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Effect of phenytoin on the pharmacokinetics of doxorubicin and doxorubicinol in the rabbit

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Summary. Doxorubicin is metabolized extensively to doxorubicinol by the ubiquitous aldoketoreductase enzymes. The extent of conversion to this alcohol metabolite is important since doxorubicinol may be the major contributor to cardiotoxicity. Aldoketoreductases are inhibited in vitro by phenytoin. The present study was conducted to examine the effect of phenytoin on doxorubicin pharmacokinetics. Doxorubicin single-dose pharmacokinetic studies were performed in 10 New Zealand White rabbits after pretreatment with phenytoin or phenytoin vehicle (control) infusions in crossover fashion with 4–6 weeks between studies. Infusions were commenced 16 h before and during the course of the doxorubicin pharmacokinetic studies. Phenytoin infusion was guided by plasma phenytoin estimation to maintain total plasma concentrations between 20 and 30 μg/ml. Following doxorubicin 5 mg/kg by i.v. bolus, blood samples were obtained at intervals over 32 h. Plasma doxorubicin and doxorubicinol concentrations were measured by HPLC. The mean plasma phenytoin concentrations ranged from 17.4 to 33.9 μg/ml. Phenytoin infusion did not alter doxorubicin pharmacokinetics. The elimination half-life and volume of distribution were almost identical to control. Clearance of doxorubicin during phenytoin administration (60.9 ± 5.8 ml/min per kg, mean ± SE) was similar to that during vehicle infusion (67.5 ± 5.4 ml/min per kg). Phenytoin administration was associated with a significant decrease in doxorubicinol elimination half-life from 41.0 ± 4.8 to 25.6 ± 2.8 h. The area under the plasma concentration/time curve (AUC) for doxorubicinol decreased significantly from 666.8 ± 100.4 to 491.5 ± 65.7 n.h.ml^{-1}. These data suggest that phenytoin at clinically relevant concentrations does not alter the conversion of doxorubicin to doxorubicinol in the rabbit. The reduction in the AUC for doxorubicinol caused by phenytoin appears to be due to an increased rate of doxorubicinol elimination. Phenytoin or similar agents may have the effect of modifying doxorubicinol plasma concentrations by induction of doxorubicinol metabolism rather than by inhibition of aldoketoreductase enzymes.

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Introduction

Cumulative dose-related chronic cardiotoxicity is a major adverse reaction of doxorubicin which in many cases limits the chemotherapeutic application of this agent [22]. The mechanism of cardiotoxicity is not established but may be mediated by free radical production with lipid peroxidation [7, 9, 10, 17, 18, 26], disturbance of cellular calcium flux [15, 25], or vasoactive substance release [6]. It is not or known whether cardiotoxicity is due to the parent molecule alone or whether the metabolites also contribute to the cardiotoxicity. Recent data from in vitro studies in this laboratory indicate that the primary alcohol metabolite doxorubicinol is much more highly cardiotoxic than doxorubin in rabbit myocardium [16]. There is also evidence that during chronic doxorubicin administration doxorubicinol accumulates selectively in cardiac tissue in the rat [8, 20], raising the possibility that the metabolite may be involved in the pathophysiology of chronic cardiotoxicity. Doxorubicin is reduced to doxorubicinol by ubiquituous cytosolic aldoketoreductase enzymes [11]. These belong to the class of alcohol:NADP$^+$ oxidoreductases that are inhibited in vitro by many agents, including barbiturates and phenytoin [27]. This study was designed to ascertain the effect of phenytoin on doxorubicin pharmacokinetics in the rabbit. It was hypothesized that phenytoin would inhibit aldoketoreductases, resulting in decreased doxorubicinol plasma concentrations in comparison to the parent drug.

Materials and methods

Animals. The study was designed to compare doxorubicin single-dose pharmacokinetics in each rabbit during infusion of phenytoin and infusion of phenytoin vehicle in randomized order with 4–6 weeks between studies. Ten New Zealand White male rabbits aged 6 months were studied. Animals were obtained from a local licensed supplier and kept for an observation period of at least 3 days prior to study. Animals were fed standard rabbit chow ad libitum. The rabbits had a mean weight of 3.1 ± 0.2 kg (± SE) during the first and 3.2 ± 0.2 kg during the second single-dose study.

Protocol. Using 4% topical lidocaine (Astra Pharmaceutical Products, Inc., Worcester, MA) as a local anesthetic, a 12-in. 21 G intravenous cannula (1-Cath, Delmed, Canton, MA) was inserted in the marginal or central vein of each ear and advanced about 8 in. Infusion with phenytoin so-

dium 25 mg/ml (Dilantin, Parke-Davis, Morris Plains, NJ) was commenced with a loading dose of 20 mg/kg over 5 min, followed by a maintenance infusion of 80 mg/kg per day, using a Harvard Apparatus Model 975 infusion pump. A saline solution containing heparin 20 units/ml was also infused at a matching rate through the intravenous cannula in parallel with the phenytoin to decrease the pH of the infusate and reduce the risk of thrombophlebitis. The phenytoin infusion rate was guided by plasma phenytoin concentration estimations at approximately 16, 20, 24, 40, and 44 h after the beginning of infusion. The maintenance rate was adjusted to maintain plasma concentrations in the range of 20–30 μg/ml. Phenytoin vehicle, made up of 40% propylene glycol and 10% alcohol in water and adjusted to pH 12.0 with sodium hydroxide, was prepared in the laboratory and sterilized prior to administration by drawing through a 0.22-μm filter (Trimed, Kendall McGaw, Santa Ana, CA). The vehicle was infused with heparinized saline in the same manner as described for the phenytoin infusion at a rate of 3.2 ml/kg per day (equivalent volume to that of phenytoin sodium infusion at 80 mg/kg per day).

At 8:00 a.m., 16 h after commencement of the phenytoin or vehicle infusion, an intravenous catheter for blood sampling was inserted in the contralateral ear as described previously, and was kept patent with bolus administration of heparinized saline (65 units/ml). Doxorubicin 5 mg/kg was administered over 2 min through the infusion catheter, and flushed with 5 ml saline. Blood samples were obtained before dosing and at 5, 15, 30 and 60 min and 2, 4, 8, 12, 24, 28 and 32 h after doxorubicin administration. Samples were collected in heparinized tubes and immediately centrifuged at 3000 rpm at 4° C for 10 min, after which the separated plasma stored at −20° C in polypropylene tubes.

Analytical methods. Doxorubicin plasma concentrations were measured using an assay developed in this laboratory. Plasma samples 1 ml in volume were spiked with 10 μl daunorubicin (internal standard) in varying concentrations and mixed. Octadecyl solid-phase extraction columns (SPE, JT Baker Chemical Company, Phillipsburg, NJ) were conditioned with 4 ml isopropyl alcohol, 4 ml 50:50 isopropyl alcohol:water (v:v) and 4 ml 10% NaCl/ammonium formate buffer (pH 4) solution respectively. The spiked plasma samples were diluted with 4 ml 10% NaCl/ammonium formate buffer, loaded on the conditioned extraction columns and aspirated through the column. The column was washed with 3 ml 10% NaCl/ammonium formate buffer and aspirated to dryness. The sample then was eluted from the column with 7 ml isopropyl alcohol and collected in polypropylene test tubes. Using a model SVC100H speed vacuum evaporator with a Model RT-400A refrigerated condensation trap (Savant Instruments, Faemingdale, NY), samples were dried at room temperature, elutriated with 3 ml isopropyl alcohol and dried at 45° C in the evaporator. Samples were reconstituted in mobile phase for analysis by high-performance liquid chromatography (HPLC). These procedures were performed with protection from direct exposure to light. Samples were injected on the HPLC system using a Waters (Milford, Mass.) automatic injector (WISP 710B). The method employed a Waters 4 μm phenyl Radial-Pak reversed-phase column, Waters M45 and 6000A pumps, a Waters automated gradient controller, a Kratos Spectroflow 980 fluorescence detector (Ramsey, NJ) equipped with a xenon lamp and a Hewlett Packard 3390A Integrator (Avondale, PA). The excitation wavelength was 470 nm and a 550 nm wavelength filter was used. The mobile phase consisted of ammonium formate buffer (pH 4.0) and acetonitrile. A 20-min gradient was used. The initial ratio was 72:28 ammonium formate:acetonitrile (v:v), which continued until 6 min, progressed to 66:34 by 6.5 min and returned to 72:28 at 11.5 min. The flow rate was 3.0 ml/min. A standard curve with doxorubicin concentrations ranging from 1 to 200 ng/ml was prepared using human plasma. The mean correlation coefficient of plasma doxorubicin concentration-to-peak height ratio in ten standard curves was 0.99. The intraassay coefficient of variation of plasma doxorubicin was 2.4% at 10 ng/ml and 3.9% at 100 ng/ml, with an interassay value of 4.3% at 60 ng/ml. The accuracy of the assay at 10 ng/ml was within 3.5%. Plasma samples obtained from both single dose doxorubicin studies in each rabbit were assayed on the same day. Doxorubicinol concentrations were expressed in doxorubicin equivalents.

Total plasma phenytoin concentrations were measured using an enzyme immunoassay (EMIT). The system used was a Syva CP-5000 EMIT Clinical Processor (Palo Alto, CA) and a Gilford 8001 spectrophotometer (Oberlin, OH). In this laboratory, the within-assay variability was 2.4% with an accuracy within 4.8% at 15 μg/ml.

Data analysis. The slopes of the terminal portions of the plasma doxorubicin and doxorubicinol concentration/time curves were fitted by least-square linear regression. Pharmacokinetic parameters were calculated using standard model-independent techniques [13]. Group values on both study days were compared using the Wilcoxon signed-ranks test. The null hypothesis was rejected at $P < 0.05$. Values are reported as means ± SE.

Results

The mean values of total plasma phenytoin concentrations obtained from each rabbit are shown in Table 1. The mean values ranged from 17.4 to 33.9 μg/ml. Individual values ranged from 6.9 to 48.6 μg/ml.

The effect of phenytoin on doxorubicin pharmacokinetics is shown in Table 2. The elimination half-life ($t_{1/2}$) of doxorubicin during phenytoin infusion (20.5 ± 2.3 h) was

Table 1. Total plasma phenytoin concentrations during continuous infusion in the rabbit

Rabbit no.	Plasma phenytoin concentration (μg/ml)		
	Mean[a]	SE	Range
1	20.9	7.2	6.9–46.6
2	22.4	2.9	13.9–29.9
3	30.0	0.7	27.8–31.9
4	23.5	3.9	11.9–34.2
5	33.7	3.7	21.1–48.6
6	25.9	3.4	14.1–40.0
7	26.1	0.9	22.2–28.5
8	24.0	4.4	9.9–33.8
9	33.9	6.0	13.4–44.0
10	17.4	2.6	7.0–26.9

[a] Each value represents the mean of five or six samples obtained during the period of infusion

Table 2. Effect of phenytoin infusion on doxorubicin pharmacokinetics in the rabbit following doxorubicin 5 mg/kg by i.v. bolus administration

Rabbit no.	β (h^{-1})		$t_{1/2}$ (h)		Vd_β (l/kg)		Cl (ml/min per kg)	
	Control	Phenytoin	Control	Phenytoin	Control	Phenytoin	Control	Phenytoin
1	0.048	0.031	14.4	22.4	103.69	116.16	83.3	60.0
2	0.027	0.059	25.7	11.7	85.46	46.14	39.0	45.5
3	0.076	0.052	9.1	13.3	70.75	65.75	89.6	56.6
4	0.032	0.035	21.7	19.8	166.19	189.96	89.3	109.5
5	0.034	0.028	20.4	24.8	102.60	133.39	57.8	62.3
6	0.031	0.032	22.4	21.7	106.40	108.75	54.6	57.4
7	0.039	0.056	17.8	12.4	113.63	64.07	74.4	60.0
8	0.032	0.020	21.7	34.7	94.18	152.71	50.6	50.7
9	0.033	0.042	21.0	16.5	116.47	91.62	64.8	63.5
10	0.037	0.025	18.7	27.7	116.93	105.05	72.7	43.9
Mean	0.039	0.038	19.3	20.5	107.63	107.63	67.5	60.9
SE	0.004	0.004	1.5	2.3	7.96	13.84	5.4	5.8
P	NS		NS		NS		NS	

similar to control (19.3 ± 1.5 h) and the corresponding slopes (β) of the terminal elimination phase were almost identical (0.038 ± 0.004 and 0.039 ± 0.004 h^{-1}). Phenytoin administration did not alter the apparent volume of distribution (Vd_β) of doxorubicin (107.36 ± 13.84 l/kg) in comparison to that during vehicle infusion (107.63 ± 7.96 l/kg). The systemic clearance (Cl) of doxorubicin was unaltered by phenytoin (60.9 ± 5.8 ml/min per kg) compared with control (67.5 ± 5.4 ml/min per kg).

In contrast to the doxorubicin response, phenytoin administration was associated with a significant increase ($P < 0.05$) in the slope of the terminal phase of the doxorubicinol plasma concentration/time curve (0.031 ± 0.004 h^{-1}) compared with vehicle infusion (0.020 ± 0.003 h^{-1}) as shown in Table 3. Phenytoin administration caused a significant decrease ($P < 0.05$) in the terminal elimination half-life of doxorubicinol from 41.0 ± 4.8 h to 25.6 ± 2.8 h.

The AUC^∞ of doxorubicin was unaffected by phenytoin (1337.8 ± 105.7 ng.h.ml^{-1}) in comparison with control (1223.2 ± 98.7 ng.h.ml^{-1}), as shown in Fig. 1. However, phenytoin decreased the AUC^∞ of doxorubicinol from 666.8 ± 100.4 to 491.5 ± 65.7 ng.h.ml^{-1} (Fig. 1; $P < 0.05$). As a result, the ratio of the AUC^∞ for doxorubicinol/AUC^∞ for doxorubicin was decreased during phenytoin infusion from 0.53 ± 0.06 to 0.36 ± 0.04 ($P < 0.01$).

Table 3. Effect of phenytoin infusion on doxorubicinol pharmacokinetics in the rabbit following doxorubicin 5 mg/kg by i.v. bolus administration

Rabbit no.	β (h^{-1})		$t_{1/2}$ (h)	
	Control	Phenytoin	Control	Phenytoin
1	0.019	0.027	36.5	25.7
2	0.011	0.054	63.0	12.8
3	0.033	0.040	21.0	17.3
4	0.017	0.029	40.8	23.9
5	0.012	0.020	57.8	34.7
6	0.012	0.018	57.8	38.5
7	0.019	0.043	36.5	16.1
8	0.017	0.023	40.8	30.1
9	0.018	0.031	38.5	22.4
10	0.040	0.020	17.3	34.7
Mean	0.020	0.031	41.0	25.6
SE	0.003	0.004	4.8	2.8
P	< 0.05		< 0.02	

Discussion

Since doxorubicin elimination was not altered, this study suggests that phenytoin at probable clinically relevant plasma concentrations does not cause significant inhibition of the metabolism of doxorubicin to doxorubicinol in

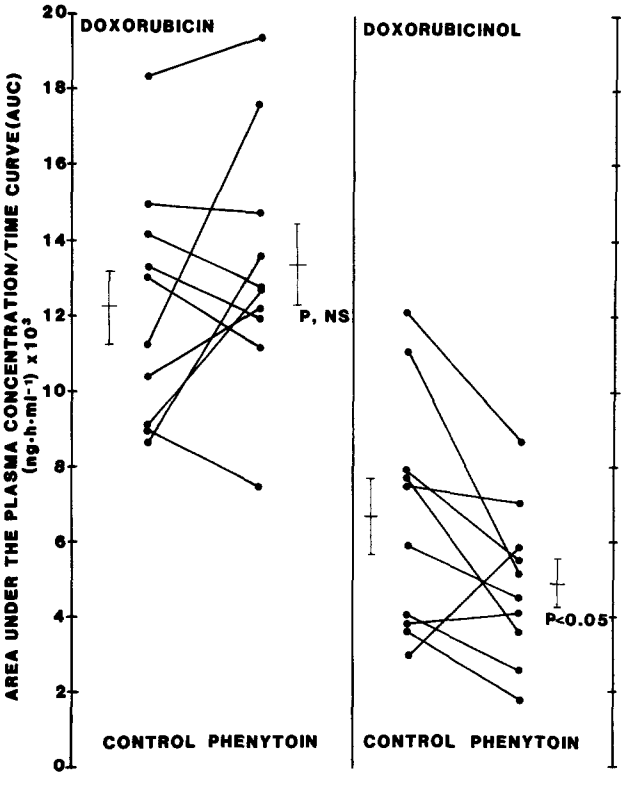

Fig. 1. Doxorubicin and doxorubicinol areas under the plasma concentration/time curves in 10 New Zealand White rabbits following administration of doxorubicin 5 mg/kg by i.v. bolus during vehicle (control) and phenytoin infusions. *Bars*, means ± SE

vivo in the rabbit. Studies in vitro have demonstrated that phenytoin at a concentration of 10 μM inhibits the activity of bovine heart aldoketoreductase by 77% [27]. Assuming a free fraction of 0.05 for phenytoin in rabbit plasma, the mean free phenytoin concentrations available for tissue uptake in the present studies ranged from 0.87 to 1.7 μg/ml, equivalent to 3.5 to 6.7 μM phenytoin. Hence, free phenytoin concentrations in this study were in the range that would be expected to inhibit aldoketoreductases by up to 77%, but the amount of enzyme inhibition in vivo required to alter doxorubicin metabolism to doxorubicinol is not known. Additionally, aldoketoreductases are a heterogeneous group of enzymes with highly variable sensitivity to phenytoin inhibition. For example, 100 times higher concentrations of phenytoin are required to inhibit brain than heart enzyme [27]. Thus, inhibition of aldoketoreductases that determine plasma drug and metabolite concentrations may not have occurred at the phenytoin concentrations attained in this study. Furthermore, in rabbit liver there are at least two groups of pH-dependent doxorubicin reductases, with optimal activity at pH 6.0 and 8.5 [1]. Reductases with maximal activity at pH 8.5 are inhibited by the anticonvulsant phenobarbital [11] and also presumably by phenytoin. In view of the observations in this study it is possible that the other group of doxorubicin reductases in rabbit liver (with a pH optimum of 6.0) may not be inhibited by phenytoin.

It is of interest that the AUC for doxorubicinol declined during phenytoin administration. This could have been due to altered volume of distribution, decreased formation, or increased elimination of doxorubicinol. The volume of distribution of doxorubicinol could not be determined because the total amount of this metabolite formed was not known. The change in AUC was accompanied by an increase in the terminal slope and a decrease in the terminal elimination half-life. This suggests that the reduction in doxorubicinol AUC was due to increased metabolism. If that is the case, doxorubicinol, rather than doxorubicin, is the main substrate for microsomal metabolism in the rabbit, possibly by reductive glycosidase, a microsomal enzyme that cleaves anthracyclines to aglycones [2].

The effects of phenobarbital on doxorubicin metabolism in mice have been studied [22]. Phenobarbital pretreatment resulted in an increased rate of disappearance of total fluorescence of doxorubicin and metabolites with a half-life of 16 h as against 26 h in control mice. The rate of appearance of aglycones in the liver was increased although the liver doxorubicin concentrations did not appear to change significantly. Consistent with the present study, liver microsomal enzyme activity in vitro was increased, associated with a greater capacity to produce aglycones than in controls. Their study also showed that aldoketoreductase activity was unaltered, an observation in agreement with the present results. It appears therefore that in vivo, contrary to observations in vitro [27], anticonvulsants such as phenytoin or phenobarbital do not produce a significant reduction in aldoketoreductase activity, but can induce the microsomal pathway of anthracycline metabolism.

There is evidence to indicate that doxorubicinol causes acute cardiotoxicity. The metabolite can stimulate superoxide anion and lipid peroxide formation in cardiac sarcosomes and mitochondria, a step that is widely considered to be important in cardiac toxicity [12, 17]. In addition, it has been shown in our laboratory that the acute in vitro cardiotoxic effect of doxorubicinol is considerably greater than that of the parent drug. Doxorubicinol was 30 times more potent than doxorubicin in inhibiting cardiac contractility in rabbit papillary muscles [16, 19]. The mechanismen of cardiac dysfunction may relate to ATPase inhibition, since doxorubicinol, but not doxorubicin, is a potent inhibitor of Ca-Mg ATPase of sarcoplasmic reticulum, Mg ATPase of mitochondria, and Na-K ATPase activity of sarcolemma [15, 28]. It is also important to consider that acute cardiotoxicity from anthracyclines produces histological lesions and other effects similar to those seen with chronic drug administration [5, 28], and some authors consider that the two forms of cardiotoxicity may have a common mechanism [24].

Increased attention has recently been focused on doxorubicinol as contributing to chronic cardiotoxicity. Following single doses of doxorubicin in the rat, considerably higher cardiac concentrations of doxorubicin than doxorubicinol were observed in cardiac tissue [20, 23]. However, chronic doxorubicin administration was accompanied by a 300% rise in cardiac doxorubicinol concentrations from 3.6 ± 0.7 to 11.1 ± 2.1 ng/g tissue between days 7 and 21 of treatment, although plasma and pulmonary doxorubicinol concentrations remained unaltered [8]. Cardiac doxorubicin concentrations during this period were unchanged. Peters et al. [20] have also demonstrated a selective accumulation of doxorubicinol in rat myocardium following chronic administration. It is not clear how doxorubicinol accumulation in the heart may occur. It may be due to transport from the plasma, although it has been stated that cellular uptake of this relatively polar metabolite does not occur readly [3]. The alternative hypothesis is that of continued intracardiac metabolism of doxorubicin to doxorubicinol by aldoketoreductases, which have been described in this tissue [14, 27].

The data in this study are consistent with phenytoin causing induction of doxorubicinol metabolism. In view of the possible role of the alcohol metabolite in the development of chronic cardiotoxicity, the ability of phenytoin to increase doxorubicinol elimination may have therapeutic potential. In this regard, preliminary studies in our laboratory show that phenytoin pretreatment significantly protects mice from the acute lethal effects of doxorubicin administration [16]. Thus, the potential protective effect of phenytoin and similar enzyme-inducing agents merits further evaluation.

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