Cancer Chemother Pharmacol (1999) 44: 74–80

ORIGINAL ARTICLE

J.R. Waddle · R.L. Fine · B.C. Case · M.L. Trogdon
K. Tyczkowska · D. Frazier · R.L. Page

Phase I and pharmacokinetic analysis of high-dose tamoxifen and chemotherapy in normal and tumor-bearing dogs

Received: 14 July 1998 / Accepted: 7 October 1998

Abstract *Purpose*: To determine whether tamoxifen plasma concentrations capable of blocking P-glycoprotein (Pgp) in vitro can be safely achieved in dogs and whether doxorubicin pharmacokinetic alterations occur when tamoxifen is coadministered. *Methods*: Tamoxifen dose escalation studies were conducted in 7 normal dogs and in 19 tumor-bearing dogs receiving full-dose chemotherapy. Plasma tamoxifen and serum doxorubicin disposition were analyzed for putative drug interactions. *Results*: Steady-state plasma concentrations of tamoxifen and *N*-desmethyl tamoxifen (NDMT) were 5–10 μ*M* following oral tamoxifen administration at 600 mg/m^2 every 12 h for 7 days to normal and tumor-bearing dogs. Mild-moderate gastrointestinal toxicity (diarrhea, anorexia) and reversible neurotoxicity were observed in dogs receiving chemotherapy plus high-dose tamoxifen. Myelosuppression was not affected by combined treatment in tumor-bearing dogs. High-dose tamoxifen decreased the clearance and volume of distribution of full-dose doxorubicin. *Conclusions*: Concentrations of tamoxifen/NDMT sufficient to inhibit Pgp may be achieved in dogs receiving full-dose chemotherapy with a moderate but acceptable increase in gastrointestinal toxicity. Tamoxifen affects doxorubicin metabolism in dogs at high doses resulting in increased serum exposure. Pharmacologic manipulation of Pgp expression or function in normal and tumor tissue in dogs may facilitate investigation of novel anticancer treatment strategies in humans.

Key words P-glycoprotein 170 · Multidrug resistance
Tamoxifen · Doxorubicin · Canine

Introduction

In addition to antiestrogenic activity, tamoxifen induces numerous direct and indirect nonhormonal antineoplastic effects. Some of these effects include: alteration of anthracycline partitioning between hydrophilic/hydrophobic cellular compartments [17]; inhibition of protein kinase C pathways; inhibition of calmodulin, insulin growth factor, and transforming growth factors alpha and beta activity [8, 18, 20, 27]; augmentation of natural killer cell [1, 6] and interferon-alpha activity [21]; and inhibition of angiogenesis [16]. Clinically, tamoxifen has reproducibly enhances activity in platinum/DTIC regimens for human melanoma although the mechanism is not yet known [25].

Tamoxifen is also a substrate of the multidrug resistance (MDR) transporter and considerable preclinical data support a modulatory effect of tamoxifen and its metabolites on drug retention mediated by P-glycoprotein 170 (Pgp). Tamoxifen, in some models, is similar to other MDR-modulating compounds (verapamil, cyclosporine and PSC 833) in its ability to bind Pgp, stimulate ATPase, inhibit drug efflux and enhance cytotoxicity in MDR-resistant cell lines [11]. Both the parent compound and the major metabolite (*N*-desmethyl tamoxifen, NDMT) have been shown to be active Pgp modulators [4, 11]. We

This work was supported by the State of North Carolina and PHS grant CA 29582

J.R. Waddle[1] · B.C. Case · M.L. Trogdon · K. Tyczkowska
R.L. Page (✉)[2]
North Carolina State University,
College of Veterinary Medicine,
4700 Hillsborough St., Raleigh,
NC 27606, USA
e-mail: rodney_page@ncsu.edu
Tel.: +1-919-829-4349; Fax: +1-919-829-4336

R.L. Fine
College of Physicians and Surgeons, Columbia University,
650 W. 168th St., NYC, NY, USA

D. Frazier
University of Tennessee, School of Veterinary Medicine,
Knoxville, TN, USA

[1] *Present address*:
Veterinary Specialty Hospital, 305 C Ashville Ave,
Cary, NC 27511, USA
[2] *Present address until 13 June 1999*:
Dartmouth-Hitchcock Medical Center,
1 Medical Center Dr., 532 E Borwell,
Lebanon, NH 03756, USA

have recently demonstrated that tamoxifen is an active Pgp modulator in an MDR-selected canine cell line [19].

The pharmacodynamic effect of Pgp chemomodulation in vivo is a result of several mechanisms. Although Pgp chemomodulators increase intracellular concentrations of antineoplastic agents both in vitro and in vivo, increased drug retention in vivo may also result from altered metabolism of the antineoplastic compounds that share similar detoxification pathways [29]. Many MDR-class anticancer agents and Pgp modulators are metabolized by CYP3A4, an isoform of the P450 system. Simultaneous administration of multiple CYP3A4 substrates may overwhelm the system and result in decreased clearance of one or both compounds. However, even anticancer agents primarily metabolized by non-P450 mechanisms, such as doxorubicin, may be affected when CYP3A4 substrates are coadministered at high doses [3]. In vivo, altered doxorubicin tissue disposition has been reported in rodents when cyclosporine and PSC 833 are administered simultaneously although serum profiles are unaffected [9, 15]. Doxorubicin disposition is particularly affected in tissues with a high Pgp expression (i.e. adrenal, intestine, kidney). In human clinical studies, cyclosporine and verapamil have been shown to reduce doxorubicin clearance [2, 24, 30].

Phase I studies investigating the effect of high-dose tamoxifen as an MDR modulator in patients with refractory cancers have also been recently reported [5, 26, 33, 35]. Tamoxifen and NDMT plasma concentrations which produced maximum inhibition of drug efflux in vitro (6–9 μM) were achieved in all studies. Limiting toxicity of high-dose tamoxifen combined with chemotherapy in the above studies were reversible cerebellar abnormalities (gait disturbance, tandem walk) [33, 35] and gastrointestinal symptoms [5, 26, 33, 35]. In addition to toxicity data, Berman et al. evaluated daunorubicin disposition in 14 patients with refractory leukemia treated with increasing doses of tamoxifen [5]. There was no apparent affect of tamoxifen on daunorubicin or daunorubicinol serum area-under-the-concentration-time curve at the doses administered.

This study was conducted as a prelude to more thorough evaluation and refinement of chemomodulation strategies in tumor-bearing dogs. Due to the broad role that tamoxifen may play in a variety of cancers more information on its function in vivo is needed. Our aims were to: (1) determine whether serum concentrations of tamoxifen which are capable of maximally blocking Pgp can be safely achieved in normal and tumor-bearing dogs, (2) characterize the toxicity associated with high-dose tamoxifen in dogs and (3) determine whether tamoxifen induces pharmacokinetic alterations in serum doxorubicin disposition in dogs.

Methods

Tamoxifen citrate was obtained as a gift from Zeneca Pharmaceuticals, Wilmington, Del. Pharmaceutical grade tamoxifen citrate was purchased from Sigma Chemical Company as a standard for the HPLC assay. In order to make the administration of tamoxifen to the dogs easier, 10- and 20-mg tablets were pulverized and reformulated into 50-, 100- and 150-mg gelatin capsules. Thus, a 1 m^2 surface area dog (33 kg body weight) receiving tamoxifen at a dose of 600 mg/m^2 every 12 h would receive four 150-mg gelatin capsules twice daily.

Tamoxifen dose escalation was accomplished in seven normal, nontumor-bearing dogs (five male, two female). Pharmacokinetic evaluation and toxicity assessment was conducted at 150, 300 and 600 mg/m^2 tamoxifen orally every 12 h for 7 consecutive days at each dose. A 3-week period preceded the next dose escalation. Serum samples were collected prior to the morning dose and at 2, 4, 6 and 8 h after administration from each dog on days 1, 5 and 7. Samples were collected into heparinized tubes and plasma was separated immediately and frozen at −85 °C until assayed for tamoxifen and metabolites. A physical examination and complete blood count were evaluated every week following tamoxifen dosing for 3 weeks. A serum biochemical panel was evaluated prior to each dose escalation. Appetite, vomiting and diarrhea were assessed daily.

The same seven normal dogs which had received tamoxifen alone were used 2 months later to evaluate the effects of tamoxifen on serum doxorubicin disposition. They received no drug treatment during the 2-month hiatus. Dogs were treated with 1.25 mg/kg doxorubicin intravenously (i.v.) over 1 h every 3 weeks alone or combined with 300 or 600 mg/m^2 tamoxifen orally every 12 h for 7 days prior to doxorubicin dosing. Doxorubicin was administered, at the prescribed dose, on day 4 of the 7-day tamoxifen administration regimen. A fourth dose of doxorubicin was administered at 0.625 mg/kg (50% reduction) plus tamoxifen at 600 mg/m^2 every 12 h for 7 days, 3 weeks following the third dose of doxorubicin. This treatment was administered to assess any pharmacokinetic alterations or toxicity following an empirical 50% reduction in doxorubicin dose that has been suggested previously for combined doxorubicin/chemomodulator treatment [24]. Pre-doxorubicin serum panel and CBC were evaluated in all dogs and a CBC was evaluated on days 7 and 14 following doxorubicin administration for each treatment course. Any neurologic or gastrointestinal signs were recorded and monitored daily. Heparinized samples for tamoxifen analysis were obtained 2–4 h following the morning tamoxifen dose just prior to doxorubicin infusion. Doxorubicin was infused over 1 h and serum samples were obtained for doxorubicin determination at 0, 30, 60, 65, 70, 75, 90 and 120 min from the beginning of the infusion. Serum was immediately frozen at −85 °C for later analysis.

A tamoxifen dose escalation study was also conducted in 19 tumor-bearing dogs that presented to the NCSU Veterinary Teaching Hospital. Criteria for eligibility included: (1) histologically proven cancer, (2) refractory to conventional therapy, (3) CBC and serum biochemical panel within normal reference ranges, (4) no major concurrent medical problems, (5) life expectancy of >3 months, and (6) no chemotherapy for 1 month prior to treatment with tamoxifen. All dogs were treated with maximum, clinically tolerated doses of single-agent chemotherapy as determined to be most appropriate for the tumor type, i.e. doxorubicin (1 mg/kg or 30 mg/m^2 i.v. over 1 h, mitoxantrone (6 mg/m^2 i.v. over 1 h), carboplatin (250–300 mg/m^2 over 30 min), cisplatin (70 mg/m^2 over 1 h) or vincristine (0.7 mg/m^2 i.v. bolus). Chemotherapy was administered on day 4 of a 7-day tamoxifen oral dosing period. Tamoxifen dose escalation was conducted in cohorts of three dogs starting at 150 mg/m^2 orally every 12 h for 7 days based on prior studies conducted in humans [35]. Escalation to 300, 600 and 1200 mg/m^2 orally every 12 h for 7 days was based on the severity and frequency of toxicity according to previously established criteria for tumor-bearing dogs. Assessment of toxicity was based upon entry of more than three previously untreated dogs in each dose group. The effect of tamoxifen on doxorubicin serum disposition was evaluated in three dogs. These dogs were treated with doxorubicin alone (1.0 mg/kg i.v. over 1 h) and 3 weeks later were treated with doxorubicin at the same dose plus tamoxifen

at 600 mg/m^2 orally every 12 h for 7 days. Plasma/serum sampling and processing were as previously described.

Plasma tamoxifen was determined at the NCSU Analytical Pharmacology Laboratory using the technique of Langan-Fahey and Jordan with modification [22]. Due to a lack of an NDMT standard, this analyte was estimated as a fraction of the tamoxifen peak and compared to canine standards obtained following validation of NDMT in the first three normal dogs at a laboratory capable of measuring NDMT (V.C. Jordan, University of Wisconsin). Plasma samples were diluted 1:1 with 100% acetonitrile, vortexed and centrifuged at 10 000 *g* for 5 min to release protein-bound tamoxifen. Recoveries in spiked canine serum ranged from 95% to 107% (coefficient of variation 3.8–9.7%) over a concentration range of 5000–50 ng/ml. A Waters Model 600 W multisolvent delivery system with a Waters U6 K injector was coupled to a Model 996 UV-Vis PDA detector (Waters Chromatography Division, Milford, Mass.). The LC separations were performed using a mobile phase of acetonitrile-methanol-triethylamine-water (70:20:0.01:9.99 v/v/v/v). The pH of the mobile phase was 7.5 and the mobile phase flow rate was 1.3–1.5 ml/min. giving a retention time of 7–9 min for tamoxifen and 6–7 min for NDMT on Nova Pack C18 columns (150–3.9 mm ID; Waters Chromatography Division). The column effluent was analyzed in the wavelength range 220–340 nm with a photodiode-array detector. Peak area measurements were computed on a Millenium 2010 Work Station (Waters Chromatography Division). The UV-Vis limit of detection for tamoxifen in canine serum was approximately 4–5 ng/ml.

Doxorubicin was analyzed by HPLC-derived methodologies as previously described in canine serum at the laboratory of one of the investigators [10]. Serum doxorubicin values were fitted to a two-compartment model for an i.v. infusion, with no lag time and first-order elimination (PK Analyst; Micromath Scientific Software, Salt Lake City, Utah). The equation used to fit the data was:

$$C(T) = Ae^{-\alpha Tstar} + Be^{-\beta Tstar} - A^{-aTime} - B^{-\beta Time}$$

where Tstar (any time point greater than i.v. (infusion time)) = Time – Ti.v. and Tstar = 0 for T < Ti.v. Derived quantities included AUC (ng/ml min^{-1}), and AUMC (ng/ml min^{-1} min^{-1}). Calculated quantities included MRT (AUMC/AUC, min), Cl (dose/AUC, ml/min kg^{-1}), Vss (MRT*Cl, ml/kg), half-life ($T_{1/2}$) (0.693* Vd/Cl, min), and C_{max} (C(TI), ng/ml).

A two-sided Student's *t*-test was used to determine the likelihood that the pharmacokinetic parameters were different between the various treatment groups. A Boneferroni correction for repeated measures was applied to the data. *P*-values of <0.05 were considered statistically significant.

Results

Peak plasma tamoxifen and NDMT concentrations increased linearly with dose in the dogs (Fig. 1). Plasma concentrations of these two metabolites combined were 6–11 μ*M* at an oral dose of 600 mg/m^2 twice daily for 7 days. Peak plasma tamoxifen concentrations varied between dogs but reached a steady state in each individual by 4–7 days (data not shown). The only toxicity observed in this group was transient diarrhea in two dogs.

Mean doxorubicin AUC values were not significantly different in the first three treatment groups (doxorubicin alone, or combined with tamoxifen at 300 mg/m^2 or 600 mg/m^2 every 12 h for 7 days), although serum concentration-time profiles suggested an increase in doxorubicin retention when tamoxifen was administered at 600 mg/m^2 every 12 h (Fig. 2). Tamoxifen administered at 600 mg/m^2 every 12 h decreased the Cl and Vd

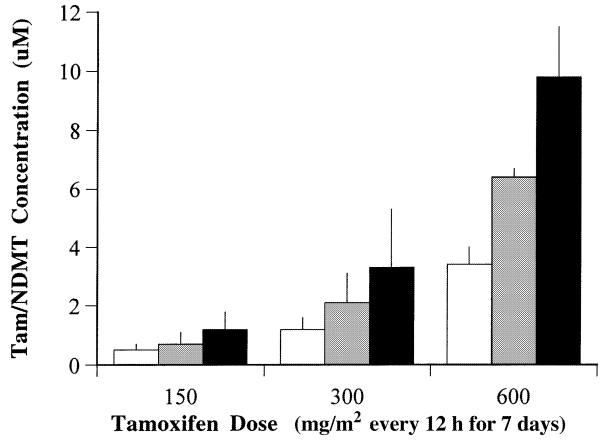

Fig. 1 Peak plasma tamoxifen and NDMT following oral administration of tamoxifen citrate to seven dogs at the doses indicated (*clear bars* NDMT, *gray bars* tamoxifen, *black bars combined values*) Values are means (± SD)

of doxorubicin (Table 1). The half-life of doxorubicin was also decreased as a result of a substantially greater decline in Vd relative to Cl. The AUC of doxorubicin at 0.625 mg/kg (a 50% dose reduction) in combination with tamoxifen at 600 mg/m^2 every 12 h for 7 days was significantly reduced from that of doxorubicin administered alone at 1.25 mg/kg or administered at 1.25 mg/kg with tamoxifen (300 or 600 mg/m^2 every 12 h for 7 days). Plasma concentrations of tamoxifen/NDMT in the tumor-bearing dogs were similar to values obtained in normal dogs not treated with doxorubicin (Fig. 1 and Table 1). No significant enhancement of doxorubicin toxicity other than partial anorexia and diarrhea was noted in the normal dogs. Neutropenia remained consistent throughout all courses of treatment in these normal dogs with the exception of the low-dose doxo-

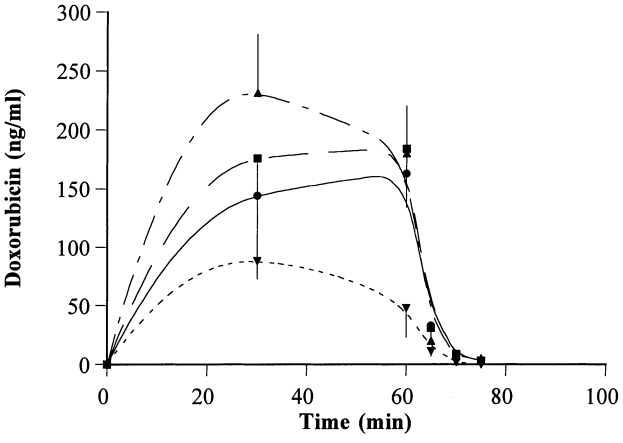

Fig. 2 Serum doxorubicin profiles for seven normal dogs treated with doxorubicin alone, and doxorubicin combined with tamoxifen. (*solid line* 1.25 mg/kg doxorubicin alone i.v. over 1 h), *long-dashed line* 1.25 mg/kg doxorubicin i.v. over 1 h plus 300 mg/m^2 tamoxifen orally every 12 h for 7 days, *short-dashed line* (1.25 mg/kg doxorubicin i.v. over 1 h plus 600 mg/m^2 tamoxifen, *dotted line* (0.625 mg/kg doxorubicin i.v. over 1 h plus 600 mg/m^2 tamoxifen

Table 1 Pharmacokinetic disposition of doxorubicin administered alone (1.25 mg/kg as a 1-h i.v. infusion) or doxorubicin (1.25 mg/kg or 0.625 mg/kg as a 1-hr i.v. infusion) combined with tamoxifen (300 or 600 mg/m^2 orally every 12 h for 7 days) in seven normal dogs. Samples for doxorubicin and tamoxifen were taken on day 7 of the tamoxifen oral dosing schedule. Values are means (±SD)

	Doxorubicin (1.25 mg/kg)	Doxorubicin (1.25 mg/kg) + tamoxifen (300 mg/m^2)	Doxorubicin (1.25 mg/kg) + tamoxifen (600 mg/m^2)	Doxorubicin (0.625 mg/kg) + tamoxifen (600 mg/m^2)
AUC (mg/ml min^{-1})	14.1 (7.8)	10.8 (2.7)	18.9 (8.7)	3.8 (1.1)*
MRT (min)	3.64 (0.5)	2.95 (0.5)	2.47 (.45)	2.94 (0.5)
CL (ml/min kg$^{-1)}$	107.6 (43)	121 (26)	78.7 (31)*	179 (44)*
Vd-ss (ml/kg)	377.7 (122)	357 (95)	189 (72)*	527 (161)*
Cmax (ng/ml)	235.2 (130)	179.6 (45)	313 (145)	62 (19)*
Half-life (min)	2.52 (.33)	2.1 (0.34)	1.7 (0.3)*	2.0 (0.4)
Tamoxifen + NDMT (μmol)	–	2.5 (1.1)	6.8 (2.0)	6.4 (3.4)

* $P < 0.05$ vs doxorubicin-alone group

rubicin treatment (treatment four) where, as expected, nadir neutrophil counts were not reduced as much as with the three previous, full-dose treatments. Thus, there was no evidence for cumulative neutrophil toxicity or irreversible bone marrow stem cell depletion.

The median age of 19 dogs enrolled in the phase I study was 9.5 years; there were 11 males and 8 females representing numerous breeds. Of these 19 dogs, 13 had nonHodgkins lymphoma, 2 had metastatic melanoma and 1 each had squamous cell carcinoma, prostatic carcinoma, osteosarcoma and mast cell tumor. Doxorubicin was administered to nine dogs, mitoxantrone to four, cisplatin to three, carboplatin to two and vincristine to one. Five dogs were treated with tamoxifen at 150 mg/m^2 every 12 h for 7 days, 5 were treated at 300 mg/m^2, 12 were treated at 600 mg/m^2 and 1 was treated at 1200 mg/m^2. Four dogs had the dose of tamoxifen increased to the next dose level and were re-treated. Dose-limiting gastrointestinal toxicity occurred in approximately 50% of dogs treated at 600 mg/m^2. Diarrhea accompanied by anorexia developed 3–5 days following chemotherapy administration. All affected dogs required symptomatic management of diarrhea and two required fluid replacement therapy. Myelosuppression was consistent between tamoxifen dose groups in frequency and no dog developed severe myelosuppression. One dog developed ataxia and paresis following tamoxifen at 1200 mg/m^2 which resolved after the drug was discontinued.

Similar plasma concentrations of tamoxifen and NDMT were observed in the tumor-bearing dogs as were observed in the normal dogs, although greater variability existed (Fig. 3). Doxorubicin serum concentration vs time curves are shown for three dogs treated first with doxorubicin alone followed by combined treatment with doxorubicin and tamoxifen (Fig. 4).

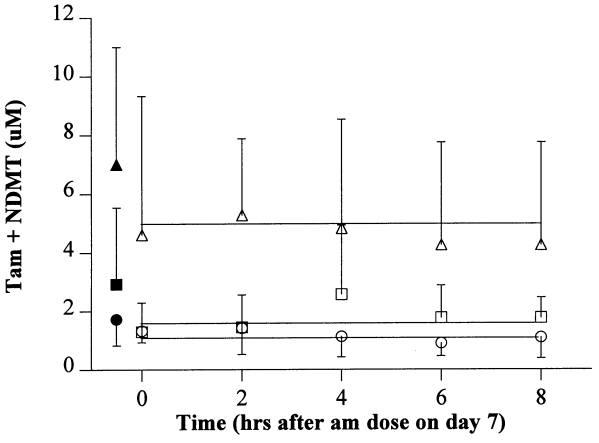

Fig. 3 Peak plasma tamoxifen and NDMT in tumor-bearing dogs following oral administration of tamoxifen every 12 h for 7 days at the following doses (*closed circles* 150 mg/m^2, $n = 3$; *closed squares* 300 mg/m^2, $n = 5$; *closed triangles* 600 mg/m^2, $n = 6$; *open symbols* mean values obtained throughout the 7th day of dosing for each dose group). Values are means (±SD)

Discussion

We determined that oral administration of tamoxifen to normal dogs and to tumor-bearing dogs receiving full-dose chemotherapy resulted in plasma values sufficient to modify Pgp function in vitro. Steady-state accumulation of tamoxifen occurred between days 2 and 5 of a 7-day dosing regimen in normal dogs and future studies should include similar oral dosing periods at 600 mg/m^2 every 12 h to achieve tamoxifen concentrations compatible with in vitro Pgp inhibition. Greater variability in plasma values were observed in tumor-bearing dogs compared with normal dogs due, most likely due to decreased bioavailability in some of these older dogs. The tamoxifen/NDMT values are generally within the range required for in vitro Pgp modulation, although dose adjustment based on individual tamoxifen/NDMT plasma monitoring would be ideal.

Gastrointestinal toxicity and reversible neurotoxicity limit high-dose (e.g. 600 mg/m^2 every 12 h) oral tamoxifen escalation in dogs. Although similar dose-limiting neurotoxicity has been observed in humans, gastrointestinal signs have not been reported to be as frequent as observed here. Tamoxifen administered orally at less than maximal doses (< 600 mg/m^2 every 12 h) may interact through non-MDR pathways and influence cytokine or immune activities in the host. Tamoxifen doses required to produce such effects appear

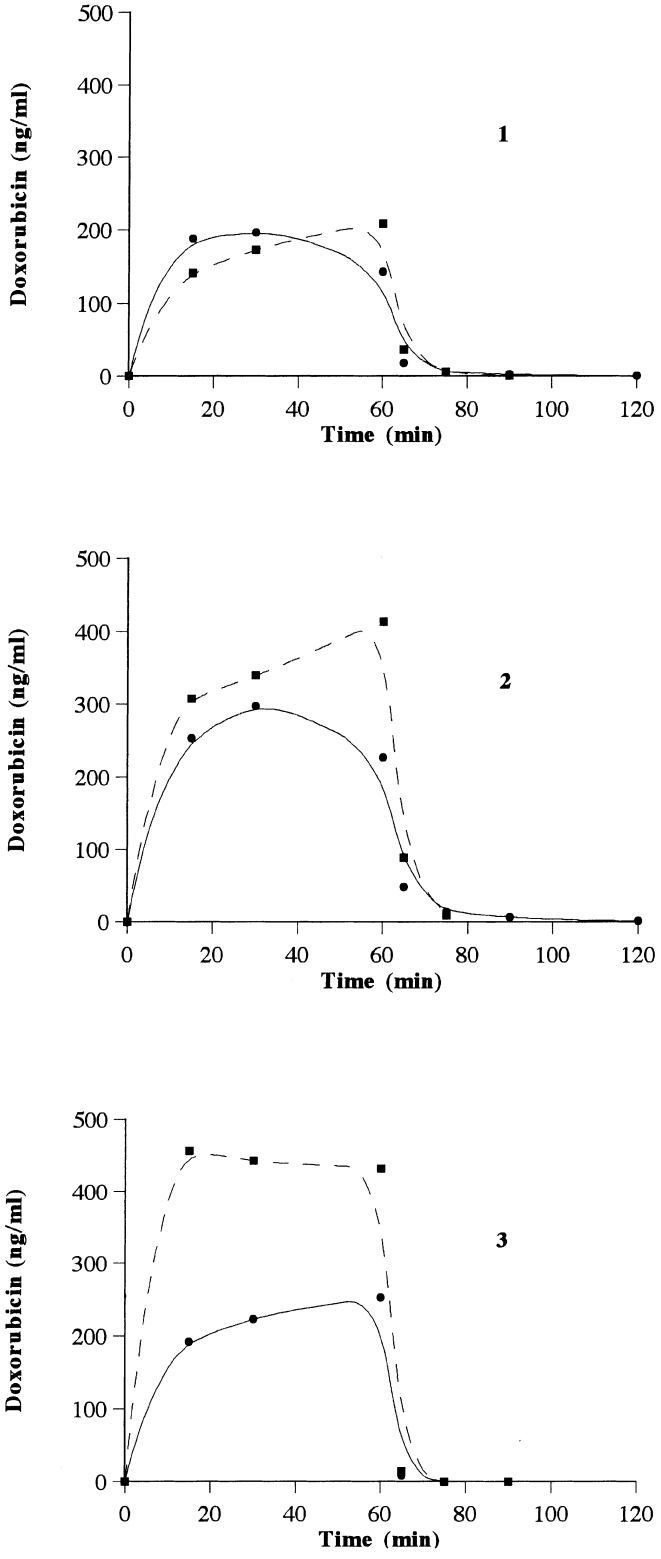

Fig. 4 Serum doxorubicin profiles in three tumor-bearing dogs following administration of 1.25 mg/kg doxorubicin alone (*solid lines*) or combined with 600 mg/m^2 tamoxifen every 12 h for 7 days (*dashed lines*)

to be safe in dogs when used in short dose cycles. Chronic, high-dose oral tamoxifen should be evaluated further.

The oral dose of tamoxifen associated with plasma tamoxifen/NDMT concentrations sufficient to inhibit Pgp in humans is reported to be approximately 150–250 mg/m^2 every 12 h, approximately 25–40% the dose required in dogs [26, 35]. The difference in prescribed dose necessary to achieve similar plasma exposure may be due to differences in gastrointestinal physiology in dogs compared to humans (reduced absorption due to more rapid gastrointestinal transit) or a more rapid biometabolism and elimination of tamoxifen. Tamoxifen metabolism has been reported in normal dogs and appears to be metabolized and excreted by pathways similar to those reported for humans [12]. In both species the compound is metabolized by hydroxylation and methylation, glucuronide conjugation and biliary excretion followed by extensive enterohepatic circulation. Therefore, reduced gastrointestinal absorption of tamoxifen would most likely account for the large difference in maximally tolerated dose in dogs compared to that in humans. It is interesting to speculate that the Pgp expression of the intestinal mucosa may be greater in dogs than in humans and thus, a higher quantity of drug would be required to saturate and overcome the Pgp barrier of the mucosal lining cells in dogs.

The pharmacologic implications of Pgp expression in the intestinal tract has been the subject of recent research. Some MDR compounds administered orally have low bioavailability that may be due, in part, to intestinal Pgp activity. For example, Schinkel has reported that the addition of PSC 833, a competitive inhibitor of Pgp, substantially increases serum concentrations of orally administered Taxol in mice [31]. Schinkel demonstrated that modification of Pgp-specific metabolism results in decreased intestinal and biliary clearance. Thus, the manipulation of Pgp function in normal tissue may be useful for numerous compounds with limited bioavailability.

Modulation of Pgp in the blood-brain barrier by tamoxifen and other classic chemomodulators is also likely. One dog treated with tamoxifen at 1200 mg/m^2 every 12 h for 7 days developed CNS signs consistent with those reported in humans following high-dose chemomodulator administration [35]. In addition to tamoxifen, PSC 833 has been reported to produce CNS toxicity at high doses when used as a chemomodulator [7]. These neurologic signs are similar to central pontine myelinolysis that has been reported following cyclosporine administration for post-transplant immunosuppression [13]. The mechanism of this disorder is not known but is associated with large fluctuations in cyclosporine levels and signs generally resolve following adjustment of the cyclosporine dose. Schinkel et al. have demonstrated that MDR1a knockout mice exhibit unexpected neurotoxicity after normal doses of ivermection [32]. Collie dogs are likewise susceptible to ivermection neurologic toxicity, preventing the use of this compound in some dogs of that breed [28]. Interestingly, it has been suggested that Collie dogs sensitive to ivermectin possess a mutated, nonfunctional Pgp

(Thierry Pineau, personal communication). The neurologic toxicity following Pgp inhibition from the examples cited above suggests that dogs are a useful model for further investigation of the pharmacologic consequences of Pgp-blocking agents.

The effect of tamoxifen on the pharmacokinetic disposition of full-dose doxorubicin in normal dogs is only apparent at high oral doses of tamoxifen ($\sim$600 mg/m^2 every 12 h). Reduced doxorubicin clearance did not occur when tamoxifen was administered at lower doses. The specific interactions between tamoxifen and doxorubicin are not known, although both compounds have complex effects on both P450 and non-P450 pathways. It is likely that the reduced Vd was due to competing protein binding between doxorubicin and tamoxifen.

The doxorubicin AUC was not significantly altered by high-dose tamoxifen although both Cl and Vd were reduced significantly. However, in this group of normal dogs, the doxorubicin AUC increased approximately 30% in the high-dose tamoxifen group and individually, AUC values increased in six of seven dogs. In the tumor-bearing dogs marked variation in serum doxorubicin profiles resulted from tamoxifen cotreatment even though the doxorubicin profiles observed without tamoxifen administration were similar (Fig. 4). As mentioned above, studies in rodents have failed to demonstrate changes in doxorubicin serum profiles when cyclosporine and PSC 833 are administered concurrently [9, 15]. Doxorubicin was administered as a bolus i.v. injection in those studies and the modulator was administered intraperitoneally. Tissue doxorubicin concentrations, however, were altered substantially, particularly in the intestines, adrenal, and liver. These findings should be confirmed using a more clinically relevant drug administration scheme. Such studies would be possible in dogs.

It has been recommended that future Pgp chemomodulation trials evaluate equivalent AUC values of the chemotherapeutic agent in order to directly assess effects of the modulator on tumor response avoiding confounding pharmacologic interference [24]. Therefore, we evaluated an empirical 50% doxorubicin dose reduction with high-dose tamoxifen. The resulting doxorubicin AUC value was significantly less than that associated with the full-dose doxorubicin. Any interaction between doxorubicin and tamoxifen was not apparently sufficient to result in equivalent doxorubicin AUC. A 25% doxorubicin dose reduction may be a more accurate initial dose adjustment for future studies in dogs when doxorubicin is combined with high-dose tamoxifen.

The results presented here indicate that dogs are a potential model for evaluation and refinement of Pgp modulation strategies. Dogs are large enough to permit extensive serum and tissue sampling and may be easily imaged with radiolabeled Pgp substrates such as sestamibi for noninvasive determination of normal tissue Pgp modulation. The canine MDR1 gene is 95% homologous with the human MDR1 gene [34] and is similarly expressed in some forms of cancer including treatment-naive and relapsed nonHodkins lymphoma [14, 23]. Carefully designed studies in this model could obviate the pharmacologic issues and therefore directly assess the primary Pgp inhibitory activity of chemomodulators.

References

1. Baral E, Nagy E, Berczi I (1995) Modulation of natural killer cell-mediated cytotoxicity by tamoxifen and estradiol. Cancer 75: 591–599
2. Bartlett NL, Lum BL, Fisher GA, Brophy NA, Ehsan MN, Halsey J, Sikic BI (1994) Phase I trial of doxorubicin with cyclosporine as a modulator of multidrug resistance. J Clin Oncol 12: 1635–1642
3. Berg SL, Cowan KH, Balis FM, Fisherman JS, Denicoff AM, Hillig M, Poplack DG, O'Shaughnessy JA (1994) Pharmacokinetics of taxol and doxorubicin administered alone and in combination by continuous 72-hour infusion. J Natl Cancer Inst 86: 143–145
4. Berman E, McBride M, Tong W (1994) Comparative activity of tamoxifen and N-desmethyltamoxifen in human multidrug resistant leukemia cell lines. Leukemia 8: 1191–1196
5. Berman E, McBride M, Lin S, Menedez-Botet C, Tong W (1995) Phase I trial of high-dose tamoxifen as a modulator of drug resistance in combination with daunorubicin in patients with relapsed or refractory acute leukemia. Leukemia 9: 1631–1637
6. Berry J, Green BJ, Matheson DS (1987) Modulation of natural killer cell activity by tamoxifen in stage I post-menopausal breast cancer. Eur J Clin Oncol 23: 517–520
7. Boote DJ, Dennis IF, Twentyman RJ, Osborne C, Laburte S, Hensel JF, Smyth JF, Brampton MH, Bleehen NM (1996) Phase I study of etoposide with SDZ PSC 833 as a modulator of multidrug resistance in patients with cancer. J Clin Oncol 14: 610–618
8. Butta AB, MacLennen K, Flanders KC, Sacks NPM, Smith I, Mckinna A, Dowsett M, Wakefield LM, Sporn MB, Baum M, Coletta AA (1992) Induction of transforming growth factor beta-1 in human breast cancer in vivo following tainoxifen treatment. Cancer Res 52: 4261–4264
9. Colombo T, Zucchetti M, D'Incalci M (1994) Cyclosporin A markedly changes the distribution of doxorubicin in mice and rats. J Pharmacol Exp Ther 269: 22–27
10. Cox SK, Wilke AV, Frazier DL (1991) Determination of adriamycin in plasma and tissue biopsies. J Chromatogr 564: 322–329
11. Fine RL, Sachs CW, Albers ME, Safa A, Rao US, Scarborough G, Burchette J, Jordan C, Trump DL (1993) Inhibition of multidrug resistance in human cancer cells by tamoxifen: laboratory to clinical studies. In: Miyazaki P, et al. (eds) The mechanisms and new approaches on drug resistance of cancer cells. Excerpta Medica, Amsterdam, pp 323–332
12. Fromson JM, Peason S, Bramah S (1973) The metabolism of tamoxifen (ICI 46474). Part I: In Laboratory Animals. Xenobiotica 3: 693–709
13. Fryer JP, Fortier MV, Metrakos P, Verran DJ, Asfar SK, Pelz DM, Wall WJ, Grant DR, Ghent CN (1996) Central pontine myelinolysis and cyclosporine neurotoxicity following liver transplantation. Transplantation 61: 658–661
14. Ginn PE (1996) Immunohistochemical detection of P-glycoprotein in formalin-fixed and paraffin-embedded normal and neoplastic canine tissues. Vet Pathol 33: 533–541
15. Gonzalez O, Colombo T, DeFusco M, Imperatori L, Zucchetti M, D'Incalci M (1995) Changes in doxorubicin distribution and toxicity in mice pretreated with the cyclosporin analogue SDZ PSC 833. Cancer Chemother Pharmacol 36: 355–360
16. Haran EF, Maretzek AF, Goldberg I, Horowitz A, Degani H (1994) Tamoxifen enhances cell death in implanted MCF7

breast cancer by inhibiting endothelium growth Cancer Res 54: 5511–5514
17. Hindenburg AA, Baker MA, Gleyzer E, Stewart VJ, Case N, Taub RN (1987) Effect of verapamil and other agents on the distribution of anthracyclines and on reversal of drug resistance. Cancer Res 47: 1421–1425
18. Horgan K, Cooke E, Hallett MB, Mansel RE (1986) Inhibition of protein kinase C mediated signal transduction by tamoxifen. Biochem Pharmacol 35: 4463–4465
19. Hughes CS, Huyan S, Trogdon M, Page RL (1995) Comparison of dye retention and cytotoxic enhancement of MDR modulators in MCF-7/dx and 4TG11-50c cells. Proceedings of the 86th Annual Meeting of the American Association of Cancer Research, Toronto 36, p. 343 (abstr.)
20. Huynh HT, Tetenes E, Wallace L, Pallak M (1993) In vivo inhibition of insulin-like growth factor I gene expression by tamoxifen. Cancer Res 53: 1727–1730
21. Kang Y, Perry RR (1993) Modulatory effects of tamoxifen and recombinant human alpha-interferon on doxorubicin resistance. Cancer Res 53: 3040–3045
22. Langan-Fahey SMTDC, Jordan VC (1990) Tamoxifen metabolises in patients on long-term adjuvant therapy for breast cancer. Eur J Cancer 26: 883–888
23. Lee JJ, Hughes CS, Fine RL, Page RL (1996) P-glycoprotein expression in canine lymphoma. A relevant, intermediate model of multidrug resistance. Cancer 77: 1892–1898
24. Lum BL, Fisher GA, Brophy NA, Yahanda AM, Adler KM, Kaubisch S, Halsey J, Sikic BI (1993) Clinical trials of modulation of multidrug resistance: pharmacokinetic and pharmacodynamic considerations. Cancer 72: 3502–3514
25. McClay EF, McClay MT, Winski PJ, Christen RD, Howell SB, Hall PD (1996) A phase I and pharmacokinetic study of high dose tamoxifen and weekly cisplatin in patients with metastatic melanoma. Cancer 79: 1037–1043
26. Millward MJ, Lien EA, Robinson A, Cantwell BM (1994) High-dose (480 mg/day) tamoxifien with etoposide: a study of a potential multidrug resistance modulator. Oncology 51: 79–83
27. Noguchi S, Motomura K, Inaji H, Imaoka S, Koyama H (1993) Down-regulation of transforming growth factor-alpha by tamoxifen in human breast tissue. Cancer 72: 131–136
28. Paul AJ, Tranquilli WJ, Seward RL, Todd KS, DiPietro JA (1987) Clinical observations in collies given ivermectin orally. Am J Vet Res 48: 684–685
29. Relling RV (1996) Are the major effects of P-glycoprotein modulators due to altered pharmacokinetics of anticancer drugs? Ther Drug Monit 18: 350–356
30. Rushing DA, Raber SR, Rodvold KA, Piscitelli SC, Plank FS, Tewksbury DA (1994) The effects of cyclosporine on the pharmacokinetics of doxorubicin in patients with small cell lung cancer. Cancer 74: 834–841
31. Schinkel AH (1998) Pharmacological insights from P-glycoprotein knockout mice. Int J Clin Pharmacol Ther 36: 9–13
32. Schinkel AH, Mayer U, Wagenaar E, Mol CAAM, Deemter L van, Smit JJ, Valk MA van der, Voordouw AC, Spits H, Tellingen O van, Zijlmans JM, Fibbe WE, Borst P (1997) Normal viability and altered pharmacokinetics in mice lacking MDR l-type (drug-transporting) P-glycoproteins. Proc Natl Acad Sci U S A 94: 4028–4033
33. Smith DC, Trump DL (1995) A phase I trial of high-dose oral tamoxifen and CHOPE. Cancer Chemother Pharmacol 36: 65–68
34. Steingold SF, Sharp NJ, McGahan MC, Hughes CS, Dunn SE, Page RL (1998) Characterization of canine NMR1 mRNA: its abundance in drug resistant cell lines and in vivo. Anticancer Res 18: 393–400
35. Trump DL, Smith DC, Ellis PG, Rogers MP, Schold SC, Winer EP, Panella TJ, Jordan VC, Fine RL (1992) High dose oral tamoxifen, a potential multidrug resistance reversal agent: phase I trial in combination with vinblastine. J Natl Cancer Inst 84: 1811–1816