Effect of Sublethal Liver Injury on Doxorubicin Metabolism

David A. August, Susan Halter, and Dean E. Brenner

The Cancer Institute of New Jersey, New Brunswick, New Jersey 08901 [D. A. A.]; Departments of Internal Medicine and Pharmacology, University of Michigan Medical School and Ann Arbor Veterans Administration Medical Center, Ann Arbor, Michigan 48109 [D. E. B.]; and Department of Clinical Pharmacology and Therapeutics, Roswell Park Memorial Institute, Buffalo, New York 14263 [S. H.]

ABSTRACT

Centrilobular hepatocyte contribution to doxorubicin (DOX) metabolism and myelotoxicity was probed with bromobenzene (BRB), a known centrilobular hepatotoxin. New Zealand White rabbits were given DOX, 3 mg/kg i.v. After 4 weeks, the rabbits were pretreated i.p. with 2.6 ml/kg 40% solution of BRB in corn oil followed 72 h later with a 3-mg/kg dose of DOX. Pharmacokinetics of DOX after BRB pretreatment was mildly changed from control. Significantly increased plasma concentrations of doxorubicinol and its aglycone product, 7-deoxydorubicinol aglycone, were detected. Treatment with BRB alone was not lethal; however, in three of seven rabbits, the combination of DOX and BRB was. The mortality appeared to be related to myelosuppression. We conclude that toxin-induced hepatocellular necrosis causes increased DOX-induced myelotoxicity. Following BRB pretreatment, the relatively small pharmacokinetic changes of parent compound concentrations as compared with greater changes in plasma pharmacokinetics of its alcohol metabolites suggest systemic changes in drug metabolism and distribution in the setting of hepatic disease may be the cause of increased toxicity.

INTRODUCTION

There is substantial experimental evidence as well as some clinical experience suggesting that antitumor agents demonstrate steep dose-response relationships (1–3). Acceptance of this concept is embodied by the clinical practice of administering maximally tolerated doses of antineoplastic drugs, aiming to achieve maximum efficacy while avoiding life-threatening toxicity. Logical extension of this approach leads to consideration of interindividual differences in the pharmacokinetics of drug metabolism as caused by the effects of disease, the effects of concurrent chemotherapy, radiation therapy, and surgery, and the effects of age, gender, environment, and heredity. The increasing use of chemotherapeutic agents during the preoperative and perioperative periods makes these issues more problematic. Little is known of the alterations in drug metabolism caused by sepsis, cholestasis, tissue injury, anesthesia, and other common sequelae of cancer and its treatment. Only by understanding the influence of these various factors on drug metabolism can chemotherapeutic agents be dosed optimally for a given patient and given safely to patients with multiple comorbidities.

DOX, an anthracycline antineoplastic agent, is frequently used during the preoperative and perioperative period, as it is often incorporated into the treatment of malignancies such as breast cancer, lymphoma, leukemia, ovarian cancer, hepatocellular carcinoma, and soft tissue sarcoma. DOX is reduced in cellular cytoplasm by aldehyde and keto reductases and detoxified, most probably by NADPH cytochrome P450 reductase catalyzed reduction of the oxygen-linked glycoside to deoxyglycone forms (4–11). DOX and its primary alcohol metabolite DOXOL are excreted unchanged in the bile and to a lesser extent in the urine. Accurate prediction of acute human clinical toxicity from DOX by empiric determination of plasma pharmacokinetics of DOX or its metabolites has not yet proved feasible (12–14). Nevertheless, some studies have suggested that DOX-induced toxicity correlates with deranged hepatic function as measured by bromsulphthalein time, bilirubin, or indocyanine green clearance (12, 15–17). Because of the prevalence of hepatic dysfunction in cancer patients due to factors such as hepatic metastases, drug-induced toxicity, and viral and bacterial infections, we have developed a model of antineoplastic pharmacokinetics in the rabbit (13, 18, 19) to study the effect of hepatic dysfunction on DOX pharmacokinetics and toxic end points.

On the basis of previous rabbit and human data (12, 13, 16, 18, 19), we hypothesized that the plasma pharmacokinetics of the alcohol metabolite of DOX, DOXOL, can be used as a marker of impending drug toxicity in the face of hepatic dysfunction. To test this hypothesis and to further evaluate the consequences of hepatic dysfunction on DOX pharmacodynamics, we have induced hepatic lesions with BRB, and evaluated the resultant pharmacokinetics and pharmacodynamics of DOX and its metabolites. BRB was selected because it is a commonly used aromatic hepatotoxin which reproducibly produces sublethal centrilobular hepatic lesions in animal models (20). These lesions are similar to the primarily hepatocyte injuries seen in humans in response to toxic and ischemic hepatocellular insults.

MATERIALS AND METHODS

Materials. Doxorubicin hydrochloride was obtained as a gift from Adria Laboratories (Columbus, OH). HPLC grade tetrahydrofuran and certified grade ammonium formate, chloro-
Liver Injury and Drug Metabolism

Takanashi and Bachur (21): DOXOL, doxorubicin aglycone, infusion over daily for 3 days, and then received DOX, 3 mg/kg, by i.v. assessment of hepatic function by aminopyrine breath testing were treated as indicated in Fig. 1. These rabbits had an initial histological analysis. Seven other rabbits (experimental group) ples of liver tissue were obtained at the time of sacrifice for blood sampling, and were sacrificed in pairs to assess the effects of BRB on blood chemistry, blood counts, and liver function during the period 1–10 days following BRB administration. Seven rabbits (experimental group) had an initial assessment of hepatic function by aminopyrine breath testing daily for 3 days, and then received DOX, 3 mg/kg, by i.v. infusion over 5 min. Blood for pharmacokinetic analysis of DOX and metabolites was sampled at 5 min to 48 h after completion of the DOX infusion. Complete blood counts were obtained following the DOX infusion to assess the degree of myelosuppression induced. Twenty-eight days after the first DOX infusion, the experimental animals received BRB, 2.6 ml/kg 40% solution in corn oil i.p. Three days later, DOX was administered and liver function and blood measurements were repeated as described before. In this fashion, each animal in the experimental group served as its own pharmacokinetic control; a baseline DOX pharmacokinetic study was performed first, then repeated 3 days after BRB administration.

Rabbits. Female, white, specific pathogen-free New Zealand rabbits, were obtained from Hazleton Laboratories (Denver, PA). The animals were kept in a clean separate room from other rabbits and were maintained in individual cages according to accepted American Association for Accreditation of Laboratory Animal Care Standards. The quarters were supervised by the local Institutional Animal Care and Use Committee and a veterinarian as required by United States federal regulations. Study procedures were reviewed by the veterinarian and the Institutional Animal Care and Use Committee prior to study. Care was taken to minimize animal discomfort.

The rabbits were fed ad libitum and allowed unlimited water and food supply. They were sacrificed by i.v. injection of 0.8 ml T-61 euthanasia solution (Hoechst, Somerville NJ) which caused death within 15 s of injection.

Experimental Procedure. A total of 15 rabbits were studied (Fig. 1). Eight received BRB only, 2.6 ml/kg 40% solution in corn oil i.p. (BRB control). The BRB control rabbits underwent aminopyrine breath testing to assess liver function and had blood sampled for blood chemistry and blood count analysis during the week prior to receiving BRB. After receiving BRB, the control rabbits underwent periodic breath tests and blood sampling, and were sacrificed in pairs to assess the effects of BRB on blood chemistry, blood counts, and liver function during the period 1–10 days following BRB administration. Seven rabbits (experimental group) had an initial assessment of hepatic function by aminopyrine breath testing daily for 3 days, and then received DOX, 3 mg/kg, by i.v. infusion over 5 min. Blood for pharmacokinetic analysis of DOX and metabolites was sampled at 5 min to 48 h after completion of the DOX infusion. Complete blood counts were obtained following the DOX infusion to assess the degree of myelosuppression induced. Twenty-eight days after the first DOX infusion, the experimental animals received BRB, 2.6 ml/kg 40% solution in corn oil i.p. Three days later, DOX was administered and liver function and blood measurements were repeated as described before. In this fashion, each animal in the experimental group served as its own pharmacokinetic control; a baseline DOX pharmacokinetic study was performed first, then repeated 3 days after BRB administration.

Measurement of Blood Counts and Blood Chemistries. Complete blood counts were performed on a Coulter Counter. To measure serum liver function tests (total bilirubin, alkaline phosphatase, ALT, and AST), plasma samples were assayed on an automated Kodak Ektachem 700 analyzer. Differences between baseline measurements and subsequent measurements were analyzed for significance using two-tailed t tests.

Measurement of Hepatic Function. Hepatic function was assessed by the rabbit liver’s ability to demethylate aminopyrine and standard liver function tests. The aminopyrine breath test is described in detail in a previous publication (18). Briefly, rabbits were given 0.5 μCi of 14C-aminopyrine i.v. diluted in 0.5 ml normal saline. Their breath was collected by a glass hood attached to the restrainer rack connected to suction. The exhaled
14\(^{14}\)C\(_2\)O\(_2\) was collected in 10 ml methanol:ethanolamine (2:1, v/v) solution over 20-min increments for up to 240 min. The \(K_{d1}\) was obtained by calculating the slope from the logarithmic linear regression of cpm against time. Half-life was calculated as \(1/(\text{concentration})^2\) weighting function was used for curve fitting.

**DOX Assay.** Specimens were assayed by HPLC after a chloroform:isopropanol (1:1, v/v) extraction according to a previously published procedure (21). The technique was modified by the use of a 15-cm \(\mu\)Bondapak phenyl column (Waters Associates, Millipore Corp., Milford, MA) and a Shimadzu fluorescence flow spectrophotometer. The excitation frequency was 470 nm and emission was measured at 550 nm. These modifications resulted in a lower limit of detection of DOX in methanol of 0.0005 nm; the lower limit of detection of DOX extracted from 1 ml pooled human plasma was 0.005 nm.

**Data Analysis and Pharmacokinetics.** DOX and metabolite plasma concentrations were calculated, stored, and statistically analyzed on RS/1 (BBN Software, Cambridge, MA).

For DOX, plasma time concentration data were fit to the equation:

\[ C(t) = A e^{-\alpha t} + B e^{-\beta t} \]

where \(C(t)\) is the plasma concentration at time \(t\), \(A\) and \(B\) are constants, and \(\alpha\) and \(\beta\) are linear elimination rate constants. A \(1/(\text{concentration})^2\) weighting function was used for curve fitting. The AUC was calculated by the log trapezoidal rule to infinity using \(\beta\) as the terminal rate constant. Total body clearance was calculated by:

\[ D_0/AUC \]

where \(D_0\) is the administered dose. Volume of distribution was calculated by a noncompartmental method (22):

\[ V_{\text{dss}} = (D_0)(AUMC)/(AUC)^2 \]

where \(V_{\text{dss}}\) is volume of distribution at steady state and AUMC is the area under the moment curve.

AUCs for metabolites were calculated by the log trapezoidal rule. Because metabolite concentrations were very low or undetectable beyond 48 h, metabolite AUCs were computed for the period 0–48 h.

After checking the data for normality using the Wilks-Shapiro test, paired \(t\) test parameters were calculated for paired means to test for statistical significance.

**Liver Histology.** Liver specimens from sacrificed rabbits were obtained from right and left lobes, fixed in 10% neutral buffered formalin, dehydrated in graded alcohol, and embedded in paraffin. Five-\(\mu\)m sections were cut and stained with hematoxylin and eosin and examined by one observer without knowledge of the treatment. Each slide was graded for the presence of degeneration and necrosis using the following grading schemes: (a) degeneration: 1+, fine cytoplasmic vacuolation; 2+, vacuoles of a size occupying less than one-half of the cytoplasm of the cells; 3+, vacuoles of a size occupying the entire cytoplasm of the cells; (b) degree of degeneration: 1+, 1–2 cell layers involved; 2+, 3–5 cell layers involved; 3+, 6–10 cell layers involved; 4+, diffusely involves the liver lobule; and (c) necrosis: 1+, 1–2 acidophil bodies/10 high-power fields; 2+, 3–5 acidophil bodies/10 high-power fields; 3+, 5–10 acidophil bodies/10 high power fields; 4+, >10 acidophil bodies/10 high-power fields. The extent of degeneration was determined by adding the grades of degeneration and degree of degeneration.

**RESULTS**

Effects of BRB on Hepatic and Hematopoietic Function. Intraperitoneal administration of BRB, 2.6 ml/kg 40% solution in corn oil, had profound effects on the liver as assessed by liver function tests, liver histology, and ability to demethylate \(14\)C-aminopyrine. BRB caused changes in the hepatocellular enzymes ALT and AST within 2 days of administration (Table 1). These changes peaked on day 4 and were totally resolved by day 10 following administration. The mean ALT and AST were significantly different from baseline on days 1, 3, 4, and 5 and days 4 and 5 (\(P < 0.05\)), respectively. That no changes occurred in total bilirubin or alkaline phosphatase following BRB administration confirms that the hepatic damage was primarily hepatocellular in nature. \(14\)C-aminopyrine breath testing revealed significant impairment in the ability to demethylate aminopyrine as early as 2 days after administration of BRB. Peak hepatic dysfunction as assessed by breath testing occurred 2–4 days after exposure to BRB; liver function returned to normal by day 10. BRB alone had no effect on hematopoiesis as assessed by total white blood cell count and platelet count.

Effect of BRB on Liver Histology. The \(14\)C-aminopyrine breath test results correlated with histological findings from liver tissue obtained from control rabbits at the time of sacrifice (Fig. 2). Hepatocellular degeneration and necrosis were not
Liver Injury and Drug Metabolism

Fig. 2 Liver histology after BRB treatment. Eight rabbits received BRB, 2.6 ml/kg 40% solution in corn oil i.p. Animals were sacrificed on days 1, 3, 5, and 10 after receiving BRB. Histological sections of liver tissue were evaluated and scored for extent of degeneration and necrosis. Each slide was graded for the presence of degeneration and necrosis using the following grading schemes: (a) degeneration: 1+, fine cytoplasmic vacuolation; 2+, vacuoles of a size occupying less than one-half of the cytoplasm of the cells; 3+, vacuoles of a size occupying the entire cytoplasm of the cells; (b) degree of degeneration: 1+, 1–2 cell layers involved; 2+, 3–5 cell layers involved; 3+, 6–10 cell layers involved; 4+, diffusely involves the liver lobule; (c) necrosis: 1+, 1–2 acidophil bodies/10 high-power fields; 2+, 3–5 acidophil bodies/10 high-power fields; 3+, 5–10 acidophil bodies/10 high-power fields; 4+, >10 acidophil bodies/10 high-power fields. The extent of degeneration was determined by adding the grades of degeneration and degree of degeneration.

Effect of DOX and BRB on Hepatic Function. Previous experiments in our laboratory have shown that administration of DOX, 3 mg/kg, by i.v. infusion over 5 min has no demonstrable effect on either liver function tests or liver histology over a 10-day period (19). Similarly, DOX alone did not impair hepatic ability to demethylate 14C-aminopyrine (Fig. 3). When DOX was administered 3 days after i.p. instillation of BRB, 2.6 ml/kg 40% solution in corn oil, hepatic demethylation of 14C-aminopyrine was impaired on days 1 and 3 following DOX administration; the magnitude of this impairment was similar to that observed following the administration of BRB alone.

Effect of DOX and BRB on Hematopoiesis. DOX alone affected hematopoiesis. The nadir white blood cell count, observed 5 days after DOX infusion, was 65% of the baseline count (Fig. 4). Thrombocytopenia was observed on days 5, 8, 9, and 10; the nadir occurred on day 8 and was 12% of baseline. Administration of BRB 3 days prior to the DOX infusion did not affect the severity of DOX-induced thrombocytopenia as measured in surviving rabbits, but such an effect would have likely been observed if all BRB rabbits had survived to the platelet nadir at 8 days. BRB pretreatment did increase both the severity and duration of DOX-induced leukopenia.

Effect of BRB on the Pharmacokinetics of DOX and Its Metabolites. BRB, presumably as a result of its effects on hepatic function, altered the metabolism of DOX as assessed by pharmacokinetic parameters. Administration of BRB, 2.6 ml/kg 40% solution in corn oil, 3 days prior to administration of DOX, 3 mg/kg, i.v. over 5 min resulted in increased DOX exposure.
The relationship between percentage of change of WBCs from baseline on day 5 and DOXOL AUC ($R^2 = 0.63, P = 0.027$). The deaths occurred at the time of maximum myelosuppression as suggested by our data (Fig. 4). There were no observed synergistic effects between DOX and BRB on liver function tests. DOX, DOXOL, and 7-deoxydoxorubicinol aglycone AUCs were all statistically significantly increased in rabbits that died from DOX-induced toxicity following BRB pretreatment when compared to surviving rabbits that did or did not receive BRB pretreatment (Table 5). Comparing rabbits that died and survivors only among animals that received DOX after BRB pretreatment, only the mean DOXOL AUC was significantly increased in rabbits that died. In conjunction with data regarding increased severity of DOX-induced neutropenia following BRB pretreatment (Fig. 4), these data suggest that BRB-induced impairment of DOX metabolism increased clinical DOX toxicity via effects on hematopoiesis.

**DISCUSSION**

The liver is a central metabolizing and excretory organ, yet little is known of the effects of hepatic disease on the pharmacodynamics of anticancer drugs. Our goal was to determine the effects of hepatic dysfunction in a controlled model on the pharmacokinetics and pharmacodynamics of a toxic drug used in the treatment of cancer. The ultimate aim of this work is to use this information to help design individualized dosing algorithms for chemotherapy.

This study characterized some of the effects of a hepatotxin, BRB, on the clinical pharmacology and toxicology of DOX and its metabolites. BRB reproducibly causes a sublethal anatomic injury (centrilobular necrosis) to the liver similar to that which may be seen in response to other drugs or ischemia. Sublethal hepatocellular necrosis may be a common clinical event.

The data presented confirm the histological, biochemical, and functional (14C-aminopyrine demethylation) effects of BRB on hepatic function. These parameters were abnormal during the period 1–5 days following BRB administration, but returned to baseline by day 10 (Table 1; Figs. 2 and 3). BRB alone had no effect on hematopoietic function. DOX alone had no effect on biochemical, histological, or functional measures of hepatic function, but did suppress myelopoiesis and thrombopoiesis (Fig. 4). The combination of BRB and DOX administration did not appear to increase hepatic dysfunction as compared with administration of BRB alone, but pretreatment with BRB did increase the severity of DOX-induced myelosuppression (Fig. 4). This effect of BRB on DOX-induced myelosuppression was clinically significant in our model; 3 of 7 rabbits died when they received DOX following BRB pretreatment whereas none (0/7) died after receiving only DOX (Table 4). In that BRB increased the severity of DOX-induced neutropenia, the deaths occurred on days 5 and 6 following DOX administration (at the time of white blood cell count nadir), and BRB alone did not cause myelopoietic dysfunction, we hypothesized that BRB-induced alterations in DOX metabolism resulted in more severe bone marrow toxicity due to increased exposure to either native drug or a metabolite. Furthermore, because the histological, biochemical, and functional effects of BRB in the rabbit appear to be specific for the centrilobular hepatocytes (without evident effect

![Graph](image-url)
Liver Injury and Drug Metabolism

rabbits received BRB, 2.6 ml/kg 40% solution in corn oil i.p., and the pharmacokinetic parameters were again measured after DOX, 3 mg/kg iv.

rabbits received BRB, 2.6 ml/kg 40% solution in corn oil i.p. Three days

be a specific result of alteration of hepatic processing of DOX.

Increased exposure to either native drug or a metabolite is correct.

more severe bone marrow toxicity and mortality due to in-

versus

ment

5).

creased exposure to DOXOL (Table 5).

only, 3 mg/kg iv., over 5 min. Twenty-eight days later, the same

rabbits received BRB, 2.6 ml/kg 40% solution in corn oil i.p., and the pharmacokinetic parameters were again measured after DOX, 3 mg/kg i.v. Alternatively, enhanced intravascular protein binding of DOXOL, which may limit its ability to traverse cell membranes, DOXOL is 5-50-fold more toxic (27, 28). Such a discrepancy in activity may in part be explained by the polarity of the metabolite as assessed by serum AUCs, was increased compared to baseline following BRB pretreatment; for DOXOL and 7-deoxydoxorubicinol aglycone, the increases in AUCs were statistically significant. While death following exposure to DOX, with or without BRB pretreatment, correlated with elevation of DOX, DOXOL, and 7-deoxydoxorubicinol aglycone AUCs, the strongest association of rabbit death appeared to be with increased exposure to DOXOL (Table 5). Only DOXOL AUCs differed significantly between rabbits surviving BRB pretreatment versus those that died. Thus it appears that the hypothesis that BRB-induced alterations in DOX metabolism resulted in more severe bone marrow toxicity and mortality due to increased exposure to either native drug or a metabolite is correct.

DOXOL is an important metabolic product of DOX. It is less cytotoxic in vitro (24–26) than DOX; however, in a model of papillary muscle function and a model of isolated ion channels, DOXOL is 5–50-fold more toxic (27, 28). Such a discrepancy in activity may in part be explained by the polarity of DOXOL, which may limit its ability to traverse cell membranes (25, 26). Alternatively, enhanced intravascular protein binding of DOXOL compared to DOX may contribute to its toxicity. The enhanced myelosuppression associated with increased pharmacokinetic DOXOL (and 7-deoxydoxorubicinol aglycone) exposure in this study suggests that either metabolite may be directly responsible for the additional myelosuppression and toxicity seen following BRB-induced sublethal hepatocellular necrosis, or that increased metabolite concentrations may be a marker of site-specific redistribution of DOX metabolism in the face of hepatocellular dysfunction. The association of increased DOXOL exposure (as demonstrated by the increased AUC in the face of hepatic dysfunction) supports our prior work with allyl alcohol-induced liver dysfunction (19). Allyl alcohol, which causes a sublethal, mixed perportal and centrilobular hepatic injury, resulted in hematological, toxicological, and pharmacokinetic sequelae similar to those observed in the current study. These findings may well be applicable to humans. Studying DOX pharmacokinetics in hepatoma patients, Chan et al. (29) found that cirrhotic patients had elevated DOXOL concentrations and increased drug-induced toxicity.

Our data may be interpreted in a number of ways. First, the anatomic location of hepatocellular necrosis (e.g., centrilobular versus periporal sites of cell damage) may be a less important factor in the alteration of drug pharmacodynamics than the total number of functional hepatocytes (30). This hypothesis is supported by the similarity of the findings in this BRB model and in an allyl alcohol model of the effects of liver function on DOX metabolism (19). Changes in DOX pharmacodynamics may be related to functional hepatic cell mass rather than to a specific anatomic lesion since the metabolizing enzymes for DOX do not appear to be anatomically localized. This argument is attractive since it is analogous to the intact nephron hypothesis, which has been clinically useful. Thus, despite the fact that DOX is reduced by cytosolic and microsomal reductase enzymes (31–33) and breath tests measure oxidative demethylation, sensitive measures of hepatic mass function such as aminopyrine or

Table 2 DOX pharmacokinetic parameters

<table>
<thead>
<tr>
<th></th>
<th>AUC (µM h)</th>
<th>t1/2 (h)</th>
<th>Clb (l/min/kg)</th>
<th>Vss (liter/kg)</th>
<th>Css (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DOX</td>
<td>1.42 ± 0.32</td>
<td>18.7 ± 6.1</td>
<td>0.067 ± 0.013</td>
<td>74.5 ± 19.3</td>
<td>2.36 ± 2.06</td>
</tr>
<tr>
<td>DOX after BRB pretreatment</td>
<td>2.45 ± 1.17</td>
<td>25.5 ± 7.7</td>
<td>0.049 ± 0.036</td>
<td>77.4 ± 72.1</td>
<td>2.65 ± 1.83</td>
</tr>
</tbody>
</table>

Mean ± SD

* Pharmacokinetic parameters were determined for seven rabbits receiving DOX only, 3 mg/kg iv. over 5 min. Twenty-eight days later, the same rabbits received BRB, 2.6 ml/kg 40% solution in corn oil i.p., and the pharmacokinetic parameters were again measured after DOX, 3 mg/kg iv. t1/2, terminal half-life; Clb, total body clearance; Vss, volume of distribution of steady state; Css, theoretical plasma concentration at time zero.

Table 3 DOX metabolite AUCs

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>DOX (µM h)</th>
<th>DOX after BRB (µM h)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>DOXOL</td>
<td>0.47 ± 0.19</td>
<td>1.79 ± 0.73</td>
<td>0.004</td>
</tr>
<tr>
<td>7-deoxydoxorubicinol aglycone</td>
<td>0.34 ± 0.12</td>
<td>2.32 ± 1.46</td>
<td>0.01</td>
</tr>
<tr>
<td>7-deoxydoxorubicinol aglycone</td>
<td>0.089 ± 0.12</td>
<td>0.17 ± 0.009</td>
<td>NS</td>
</tr>
<tr>
<td>Doxorubicinol aglycone</td>
<td>0.41 ± 0.40</td>
<td>0.15 ± 0.068</td>
<td>NS</td>
</tr>
</tbody>
</table>

Mean ± SD

* Metabolite AUCs were determined for seven rabbits receiving DOX only, 3 mg/kg iv., over 5 min. Twenty-eight days later, the same rabbits received BRB, 2.6 ml/kg 40% solution in corn oil i.p. Three days following BRB, AUCs were again measured following DOX, 3 mg/kg iv.

Table 4 DOX-related mortality

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No.</th>
<th>Deaths</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRB alone</td>
<td>8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>DOX alone</td>
<td>7</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>DOX after BRB pretreatment</td>
<td>7</td>
<td>3</td>
<td>43</td>
</tr>
</tbody>
</table>

* Rabbits received either BRB (2.6 ml/kg 40% solution in corn oil i.p.), DOX (3 mg/kg i.v.), or DOX 3 days after BRB and were observed for at least 10 days after the last drug administration. Significantly different from DOX after BRB pretreatment group, P < 0.05 by χ² test.
erythromycin breath tests may provide important DOX pharmacodynamic information to individualize dosing regimens.

Second, changes observed in DOX, DOXOL, and 7-deoxydoxorubicinol aglycone pharmacokinetics following administration of a hepatocellular toxin suggest a greater role for hepatic clearance of DOX than previously thought (34, 35). Data from a pig model in our laboratory suggest that at moderate concentrations, hepatic extraction of DOX may exceed 50% (36). Hepatic distribution and metabolism appear to play an important role in the ultimate metabolic disposition and pharmacodynamic effects of DOX. It is also possible that some of the pharmacokinetic changes observed in this work may be a reflection of a complex set of changes in multiple organs reacting to compromised hepatic function.

Third, the work of Levy and associates has emphasized the disparity between measured plasma drug concentration and the intensity of pharmacological effects in disease states. Their work in a rat model demonstrated a wide variability in plasma drug concentrations, target organ drug concentrations, and locally effective drug concentrations for some central nervous system-acting drugs (37, 38) but not for others (39, 40). Disease states, particularly renal disease, may cause dissociation of effect and tissue measured drug concentrations (37, 41). Reasons for such dissociation may be drug, disease, and tissue specific (e.g., enhanced receptor sensitivity in uremia, or increased drug delivery due to reduced plasma protein concentrations in nephrotic syndrome; Refs. 42 and 43).

Ultimately, it is desirable to predict individual clinical toxicity for cytotoxic antineoplastic agents. Recent work has correlated human plasma pharmacokinetics with toxicological end points using nonlinear curve-fitting techniques (44-46) or with response using plasma concentration (47, 48). In the case of two anthracyclines evaluated in such a manner, pharmacokinetic correlations with toxicological end points remained poor (12, 49, 50). As the current work suggests, it is likely that disease states alter the pharmacokinetics and pharmacodynamics of DOX metabolism. Thus, dosing algorithms that do not incorporate the pharmacological and pharmacodynamic consequences of disease states may not be useful in humans.

ACKNOWLEDGMENTS

Aruna Arakali, David Boudreau, Michael Murphy, Tommy Yu, Mary Hensen, Mary Ann Vaerten, and Nick Verma provided valuable technical assistance. Leesa Knox provided valuable assistance with manuscript preparation.

REFERENCES


Effect of sublethal liver injury on doxorubicin metabolism.

D A August, S Halter and D E Brenner