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Effect of a low-protein diet on doxorubicin pharmacokinetics in the rabbit*

Barry J. Cusack[1, 2], Stephan P. Young[1], Vicki L. Loseke[1], Michelle R. Hurty[1], Loring Beals[1], and Richard D. Olson[1, 2]

[1] Gerontology and Clinical Pharmacology Research Unit, VA Medical Center, Boise, Idaho, USA
[2] School of Medicine, University of Washington, Seattle, Washington, USA

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Summary. Malnutrition involving protein deficiency, which commonly occurs in cancer patients receiving anthracycline treatment, is considered to be a risk factor for the development of cardiotoxicity. Protein deficiency has been shown to impair the metabolism of drugs such as theophylline and acetaminophen. If protein deficiency also impairs anthracycline metabolism, it could explain at least in part the enchanced anthracycline toxicity associated with malnutrition. We tested this idea by determining the effect of a low-protein, isocaloric diet on doxorubicin pharmacokinetics in rabbits. The animals were randomized into two groups for 8–12 weeks. Rabbits in group 1 received a low-protein (5%), isocaloric diet, whereas those in group 2 received a normal-protein (15%) diet. Both groups (group 1, $n = 15$; group 2, $n = 14$) were given 5 mg/kg doxorubicin by i.v. bolus. After doxorubicin injection, blood samples were obtained over the next 52 h for the measurement of doxorubicin and doxorubicinol plasma concentrations by high-performance liquid chromatography (HPLC) with fluorometric detection. The low-protein diet significantly decreased doxorubicin clearance (48 ± 3 vs 59 ± 4 ml min^{-1} kg^{-1}; $P < 0.05$), prolonged the terminal elimination half-life (28 ± 2 vs 22 ± 2 h; $P < 0.05$), and increased the area under the plasma concentration/time curve extrapolated to infinity (1722 ± 122 vs 1405 ± 71 ng h ml^{-1}; $P < 0.05$) as compared with the values determined for rabbits fed the standard rabbit chow (15% protein). The volume of distribution for doxorubicin was not altered by the low-protein diet. In addition, in rabbits fed the the low-protein diet, the terminal elimination half-life of the alcohol metabolite, doxorubicinol was prolonged (52 ± 5 vs 40 ± 2 h; $P < 0.05$). Thus, a low-protein diet causes a reduction in the ability of rabbits to eliminate doxorubicin and possibly its alcohol metabolite doxorubicinol. If a similar alteration in anthracycline pharmacokinetics occurs in malnourished cancer patients, this phenomenon may contribute to their increased risk of developing cardiotoxicity associated with anthracycline therapy.

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Offprint requests to: Barry J. Cusack, VA Medical Center (111), 500 W. Fort St., Boise, ID 83702-4598, USA

Introduction

Doxorubicin, a widely used anthracycline antineoplastic agent, causes cumulative, dose-related cardiotoxicity, which may limit its therapeutic potential [24]. Doxorubicin undergoes complex metabolism [22], including reduction of the C-13 carbonyl group to the alcohol metabolite, doxorubicinol, a potent cardiotoxic agent [4, 19].

Many patients undergoing anthracycline chemotherapy suffer from anorexia and undernutrition [8]. In all, 15% of cancer patients lose over 10% of their body weight prior to the initiation of chemotherapy [10], and over 80% of hospitalized cancer patients display reduced serum albumin concentrations [17]. Nutritional factors play a role in the management of these individuals. For example, there is clinical evidence that malnutrition is a risk factor for chronic anthracycline cardiotoxicity [18]. The reason why malnutrition increases the severity of anthracycline cardiotoxity remains unknown. Specific nutritional deficits may increase the susceptibility of the myocardium to the toxic effects of anthracyclines [12]. Malnutrition, including protein deficiency, is known to impair hepatic drug metabolism both in animals and in man [3, 13, 14, 23]. Thus, protein deficiency may be associated with a decrease in the metabolism of anthracyclines or their metabolites, which increases the duration of exposure of the heart to toxic substances. We tested this hypothesis by evaluating the effect of a low-protein, isocaloric diet on the pharmacokinetics of doxorubicin and its primary metabolite doxorubicinol following single-dose administration of the parent compound to the rabbit.

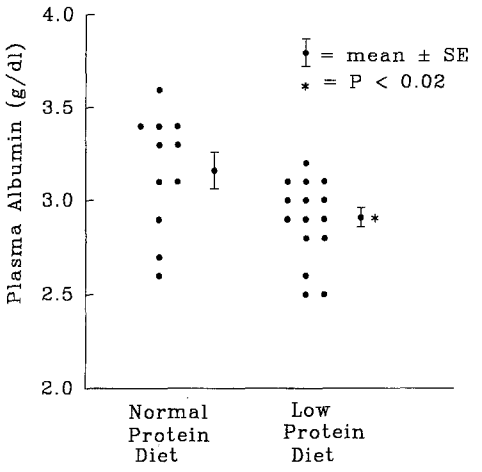

Fig. 1. Comparison of plasma albumin concentrations in rabbits fed low-protein (5%) and normal-protein (15%) diets for 8–12 weeks

Materials and methods

Protocol. Healthy New Zealand White rabbits of either sex (2.5–3.5 kg), obtained from a licensed supplier, were observed for a period of 1 week to ensure that they were in good health prior to their inclusion in the study. They were then entered into the protocol, receiving either a standard diet containing 15% protein (Purina, Richmond, Ind.) or a modified diet containing 5% protein (Purina). The modified diet also contained 5.9% fat, 12.8% fiber, and 69.7% carbohydrate along with standard vitamin and mineral supplementation. This diet was isocaloric, with an energy equivalent of 3.52 kcal/g. Control and study animals were fed 4 oz normal- and low-protein feed per day, respectively, for 8–12 weeks prior to and during the course of the pharmacokinetic studies.

On the morning of the study, following the application of 4% topical lidocaine (Astra Pharmaceutical Products, Inc., Worcester, Mass.) as a local anesthetic, a 12-in., 21-gauge i. v. cannula (1-Cath; Delmed, Canton, Mass.) was inserted in a marginal or central ear vein and advanced about 8 in. for serial blood sampling. A polyethylene cannula was inserted in a marginal vein in the other ear for drug administration. The catheter was kept patent by the bolus administration of heparinized saline (65 USP units/ml). Following the removal of a baseline blood sample, 5 mg/kg doxorubicin (Adriamycin; Adria Laboratories, Columbus, Ohio) was injected i. v. over 2 min and the cannula was flushed with 5 ml saline. Blood sampling (2–3 ml) was performed before dosing and at 5, 30, and 60 min and 2, 4, 7, 12, 24, 28, 32, 48, and 52 h after drug administration. The blood samples were placed in heparinized polypropylene tubes, and the separated plasma was stored at –20° C prior to drug analysis.

Analytical methods. The plasma concentrations of doxorubicin and doxorubicinol were measured by gradient high-performance liquid chromatography (HPLC) with fluorometric detection following solid-phase extraction. A modification of the solid-phase extraction procedure described by Robert [21] was used. Sep-Pak extraction cartridges (Waters, Milford, Mass.) were conditioned with 4 ml methanol, 4 ml methanol/water (50:50, v/v) and 5 ml 0.1% ammonium formate buffer (AFB), pH 4. Next, the plasma samples were diluted in 4 ml AFB and passed through cartridges followed by 4 ml 0.1% AFB and 1 ml heptane. The cartridges were then aspirated to dryness, and the samples were eluted from the Sep-Pak cartridges with 7 ml methanol, collected, and dried at 45° C using a SVC100 speed vacuum evaporator (Savant Instruments, Farmingdale, N. Y.). Samples reconstituted in 0.1% AFB were injected on the HPLC column. During the procedures, the samples were protected from direct light. The HPLC analysis was performed as previously described [9]. A Monarch 2000 autoanalyser (Instrumentation Laboratories, Fisher Scientific, Lexington, Mass.) was used to measure albumin concentrations by a standard spectrophotometric method using bromocresol green [20].

Table 1. Effect of a low-protein diet on doxorubicin pharmacokinetics in the rabbit

Group	$t_{1/2}$ (h)	λ (h^{-1})	V_{area} ($l\ kg^{-1}$)	AUC_∞ ($ng\ h\ ml^{-1}$)
Control:				
Mean	21.5	0.034	107.8	1405
SE	1.5	0.002	7.5	71
Low-protein diet:				
Mean	27.9	0.027	113.7	1722
SE	2.3	0.002	9.4	122
P	<0.05	<0.05	NS	<0.05

NS, Not significant

Data analysis. The slopes of the terminal portions of the plasma doxorubicin and doxorubicinol concentration/time curves were fitted by linear regression. Pharmacokinetic parameters were calculated using standard model-independent techniques [11]. Group parameters were compared using Student's two-tailed *t*-test for unpaired data. The null hypothesis was rejected at $P < 0.05$. The results are reported as mean values ± SE.

Results

Rabbits fed an isocaloric, low-protein diet did not exhibit statistically significant differences in body weight as compared with controls (3.06 ± 0.13 vs 3.28 ± 0.07 kg). However, serum albumin concentrations measured in the low-protein-diet group were lower than those observed in controls (2.9 ± 0.05 vs 3.2 ± 0.1 g/dl; $P < 0.02$; Fig. 1). There were differences in doxorubicin pharmacokinetics between the two groups (Table 1). The plasma elimination half-life ($t_{1/2}$) was prolonged by 30% ($P < 0.05$) in rabbits fed a low-protein diet as compared with control animals fed a standard diet. The slope of the terminal elimination-rate constant (λ) was 21% less in the low-protein-diet group than in the controls ($P < 0.05$). There was no statistical difference in the apparent volume of distribution (V_{area}) between the two groups of rabbits. However, the systemic clearance (C) of doxorubicin was 19% lower in rabbits receiving the low-protein diet than in the controls (48 ± 3 vs 59 ± 4 ml min^{-1} kg^{-1}; $P < 0.05$; Fig. 2). Similarly, the area under the plasma concentration-time curve (AUC_∞) for doxorubicin was increased by 23% ($P < 0.05$) in the experimental group as compared with the control animals (Table 1).

The low-protein diet also had statistically significant effects on the kinetics of doxorubicinol, the primary metabolite of doxorubicin. Feeding rabbits an isocaloric, low-protein diet prolonged the half-life ($t_{1/2}$) of doxorubicinol by 30% ($P < 0.05$) but did not alter the terminal elimination-rate constant (λ) or the AUC_∞ (Table 2). The clearance and the apparent volume of distribution could not be computed because the total amount of doxorubicinol generated was unknown.

Discussion

Anthracyclines, particularly doxorubicin and daunorubicin, produce exposure-dependent cardiotoxicity [24].

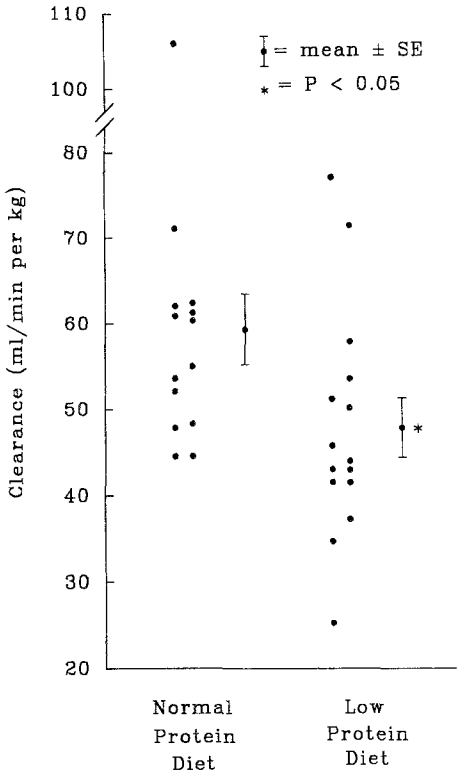

Fig. 2. Effect of a low-protein (5%), isocaloric diet on the systemic clearance of doxorubicin following the administration of 5 mg/kg by i. v. bolus

Thus, interventions that increase the duration of the exposure of cardiac tissue to doxorubicin may increase the extent of cardiac injury and be a risk factor for doxorubicin-induced cardiotoxicity. For example, liver dysfunction is a risk factor for systemic doxorubicin toxicity because it can decrease doxorubicin clearance and elevate plasma levels of the parent drug and its metabolites for prolonged periods [2, 7]. Thus, protein deficiency in malnourished patients receiving anthracycline chemotherapy might also represent a risk factor for anthracycline-induced cardiotoxicity by decreasing anthracycline metabolism. The purpose of the present study was to determine whether protein-deficient rabbits (fed an isocaloric diet containing 67% less protein than the standard laboratory diet given to control rabbits) would exhibit changes in their metabolism of doxorubicin or its primary circulating metabolite doxorubicinol.

A low-protein diet has been demonstrated to inhibit both microsomal and nonmicrosomal drug metabolism [6, 13, 14]. For example, as compared with a standard-protein (23%) laboratory diet, a low-protein (5%)diet reduced cytochrome P-450 activity by 65% and decreased the clearance of theophylline by 39% [13]. (Theophylline is a well-documented substrate for microsomal drug metabolism.) Similarly, a low-protein diet decreased the clearance of acetaminophen through nonmicrosomal metabolic processes [14]. In the current study we found that the metabolism of doxorubicin and possibly its primary circulating metabolite doxorubicinol was also decreased by a low-protein diet.

Table 2. Effect of a low-protein diet on doxorubicinol pharmacokinetics after the administration of 5 mg/kg doxorubicin by i. v. bolus

Group	$t_{1/2}$ (h)	λ (h^{-1})	AUC_∞ (ng h ml^{-1})
Control:			
Mean	39.7	0.019	1182
SE	2.5	0.001	60
Low-protein diet:			
Mean	51.6	0.015	1455
SE	4.5	0.001	128
P	<0.05	NS	NS

NS, Not significant

How might a low-protein diet decrease the metabolism of doxorubicin and doxorubicinol? Doxorubicin is metabolized by two main pathways [22]. The primary pathway involves reduction of the C-13 carbonyl moiety to an alcohol by cytoplasmic aldoketo reductases (termed anthracycline reductases) to form doxorubicinol. A secondary and less extensive pathway of doxorubicin metabolism (but the primary pathway of doxorubicinol metabolism) involves cleavage of the daunosamine sugar by microsomal oxidases to form aglycones [15]. Which of these two pathways is most affected by a low-protein diet remains uncertain. However, data obtained from the present study suggests that protein deficiency may prefentially affect the microsomal rather than the anthracycline reductase pathway of doxorubicin metabolism. This interpretation is suggested by two observations. First, doxorubicinol plasma concentrations were not decreased in the protein-deficient group. If the protein-deficient diet had substantially inhibited anthracycline reductase activity, less doxorubicinol would have been formed, leading to lower doxorubicinol plasma concentrations (assuming only a slight effect, if any, on doxorubicinol kinetics). Second, the prolongation of the elimination half-life of doxorubicinol observed in rabbits fed the low-protein diet is consistent with a decrease in the microsomal oxidation of doxorubicinol to doxorubicinol aglycone. Thus, the effects of the low-protein diet on doxorubicin and doxorubicinol metabolism support the idea that microsomal rather than nonmicrosomal metabolism was preferentially perturbed.

In contrast to its effects on clearance, the low-protein diet did not change the volume of distribution of doxorubicin. Thus, the prolonged elimination half-life and increased plasma concentrations of doxorubicin were attributable to a decrease in the drug's clearance. In accordance with previous data [1, 9, 16], we report that only a small fraction of the doxorubicin dose remains in plasma (i.e., large volume of distribution). Of the fraction that persists in blood, only 70% is bound to plasma proteins, leaving 30% unbound [16]. Because of the large volume of distribution and the low protein binding of doxorubicin, dietary changes in plasma protein content such as those seen in this study are unlikely to result in large changes in plasma concentrations of free doxorubicin. Thus, we did not attempt to determine whether a low-protein diet would alter the plasma protein binding of doxorubicin.

We also evaluated the effect of a low-protein diet on doxorubicinol metabolism because recent studies suggest that this metabolite may contribute to the cardiotoxicity of doxorubicin therapy [4, 19]. Doxorubicinol is more potent than doxorubicin in its inhibition of cardiac contractility and of ATPase activity in sarcolemma, mitochondria, and sarcoplasmic reticulum [4, 19]. Thus, the effect of low-protein diet on doxorubicinol metabolism may be as meaningful as the effect on doxorubicin metabolism. However, since the total doxorubicinol dose or load is unknown, some kinetic parameters that were determined for doxorubicin (such as volume of distribution and clearance) could not be calculated for doxorubicinol.

The low-protein diet significantly ($P < 0.05$) increased the terminal elimination half-life of doxorubicinol by 23% as compared with the values obtained for rabbits fed standard rabbit chow (Table 2). This observation is consistent with the idea that the low-protein diet decreased the metabolism of doxorubicinol. However, it must be emphasized that an impairment of the elimination of the metabolite is not the only interpretation of the data. Other possible explanations include an alteration in the volume of distribution or a delay in the appearance of doxorubicinol in the plasma. Whatever the reason for the increased elimination half-life of doxorubicinol, a low-protein diet prolonged the cardiac exposure to this toxic metabolite.

This study demonstrates for the first time that dietary protein deprivation impairs the elimination of doxorubicin and prolongs the exposure of the heart to doxorubicin and its toxic metabolite doxorubicinol. The altered anthracycline pharmacokinetics observed in rabbits fed a low-protein diet probably resulted at least in part from a decrease in microsomal oxidase activity. For several reasons, however, these findings do not imply that altered anthracycline metabolism occurs in malnourished cancer patients undergoing chemotherapy. This model of protein undernutrition may differ in character and in etiology from the malnutrition seen in cancer patients, which is not simply due to dietary protein deficiency. Although the rabbit is a good model for the study of human anthracycline pharmacokinetics [5], the effects of protein deficiency on anthracycline metabolism could possibly differ between the two species. Furthermore, conditions in cancer patients are often complicated by the presence of concomitant drug therapy, tumor burden, and other disease states, none of which exist in the present model. It is therefore clear that further study is required to evaluate the clinical importance of these observations and to determine whether doxorubicin dose adjustment is required in nutritionally deprived patients.

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