TOR has been given to cancer patients in combination with cytotoxic treatment and has produced acceptable toxicity and encouraging results [6].

The aim of this study was to determine the optimal dose of TOR for clinical MDR reversal in a 7-day treatment course in patients with operable lung tumors [either small-cell lung cancer (SCLC) or non-small-cell lung cancer (NSCLC)]. The concentrations of TOR and its metabolites were assessed in serum, in lung tissue, and in tumor tissue after the administration of 240-, 480-, or 600-mg/day regimens in this dose-range-finding study. Observed tumor concentrations were compared with the concentrations concomitantly obtained in serum and with those needed to reverse MDR in vitro.

Patients and methods

This study was an open, nonrandomized dose-finding study. It was conducted according to the standard for good clinical trial practice. For determination of the optimal dose of TOR, e.g., the dose resulting in acceptable toxicity and in vivo TOR concentrations known to reverse MDR, 18 lung-tumor patients were included, 17 of whom were men. After the surgery the histology of the tumor was NSCLC in 16 patients, 13 of the patients had squamous-cell carcinomas, and 3 had adenocarcinomas. In two patients the tumor-biopsy verification showed a benign histology: one lesion was a carcinoid tumor and the other was a hamartoma. The mean age of the patients was 61.7 (range 42–70) years. The preoperative performance status of the patients was normal. In all patients, hematology was carried out and liver and renal functions were measured. The life expectancy of all patients was more than 2 months. Witnessed informed consent was obtained from all patients, and the study was approved by the ethics committee of Turku University Hospital.

The patients were given TOR at 240, 480, or 600 mg/day in two daily doses as 60-mg tablets for 7 days before surgical removal of the lung tumor. On the morning of the operation day the patients received only the first dose (half of the daily dose). Five patients were scheduled to be included at each dose level such that all five must have received the full course of therapy before escalation of the dose. The patients were given normal hospital meals or were instructed to eat normally when they stayed at home before the operation.

The patients were urged to report all adverse events immediately after their appearance. Adverse events were assessed on the WHO scale and their causal relationship to the study treatment was determined by the investigator. No control treatment was used in this survey.

Blood samples of 10 ml for serum TOR and metabolite concentration measurements were drawn on days 0 and 7, i.e., before the medication and at the operation. Approximately 0.5 g of tumor tissue was sampled, placed in a dry test tube, and kept frozen ($-20\,^{\circ}C$) until analyzed. Before analysis, weighed tissue samples were homogenized in 1.5 ml of pH 9.5 buffer, and 0.5 ml of homogenate was analyzed as the serum sample. A sample from the removed healthy lung tissue was also taken and frozen as described above. Serum samples were kept frozen ($-20^{\circ}C$) until all samples had been collected. The serum, lung, and tumor tissue concentrations of TOR and its metabolites at different dose levels were determined using high-power liquid chromatography (HPLC) [1]. The HPLC system (Waters Chromatography Division, Millipore, Milford, Mass) consists of an isocratic solvent-delivery pump, an automatic sampling injector, a postcolumn ultraviolet activator (where the analytes are converted into fluorescing compounds), and a fluorescence detector. The mobile phase consists of methanol, water, and diethylamineacetate run at a flow rate of 2 ml/min. The interassay and intraassay quality-control data show that the assay is accurate and precise, with the relative standard deviation generally falling below 10%. Clinical data were recorded on case-report forms and entered into a data-base system. One-way analysis of variance and linear regression analysis were used in the statistical evaluation of the data.

Results

Altogether, 18 patients with operable lung tumors were included in the study. Three of the patients were considered ineligible; two of them had a tumor of benign histology and one patient had an increased serum creatinine level at baseline. No sample was obtained for pharmacokinetics study from five patients, who were thus considered inevaluable; two of these inevaluable

Table 1 Serum concentrations of TOR and metabolites measured in patients after 7 days of treatment with oral TOR

Dose (mg/day)		Concentration (μmol/l)				
		TOR	DMT	DAHT	4-HT	DDMT
240	Mean (SD)	2.5 (0.6)	3.8 (1.0)	0.3 (0.2)	0.1 (0.1)	0.1 (0.1)
	Range	1.9–3.2	2.7–4.7	0.1–0.5	0.0–0.3	0.0–0.2
	n	4	4	4	4	4
480	Mean (SD)	3.8 (0.7)	6.9 (1.0)	0.9 (0.3)	0.2 (0.2)	0.4 (0.1)
	Range	2.6–0.6	5.8–8.2	0.6–1.4	0.1–0.4	0.2–0.5
	n	5	5	5	5	5
600	Mean (SD)	4.9 (0.8)	7.2 (1.0)	0.9 (0.1)	0.1 (0.0)	0.2 (0.1)
	Range	4.2–5.7	6.3–8.3	0.8–1.0	0.1–0.1	0.1–0.3
	n	3	3	3	3	3

patients, one with a benign tumor and one with an increased serum creatinine value, were also ineligible. Two patients were also regarded as inevaluable due to premature study discontinuation. One of the ineligible patients had a benign carcinoid tumor; however, he was treated according to the protocol and was subsequently considered evaluable. Overall, 12 patients were evaluable for TOR tumor pharmacokinetics. Of these patients, 4 were treated at the 240-mg/day level; 5, at the 480-mg/day level; and 3, at the 600-mg/day dosing level.

Serum concentrations

The mean serum concentrations determined for TOR and its metabolites on the 7th day of drug administration are presented in Table 1 and in Fig. 1. The highest concentrations observed among all dosing regimens were those of DMT, followed by those of TOR. The proportion of TOR in serum in relation to the metabolites was similar at all dose levels, suggesting a non-dose-dependent metabolism of the drug.

Tissue concentrations

The mean tissue concentrations determined at different dose levels are presented in Tables 2 and 3 and in Fig. 1. Concentrations of TOR and DMT measured in lung tissue were high and exceeded 100 μmol/g at each TOR dose used in the study. The concentrations increased with the dose of TOR, and the values of DMT were higher than those of TOR at each dose step. The mean tumor concentrations of TOR and DMT were 20 μmol/g or more at each dose level, and on dose escalation they reached mean concentrations of 123 (TOR) and 108 μmol/g (DMT) at the highest dose level (TOR 600 mg/day). Tissue concentrations measured for other metabolites were clearly lower than those found for TOR or DMT (Table 3). Lung-tissue concentrations determined for TOR were 44.8%, 36.3% and 35.7% of the corresponding serum concentrations measured in the groups treated at 240, 480, and 600 mg/day, respectively. Thus, a similar proportion of TOR was found in healthy lung tissue at all doses used as compared with serum concentrations. However, the

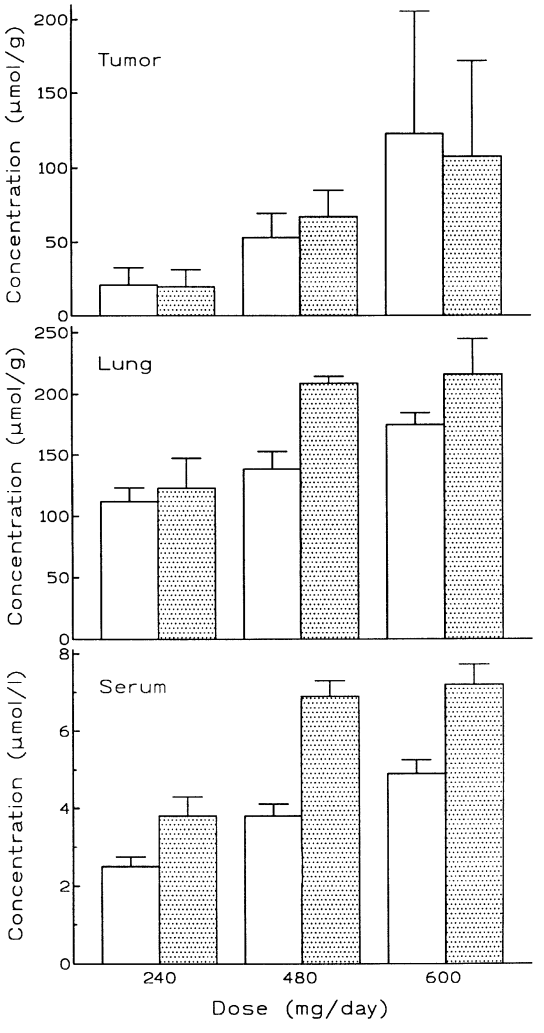

Fig. 1 Concentrations of TOR and DMT measured in serum, lung, and tumor tissues on the 7th day of TOR administration. Results are shown as mean values ± SE, $n = 3$–5 (*White bars* TOR, *shaded bars* DMT)

Table 2 Tissue concentrations of TOR and metabolites measured in normal lung tissue

Dose (mg/day)		Concentration (μmol/g)				
		TOR	DMT	DAHT	4-HT	DDMT
240	Mean (SD)	111.9 (23.0)	122.7 (50.2)	1.2 (0.1)	5.8 (1.5)	2.4 (1.8)
	Range	88.2–134.0	85.7–179.9	1.1–1.3	4.3–7.3	1.1–4.5
	n	3	3	2	3	3
480	Mean (SD)	138.2 (29.7)	208.7 (11.6)	1.2 (0.1)	6.7 (1.2)	6.2 (2.6)
	Range	120.4–172.4	200.5–221.7	1.1–1.3	5.7–8.0	3.4–8.2
	n	3	3	3	3	3
600	Mean (SD)	175.0 (22.1)	215.8 (67.1)	2.4 (1.5)	8.8 (1.3)	5.1 (2.8)
	Range	159.4–190.6	168.4–263.3	1.3–3.4	7.8–9.7	3.2–7.1
	n	2	2	2	2	2

Table 3 Tissue concentrations of TOR and metabolites measured in lung tumor

Dose (mg/day)		Concentration (μmol/g)				
		TOR	DMT	DAHT	4-HT	DDMT
240	Mean (SD)	20.8 (26.0)	19.5 (26.0)	4.0 (–)	2.7 (1.2)	4.8 (–)
	Range	4.8–59.6	4.8–58.4	–	1.1–3.6	–
	n	4	4	1	4	1
480	Mean (SD)	53.2 (40.2)	67.3 (39.9)	1.6 (0.5)	3.7 (1.4)	1.5 (0.5)
	Range	12.3–106.6	17.8–116.6	1.1–2.1	2.1–5.9	1.1–2.1
	n	5	5	3	5	4
600	Mean (SD)	122.7 (169.6)	107.6 (131.0)	1.8 (–)	5.4 (5.8)	1.1 (–)
	Range	14.8–318.2	10.7–256.7	–	0.9–13.3	–
	n	3	3	1	3	1

respective proportions of TOR found in tumor tissue were 8.3%, 14.0%, and 22.0%, suggesting the possibility of a dose-dependent accumulation of TOR and its metabolites in tumors. We could not show any correlation between the tumor size and the tumor TOR concentration.

Adverse events

Ten patients were reported to have adverse events. Most of the latter were judged as being mild or moderate in severity, and their causal relationship to the study treatment was undetermined. One patient treated at 600 mg/day discontinued the treatment after 3 days due to possibly treatment-related headache and nausea. Two patients died during the study; one patient experienced possibly treatment-related atrial fibrillation, cerebral infarction, and subsequent death on the 6th postoperative day, and another patient had pneumonia and acute distress syndrome followed by death at 2 days after surgery. No significant change in hematological or chemical laboratory variables was observed during the study period.

Discussion

It has been shown in human NSCLC cell-line studies that resistance against cytotoxic agents can be completely reversed by TOR. In these studies, TOR has caused a 6-fold modification of MDR in vinblastine-resistant cells [10] Kirk et al. [11] have shown that TOR can also modify doxorubicin toxicity to some lung-cancer cell lines. Poupon et al. [13] have shown that SCLC tumors expressing the *mdr1* (PG-170) gene are often coincident with ineffective chemotherapy and that therapeutic benefit could therefore be expected from chemotherapy combined with MDR1 inhibitors. When the in vitro-active MDR modulators verapamil and tamoxifen were combined with doxoroubicin, vincristine, and etoposide in the chemotherapy of SCLC patients, slightly more responses were seen in patients receiving higher doses of the modulators [8]. Due to its good tolerability and MDR-reversal properties, TOR would be a good candidate for in vivo studies as an MDR-modulating agent in patients with drug-resistant lung tumors. Our aim was to find out if sufficient tissue concentrations known to reverse. MDR in vitro could also be achieved in vivo in normal lung and lung tumor tissue with acceptable toxicity. Because the prognosis of lung cancer patients has been dismal for years, there is a constant need for better therapy, especially for those who have inoperable NSCLC. A favorable result of this in vivo study would determine the optimal dose of TOR for larger therapeutic clinical trials in the future. TOR was given orally at three different dose levels for 7 consecutive days. The dose level generating a sufficient tumor concentration without producing any significant toxicity would be called optimal.

In our study the total concentrations of TOR and its main metabolite, DMT, were greater in serum than the concentration shown to reverse MDR in vitro [2, 4]. Although TOR is extensively bound to plasma proteins, its concentration in healthy lung tissue was surprisingly high and was clearly above the respective concentration measured in serum. In tumor tissue the concentrations measured for TOR and its metabolites were lower than those found in healthy lung tissue but were clearly higher than those determined in serum. When the daily dose of TOR was increased, the tumor concentrations of the drug and its metabolites increased more than the serum concentrations. In our study we could not find any difference in tumor TOR levels between the squamous carcinomas and the adenocarcinomas.

The adverse events reported by the patients were equivalent to those usually connected with antiestrogen treatment. TOR doses over 600 mg/day have been reported to cause dizziness, nausea, and vomiting [3]. However, the study drug was well tolerated, and only one patient discontinued treatment at the dose level of 600 mg/day due to headache and nausea, which may have been treatment-related.

We conclude that TOR given at daily oral doses ranging from 480 to 600 mg for 7 days will produce concentrations of the parent drug and its main metabolite in serum, lung, and lung-cancer tissue that have been shown to reverse MDR in cancer cells in vitro. The concentrations of TOR achieved in tumor tissue are theoretically sufficient to overcome the effect of high-level protein binding and to reverse MDR in cancer cells [19]. Although after 7 days of 240 mg/day dosing the mean tumor concentration of TOR was high, three of the four tumor samples analyzed showed concentrations of 10 μmol/g tissue or lower. This variation may reflect differences in tumor vascularization, and a 240-mg daily dose may thus represent a borderline dose for the achievement of tumor concentrations of TOR high enough to cause MDR reversal. At 480 mg/day, TOR produced tumor concentrations in all five patients sufficient to reverse MDR, and as the 600-mg/day dose, may represent a borderline for the occurrence of dose-dependent toxicity, we recommend a dose of 480 mg/day for 7 days for foreseen clinical studies to assess the therapeutic efficacy of TOR in the reversal of MDR.

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