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ORIGINAL ARTICLE

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Effect of tamoxifen pretreatment on the pharmacokinetics, metabolism and cardiotoxicity of doxorubicin in female rats

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Abstract The purpose of this study was to examine the effect of tamoxifen pretreatment on the metabolism and pharmacokinetics of doxorubicin. We tested the hypothesis that the pretreatment would counteract the side effects of doxorubicin and modify the disposition of the drug. The concentration-time profiles of doxorubicin in plasma and blood cells were determined in conjunction with the cumulative amount of renal and hepatobiliary elimination of unchanged drug and metabolites following a 10-day tamoxifen pretreatment at a dose of 1 mg/kg per day. Furthermore, under the same experimental protocol the serum concentration-time profile of endothelin was determined as a biomarker of toxicity. *Methods*: Female Sprague Dawley rats (225–275 g), pretreated orally for 10 days with corn oil or tamoxifen in corn oil (1 mg/kg per day), received ^{14}C-doxorubicin (specific activity 0.4 μCi/mg, 10 mg/kg) intravenously. Plasma, blood cells, bile and urine were collected periodically and analyzed for doxorubicin and its metabolites. Four other groups of animals received the same pretreatment and non-labeled doxorubicin. Their serum samples were analyzed for endothelin. Two additional groups were also used to examine the effect of tamoxifen on the in vitro metabolism of doxorubicin by the cytosolic enzyme aldo-keto reductase. *Results*: Tamoxifen pretreatment reduced the total protein of the cytosolic fraction by 50% and reduced the formation of doxorubicinol both in vitro and in vivo. The pretreatment resulted in a notable increase in the area under plasma and blood cells concentration-time curves of doxorubicin and a significant reduction in mean residence time, apparent volume of distribution and serum endothelin levels. *Conclusions*: We attributed the increase in the area under the curves of plasma and blood cells following tamoxifen pretreatment to a reduction in the uptake of doxorubicin by peripheral tissues. This conclusion was consistent with the reduction in the volume of distribution of plasma, mean residence time and higher availability of the parent compound for excretion. An interesting observation was that the increase in concentration of doxorubicin in plasma was not concomitant with an increase in concentration of doxorubicinol. The levels of this toxic metabolite and its corresponding biliary rate constant were reduced by approximately 50%. The results demonstrate that tamoxifen, in addition to being a modulator of P-glycoprotein and counteracting the effects of doxorubicin at the cellular level, also alters the metabolic profile of doxorubicin either by inhibiting the formation of the toxic metabolite doxorubicinol or by reducing the enzyme responsible for the biotransformation. The change in metabolism may well be a contributing factor to reduction of serum endothelin levels.

Key words Tamoxifen · Doxorubicin · Pharmacokinetics · Doxorubicinol · Endothelin

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Introduction

Tamoxifen, a synthetic nonsteroidal antiestrogen, in addition to its application in treatment and prevention of breast cancer, is also a modulator of multidrug resistance and an antioxidant [7, 8, 15]. This compound and its major metabolite, 4-hydroxytamoxifen, are known to inhibit both metal ion-dependent enzymatic and nonenzymatic lipid peroxidation in a number of model membranes [22, 28, 29]. It has been reported that both the parent compound and its metabolite induce enzymes such as glutathione peroxidase, catalase and superoxide dismutase in postmenopausal women [22].

They also inhibit nitric oxide synthase, which reduces NADPH and forms nitric oxide/superoxide ion in various tissues [16]. Tamoxifen, being a highly lipophilic compound, can physically partition into the cell membrane, and the lipophilic moiety of the molecule interacts with the saturated, monounsaturated and polyunsaturated residues of phospholipids. This interaction stabilizes the cell membrane and prevents lipid peroxidation [27]. In its in vitro interaction with erythrocytes, tamoxifen changes the discoid cells to stomatocytes and remains in the inner leaflet of phospholipid bilayer of the membrane [21]. This change, however, may not be observed at therapeutic doses of tamoxifen. Other findings have indicated that tamoxifen reduces the number of erythrocytes and hemoglobin concentration [12], reduces the inward flux of Ca^{2+} and inhibits ceramide glycosylation in human cancer cells [19, 25]. It has also been reported to potentiate the doxorubicin-induced apoptosis of hepatocellular carcinoma cell culture at a concentration of 2.5 μM [6]. It is worth noting that caution must be exercised in extrapolating data from in vitro model systems that are based on very high concentrations of tamoxifen to in vivo conditions.

The interaction of tamoxifen with doxorubicin has not been fully elucidated. The published data, however, indicate that these two compounds may have opposing properties in a number of physiological processes. For example, doxorubicin and its alcohol metabolite doxorubicinol increase cytoplasmic calcium by inhibiting Na^{+}/Ca^{2+} exchange or the Ca^{2+} ATPase of sarcolemma [18], and tamoxifen reduces the inward flux of Ca^{2+} by inhibiting the calcium channels and negates the effect of doxorubicin [19]. Doxorubicin renders the membranes of organelles leaky whereas tamoxifen, as mentioned above, increases the rigidity of the membrane [27]. Doxorubicin reduces the levels of certain cardiac detoxifying enzymes such as glutathione peroxidase and glutathione reductase [13] and increases the levels of cholesterol and LDL [20]. Tamoxifen, on the other hand, increases the levels of the antioxidant enzyme [22] and significantly decreases the levels of cholesterol and LDL [25]. Finally, doxorubicin forms hydroxyl free radicals whereas tamoxifen has a free radical scavenging effect. The in vivo investigation of the effect of tamoxifen on metabolism and pharmacokinetics of doxorubicin is very limited [9].

Based on the aforementioned properties of tamoxifen, we hypothesized that pretreatment with tamoxifen would be effective in reducing the side effects of doxorubicin. To test this hypothesis, we investigated the concentration-time profiles of doxorubicin in plasma and blood cells, its cumulative renal and hepatobiliary elimination and its in vitro and in vivo metabolism following a 10-day tamoxifen pretreatment at a dose 1 mg/kg per day. Furthermore, we evaluated the serum concentration-time profile of endothelin as a cardiotoxicity biomarker in response to the pretreatment [30, 31].

Materials and methods

Chemicals

Tamoxifen (citrate salt), Trizma HCl and the total protein reagent were obtained from Sigma Chemical Co. (St. Louis, Mo.). Doxorubicin (HCl salt), doxorubicinol, doxorubicin aglycone and doxorubicinol aglycone were gifts from Pharmacia Upjohn (Albuquerque, N.M.). Radiolabeled doxorubicin (^{14}C, specific activity 91.1 μCi/mg) was purchased from Amersham Corporation (Arlington Height, Ill.). Beckman Instruments (Fullerton, Calif.) provided tissue solubilizer (BTS-450). Scintillation cocktail (Scintiverse E), corn oil and all chemicals of analytical grade were purchased from Fisher Scientific (Springfield, N.J.). HPLC-grade solvents, polycarbonate and polypropylene ultracentrifuge tubes and hydrogen peroxide were obtained from VWR Scientific (Boston, Mass.). Sodium pentobarbital (Nembutal, 50 mg/ml) was purchased from Henry Shein Co. (Port Washington, N.Y.), heparin (5000 U/ml) from Elkins-sinn (Cherry Hill, N.J.) and endothelin enzyme reagents from Peninsula Laboratories (San Carlos, Calif.). All surgical instruments including silk suture, polyethylene tubing (PE 10) and syringes were obtained from Becton Dickinson and Company (Sparks, Md.).

Protocol of in vitro metabolism

Two groups of female Sprague Dawley rats ($n = 6$ per group) weighing 150–220 g (Taconic Farms, Germantown, N.Y.), in parallel design, received tamoxifen in corn oil (1 mg/kg body weight per day) or an equal volume of corn oil by oral intubation for ten consecutive days. During this time the animals were kept, two per cage, in a clean environment under a 12-hour light/dark cycle and had access to food and water ad libitum. The animals were weighed daily after each dose and the amount of food consumed was recorded. The animals maintained a normal increase in weight that was comparable to that of the control group. On the 11th day after the last pretreatment dose, the animals were killed and the liver was removed, washed in ice-cold normal saline, weighed and immediately frozen in liquid nitrogen and stored in at −20 °C. No significant changes were observed in the weight of the liver. The liver microsome and cytosol were prepared according to the method of Boyd et al. [4]. Briefly, 2 g liver was homogenized in 10 ml ice-cold Tris buffer (20 mM, pH 7.4) containing 15% KCl and 10% glycerin, and centrifuged at 25,000 g for 20 min. The supernatant was filtered through wet gauze into a precooled centrifuge tube and centrifuged (Beckman, Fullerton, Calif.) at 100,000 g for 60 min to separate the microsomal and cytosolic fractions. After determining the protein content of both fractions by biuret assay [11], 2 mg proteins from each fraction were incubated with 0.5 mM NADPH, 0.05 M Tris buffer, pH 7.4, and different concentrations of doxorubicin in a total volume of 2 ml at 37 °C for 60 min. The control samples contained all the additives of the incubation except the proteins, and their incubation was also carried out simultaneously under the same conditions. After stopping the enzymatic reactions by the addition of 0.5 ml acetone, the samples were centrifuged at 7,500 g for 15 min and the supernatants were analyzed by HPLC for doxorubicin and its metabolites. The total length of the experiment from sampling to HPLC analysis was about 3.5 h.

Protocol of in vivo experiment

The dose was prepared by the addition of non-labeled doxorubicin to radiolabeled drug in sterile normal saline to obtain a final specific activity of 0.4 μCi/mg. Four groups of female Sprague Dawley rats weighing 225–250 g ($n = 6$/group) were housed, two per cage, in a clean environment, allowed acclimatizing for at least 1 week prior to experimentation and used in a parallel design for the in vivo experiment. Two groups (I and II) received an equal volume of corn oil daily and the remaining two groups (IV and III) received

tamoxifen in corn oil (0.5 mg/ml, 1 mg/kg per day) by oral intubation. The treatments continued for ten consecutive days and the animals had free access to food and water under a 12-h light/dark cycle. On the tenth night food was withheld and on the 11th day after the last dose, the animals were anesthetized by i.p. injection of sodium pentobarbital (Nembutal, 45 mg/kg) and their bile duct and tail vein were cannulated. The animals were kept under constant anesthesia during the 10-h experiment. The cumulative dose of sodium pentobarbital did not exceed 50 mg. Groups III and I (controls) received normal saline and groups II and IV received ^{14}C-doxorubicin (specific activity 0.4 μCi/mg, 10 mg/kg). Both injections were via a femoral vein. Blood samples (about 200 μl) were collected in heparinized tubes at 5, 15, 30 and 45 min then every hour. Bile samples were collected periodically and urine was collected during and at the end of the experiment by puncturing the bladder. The blood samples were centrifuged at 5000 *g* for 15 min to separate blood cells from plasma. The plasma, bile and urine samples were frozen in liquid nitrogen immediately and stored at −20 °C until further analysis. The blood cells were stored at 4 °C.

Protocol of endothelin assay

Four other groups of female Sprague Dawley rats (groups V–VIII, $n = 6$ per group) received either corn oil (groups V and VI) or tamoxifen in corn oil (groups VII and VIII) by oral intubation for ten consecutive days under the same condition as described above. On the 11th day, following the last dose of pretreatments and tail vein cannulation, groups V and VII received normal saline and groups VI and VIII received doxorubicin. Both injections were via a femoral vein 1 h after the cannulation. The 1-h delay was necessary to attain the basal levels of the biomarker. Blood samples (about 250 μl), collected periodically without heparin, were centrifuged at 5000 *g* for 15 min, and the serum portion was separated, frozen immediately in liquid nitrogen and stored at −20 °C.

Analytical methodology

The total radioactivity of bile, urine, plasma or blood cells of all biological samples was measured by a liquid scintillation counter (Wallac, Gaithsburg, Md.). The samples of blood cells, normalized with respect to volume, were treated with tissue solubilizer at room temperature for 24 h followed by the addition of hydrogen peroxide (300 μl) to remove the color, and glacial acetic acid (100 μl) to prevent quenching. After the addition of liquid scintillation counting fluid, the samples were kept in a refrigerator overnight and the total radioactivity was determined the next day. Bile and urine samples were also analyzed by HPLC for doxorubicin and its major metabolites.

The HPLC system consisted of a Waters solvent delivery system, Novapak C-18 cartridge, C-18 Novapak Sentry guard column (Waters Corporation, Milford, Mass.) and a Hitachi AS-2000 autosampler (Danbury, Ct.). The mobile phase was 0.1% v/v ammonium formate buffer/methanol (30:70% v/v), pH 4.0. The detectors were a fluorometer (Gilson, Middleton, Wis.) with excitation and emission wavelengths of 480 nm and 540 nm, respectively, and an online scintillation counter (Beta Flo, Packard, Meriden, Ct.). The guard column was replaced regularly and the retention time of doxorubicin and each metabolite determined and confirmed daily with authentic standards and daunorubicin as internal standard. A typical HPLC separation of doxorubicin and its major metabolites using the aforementioned methodology is presented in Fig. 1.

The levels of endothelin in serum samples of groups V–VIII were determined by an enzyme immunoassay procedure. Briefly, the procedure involved placing in a coated 96-well plate the anti-peptide antibody, the standard serum samples or serum samples from groups V–VIII and biotinylated peptide. The biotinylated peptide competes with the standard peptide or the peptide in the sample for binding sites on the antibody. The mixture was incubated overnight at room temperature. The wells were then washed five times to remove the excess unbound biotinylated peptide.

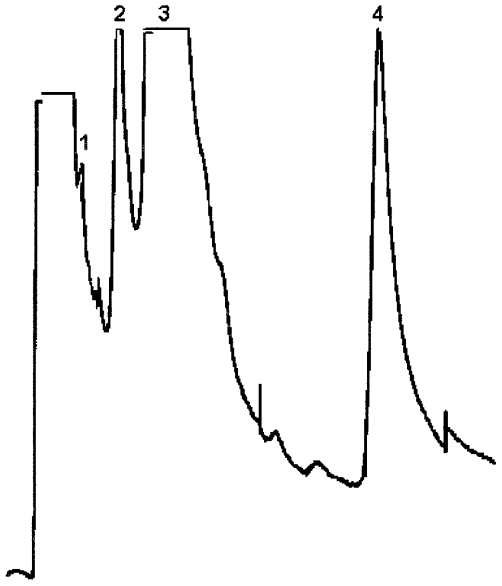

Fig. 1 A typical HPLC separation profile of doxorubicin and its major metabolites using 0.1% v/v ammonium formate buffer/methanol (30%/70% v/v), pH 4.0, as mobile phase and Novapak C-18 cartridge as the column at excitation and emission wavelengths of 480 nm and 540 nm, respectively (*peak 1* doxorubicin aglycone, *peak 2* doxorubicinol aglycone, *peak 3* doxorubicin, *peak 4* doxorubicinol)

A solution of streptavidin-horseradish peroxidase conjugate was added to the wells and the plates were incubated at room temperature for 60 min to allow the conjugate to bind to the immobilized primary antibody-biotinylated peptide complex. After washing the excess conjugate, a solution of 3,3′,5,5′-tetramethyl benzidine dihydrochloride was added at room temperature and 50 min later 2 *N* HCl was added to stop the formation of colored complex. The intensity of the color was measured at 450 nm with a microtiter plate reader (Bio-Tek Instruments, Winooski, Vt.). The concentration of peptide was determined from the inverse relationship between the concentration of the peptide and the intensity of the colored samples [30, 31].

Data analysis

The pharmacokinetic analysis of doxorubicin in plasma or associated with blood cells was achieved by non-compartmental analysis with the following equations:

$$\mathrm{Cl} = \mathrm{Dose}/\mathrm{AUC} \tag{1}$$

$$\mathrm{MRT} = \mathrm{AUMC}/\mathrm{AUC} \tag{2}$$

$$\mathrm{Vd_{ss}} = \mathrm{MRT} \cdot \mathrm{Cl} \tag{3}$$

where Cl is the clearance, AUC is the area under the concentration-time curve, MRT is the mean residence time, AUMC is the area under the first moment curve and $\mathrm{Vd_{ss}}$ is the volume of distribution.

The simultaneous curve fitting of biliary elimination and plasma concentration was achieved by using the following equations of two-compartment model:

$$\text{Plasma: } \mathrm{C_p} = \mathrm{A}e^{-\alpha_t} + \mathrm{B}e^{-\beta_t} \tag{4}$$

$$\text{Bile: } \mathrm{A_B} = \mathrm{k_b}[(\mathrm{A}\beta + \mathrm{B}\alpha)/\alpha\beta] - \mathrm{k_b}[(\mathrm{A}/\alpha)e^{-\alpha_t} + (\mathrm{B}/\beta)e^{-\beta_t}] \tag{5}$$

where $\mathrm{C_p}$ is the plasma concentration with units of mass/time, $\mathrm{A_B}$ is the cumulative amount eliminated in bile at time t, A and B are the coefficients of the exponential terms, α and β are the hybrid rate

constants associated with the transfer of drug between the two compartments and the elimination of the drug from the central compartment, and k_b is the first-order rate constant for biliary excretion.

At time $t = \infty$,

$$A_B^{\infty} = k_b[(A\beta + B\alpha)/\alpha\beta]. \quad (6)$$

where A_B^{∞} corresponds to the total amount that ultimately will be eliminated in the bile.

The change in endothelin concentration was used as a toxicodynamic parameter and the following sigmoidal E_{max} model was applied to the data:

$$E = (E_{max} \cdot T^{\gamma})/(ET_{50}^{\gamma} + T^{\gamma}) \quad (7)$$

where E is the serum endothelin level, E_{max} is the maximum serum endothelin level, T is the time, ET_{50} is the time required to reach 50% of maximum endothelin level and γ is the degree of sigmoidicity [10].

Statistical comparisons were carried out using the two-tailed paired and unpaired Student's *t*-tests as needed. WinNonlin (Scientific Consulting, Cary, N.C.) was used for pharmacokinetic analysis.

Results

In vitro metabolism

Tamoxifen is known to induce the cytochrome P450 enzyme system in a similar manner to phenobarbital [26]. However, unlike phenobarbital the effect of tamoxifen on cytosolic enzyme systems has not been investigated. Doxorubicin is metabolized mainly by aldo-keto reductases of the cytosolic fraction. The pretreatment of female Sprague Dawley rats with tamoxifen in the present study resulted in a significant increase in the total protein content of the microsomal fraction of the liver. This was consistent with the effect of tamoxifen on the induction of CYP450 enzyme systems. However, the intriguing observation was a 50% reduction in total protein content of the cytosolic fraction (Fig. 2). Further analysis revealed that among the primary metabolites of doxorubicin only doxorubicinol was reduced from 15.78 μg/g of liver in the control group to approximately 8 μg/g of liver in tamoxifen pretreated animals, a reduction of approximately 50% (Fig. 1). Evaluation of the minor metabolites of doxorubicin such as glucuronide and sulfate conjugates or deoxydoxorubicin aglycone and deoxydoxorubicinol aglycone was not a part of this experiment.

In vivo data

As described in the Methods, each blood sample was separated into plasma and blood cells and the radioactivity of each portion was measured independently. By using the specific activity of the dose, the measured radioactivity of each sample was converted to units of micrograms per milliliter. Table 1 summarizes the pharmacokinetic parameters of plasma, analyzed by noncompartmental analysis and using WinNonlin. The pretreatment of animals with tamoxifen increased the

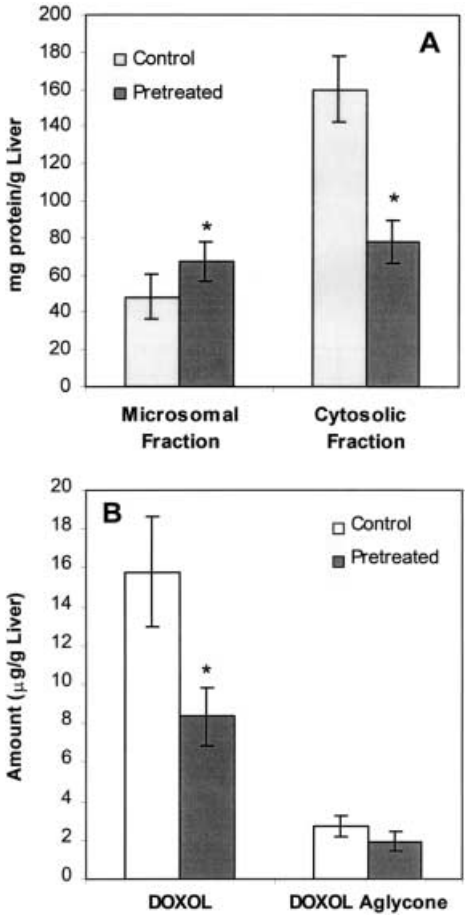

Fig. 2A,B Effects of tamoxifen pretreatment on the total protein content of liver microsomes and cytosol (**A**) and on the formation of the alcohol metabolite, doxorubicinol and its aglycone (**B**). The incubation of the proteins with doxorubicin was carried out in the presence of 0.5 m*M* NADPH, 0.05 *M* Tris buffer, pH 7.4, at 37 °C for 60 min followed by 15 min of centrifugation at 7500 *g*. Data are means ± SD from triplicate measurements in six rats. The total length of the experiment from sampling to HPLC analysis was 3.5 h. * Significantly deceased vs control group

area under plasma concentration-time curve (AUC_{plasma}) from 30.04 mg · h/l to 40.25 mg · h/l, reduced the mean residence time (MRT) from 11.39 h to 4.36 h and reduced the volume of distribution (Vd_{ss}) from 3.79 l to 1.04 l. The change in plasma clearance was not significant. Table 2 shows the pharmacokinetic parameters related to the amount of doxorubicin associated with blood cells, also analyzed by noncompartmental analysis and using WinNonlin. Similar to the plasma data, pretreatment with tamoxifen increased the area under blood cells concentration-time curve ($AUC_{blood\ cells}$) from 63.41 mg · h/l to 84.22 mg · h/l. This increase was consistent with a reduction in clearance from 3.4 ml/min to 2.00 ml/min and an increase in MRT from 5.39 l to 6.72 h. There was no significant change in the volume of distribution of blood cells.

Based on the criteria for model selection of WinNonlin, such as the Akaike criteria and *F*-test, a two-compartment model was selected for simultaneous curve

Table 1 Effect of pretreatment with tamoxifen on the pharmacokinetic parameters and constants of doxorubicin in plasma using non-compartmental analysis. Data were calculated using WinNonlin and are presented as mean ± SD ($n = 6$). The dose of doxorubicin was 10 mg/kg and the daily dose of tamoxifen was 1 mg/kg per day for ten consecutive days (AUC area under the plasma concentration–time curve, MRT mean residence time, V_{ss} volume of distribution)

Treatment	AUC (mg · h/l) kg^{-1}	MRT (h)	V_{ss} (l)	Clearance (ml/min)
Corn oil + doxorubicin	30.04 ± 2.25	11.39 ± 1.33	3.79 ± 0.42	5.50 ± 0.81
Tamoxifen + doxorubicin	40.25 ± 1.54*	4.36 ± 1.79*	1.04 ± 0.06*	4.10 ± 0.57

* $P < 0.05$

Table 2 Effect of pretreatment with tamoxifen on the pharmacokinetic parameters and constants of doxorubicin associated with blood cells using non-compartmental analysis. Data were calculated using WinNonlin and are presented as means ± SD ($n = 6$). The dose of doxorubicin was 10 mg/kg and the daily dose of tamoxifen was 1 mg/kg per day for ten consecutive days (AUC area under the blood cells concentration–time curve, MRT mean residence time, V_{ss} volume of distribution)

Treatment	AUC (mg · h/l) kg^{-1}	MRT (h)	V_{ss} (l)	Clearance (ml/min)
Corn oil + doxorubicin	63.41 ± 5.61	5.39 ± 0.98	1.08 ± 0.02	3.40 ± 0.22
Tamoxifen + doxorubicin	84.22 ± 2.64*	6.72 ± 0.55*	0.80 ± 0.09	2.00 ± 0.17*

* $P < 0.05$

fitting of the plasma and bile data. Figure 3 shows the observed cumulative amount of doxorubicin and its metabolites excreted in the bile. The lines were generated using Eqs. 4 and 5, and simultaneously fitted to plasma and bile data. The total biliary elimination of doxorubicin and metabolites in the control group was 30.82% of the dose. The predicted value on the fitted line was 31.03%, which was very close to the observed value and added to the confidence in the selected model and the fit. From the total amount eliminated in the bile of the control group as percent of the dose, about 11% was unchanged doxorubicin, 3% doxorubicinol, 1.3% doxorubicin aglycone and 1.3% doxorubicinol aglycone. Tamoxifen pretreatment increased the total amount of elimination in the bile by 40% where the parent compound constituted the major portion with an increase of 26% with respect to the control group. Doxorubicinol, however, was reduced by 50%. The amount of the aglycones remained essentially the same (Table 3). The bile flow rate fluctuated slightly, but the average remained at a plateau during the experiment. The total elimination of doxorubicin and its metabolites in 10 h in the urine of the control group was 7.80 ± 0.45% of the dose and in the tamoxifen pretreated group was 8.51 ± 0.42%. The difference was related to an increase in doxorubicin excretion (from 4.77 ± 0.48% in the control group to 6.33 ± 0.28% in the test group) and a significant reduction in elimination of doxorubicinol (from 0.36 ± 0.06% in the control group to 0.22 ± 0.03% in the test group). The aglycones remained essentially the same.

The serum endothelin level of groups V and VII remained at a mean value of 2 ng/ml throughout the experiment. The endothelin levels of the control group (group VI, pretreated with corn oil and doxorubicin) gradually elevated to a maximum level of about 20 ng/ml. The maximum endothelin level of group VIII (tamoxifen-pretreated group that received doxorubicin) was reduced to 6 ng/ml. The endothelin data were then analyzed according to eq. 7. Table 4 summarizes the parameters of this analysis.

Discussion

Doxorubicinol, the C-13 alcohol metabolite of doxorubicin, is known to be more toxic than the parent compound [2, 23]. Tamoxifen pretreatment, according to the present protocol, lowered the level of this metabolite in the cytosolic incubation of the in vitro study significantly. This decrease may well have been related to the reduction of protein content of the cytosolic fraction. However, the consistency of the reduction of doxorubicinol both in vitro and in vivo confirms that indeed tamoxifen reduces the formation of this metabolite.

Tamoxifen is also an inhibitor of P-glycoprotein (P-gp) [1, 5, 14, 17]. Theoretically, the inhibition of P-gp should increase the intracellular uptake of doxorubicin and lower the level of hepatobiliary elimination and plasma concentration. However, the observed plasma concentration and biliary elimination of doxorubicin in tamoxifen pretreated animals was significantly higher than in control animals. Since the in vivo dose-dependency of inhibition of P-gp by tamoxifen and the contribution of P-gp to the overall disposition of a compound are not known, the changes in concentration of doxorubicin in plasma and bile cannot be explained based on the inhibition of P-gp. The consistency among the calculated pharmacokinetic parameters of the pretreated group led to the conclusion that tamoxifen, under the present protocol, modified the disposition of doxorubicin significantly. The increase in biliary elimination of doxorubicin and its corresponding biliary rate constant are in agreement with the pharmacokinetic parameters of plasma and blood cells. The higher AUC of both plasma and blood cells are indicative of greater

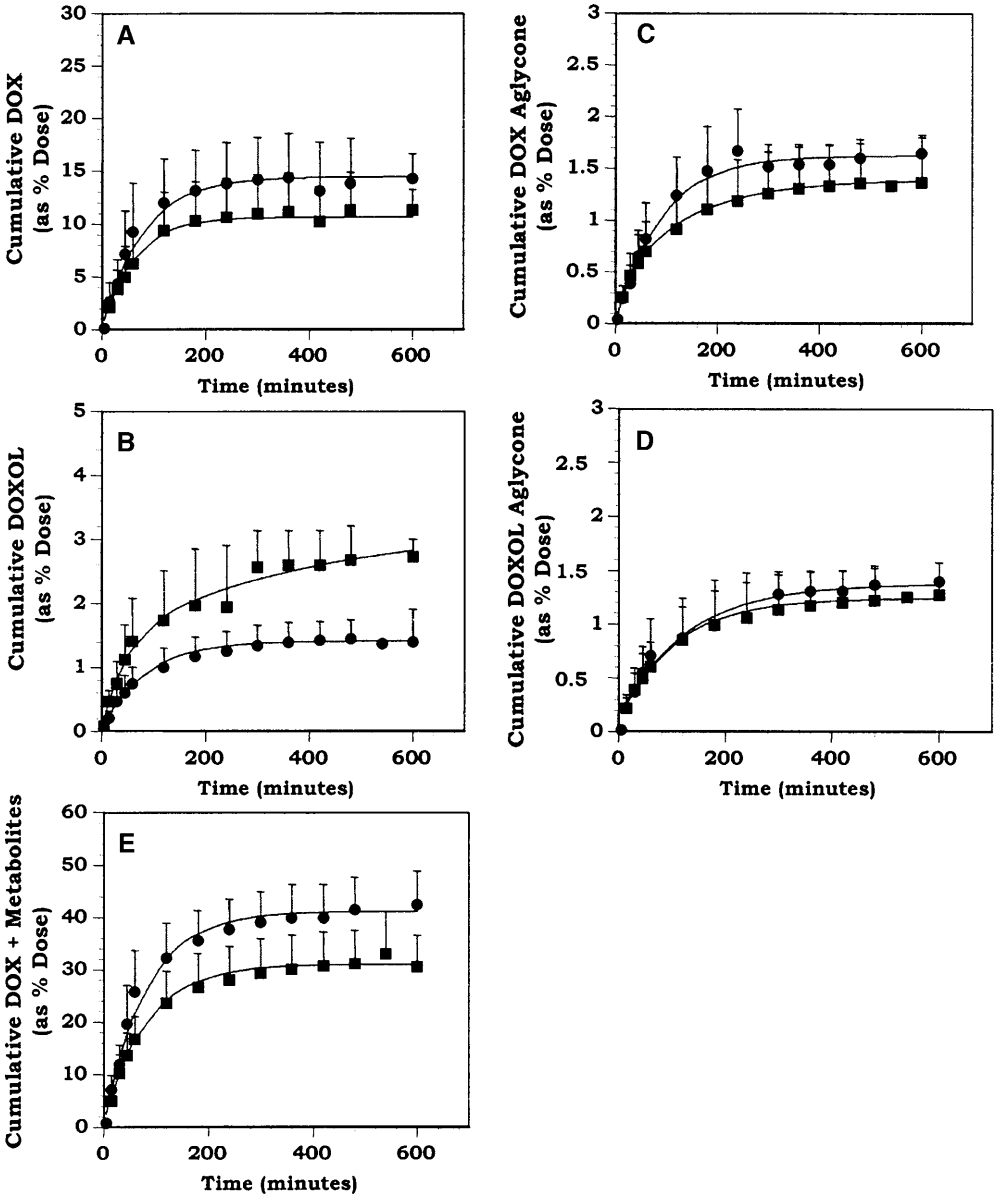

Fig. 3A–E Effect of tamoxifen pretreatment on the cumulative biliary elimination of doxorubicin (**A**), doxorubicinol (**B**), doxorubicin aglycone (**C**), doxorubicinol aglycone (**D**) and total radioactivity (parent compound and metabolites) (**E**). Data are means ± SD ($n = 6$) of the cumulative amount eliminated as percent dose of doxorubicin. The fitted lines were generated by simultaneous curve fitting of plasma concentrations and biliary cumulative amount using WinNonlin and Eqs. 4 and 5

Table 3 Total amount of biliary elimination of doxorubicin and its metabolites as percent of the dose in female rats pretreated with corn oil or tamoxifen in corn oil, and related biliary rate constants

Compound	Corn oil pretreatment			Tamoxifen pretreatment		
	k_b (h^{-1})[a]	$A_{B(observed)}$ (% of dose)[b]	$A_{B(predicted)}$ (% of dose)[a]	k_b (h^{-1})[a]	$A_{B(observed)}$ (% of dose)[b]	$A_{B(predicted)}$ (% of dose)[a]
Doxorubicin	1.92	11.45 ± 1.8	10.65	2.40	4.42 ± 2.3*	14.45
Doxorubicinol	0.48	2.75 ± 0.25	2.83	0.24	1.42 ± 0.41*	1.41
Doxorubicin aglycone	0.24	1.37 ± 0.42	1.37	0.30	1.65 ± 0.16	1.62
Doxorubicinol aglycone	0.24	1.28 ± 0.11	1.23	0.24	1.41 ± 0.16	1.36
Total[c]	4.86	30.82 ± 4.3	31.03	7.08	42.77 ± 5.3*	41.18

* $P < 0.05$
[a] Estimated by simultaneous curve fitting of plasma and bile data using WinNonlin
[b] Means ± SD ($n = 6$) of measurements over 10 h of sampling following injection of doxorubicin
[c] Parent compound and metabolites eliminated during 10 h of sampling

availability of doxorubicin for elimination. Doxorubicin is known to follow a two- or three-compartment model [3, 24, 32]. Therefore, the increase in the AUC of plasma and blood cells could be related, in part, to a reduction in the uptake of doxorubicin by peripheral tissues. The decrease in the volume of distribution of plasma data in

Table 4 Parameters of the E_{max} model based on serum endothelin levels following injection of doxorubicin (10 mg/kg) to female rats pretreated with tamoxifen (1 mg/kg per day) for ten consecutive days. The data are presented as means ± SD ($n = 6$)

Pretreatment	E_{max} (ng/ml)	ET_{50} (h)	γ
Corn oil (control)	23.45 ± 6.63	3.81 ± 1.45	2.22 ± 0.15
Tamoxifen + corn oil	6.30 ± 1.23*	5.68 ± 1.26*	2.11 ± 0.57

* $P < 0.05$

the tamoxifen-pretreated group is also indicative of a limited distribution of doxorubicin. This concept of limited distribution is in agreement with the reported interaction of tamoxifen with the cell membrane [8, 21].

It is interesting that the increase in the concentration of doxorubicin in plasma was not concomitant with an increase in the concentration of doxorubicinol in the bile or urine. The level of this metabolite and its biliary rate constant were reduced by 50%. The significant reduction in MRT, that is the time required for the overall elimination of 63.3% of the dose, is another indication of the effect of tamoxifen on the metabolism of doxorubicin. Therefore, we conclude that tamoxifen pretreatment for 10 days alters the distribution and metabolism of doxorubicin.

The serum endothelin level of the control group, that were pretreated with corn oil and received doxorubicin, after a threshold of about 6 h reached a plateau level of 20 ± 1.98 ng/ml. Tamoxifen pretreatment lowered this plateau level from 20 ± 1.98 ng/ml to 6 ± 0.48 ng/ml. The time required to reach 50% of the plateau level according to the sigmoidal E_{max} model (Table 4) was 5.68 ± 1.26 h for tamoxifen-pretreated animals and 3.81 ± 1.45 h for corn oil-pretreated animals. The lower level of endothelin in tamoxifen-pretreated animals may well be related to a reduction in doxorubicinol and/or a reduction in influx of calcium and/or the antioxidant property of tamoxifen.

In summary, the present study showed that pretreatment with tamoxifen alters the metabolism, distribution and toxicity of doxorubicin. Based on the observed reduction of doxorubicinol and possible counteraction of hydroxyl free radicals, the concept of tamoxifen pretreatment is a viable therapeutic option for improving the safety of doxorubicin.

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