J. Pharm. Pharmacol. 1999, 51: 1275–1282
Received April 15, 1999
Accepted June 7, 1999

Inhibition of Aldo–keto Reductases by Phenobarbital Alters Metabolism, Pharmacokinetics and Toxicity of Doxorubicin in Rats

KAMELIA BEHNIA* AND MEHDI BOROUJERDI

Department of Pharmaceutical Sciences, Northeastern University, Boston, MA 02115, USA

Abstract

Doxorubicin is an effective anticancer agent that is limited by numerous adverse effects, cardiotoxicity causing the most concern. Its alcohol metabolite, doxorubicinol, and free radicals have been implicated in the aetiology of this toxicity. This study was based on the premise that inhibition of aldo–keto reductases would improve the efficacy of doxorubicin by reducing its toxic metabolites and modifying its pharmacokinetics. We assessed the effect of in-vitro inhibition of aldo–keto reductases on the metabolism of doxorubicin in cytosolic fractions of heart and liver of rats in the presence of Na-phenobarbital.

The inhibition was confirmed by a significant reduction in the formation of doxorubicinol. The results of the in-vitro study were further evaluated in-vivo. The concentrations of doxorubicin in plasma, bile and urine and its major metabolites in bile and urine were measured in Na-phenobarbital-pretreated rats. Each rat received 100 mg kg^{-1}/day intraperitoneal injection of sodium phenobarbital for three days followed by a single intravenous dose of 10 mg kg^{-1} [^{14}C-14]doxorubicin (sp. act. 0·2 μCi mg^{-1}) on the fourth day. The levels of drug in all biological samples were measured by HPLC. The pretreatment resulted in an increase in biological half-life (5·8 ± 1·5 vs 3·7 ± 0·93 h control group, $P < 0.05$) and area under plasma concentration–time curve (19·6 ± 1·7 vs 14·65 ± 1·68 mg h L^{-1} control group, $P < 0.05$). The cumulative amount of doxorubicinol in the bile and urine of pretreated animals was reduced significantly. In terms of % dose, the amount in the bile declined from 4·2 ± 0·8% in control to 2·4 ± 0·3% and in urine from 0·18 ± 0·08% to 0·12 ± 0·07%. There were no significant changes in doxorubicin aglycone and doxorubicinol aglycone. Serum creatine kinase levels were measured as a biomarker of damage to cardiac muscle. The area under creatine kinase level–time curve was reduced by approximately 50% in phenobarbital-pretreated animals.

The results indicate that the inhibition of aldo–keto reductase could provide a useful approach to improve the safety of doxorubicin by reducing its alcohol metabolite. Furthermore, if the reduction in the area under the serum creatine kinase–time curve represents a reduced damage to heart muscle, it can be concluded that doxorubicinol plays an important role in this injury.

Doxorubicin is an anthracycline antibiotic with a wide spectrum of antitumour activity. Clinical use of doxorubicin is limited because of the potential for the development of side effects. Doxorubicin-induced cardiotoxicity is the greatest concern (Lefrak et al 1973; Buzdar et al 1985; Lee et al 1987). Nephrotoxicity and other adverse effects are also of clinical importance. The main metabolites of doxorubicin are a water-soluble alcohol metabolite doxorubicinol, two poorly water-soluble aglycones (doxorubicin aglycone and doxorubicinol aglycone) and a deoxy form of the aglycones. The metabolism of doxorubicin to doxorubicinol occurs by cytoplasmic NADPH dependent aldo–keto reductase, whereas aglycones are formed by cleavage of the amino sugar moiety of doxorubicin in microsomes by NADPH dependent

Correspondence: M. Boroujerdi, 206 Mugar Life Sciences Building, 360 Huntington Avenue, Northeastern University, Boston, MA 02115, USA.
E-Mail: mborouje@lynx.neu.edu
*Present address: Bristol-Myers Squibb Pharmaceutical Research Institute, Princeton, NJ 08543, USA.

cytochrome P450 reductase (Gewirtz & Yanovich 1987). It has been proposed that free radicals play a role in the aetiology of doxorubicin-induced toxicity. The formation of the free radical, either from parent compound or metabolite, is also mediated by NADPH dependent cytochrome P450 (Powis 1991; Myers 1992). The presence of such free radicals was also confirmed by the reduction of cardiomyopathy by antioxidants (Doroshow et al 1981; Vora & Boroujerdi 1996).

Phenobarbital induces a number of liver proteins, including P450, glutathione S-transferase, UDP-glucuronosyltranferase and epoxide hydrolase (Waxman 1992). Among the several forms of P450, the CYP2B isozymes are more sensitive to induction by phenobarbital in rats (Funae & Imaoka 1993). However, phenobarbital is also known to inhibit the activity of cytosolic aldehyde reductase in the heart (Kawalek & Gilbertson 1976), liver (Felsted et al 1977; Tulsiani & Touster 1977; Ahmed et al 1978), kidney (Bosron & Prairie 1972) and brain (Erwin et al 1972; Bronaugh & Erwin 1973) tissues.

It has been reported that doxorubicinol, the water soluble and circulating alcohol metabolite, is the major cause of cardiotoxicity (Del Tacca et al 1985; Danesi et al 1986; Minotti et al 1995). There is compelling evidence that doxorubicinol, but not doxorubicin, accumulates in cardiac tissue after a multiple dosing regimen in rats (Olson et al 1988). Analysis of autopsy samples of heart of patients who had received doxorubicin also corroborated that doxorubicinol was the major compound in the heart followed by the parent compound. Doxorubicinol is a more potent inhibitor of Na/Ca exchange in sarcolemma than doxorubicin (Olson et al 1988).

In this study, we studied the effect of Na-phenobarbital, an inhibitor of aldo–keto reductase enzyme systems, on the metabolism and pharmacokinetics of doxorubicin. We have hypothesized that the inhibition of aldo–keto reductase would be an appropriate approach to protect the body against the toxicity induced by doxorubicinol. The serum creatine kinase level was selected as the toxicity biomarker and the area under its serum level–time curve was chosen as the toxicodynamic parameter.

Materials and Methods

Chemicals

Doxorubicin and its major metabolites doxorubicinol, doxorubicin aglycone and doxorubicinol aglycone were gifts from Pharmacia & Upjohn (Albuquerque, NM). Radiolabelled doxorubicin (^{14}C-14, sp. act. 102 μCi mg^{-1}) was purchased from Amersham Corp. (Arlington Height, IL). Reagents of total protein and creatine kinase were purchased from Sigma Chemical Co. (St Louis, MO). Na-phenobarbital was obtained from Merck & Company, Inc. (Rahway, NJ). HPLC grade solvents, liquid scintillation fluid (Scintiverse E), polyethylene catheter (PE-10), and other analytical grade reagents and chemicals were purchased from Fisher Scientific (Springfield, NJ). Tissue solubilizer (BTS-450) was purchased from Beckman Instruments Inc. (Fullerton, CA).

Animals and experimental protocol

In-vitro study. Male Sprague–Dawley rats (250–350 g, Taconic Farms, Germantown, NY) were pretreated with Na-phenobarbital (100 mg kg^{-1}/day, i.p.) or normal saline (1 mL kg^{-1}/day, i.p.) for three days (n = 4 per group). A third group received no pretreatment. The animals were housed in a climate- and light-controlled environment and had access to food and water. All three groups of animals were killed and their microsomal and cytosolic fractions of the heart and liver were prepared according to the method of Boyd et al (1978). The microsomal or cytosolic incubation was then carried out in triplicate with 2 mg of proteins, 0·5 mM NADPH, 0·034 mM doxorubicin and 0·05 M Tris buffer pH 7·4 in a final volume of 2 mL, at 37°C in a shaking water bath for 60 min. To the incubations of the third group Na-phenobarbital (1 or 5 mM) was added directly. A solution of doxorubicin was also incubated in the absence of proteins with all ingredients of the incubation. The enzymatic reactions were stopped by addition of acetone (500 μL). Samples were then centrifuged and their supernatants were analysed by HPLC.

In-vivo study. In two groups of animals (n = 5 per group), the in-vitro pretreatment protocol was followed by the in-vivo study. On the third day, after the last pretreatment dose, the animals were anaesthetized with sodium pentobarbital (Nembutal), and were kept under anaesthesia throughout the study. The total dose of sodium pentobarbital did not exceed 50 mg during the 10-h experiment. The bile duct of both groups was catheterized using a polyethylene catheter (PE 10) (Behnia & Boroujerdi 1998). The tail vein was also catheterized for blood sampling using PE-10 and a 20-gauge needle as a trocar. The dose, 10 mg kg^{-1} [^{14}C-14] doxorubicin (sp. act. 0·2 μCi mg^{-1}), was injected via the femoral vein and serial blood samples (100 μL) were collected from the tail vein. The total

radioactivity of plasma and blood cells was determined after treating the blood cell samples with tissue solubilizer (0·5 mL) followed by de-coloration with hydrogen peroxide. Bile samples were collected periodically with a final volume of 100–200 μL. Urine samples were collected directly from the bladder at the end of the experiment.

Assessment of biomarker. The serum creatine kinase levels were measured in four separate groups of rats. After pretreatment with normal saline or Na-phenobarbital, two groups (n = 3 per group) received normal saline via the caudal vein and two groups (n = 5 per group) received an intravenous injection of non-radiolabelled doxorubicin (10 mg kg^{-1}). Since the serum creatine kinase level subsided to its basal level 1 h after the caudal vein catheterization, doxorubicin or normal saline was injected 1 h after the catheterization. The serum levels were determined at different time intervals and analysed as % basal level.

Analysis of biological samples. The concentrations of doxorubicin and its major metabolites were assayed by an HPLC system (Waters, Milford, MA). It consisted of solvent delivery pumps, an autosampler (Hitachi AS-2000 Danbury, CT), Novapak C_{18} radial compression unit, Sentry C_{18} Novapak guard column (Waters), a data module and two online detectors, a fluorometer with 480/540 nm wavelengths (Gilson, Middleton, WI) and a radioactivity detector (Beta Flo, Packard, Meriden, CT). The mobile phase consisted of methanol/ammonium formate buffer (0·1%, pH 4·0) at a ratio of 70 : 30. The isocratic chromatography was carried out at a flow rate of 2 mL min^{-1}. To confirm the retention times, authentic standard solution were routinely injected after every sixth to tenth sample injection. The guard column was replaced regularly and the column was changed when it was necessary.

Pharmacokinetic data analysis. Various models were fitted to plasma data and the appropriateness of a two-compartment model was determined using Akaike and Schwarz criteria (Ludden et al 1994) and the software PCNONLIN (Scientific Consulting Inc., Lexington, KY). The following are the general equations:

$$C = Ae^{-\alpha} + Be^{-\beta} \quad (1)$$

$$A_B = k_b[(A\beta + B\alpha)/\alpha\beta] - k_b[(A/\alpha)e^{-\alpha} + (B/\beta)e^{-\beta}] \quad (2)$$

$$A_B^{\infty} = k_b[(A\beta + B\alpha)/\alpha\beta] \quad (3)$$

Where, Cp is the plasma concentration of doxorubicin at time t. A and B are the coefficients of the exponential terms with units of mass/volume. The first-order hybrid-rate constants of α and β correspond to the first and second phases of disposition, respectively. A_B is the cumulative amount of doxorubicin eliminated in the bile at time t, A_B^{∞} is the total amount of the drug that ultimately will be eliminated unchanged in the bile, and k_b is the corresponding first-order biliary rate constant.

The pharmacokinetic parameters of doxorubicin associated with blood cells were calculated by model-independent non-compartmental analysis using the following general equations:

$$CL_T = Do\ e/AUC \quad (4)$$

$$MRT = AUMC/AUC \quad (5)$$

$$Vd = MRT \cdot CL_T \quad (6)$$

Where, CL_T is clearance, AUC is the area under the concentration–time curve, MRT is mean residence time, AUMC is the area under the first moment curve, and Vd_{ss} is the volume of distribution.

Results

In-vitro metabolism study

The weight of the liver in Na-phenobarbital pretreated animals increased significantly (15 ± 0·36 vs 11 ± 0·5 g control group). This was consistent with an elevation of total protein concentration of the liver microsomal fractions (19 ± 2·5 vs 12 ± 0·62 mg mL^{-1} control group). However, the protein content of the cytosolic fractions remained the same. Pretreatment had no effect on the weight of the heart or the total protein content of its microsomal or cytosolic fractions.

Approximately 5% (5·12% ± 0·89) of the amount of doxorubicin (0·034 mM) incubated with liver microsomes of the control group was converted to doxorubicin aglycone. The level of this metabolite increased in the Na-phenobarbital pretreated group. However, the increase was not statistically significant. The levels of this metabolite in the heart microsomal incubation were even less than the liver (2·67% ± 0·98). As expected, due to the absence of doxorubicinol in the microsomal incubation, doxorubicinol aglycone was not detected.

The cytosolic incubations of both organs catalysed the reductive conversion of doxorubicin to doxorubicinol. This conversion was approximately 19% (19% ± 2·9) for the liver and 8% (8% ± 0·98) for the heart of the control group. This conversion

was inhibited significantly by direct addition of Na-phenobarbital to the incubations. The % inhibition for 1 and 5 mM Na-phenobarbital was 20% and 40%, respectively (Table 1). We did not detect doxorubicin aglycone or doxorubicinol aglycone in cytosolic incubations.

In-vivo study

Plasma data. The plasma concentrations of doxorubicin after saline or Na-phenobarbital pretreatment were analysed for each rat by PCNONLIN (Figure 1). Na-phenbarbital pretreatment increased the parameter and constant of the initial rapid decline of plasma concentration (A and α), the distribution rate constants between the two compartments (k_{12} and k_{21}), the mean residence time (MRT) and the area under the plasma concentration–time curve (AUC). However, the parameter and constant of the disposition phase (B and β), the elimination rate constant (k_{10}) and the total body clearance (CL_T) were decreased. The calculated data are presented in Table 2.

Blood cells data. The radioactivity associated with blood cells was normalized with respect to the number of cells. The non-compartmental analysis

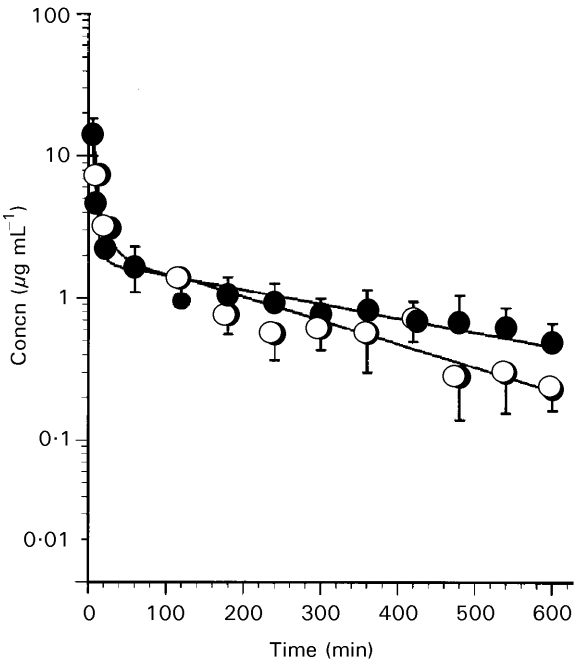

Figure 1. Comparison of the plasma concentration–time profiles of doxorubicin in saline (○) and phenobarbital (●) pretreated rats. The fitted lines were generated by the related two-compartment models and PCNONLIN. The data points are presented as mean ± s.d. (n = 5).

Table 1. Inhibitory effect of phenobarbital on reductive conversion of doxorubicin to doxorubicinol in the heart and liver cytosols.

Phenobarbital (mM)	Liver		Heart	
	% Doxorubicin	% Doxorubicinol	% Doxorubicin	% Doxorubicinol
1	97·11 ± 3·9	81.21 ± 4·1*	129·47 ± 15·5	117·17 ± 16·6
5	127·10 ± 4·2*	58·3 ± 16·4**	106·77 ± 2·0	54·68 ± 21.2**

Each value is presented as percent of control and represents mean ± s.d. *$P < 0.05$, **$P < 0.01$.

Table 2. Pharmacokinetic parameters and constants of doxorubicin in saline- (control) and phenobarbital-pretreated rats.

Parameter or constant	Saline	Phenobarbital
Coefficient of first exponential (mg L^{-1})	29·90 ± 5·83	37·39 ± 7·15
Coefficient of second exponential (mg L^{-1})	2·18 ± 0·62	1·71 ± 0·40
First hybrid rate constant (h^{-1})	9·48 ± 1.80	16·20 ± 3·00*
Second hybrid rate constant (h^{-1})	0·19 ± 0·03	0·12 ± 0·02*
Overall elimination rate constant (h^{-1})	2·40 ± 0·36	1.86 ± 0·18
Transfer rate constant to peripheral compartment from central compartment (h^{-1})	6·54 ± 0·36	13·20 ± 1.24*
Transfer rate constant to central compartment from peripheral compartment (h^{-1})	0·72 ± 0·10	1.03 ± 0·48
Total body clearance (mL min^{-1})	4·58 ± 0·78	2·30 ± 0·22*
Area under plasma concentration–time curve ($\mu g\ min\ mL^{-1}$)	654·80 ± 89·10	1146·80 ± 106·00*
Mean residence time (h)	3·40 ± 1.19	8·84 ± 1.42*

*$P < 0.05$. Dose = 10 mg kg^{-1}. The value of each parameter or constant is presented as mean ± s.d.

Table 3. Effect of Na-phenobarbital pretreatment on pharmacokinetic parameters of doxorubicin associated with blood cells using non-compartmental analysis.

Parameter	Saline (control)	Na-phenobarbital
Area under doxorubicin concentration associated with blood cells vs time (mg h L^{-1})	3·08 ± 0·19	9·32 ± 1·68*
Mean residence time (h)	11.83 ± 1.78	26·78 ± 5·02*
Volume of distribution (L)	9·60 ± 0·54	7·56 ± 0·35
Clearance (L h^{-1})	13·54 ± 1·02	4·71 ± 0·87**

Values are mean ± s.d. *$P < 0.05$, **$P < 0.01$.

revealed that the AUC and MRT of doxorubicin associated with blood cells were significantly higher in Na-phenobarbital pretreated animals. Correspondingly, the related clearance term was much smaller. The data are presented in Table 3.

Bile data. The total radioactivity recovered in the bile of control and Na-phenobarbital pretreated groups accounted for 29 ± 6·25% and 32 ± 2·70% of the dose, respectively. These values represent doxorubicin and all of its metabolites during the 10-h experiment. The difference is not statistically significant. However, the pretreatment with Na-phenobarbital increased the level of doxorubicin and reduced the level of doxorubicinol in the bile. There was no significant change in other metabolites such as aglycones and the combined polar metabolites such as conjugates. The comparison of

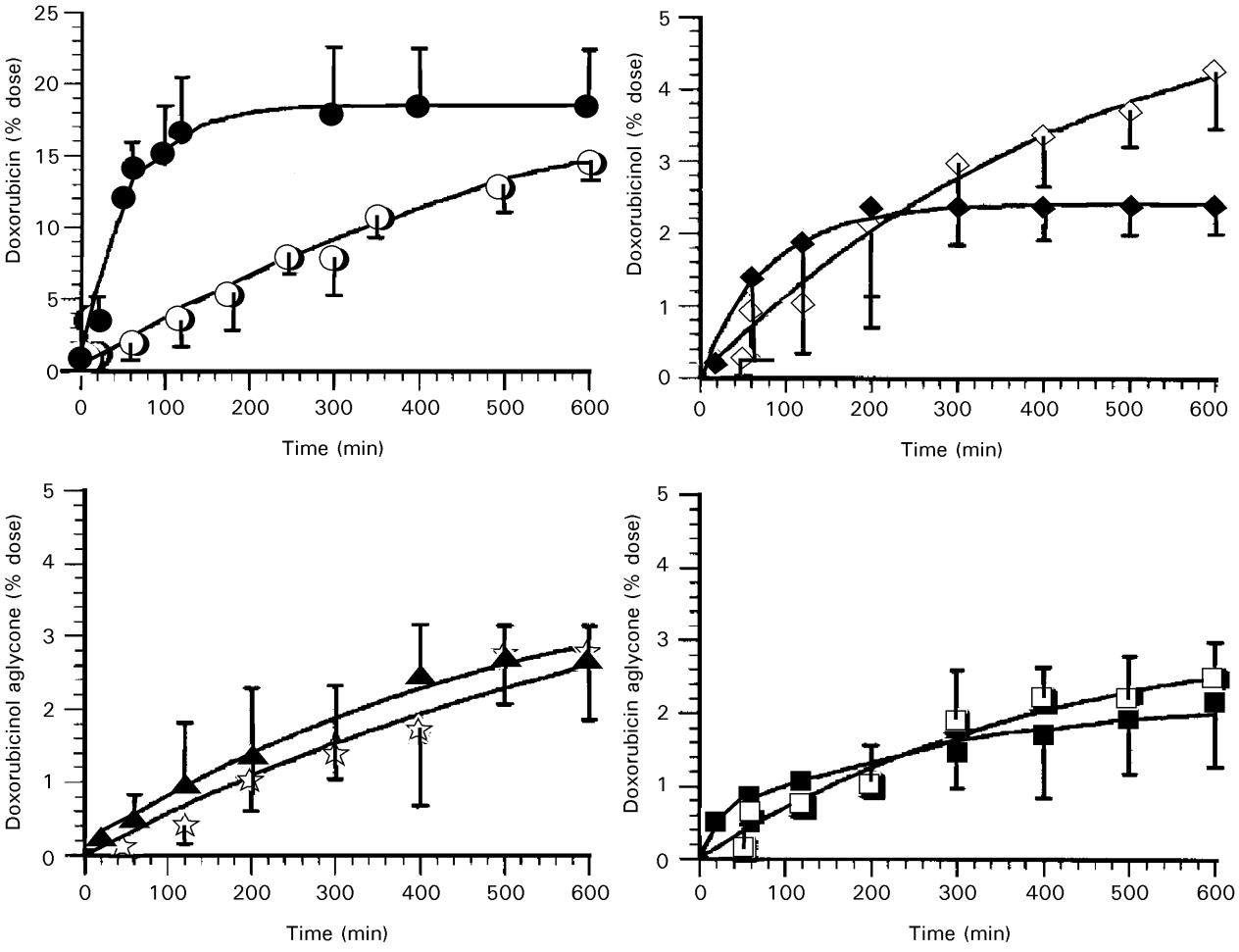

Figure 2. Effect of saline (open symbols) and phenobarbital (closed symbols) pretreatment on the elimination of doxorubicin and its metabolites in the bile. The fitted lines are generated by the equation of the biliary elimination of the two-compartment model and PCNONLIN. The data points are presented as mean ± s.d. (n = 5).

Table 4. Comparison of the effect of saline and phenobarbital pretreatment on the biliary elimination of doxorubicin and its metabolites and their related biliary rate constants.

Saline/phenobarbital	Doxorubicin	Doxorubicinol	Doxorubicin aglycone	Doxorubicinol aglycone
Total amount eliminated in 10 h as % dose (saline pretreatment)†	14·53 ± 0·81	4·25 ± 0·80	2·49 ± 0·51	2·78 ± 0·93
Total amount eliminated in 10 h as % dose (phenobarbital pretreatment)†	18·52 ± 1·50*	2·43 ± 0·39*	2·17 ± 0·68	2·71 ± 0·38
Predicted maximum amount as % dose at (saline pretreatment)	24·46	5·94	3·28	5·06
Predicted maximum amount as % dose at (phenobarbital pretreatment)	18·66*	2·49*	2·25	4·27
Predicted biliary elimination rate constant (h^{-1}) (saline pretreatment)	1.20	3·70	2·70	1.92
Predicted biliary elimination rate constant (h^{-1}) (phenobarbital pretreatment)	3·24*	2·28*	2·64	1.62

*$P < 0·05$. †Results are expressed as mean ± s.d.

the total amount of metabolites, as % dose, is presented in Table 4, and the time course of their cumulative amount eliminated in the bile is presented in Figure 2. The biliary data were analysed according to equations 2 and 3 and the related first-order biliary rate constant, k_b, of each metabolite and the predicted plateau level, A_B^∞ are reported in Table 4. The solid lines shown in Figure 2 were generated by fitting the data to equation 2.

Urine data. The total urinary excretion of the parent compound and the metabolites as % dose during the 10-h urine collection was 8·22 ± 0·91% for control group and 6·40 ± 1·37% for Na-phenobarbital group. There was a significantly higher elimination of doxorubicin in the pretreated group (3·87 ± 0·38% vs 2·34 ± 1·07% control, $P < 0·05$). Doxorubicin aglycone remained essentially the same (0·67 ± 0·15% vs 0·60 ± 0·16% control). Doxorubicinol and doxorubicinol aglycone were reduced (doxorubicinol: 0·120 ± 0·07% vs 0·182 ± 0·08% control; doxorubicinol aglycone: 0·412 ± 0·04% vs 0·66 ± 0·28% control) and the polar compounds, eluted at the beginning of chromatography, were also reduced (approx. 4% for control and 2% for pretreated group).

Biomarker data. Our preliminary data indicated that the caudal vein catheterization or the injection of anaesthetic did not alter the normal levels of creatine kinase (Figure 3). The injection of doxorubicin to the control group resulted in a significant elevation of the serum levels of creatine kinase. Na-phenobarbital pretreatment, 100 mg kg^{-1}/day, lowered both the maximum serum concentration of this biomarker and its area under the curve

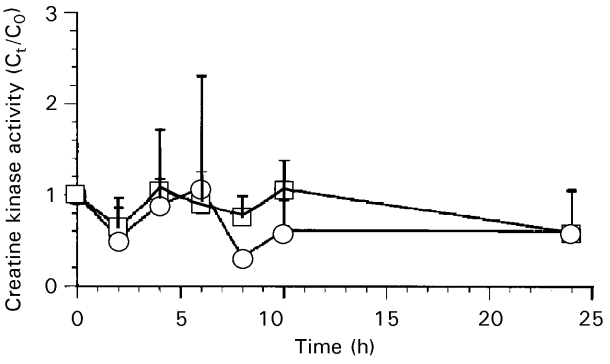

Figure 3. Serum creatine kinase activity after the injection of saline to phenobarbital (○) and saline (□) pretreated rats. The data points are normalized with respect to the basal value (C_t/C_0) and are presented as mean ± s.d. (n = 5).

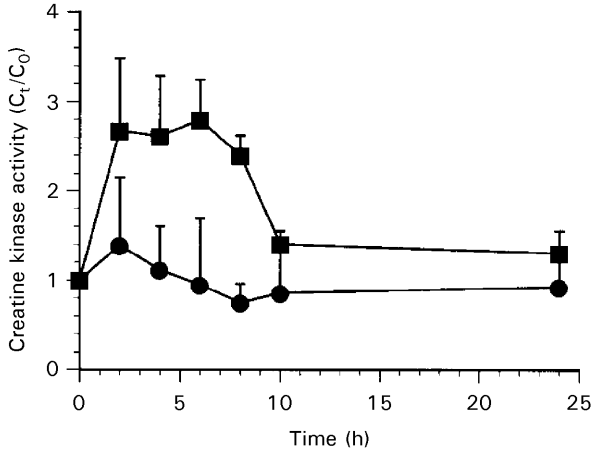

Figure 4. Serum creatine kinase activity after the injection of doxorubicin to phenobarbital (●) and saline (■) pretreated rats. The data points are presented as mean ± s.d. (n = 5).

(AUC_{CK}) by more than 50% (Figure 4). The data were plotted as % basal level. The AUC_{CX} for control and pretreated groups were $52{\cdot}95 \pm 13{\cdot}5$ and $23{\cdot}40 \pm 8{\cdot}5$, respectively ($P < 0{\cdot}05$).

Discussion

Na-phenobarbital pretreatment did not reduce the concentration of doxorubicin, suggesting that it did not elicit its protective effect by reducing the efficacy of doxorubicin. The in-vivo data demonstrated that the pretreatment increased the duration of action of doxorubicin. This was evident by the observed increase of the biological half-life and mean-residence time. The effect of the pretreatment on the disposition constants of the drug, such as reduction of overall elimination rate constant and total body clearance was very much consistent with the in-vitro observation of the inhibition of aldo–keto reductases. The direct outcome of this inhibition was the increase in the area under the curve and longer exposure to doxorubicin. We were rather surprised with the amount of drug associated with blood cells. Indeed, blood cells are a major factor in the overall disposition of doxorubicin. The pretreatment further increased the area under doxorubicin level associated with blood cells. This increase can be interpreted as either Na-phenobarbital inhibited the release of doxorubicin from blood cells, or it inhibited the metabolism of the drug and the increase in plasma concentration enhanced the uptake by blood cells. The second interpretation seems more in agreement with the plasma data and is consistent with changes of mean residence time and clearance.

Na-phenobarbital is known to increase the bile flow rate. We observed this effect by the initial increase of the total biliary elimination of doxorubicin and its metabolites. However, this effect was temporary and returned to control levels after approximately 3 h. The increase of the bile flow rate did not change the elimination of the total amount of radioactivity, i.e. doxorubicin and its metabolites, significantly. The observed cumulative amount as % dose was 31·9% vs 28·84% control. The estimated biliary rate constant of doxorubicin in the pretreated group was greater than control. We attributed this to the increase of the bile flow rate. The alcohol metabolite, doxorubicinol, was the major metabolite detected in the bile. The observed cumulative amount of this metabolite accounted for approximately 4·25% of the dose. The pretreatment reduced this amount by 50%. Despite the obvious reduction in the total amount of doxorubicinol, the biliary levels of doxorubicinol aglycone remained unaltered. The results of the in-vitro investigation also revealed a marked inhibition of doxorubicinol in the liver and heart after direct addition of Na-phenobarbital. In spite of the induction of P450 in microsomal fractions of the heart and liver by Na-phenobarbital, the levels of aglycones remained unchanged. From this observation we confirmed that CYP2B isozymes had no role in the formation of aglycones and NADPH CYP450 reductase was unaltered by the pretreatment.

An interesting result of this study was the effect of inhibition of aldo–keto reductases on the serum levels of creatine kinase after the injection of doxorubicin. The pretreatment with sodium phenobarbital had no effect on the levels of creatine kinase. However, the levels of this biomarker in corn oil-treated animals receiving doxorubicin peaked within 2 h after doxorubicin injection and remained at high levels for nearly 10 h. The serum levels of creatine kinase and its corresponding AUC_{CK} in the pretreated group were much lower than in the control group. In spite of the significant increase in concentration of doxorubicin, the level of creatine kinase remained less than control. It was concluded that doxorubicin was not as toxic as doxorubicinol and the inhibition of aldo–keto reductases was the reason for the reduction in the level of creatine kinase.

Our data clearly demonstrated the potential benefit of inhibition of aldo–keto reductases with doxorubicin therapy. The results show that by inhibiting the formation of doxorubicinol the concentration of doxorubicin in plasma increased whereas the toxicity decreased. Therefore, it can be concluded that the inhibition of aldo–keto reductases may increase the effectiveness of doxorubicin. The purpose of using Na-phenobarbital at the dose used in this study was to ensure a significant in-vivo inhibition of aldo–keto reductases after three days pretreatment. Considering the lipophilic nature of Na-phenobarbital and its very long half-life, smaller doses over a longer period of pretreatment may also provide considerable inhibition. Obviously, with a more potent, highly specific and safe inhibitor, a more convenient regimen could be designed. Among the marketed therapeutic agents there are a few compounds known to inhibit aldo-keto reductases. Compounds such as nitrazepam, chlopromazine and reserpine have been shown to inhibit this enzyme system in different organs (Inazu et al 1996). More potent inhibitors exist such as ethyl 1-benzyl-3-hydroxy-2(5H)-oxopyrrole-4-carboxylate (Mylari et al 1991), but their application requires further scrutiny before in-vivo application. In general, for the diverse purposes related to the inhibition of the

biological roles of aldo–keto reductases, further investigation is needed to identify safer and more appropriate inhibitors of this enzyme system.

References

Ahmed, N. K., Felsted, R., Bachur, N. R. (1978) Heterogeneity of anthracycline antibiotic carbonyl reductases in mammalian livers. Biochem. Pharmacol. 27: 2713–2719

Behnia, K., Boroujerdi, M. (1998) Investigation of the enterohepatic recirculation of adriamycin and its metabolites by a linked-rat model. Cancer Chemother. Pharmacol. 41: 370–376

Bosron, W., Prairie, R. (1972) Triphosphopyridine nucleotide-linked aldehyde reductase. J. Biol. Chem. 274: 4480–4485

Boyd, M. R., Burka, L. T., Benjamen, J. W., Sasame, H. A. (1978) In vitro studies on the metabolic activation of the pulmonary toxin, 4-ipomeanol by rat lung and liver microsomes 207: 677–686

Bronaugh, R. L., Erwin, G. (1973) Partial purification and characterisation of NADPH-linked aldehyde reductase from monkey brain. J. Neurochem. 21: 809–815

Buzdar, A. U., Marcus, C., Terryl-Smith, R. N., Blumenshein, G. R. (1985) Early and delayed clinical cardiotoxicity of doxorubicin. Cancer 55: 2761–2765

Danesi, R., Del Tacca, M. D., Della Torre, P., Bernardini, C. (1986) General and cardiac toxicity of adriamycinol in rat. Anticancer Res. 6: 967–972

Del Tacca, M., Danesi, R., Ducci, M., Bernardini, C., Romanini, A. (1985) Might adriamycinol contribute to adriamycin-induced cardiotoxicity? Pharmacol. Res. Commun. 17: 1073–1084

Doroshow, J. H., Locker, G. Y., Ifrim, I., Myers, C. E. (1981) Prevention of doxorubicin cardiac toxicity in the mouse by N-acetylcysteine. J. Clin. Invest. 68: 1053–1064

Erwin, V. G., Heston, W. D. W., Tabakoff, B. (1972) Purification and characterisation of an NADH-linked aldehyde reductase from bovine brain. J. Neurochem. 19: 2269–2278

Felsted, R. L., Richter, D., Bachur, R. N. (1977) Rat liver aldehyde reductase. Biochem. Pharmacol. 26: 1117–1124

Funae, Y., Imaoka, S. (1993) Cytochrome P450 in rodents. In: Schenkman, J. B., Greim, H. (eds) Cytochrome P450, Handbook of Experimental Pharmacology. Vol. 105. Springer-Verlag, pp 221–238

Gewirtz, D. A., Yanovich, S. (1987) Metabolism of adriamycin in hepatocytes isolated from the rat and the rabbit. Biochem. Pharmacol. 36: 1793–1798

Inazu, N., Hayashi, M., Hiura, M., Satoh, T. (1996) Effect of psychotropic drugs on aldo-keto reductase activity in rat ovary and adrenal gland. Biochem. Pharmacol. 52: 1581–1591

Kawalek, J. C., Gilbertson, J. R. (1976) Partial purification of the NADPH-dependent aldehyde reductase from bovine cardiac muscle. Arch. Biochem. Biophys. 173: 649–657

Lee, B. H., Goodenday, L., Muswick, G. (1987) Alterations in left ventricular diastolic function with doxorubicin therapy. J. Am. Coll. Cardiol. 9: 184–188

Lefrak, E. A., Pitha, J., Rosenheim, S., Gottlieb, J. (1973) Clinicopathologic analysis of adriamycin cardiotoxicity. Cancer 32: 302–314

Ludden, T. M., Beal, S. L., Sheiner, L. B. (1994) Comparison of the Akaike information criterion, the Schwarz criterion and the F-Test as guide to model selection. J. Pharmacokin. Biopharm. 22: 431–445

Minotti, G., Cavaliere, A. F., Moredente, A., Rossi, M., Schiavello, R., Zamparelli, R., Possati, G. (1995) Secondary alcohol metabolites mediate iron de-localisation in cystolic fraction of myocardial biopsies exposed to anticancer anthracyclines. Novel link between anthracycline metabolism and iron-induced cardiotoxicity. J. Clin. Invest. 95: 1595–1605

Myers, C. E. (1992) Anthracyclines. In: Pinedo, H. M., Longo, D. L., Chabner, B. A. (eds) Cancer Chemotherapy and Biological Modifiers Annual 13. Elsevier, New York, p. 45

Mylari, B. L., Beyer, T. A., Siegel, T. W. (1991) A highly specific aldose reductase inhibitor, ethyl 1-benzyl-3-hydroxy-2(5H)-oxopyrrole-4-carboxylate, and its congeners. 34: 1011–1018

Olson, R. D., Mushlin, P. S., Brenner, D. E., Fleischer, S., Chang, B. K., Cusack, B. J., Boucek, R. J. (1988) Doxorubicin cardiotoxicity may be caused by its metabolite doxorubicinol. Proc. Natl Acad. Sci. 85: 3585–3589

Powis, G. (1991) Toxicity of free radical forming anticancer drugs. In: Powis, G., Hacker, M. P. (eds) The Toxicity of Anticancer Drugs. Pergamon, New York, pp 106–132

Tulsiani, D. R. P., Touster, O. (1977) Resolution and partial characterisation of two aldehyde reductases of mammalian liver. J. Biol. Chem. 252: 2545–2550

Vora, J., Boroujerdi, M. (1996) Pharmacokinetic–toxicodynamic relationships of adriamycin in rat: prediction of butylated hydroxyanisole-mediated reduction in anthracyclin cardiotoxicity. J. Pharm. Pharmacol. 48: 1264–1269

Waxman, D. J. (1992) Phenobarbital induction of cytochrome P-450 gene expression. Biochem. J. 281: 577–592