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Pharmacokinetic analysis of high-dose toremifene in combination with doxorubicin

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Abstract *Purpose*: Toremifene (Fareston) is an orally administered triphenylethylene derivative with chemosensitizing activity in vitro in estrogen receptor-negative multidrug-resistant human breast cancer cells. The purpose of this study was to evaluate the effects of high-dose toremifene (600 mg/day for 5 days) on the plasma pharmacokinetics of doxorubicin in humans. The 600-mg dose had been previously established as the maximum tolerated dose in a phase I study of 35 patients. *Methods*: Doxorubicin was administered as an intravenous (i.v.) bolus over 15 min at a dose of 60 mg/m^2 to 11 patients in the absence of toremifene pretreatment to establish baseline doxorubicin pharmacokinetics. Six of these patients received 600 mg/day toremifene for 5 days 4 weeks later, followed by an i.v. bolus dose of doxorubicin (60 mg/m^2) on day 5. During toremifene pretreatment, blood specimens (5 ml) were drawn at 0, 2, 4, and 24 h after dosing to assess peak levels. Following doxorubicin administration in each cycle, blood specimens were collected over a 72-h period for determination of the terminal half-life of elimination. Plasma concentrations of doxorubicin and toremifene were assessed by high-performance liquid chromatography (HPLC). Cumulative linear areas under the time-concentration curve (AUC) for doxorubicin were calculated using a noncompartmental model. *Results*: Prior to toremifene dosing, baseline doxorubicin pharmacokinetic studies showed an average terminal half-life of elimination of 40.04 ± 7.86 h in 4 patients, and an average AUC of 135 600 ± 67 600 μg/ml · h in 11 patients. In 4 of the patients receiving 600 mg/day toremifene for 5 days, the average terminal half-life of elimination was 38.12 ± 7.81 h, and the average AUC was 141 900 ± 62 900 μg/ml · h in 6 patients, i.e. a slight increase of 4.6%. No statistically significant change in the doxorubicin elimination kinetics with or without toremifene therapy was observed. *Conclusions*: Toremifene does not appear to interfere with the elimination kinetics of doxorubicin.

Key words Toremifene · Doxorubicin · MDR · Drug interaction

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Introduction

The development of multidrug resistance (MDR) is believed to be one of the major mechanisms by which breast cancer might become refractory to chemotherapeutic agents, especially anthracyclines (doxorubicin, DOX) and vinca alkaloids (vincristine, vinblastine) [11, 18]. Reversal of MDR in vitro can be accomplished by a variety of agents, including verapamil, trifluoperazine, reserpine, quinidine, perhexiline maleate, and cyclosporin [2, 14, 16, 17, 22]. However, despite encouraging in vitro data, the plasma concentrations of these chemosensitizing agents required to reverse MDR cannot be achieved without substantial toxicity. Triparanol analogs, such as the antiestrogen tamoxifen which is commonly used to treat hormone-dependent breast cancer, also have demonstrated in vitro anti-MDR activity, apparently unrelated to their antiestrogenic effects [6, 19]. Toremifene (TOR) is a new triparanol derivative that has chemosensitizing activity in MDR-positive cells at concentrations that are achievable in humans without significant toxicity [5, 8, 10].

The hepatic enzymes collectively known as cytochrome P450 play a major role in the metabolism of many anticancer agents, including cyclophosphamide, vincristine, cyclosporin, prednisone, tamoxifen, and TOR [1, 9, 12, 13, 15, 23, 24]. Specifically, cytochrome

P450 isoform 3A (CYP 3A) has been shown to be involved in the metabolism of vincristine and vinblastine [13, 24] and is implicated in DOX, prednisone, tamoxifen, and TOR metabolism [9, 12, 15], while isoform 2B is involved in cyclophosphamide metabolism [9].

Interestingly, cyclosporin, a known MDR modulator, is a substrate of CYP 3A, and its effects on the pharmacokinetics of DOX have been studied in humans. In 12 patients, the combination of cyclosporin and DOX caused the dose-adjusted area under the time-concentration curve (AUC) of DOX and doxorubicinol to increase by 55% and 350%, respectively. The total clearance of DOX was also reduced by 50% [9]. In that study, the combination of cyclosporin and DOX was associated with increased nausea, vomiting, and myelosuppression even though the dose of DOX was reduced by 40% when cyclosporin was added.

Following a phase I study of the combination of DOX and TOR in which the maximum tolerated dose for TOR was defined (unpublished data), we conducted the present study which examined the plasma pharmacokinetics of DOX with and without TOR. The purpose of this study was to determine any potential drug interactions between these agents.

Material and methods

All solvents used in the extraction and HPLC analyses of TOR (Fareston) and DOX were obtained from Fisher (Pittsburgh, Pa.) and were of HPLC grade. TOR, 4-hydroxytoremifene, and *N*-desmethyltoremifene used in the preparation of standard calibration curves were supplied by Orion Corporation-Pharma (Finland).

Patients

A total of 11 patients with a variety of neoplasms resistant to standard chemotherapy were included in this study. They were given DOX (60 mg/m^2) by bolus i.v. infusion in the absence of TOR pretreatment to establish baseline DOX pharmacokinetics. Blood specimens (5 ml) were drawn before dosing and at the following time-points after dosing: 5 and 30 min and 1, 2, 4, 8, 24, 48, 57 and 72 h. The six patients remaining on the study received TOR (600 mg/day for 5 days) 28 days later followed by another course of DOX on day 5. TOR was supplied as 60-mg tablets of TOR citrate by Orion Corporation-Pharma. Blood specimens (5 ml) were drawn at 2, 4, and 24 h after TOR dosing each day to assess peak levels. Following the DOX infusion, blood samples were collected at the time-points listed above. All blood samples were collected in green-top heparinized tubes. Plasma was separated from whole blood by centrifugation, placed in labeled vials, and immediately frozen at −20 °C.

HPLC analysis of TOR

TOR concentrations in all patient plasma specimens were quantitated by HPLC as previously described [7]. Briefly, plasma specimens (1 ml) were placed in clean 16 mm × 125 mm glass extraction tubes (Corning, Corning, N.Y.) and internal standard (nafoxidine HCl, 200 ng; Sigma, St. Louis, Mo.) followed by 9 ml of a solution of 2% butanol in hexane were added. The samples were vortexed for 1 min and centrifuged for 10 min at 1000 *g*, and the organic layer was evaporated to dryness under a gentle stream of nitrogen at 37 °C. Samples were then reconstituted in 200 µl methanol, transferred to an Infrasil quartz cuvette, and irradiated for 1 min with high-intensity ultraviolet light (254 nm). The activated samples were removed from the cuvette and injected onto the HPLC column. The fluorescence of photochemically activated compounds was detected using an Applied Biosystems 980 Programmable Fluorescence Detector set at an excitation wavelength of 266 nm. Retention times and peak heights were recorded with a Spectra-Physics 4100 integrator. All standard curves for TOR, 4-hydroxytoremifene, and *N*-desmethyltoremifene were prepared in 1.0 ml stock human plasma and were extracted using the procedure described above. The correlation coefficient for each curve was >0.985. To ensure linearity, standards were repeated regularly. All samples were stored at −20 °C until used.

HPLC analysis of DOX

DOX was quantified in each sample using an HPLC assay as previously described [4]. Patient plasma samples (2.0 ml) were placed in clean 16 mm × 125 mm glass extraction tubes (Corning). Briefly, a daunorubicininternal standard (1 µg/20 µl; Sigma) was added to each specimen before extraction. Each sample was extracted with 7 ml of a chloroform/methanol mixture (4/1, v/v), vortexed for a 1 min, and then centrifuged at 4000 *g* for 15 min. The bottom chloroform layer was removed and evaporated to dryness under a gentle stream of nitrogen at 37 °C. The extraction efficiency was approximately 90% using this method. Dried samples were reconstituted in 200 µl mobile phase and injected onto the column. Samples were eluted isocratically with a mobile phase consisting of 50% acetonitrile, 35% water, and 15% 0.1 *M* phosphoric acid. A Whatman C18 reverse-phase column was used. Fluorescence was detected using an Applied Biosystems 980 Programmable Fluorescence detector set at an excitation wavelength of 470 nm. All samples not immediately analyzed after extraction were stored at −20 °C until used. The system consisted of two Altex Model 110 A pumps, a Model 420 controller, and a Hewlett Packard Model 3394 integrator. The flow rate was set at 1.0 ml/min. Standard calibration curves were extracted from 2.0 ml stock human plasma at final concentrations of 5, 10, 40, 80, and 160 ng/ml for terminal half-life analysis. A standard concentration range of 0.1, 0.5, 1.0, 5.0, 10.0, and 20.0 µg/ml was used for peak plasma level determination. The correlation coefficients for all standard curves were >0.985.

Results

Peak TOR levels

Of 11 patients who were entered onto the study, 6 completed both courses. Among these 6 patients, the mean peak TOR level was 13.70 ± 6.76 µ*M*, or two to four times the concentration that reverses MDR in vitro [19]. The metabolite *N*-desmethyltoremifene, which also has anti-MDR activity, peaked at a mean of 10.39 ± 6.72 µ*M*. The mean combined TOR and N-des methyltoremifene concentrations peaked at 24.09 ± 13.38 µ*M*, or four to ten times the level which is active against MDR in vitro. These concentrations are in the range of the levels present at the time DOX was administered.

DOX pharmacokinetics

The effects of TOR (600 mg/day for 5 days) on the plasma pharmacokinetics of DOX (60 mg/m^2) were examined. The mean peak DOX concentration in course 1 was 1.798 ± 0.897 µg/ml ($n = 6$) (see Fig. 1). The mean

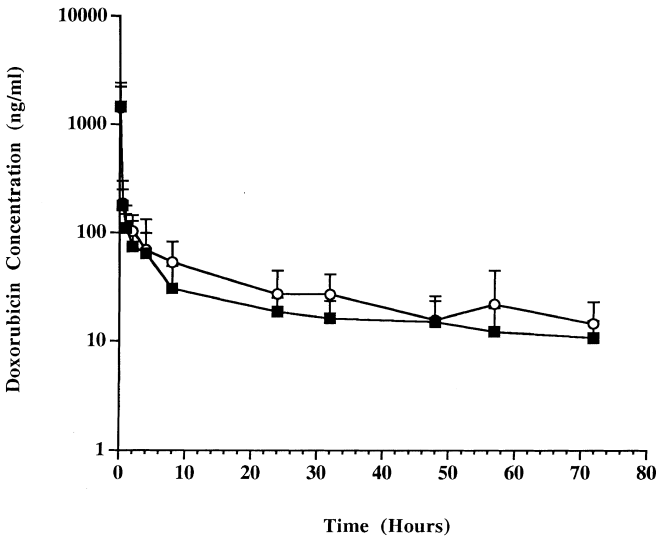

Fig. 1 DOX plasma kinetics with and without pretreatment with 600 mg/day TOR for 5 days. *Solid squares* represent average plasma concentrations following treatment with DOX alone (60 mg/m^2; $n = 11$), and *open circles* represent average plasma concentrations following TOR pretreatment ($n = 6$)

cumulative linear AUC, calculated using the trapezoidal method, was 135 600 ± 67 600 μg/ml · h ($n = 6$). The mean terminal half-life of elimination ($n = 4$) was 40.04 ± 7.86 h. In two of the six patients who completed both courses, the half-life could not be determined owing to inadequate DOX plasma levels after 24 h.

In course 2, DOX was given following TOR pretreatment. The DOX concentrations peaked at a mean of 1.411 ± 0.803 μg/ml ($n = 6$; see Fig. 1), and the mean AUC was 141 900 ± 62 900 μg/ml · h ($n = 6$). The average terminal half-life of elimination ($n = 4$) was 38.12 ± 7.81 h. The differences in these three parameters between courses 1 and 2 were not statistically significant (two-tailed Student's *t*-test; $\alpha = 0.05$). The mean peak DOX levels and the mean DOX AUC were not significantly different between course 1 ($n = 6$) and course 2 ($n = 6$) ($P > 0.10$). Likewise, the average terminal half-life of elimination was not significantly different ($P > 0.10$, $n = 4$, both courses).

There were five additional patients who did not complete course 2. With these data included, the mean peak DOX concentration in course 1 was 1.457 ± 0.957 μg/ml ($n = 11$; see Fig. 1), the mean DOX AUC in course 1 was 109 500 ± 58 400 μg/ml · h ($n = 11$), and the mean terminal half-life of elimination was 40.11 ± 9.61 h ($n = 8$). Although there appeared to be a slight trend toward increasing DOX AUC values from course 1 to course 2 with the inclusion of the additional data, the difference still was not significant ($P > 0.10$).

Discussion

TOR is a triphenylethylene antiestrogen currently being used in the treatment of patients with metastatic breast cancer. In vitro studies have shown that TOR possesses chemosensitizing activity in MDR-positive breast cancer cells that is apparently unrelated to its antiestrogenic effects [6, 19, 20]. A recently completed phase I study in 35 patients examined the combination of TOR and DOX for potential use against MDR + breast cancer. In that study, the maximum tolerated dose of TOR was found to be 600 mg/day (unpublished data). In the present study, we sought to determine whether high-dose TOR (600 mg/day for 5 days) affects the plasma pharmacokinetics of DOX (60 mg/m^2). It is known that the cytochrome P450 hepatic enzyme system plays a major role in the metabolism of many anticancer agents, including TOR [1, 9, 12, 13, 15, 23, 24]. Cytochrome P450 isoform 3A has also been implicated in DOX metabolism [9, 12, 15]. As in the case of the combination of DOX and cyclosporin [9], the potential exists for TOR to increase DOX toxicity because of their shared metabolic pathways. However, the data from our study in 11 patients provide insufficient evidence to conclude that TOR significantly affects the plasma pharmacokinetics of DOX.

Several factors potentially contribute to the failure of chemosensitizing agents, including inadequate blood flow into the tumor causing poor drug delivery, physiologic and chemical barriers which may prevent the drug from reaching its intended target, and insufficient C × T drug kinetic profiles as a result of inadequate dosing schedules [3]. High protein binding may contribute to all of these, resulting in decreased bioavailability to the target site. We have previously shown that nonspecific plasma protein binding may reduce cellular TOR concentrations in MDR-positive breast cancer cells [21]. Interestingly, the combined TOR + *N*-desmethyltoremifene concentrations in the present study peaked at four to ten times the level which shows anti-MDR activity in vitro. This may give TOR a therapeutic advantage over other chemosensitizing agents because it is well tolerated at high doses [10, 19].

In short, there do not appear to be any significant drug interactions between TOR and DOX. Further clinical studies are needed to determine whether the combination of TOR and DOX is effective in the treatment of drug-resistant cancers.

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