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Effect of low-protein diet on anthracycline pharmacokinetics and cardiotoxicity

Ebtehal El-Demerdash[a], Azza A. Ali[b], Dalia E.M. El-Taher[c] and Farid M.A. Hamada[b]

[a]Pharmacology & Toxicology Department, Faculty of Pharmacy, Ain Shams University, [b]Pharmacology & Toxicology Department, Faculty of Pharmacy, Al-Azhar University and [c]Hospitals of Ain Shams University, Cairo, Egypt

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Correspondence
Ebtehal El-Demerdash, Department of Pharmacology & Toxicology, Faculty of Pharmacy, Ain Shams University, Abasia, Cairo, Egypt.
E-mail: ebtehal_dm@yahoo.com

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Abstract

Objectives Anthracyclines are broad spectrum anticancer drugs with dose-dependent cardiotoxicity. Protein malnutrition commonly occurs in cancer patients and is considered a risk factor for development of cardiotoxicity. This study was designed to assess the modulatory effect of protein malnutrition on the pharmacokinetics and drug disposition properties of a single dose of doxorubicin and epirubicin and how these possible changes will affect the degree of cardiotoxicity of these drugs.

Methods A single interperitoneal dose of 15 mg/kg of either doxorubicin or epirubicin was injected into rats fed with either normal protein diet or low-protein diet. The plasma concentration–time profiles of doxorubicin and epirubicin and their concentrations in different tissues were determined. Serum creatine kinase level was determined at different time intervals and histopathological examination of heart tissue was carried out.

Key findings Protein malnutrition significantly altered the pharmacokinetics of doxorubicin and epirubicin, with a significant decrease in their elimination, and prolonged the exposure of the heart to these drugs. Histopathological examination and serum creatine kinase measurements supported the role of protein malnutrition in enhancement of anthracycline cardiotoxicity.

Conclusions If similar alteration in anthracyclines' pharmacokinetics occurs in malnourished cancer patients, protein malnutrition will be a risk factor for development of anthracycline cardiotoxicity and dose adjustment will be required in nutritionally deprived patients.

Introduction

Protein malnutrition is a worldwide socioeconomic problem especially in developing countries.[1] It is a common problem in patients with cancer and occurs as a consequence of an imbalance between the nutritional needs of the patient, the demands of the tumour and the availability of nutrients in the body.[2,3] The global incidence of protein malnutrition in cancer ranges from 30% to 85%, being most prevalent in patients with gastric, pancreatic, lung, prostate and colon cancer.[4,5]

Prolonged malnutrition can result in cachexia characterized by progressive, involuntary weight loss with depletion of lean body mass, muscle wasting and weakness, oedema, impaired immune responses and decline in motor and mental function. This pattern of weight loss differs from simple starvation seen in otherwise healthy patients, in which loss of body fat with sparing of skeletal muscle occurs.[6] The frequency of moderate to sever cancer cachexia varies from 15% to 40% at the initial presentation of cancer and occurs in 40–60% of patients.[7] Protein malnutrition has been associated with a decreased quality of life, considerable morbidity and mortality, reduced response to chemotherapy and an increased risk of chemotherapy-induced adverse effects and toxicity.[8] In addition, cancer-associated protein malnutrition can impair the metabolism of chemotherapeutic drugs such as anthracyclines, methotrexate and 5-fluorouracil.[9]

Anthracycline antibiotics are among the most active cytotoxic agents for treatment of a wide variety of solid tumours and haematological malignancies. They are the mainstay of a large number of clinical protocols for the treatment of adult and childhood neoplastic diseases.[10] Their anticancer activity is related to their ability to react with cellular constituents in various ways. Their aglycon moiety can intercalate between

the adjacent DNA base pair and cause single-stranded (via topoisomerase I) or double-stranded (via topoisomerase II) DNA breaks. They can also modify the ability of nuclear helicases to dissociate duplex DNA into single DNA strands.[11]

The occurrence of cardiotoxicity varies markedly within the anthracycline group, and for this reason, the pharmacokinetics and toxicodynamics of anthracyclines have been extensively investigated to identify integrated models that can be used in the clinical setting to prevent the development of serious toxicity.[10] As an important example, epirubicin is an epimer of doxorubicin that shows lower cardiotoxicity than the parent compound. This difference may be related to several pharmacokinetic factors including difference in tissue distribution, drug metabolism and conjugation as well as drug clearance.[12,13] Anthracyclines undergo extensive biotransformation in the liver to active and inactive metabolites.[14] Both doxorubicin and epirubicin are extensively metabolized to their 13-dihydro derivatives: 13-S-dihydrodoxorubicin (Dox-ol) and 13-S-dihydroepirubicin (Epi-ol).[15] It has been proposed that chronic cardiomyopathy develops after conversion of doxorubicin to Dox-ol.[16]

Since cancer protein malnutrition was associated with changes in drug metabolism,[9] protein malnutrition may induce an alteration in anthracyclines' pharmacokinetics and disposition and so their cardiotoxicity risk. In particular, a large body of evidence supports the association between hypoalbuminaemia, one finding resulting from protein malnutrition, and cardiovascular diseases.[17] Thus, this study examined for the first time the modulatory effect of protein malnutrition on the pharmacokinetics and drug disposition properties of a single dose of doxorubicin and epirubicin and how these possible changes will affect the degree of cardiotoxicity of these drugs.

Materials and Methods

Drugs

Both doxorubicin (Adriablastina) and epirubicin (Farmarubicin) were obtained from Pharmacia and Upjohn (Milan, Italy). Each vial contained 10 mg of freeze-dried powder, was dissolved in saline and injected intraperitoneally as a single dose of 15 mg/kg. The dose was chosen according to the study of Hiroe *et al*.,[18] which found it is equivalent to 500 mg/m^2 of doxorubicin in a 50-kg human. Since epirubicin is an epimer of doxorubicin it was used at the same dose.

Animals

The investigation was performed in male albino rats, 110–150 g, obtained from El-Nile Co. outbreed stock for pharmaceutical and chemical industries (Cairo, Egypt). The rats were kept in the animal house of the Faculty of Pharmacy, Al-Azhar University, under suitable laboratory condition. The rats were fed on one type of diet according to the casein content: standard protein diet (20% casein) or low-protein diet (5% casein). Handling and experimentation were conducted in accordance with the international ethical guidelines. The experimental protocol was approved by Faculty of Pharmacy, Al-Azhar University (Cairo, Egypt).

Each 100 g of standard protein diet contained casein (20 g), sucrose (70 g), salt mixture (4 g), oil and oil-soluble vitamins (5 g) and vitamin mixture in starch (0.6 g). Low-protein diet had the same composition as the standard protein diet except that the amount of casein per 100 g was reduced to 5 g and replaced by sucrose–starch mixture (15 g) according to the study of Oumi *et al*.[19]

Experimental design

Assessment of the possible modulatory effect of protein malnutrition on pharmacokinetics and drug disposition of a single dose of doxorubicin and epirubicin

Sixty male albino rats were classified randomly into two groups and subjected to one type of nourishment (either standard protein diet or low-protein diet) for three weeks, and then each group was further classified into two groups and given a single intraperitoneal injection of 15 mg/kg of either doxorubicin or epirubicin. Rats were anaesthetized by a single dose of chloral hydrate 300 mg/kg[20] and blood samples were collected from the retro-orbital sinus of the eye into Eppendorf tubes at 0, 10 and 30 min, and 1, 2, 4, 6 and 24 h after drug injection. Serum samples were immediately obtained by centrifugation at 3000 rpm for 10 min.

After withdrawal of the last blood sample, the rats were killed by cervical dislocation and selected tissues (heart, liver, kidney, spleen and lung) were dissected out immediately and washed with ice-cold saline. The tissues were homogenized (10%) in water using a Branson Sonifier. Doxorubicin and epirubicin concentrations in plasma and tissue homogenate were analysed by the method of Formelli *et al*.[21] as modified by Rhaman *et al*.[22] In brief, 0.2 ml silver nitrate (33% w/v) was added to 1 ml tissue homogenate. Plasma (0.25 ml) was diluted to 1 ml with distilled water, and added to 0.2 ml silver nitrate. The tubes were vortexed vigorously and 3 ml *n*-butyl alcohol saturated with water was added. Each tube was vortexed for 1 min and then centrifuged at 5000 rpm for 10 min. The organic layer was removed followed by further extraction of the residue with 2 ml *n*-butyl alcohol. The tubes were vortexed for 30 s and then centrifuged for 10 min again at 5000 rpm. The second organic layer was removed and pooled with the first. The butanol extract was read in a spectrophotofluorometer at 470 nm excitation and 585 nm emission. Fresh doxorubicin and epirubicin samples were prepared in n-butyl alcohol each day for calculation of the concentration of drugs in the samples.

Assessment of the possible modulatory effect of protein malnutrition on the degree of cardiotoxicity of a single dose of doxorubicin and epirubicin

Sixty male albino rats were classified randomly into two groups and subjected to one type of nourishment (either standard protein diet or low-protein diet) for three weeks. Then each group was further classified into three groups and given a single intraperitoneal injection of one of the following: doxorubicin (15 mg/kg), epirubicin (15 mg/kg) or an equal volume of saline. Rats were anaesthetized by a single dose of chloral hydrate 300 mg/kg and blood samples were collected from the retro-orbital sinus of the eye into Eppendorf tubes at 0, 10 and 30 min, and 1, 2, 4, 6 and 24 h after drug injection. Serum samples were immediately obtained by centrifugation at 3000 rpm for 10 min. After withdrawal of the last blood sample, the rats were killed by cervical dislocation and the hearts were dissected out and immersed in 10% formalin for histopathological examination. Serum creatine kinase was analysed using a ready-made kit according to the method of Szasz.[23] This depends on reactivation of creatine kinase activity with *N*-acetyl-L-cysteine. Creatine kinase specifically catalyses the transphosphorylation of adenosine diphosphate (ADP) to adenosine triphosphate (ATP). Through a series of coupled enzymatic reactions, NADH is produced at a rate directly proportional to the creatine kinase activity.

In both parts of the study, 20 rats from the normal protein diet (NF) and protein malnutrition groups were randomly selected, body weight was measured and blood samples were withdrawn and used for measurement of serum albumin and serum total protein using ready-made kits obtained from Stanbio Laboratory Inc. (San Antonio, USA).

Statistical analysis

Pharmacokinetic parameters were calculated according to the general equation for a two-compartment open model: $C_t = Ae^{-\alpha t} + Be^{-\beta t}$, Where C_t is the plasma concentration at time t, A and B are the coefficient of the exponential terms of the concentration in plasma, and α and β are the hybrid rate constants of disposition. Both hybrid rate constants (α and β) and the initial and terminal half-life were determined by least squares linear regression analysis of the log-transformed data (method of residuals).

Clearance was calculated as dose/AUC. Area under serum drug concentration-time curve was calculated using the trapezoidal rule from time 0 to $\propto$ ($AUC_{0-\propto}$). Similarly, area under the serum creatine kinase concentration–time profile was calculated using the trapezoidal rule from time 0 to 24 h (AUC_{CK0-24}) as described by Vora and Boroujerdi.[24]

Data are presented as means ± SD. Individual groups were compared using the two-tailed Student's *t*-test as appropriate. Multiple group comparisons were carried out using either one-way analysis of variance followed by Tukey–Kramer test for post-hoc analysis or two-way analysis of variance followed by Bonferroni test as appropriate. Statistical significance was accepted at a level of $P < 0.05$. The data were analysed using Prism software program (GraphPad software incorporated, version 2).

Results

Effect of protein malnutrition on body weight and serum albumin and total protein

After three weeks, feeding rats with normal protein diet (20%) significantly increased their body weight by 35% as compared with their initial body weight. On the other hand, feeding rats with low-protein diet (5%) caused a significant decrease in their body weight by 18% as compared with their initial body weight (Table 1). In addition, a significant alteration in serum albumin and total protein level was observed in rats fed 5% low-protein diet as compared with NF rats. In the protein malnutrition group, serum albumin and total protein were decreased by 26% and 11%, respectively, as compared with the NF group (Table 1).

Effect of protein malnutrition on pharmacokinetic parameters and constants of a single dose of doxorubicin and epirubicin (15 mg/kg, i.p.)

The concentrations of doxorubicin and epirubicin in serum were determined and, according to two-compartmental

Table 1 Effect of protein malnutrition on rat body weight and serum albumin and total protein

	Animal body weight			
Type of nourishment	Initial weight	Final weight	Serum albumin (g/dl)	Serum total protein (g/dl)
NF (20% casein)	126.5 ± 11.05	170.7* ± 14.89	4.83 ± 0.89	7.14 ± 0.72
PMN (5% casein)	133.8 ± 11.85	110.3* ± 6.98	3.58[a] ± 0.54	6.34[a] ± 0.54

Rats were fed either normal protein diet (NF) or low protein diet (PMN) for three weeks. Data is expressed as mean ± SD, $n = 20$. *$P \leq 0.05$ compared with the corresponding initial weight using paired Student's *t*-test. [a]$P \leq 0.05$ compared with the corresponding normally fed group at using unpaired Student's *t*-test.

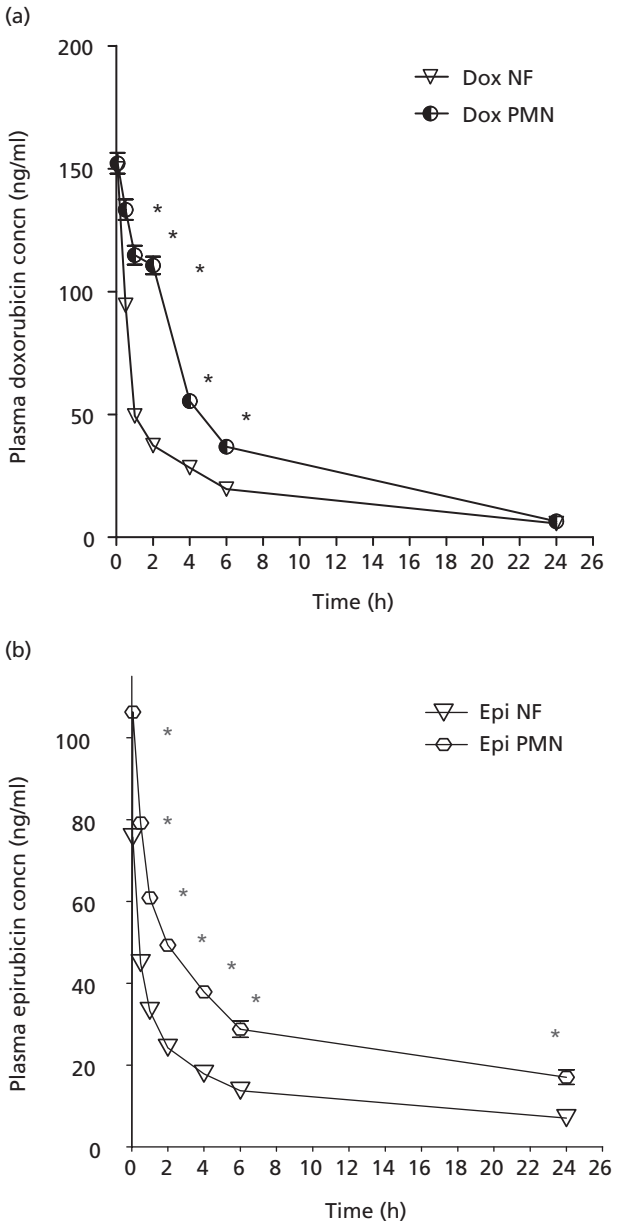

Figure 1 Comparison of the serum concentration–time profile of a single intraperitoneal dose (15 mg/kg) of either doxorubicin; Dox (a) or epirubicin; Epi (b) in normal fed (NF) and protein-malnourished (PMN) rats. Each group includes 15 rats. $*P \leq 0.05$ compared with corresponding normally fed group using unpaired Student's *t*-test.

analysis, the pharmacokinetic parameters and constants were estimated. There was a slow decline and significant increase in plasma doxorubicin and epirubicin concentration in protein-malnourished rats as compared with NF rats (Figure 1a and 1b). A significant alteration in doxorubicin and epirubicin pharmacokinetics and constants were observed in protein-malnourished rats as compared with the NF group (Table 2). Among these changes, one of the most important was the significant decrease in the total body clearance (by 50%) concomitant with a significant increase in AUC by two fold for both drugs (Table 2).

Effect of protein malnutrition on tissue distribution of a single dose of doxorubicin and epirubicin (15 mg/kg, i.p.)

Concerning doxorubicin tissue distribution, protein malnutrition significantly increased the doxorubicin level in the heart, liver and kidney by 43, 40 and 16%, respectively, as compared with the NF group. On the other hand, protein malnutrition significantly decreased doxorubicin concentration in spleen and lung by 5 and 9%, respectively, as compared with the NF rats (Table 3).

Regarding epirubicin-treated rats, protein malnutrition significantly increased epirubicin level in lung and kidney by 25 and 13%, respectively, and significantly decreased epirubicin level in spleen by 16% as compared with the NF group. In addition, there was a non-significant increase in epirubicin level in both heart and kidney (Table 3).

Effect of protein malnutrition on serum creatine kinase of rats treated with a single dose of either doxorubicin or epirubicin (15 mg/kg, i.p.)

Serum creatine kinase levels were determined at different time intervals and the area under the serum concentration–time curve (AUC_{ck}) and its maximum peak concentration was chosen as toxicodynamic parameters in accordance with the study conducted by Vora and Boroujerdi.[24]

Firstly, rats fed low-protein diet showed a significant increase in serum concentrations of creatine kinase as compared with the NF group. Also, the protein-malnourished group showed a significant increase in both maximum serum creatine kinase concentration and $AUC_{CK0–24}$ by 68 and 61%, respectively, as compared with the NF group (Figure 2a and 2b). Treatment of NF rats with a single injection of doxorubicin induced a significant increase in maximum serum creatine kinase concentration and $AUC_{CK0–24}$ by 3.5 and two fold, respectively, as compared with the NF group (Table 4). Although treatment of NF rats with a single injection of epirubicin showed a significant increase in maximum serum creatine kinase concentration and $AUC_{CK0–24}$, the effect of doxorubicin was significantly higher by approximately two fold as compared with the epirubicin group (Table 4). Treatment of protein-malnourished rats with a single injection of either doxorubicin or epirubicin induced a significant increase in cardiotoxicity indices as compared with the NF and protein-malnourished control groups (Figure 2; Table 4).

Table 2 Comparison of pharmacokinetic parameters and constants of a single dose of doxorubicin and epirubicin in normally fed (NF) and protein-malnourished (PMN) rats

Pharmacokinetic constants	Doxorubicin (NF)	Doxorubicin (PMN)	Epirubicin (NF)	Epirubicin (PMN)
Coefficient of first exponential, A (μg/l)	177.41 ± 6.16	100.60[a] ± 11.08	68.10 ± 6.00	85.22[a] ± 6.55
Coefficient of second exponential, B (μg/l)	29.35 ± 1.82	66.13[a] ± 7.75	17.20 ± 1.24	34.21[a] ± 2.32
First hybrid rate constant, α (1/h)	2.11 ± 0.31	0.59[a] ± 0.15	1.49 ± 0.23	1.15[a] ± 0.15
Second hybrid rate constant, β (1/h)	0.07 ± 0.012	0.10[a] ± 0.008	0.04 ± 0.008	0.03[a] ± 0.004
Initial half-life, $t_{1/2\alpha}$ (h)	0.33 ± 0.04	1.23[a] ± 0.31	0.48 ± 0.08	0.61[a] ± 0.08
Terminal half-life, $t_{1/2\beta}$ (h)	10.31 ± 1.55	7.17[a] ± 0.62	18.90 ± 2.67	24.20[a] ± 2.67
Overall elimination rate constant, k_{13} (1/h)	0.43 ± 0.039	0.20[a] ± 0.019	0.17 ± 0.019	0.10[a] ± 0.015
Total body clearance, CL (l/h/kg)	31.91 ± 2.91	16.81[a] ± 1.36	44.82 ± 2.36	21.61[a] ± 1.20
Area under plasma concentration-time curve, AUC (μg h/l)	473.21 ± 42.49	897.10[a] ± 70.60	335.87 ± 18.24	696.47[a] ± 38.34

Data is expressed as mean ± SD, $n = 15$. [a]$P \leq 0.05$ compared with the corresponding normally fed group using unpaired Student's *t*-test.

Table 3 Tissue distribution and disposition of doxorubicin and epirubicin in normally fed (NF) and protein-malnourished (PMN) rats

Tissue	Doxorubicin (NF)	Doxorubicin (PMN)	Epirubicin (NF)	Epirubicin (PMN)
Heart	19.7 ± 2.71	28.2[a] ± 3.76	16.5 ± 5.23	20.9 ± 6.43
Liver	20.2 ± 1.43	23.5[a] ± 0.81	12.8 ± 4.22	17.4 ± 7.71
kidney	18.6 ± 5.15	26.1[a] ± 4.57	10.6 ± 0.97	12.0[a] ± 1.55
Spleen	29.5 ± 2.25	28.1[a] ± 0.97	22.1 ± 2.32	18.5[a] ± 2.98
Lung	23.5 ± 2.29	21.4[a] ± 3.18	11.3 ± 1.28	14.1[a] ± 2.60

Each group includes 15 rats fed with either a normal protein diet (NF, 20%) or low-protein diet (PMN, 5%). Doxorubicin and epirubicin groups were given a single dose of 15 mg/kg, intraperitoneally. The rats were sacrificed after 24 h of treatment. The tissues were dissected out, immersed in ice-cold saline then homogenized (10% w/v) in saline. Data is represented as means ± SD. [a]$P \leq 0.05$ compared with the corresponding normally fed group using unpaired Student's *t*-test.

Table 4 Effect of a single injection (15 mg/kg) of doxorubicin or epirubicin on serum creatine kinase in normally fed (NF) and protein-malnourished (PMN) rats

Parameter	Treatment	NF	PMN
Peak creatine kinase concentration(U/l)	Control	298.5 ± 10.59	502.0[a] ± 11.57
	Doxorubicin	1336.9[a,b] ± 65.71	2312.0[a,b,c] ± 96.23
	Epirubicin	456.8[a,b,c,d] ± 34.50	983.8[a,b,c,d,e] ± 50.47
AUC_{CK} (U h/l)	Control	6.15 ± 0.13	9.90[a] ± 0.19
	Doxorubicin	18.16[a,b] ± 0.60	28.10[a,b,c] ± 0.66
	Epirubicin	9.10[a,b,c,d] ± 0.19	16.20[a,b,c,d,e] ± 0.63

Data was expressed as mean ± SD (each group includes 10 rats). Blood samples were collected from the retro orbital sinus of eye at 0, 1, 2, 4, 6 and 24 h after drug injection. [a,b,c,d,e]Significantly different from the corresponding control NF, control PMN, Doxorubicin NF, Doxorubicin PMN and Epirubicin NF, respectively, at $P \leq 0.05$ using two-way analysis of variance followed by Bonferroni test as a post-hoc test.

Effect of protein malnutrition on the histopathology of heart tissue of rats treated with a single dose of doxorubicin or epirubicin (15 mg/kg, i.p.)

Histopathological examination of heart tissues obtained from NF control rats showed normal histopathological structure of the myocardium (Figure 3a). In contrast, control rats fed on a low-protein diet for three weeks showed focal inflammatory cell infiltration in the hyalized myocardium (Figure 3b). Examination of heart specimens of rats fed with a normal protein diet and injected with a single dose of doxorubicin showed hyalization and degeneration of the myocardial muscle cells with focal fibrosis in between (Figure 3c and 3d). On the contrary, examination of heart specimens of rats fed with a normal protein diet and injected with a single dose of epirubicin showed no histopathological alterations (Figure 3g).

Treatment of protein-malnourished rats with a single dose of doxorubicin showed marked histopathological changes of the myocardium. There was severe congestion and dilatation of myocardial blood vessels associated with hyalinization and hypertrophy in the myocardial muscle cells (Figure 3e and 3f). Furthermore, degeneration and hyalization of myocardial muscle cells was detected in protein-malnourished rats injected with a single dose of epirubicin (Figure 3h).

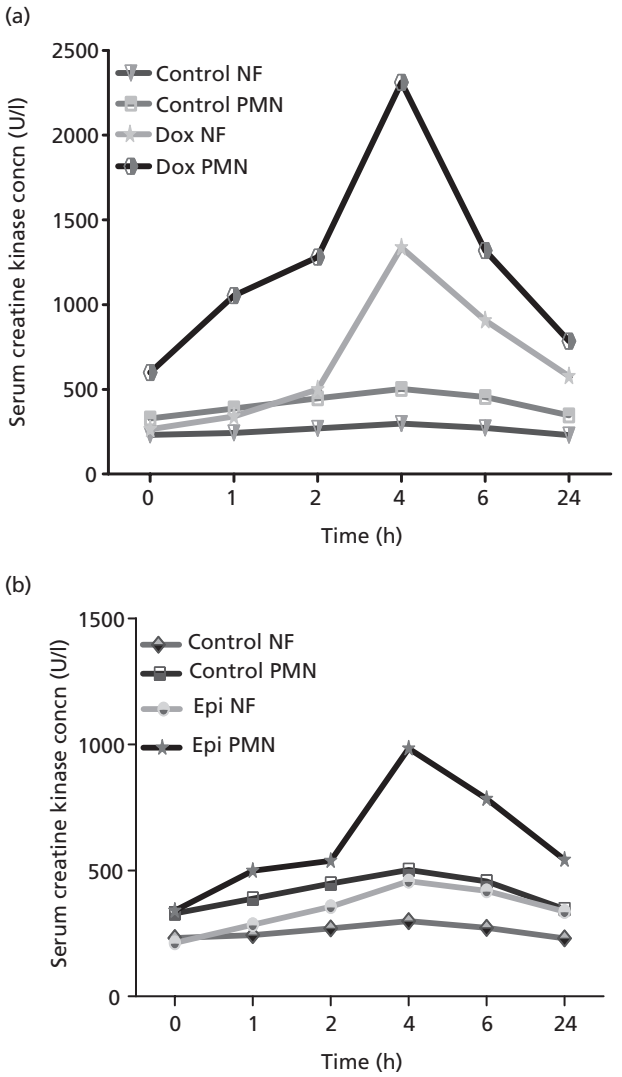

Figure 2 Profile of serum creatine kinase of normal fed (NF) and protein-malnourished (PMN) rats treated with a single intraperitoneal dose (15 mg/kg) of either doxorubicin; Dox (a) or epirubicin; Epi (b). Each group includes 10 rats.

Discussion

Initially, the model of protein malnutrition was successfully established by feeding rats a low-protein diet (5%) according to the study of Oumi *et al*.[25] The protein-malnourished group showed a significant decrease in their body weight by 18% as compared with their initial body weight and a significant decrease in serum albumin and total protein by 39 and 45%, respectively, as compared with NF rats. Our results are in accordance with previous studies.[26,27]

In the second part of the study, we used a uniform experimental protocol to evaluate the pharmacokinetic and toxicodynamic differences between doxorubicin and epirubicin. So, a single intraperitoneal dose of 15 mg/kg of doxorubicin or epirubicin was injected to rats fed with either a normal protein diet (20%) or low-protein diet (5%). Serum doxorubicin and epirubicin concentration–time profiles were analysed and showed significant alterations in pharmacokinetic parameters and constants in protein-malnourished rats compared with NF rats. Among to these changes, one of the most important was the significant decrease in the total body clearance concomitant with a significant increase in AUC, which was detected for both doxorubicin and epirubicin as compared with the corresponding NF group. These significant alterations may be explained as protein-malnourished rats were reported to exhibit 60–80% suppression in the hepatic microsomal cytochrome P450 levels as compared with NF rats.[9] In the study of Cusack *et al*.,[28] the authors found that the metabolism of doxorubicin and possibly its primary circulating alcohol metabolite doxorubicinol (Dox-ol), was decreased by low-protein diet. Also, Kim *et al*.[29] found that AUC_{0-12h} of doxorubicin tended to be greater by 42% in rats fed with 5% protein as compared with NF rats.

In addition, this study revealed a great difference between doxorubicin and epirubicin pharmacokinetic constants, with the former having higher serum concentration and AUC and a lower rate of body clearance than the latter. Ramanathan-Girish and Boroujerdi[30] found a stronger interaction between doxorubicin and blood cells compared with epirubicin and so they detected a higher AUC for multiple doses of doxorubicin compared with epirubicin. The authors suggested that the continual increase in AUC of doxorubicin associated with blood cells may play a significant role in the cardiotoxicity of doxorubicin. Further studies are warranted to explore whether a low-protein diet would alter the plasma protein binding of doxorubicin.

The changes in the pharmacokinetics of doxorubicin and epirubicin significantly reflected on their tissue distribution, particularly in the heart. It is important to mention that this study is the first one examining the effect of protein malnutrition on the tissue distribution of doxorubicin and epirubicin. Since anthracyclines produce exposure-dependent cardiotoxicity,[10] interventions that increase the duration of the exposure of cardiac tissue to anthracyclines may increase the extent of cardiac injury. Thus, the findings of an increase in the heart level of doxorubicin and, to lesser extend the level of epirubicin, could highlight an important risk factor for anthracycline-induced cardiomyopathy in protein-malnourished patients. Accordingly, the possible myocardial damage resulting from doxorubicin and epirubicin injection was investigated by measuring serum creatine kinase and determining the histopathological changes in the myocardium.

The results highlighted several important issues. Firstly, induction of protein malnutrition in normal rats caused a significant increase in maximum peak concentration of creatine kinase and its AUC_{ck}, findings that support the possible

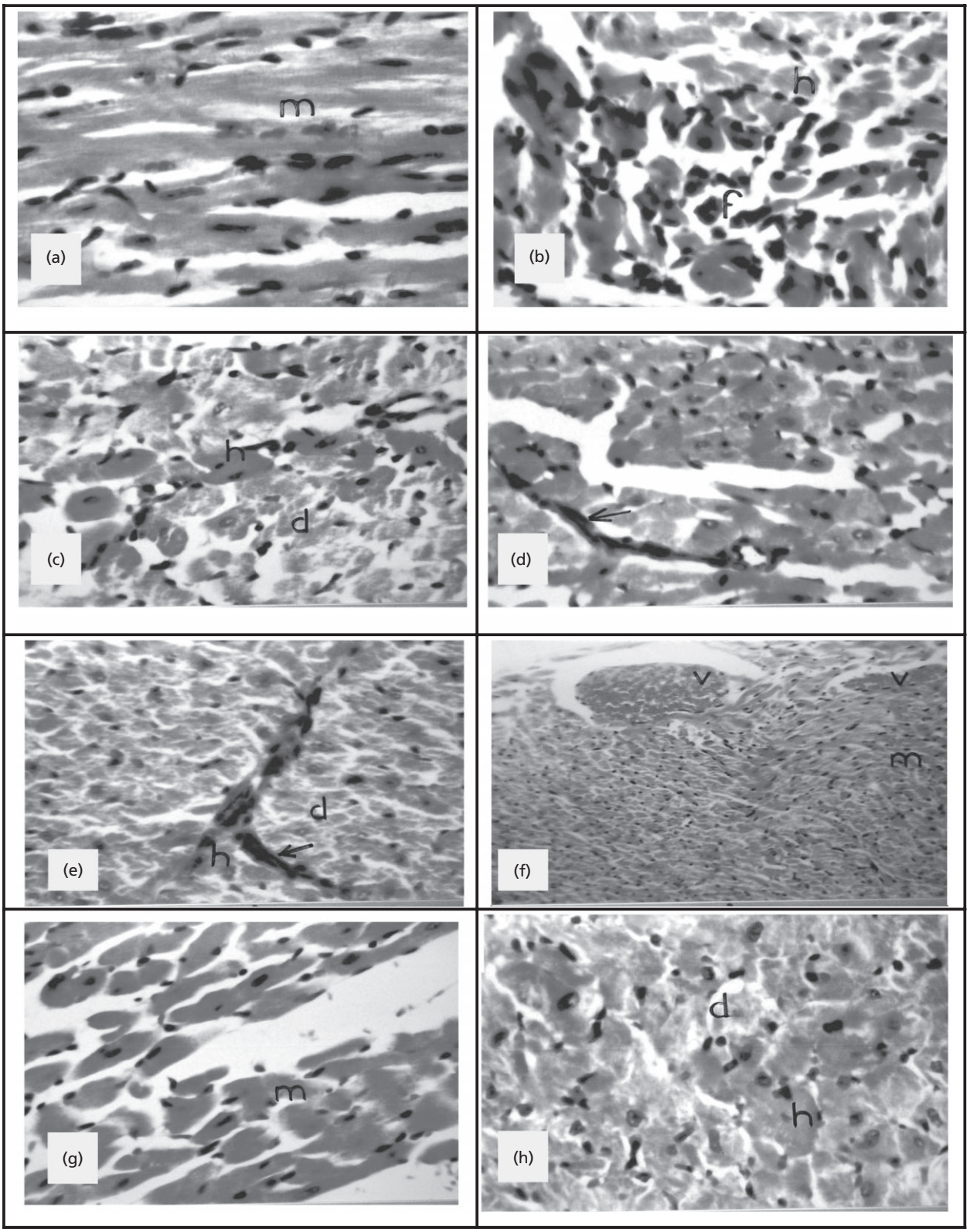

Figure 3 Photomicrographs of sections in the rat hearts stained with hematoxylin-eosin stain. (a) (×160) Representative section taken from heart of control group fed on normal protein diet (20%) for three weeks showing normal histopathological structure of the myocardium (m). (b) (×160) Representative section taken from heart of control rat group fed on low-protein diet (5%) for three weeks showing focal inflammatory cells infiltration.(f) and hyalization (h) in myocardium. (c, d) (×160) Representative sections taken from heart of rats fed on normal protein diet for three weeks and given a single intraperitoneal dose of doxorubicin (15 mg/kg). Section (c) shows hyalization (h) and degeneration (d) of the myocardium and section (d) shows focal fibrosis in myocardium (arrow). (e) (×160) & (f) (×40) Representative sections taken from heart of rat fed on low-protein diet for three weeks and given a single intraperitoneal dose of doxorubicin (15 mg/kg). Section (e) shows hyalization (h) and degeneration (d) of the myocardium and severe focal fibrosis (arrow) in between. Section (f) shows severe dilation and congestion of blood vessels (v) of the myocardium (m). (g) (×40) Representative section taken from heart of rat fed on normal protein diet for three weeks and given a single intraperitoneal dose of epirubicin (15 mg/kg). It shows normal histopathological structure of the myocardium (m). (h) (×160) Representative sections taken from heart of rat fed on low protein diet for three weeks and given a single intraperitoneal dose of epirubicin (15 mg/kg). It shows degeneration (d) and hyalization (h) of the myocardium.

role of protein malnutrition as a predisposing factor for cardiovascular disease. Indeed, a large body of evidence supports the association between hypoalbuminaemia, one of the findings resulting from protein malnutrition, and cardiovascular diseases.[17] In addition, Ramírez de Martens and coworker[31] demonstrated that rats fed with a low-protein diet showed a significant decrease in heart protein level with a significant increase in the mitochondrial proteins. These biochemical changes were correlated with ultrastructural myocardial changes such as a decrease in the myofibrils and abnormal configurations of the mitochondria.

Secondly, induction of protein malnutrition significantly increased the degree of cardiotoxicity not only for doxorubicin but also for epirubicin. Histopathological examination was performed to better observe and differentiate between doxorubicin and epirubicin cardiotoxicity regarding the type of nourishment. Doxorubicin was shown to cause a significantly greater degree of histopathological changes in the myocardium than epirubicin did. While doxorubicin-treated NF rats showed hyalization and degeneration with focal fibrosis of the myocardium, epirubicin-treated NF rats showed normal histological structure of the myocardium. So the increase in creatine kinase activity that was observed in epirubicin-treated NF rats was associated with a low degree of cardiotoxicity without any significant histological changes. The histological changes induced by doxorubicin in NF rats are in accordance with previous studies.[32,33] On the other hand, under the condition of low-protein diet, both drugs induced histopathological changes to the myocardium, which were still more severe and drastic in doxorubicin-treated rats than in epirubicin-treated rats.

Thirdly, it was obvious that the increase in serum creatine kinase for the two types of nourishment was higher in doxorubicin groups than in epirubicin groups (i.e. doxorubicin is still more cardiotoxic than epirubicin). It is important to mention that the equimolar dose ratio of doxorubicin to epirubicin for cardiotoxicity is 1 : 1.7–2.0.[34] This may explain why in our results a single dose of 15 mg/kg of doxorubicin in NF rats produced a significant change in the myocardium, while with the same dose of epirubicin, normal myocardial structure was seen.

Conclusions

This study demonstrates that dietary protein malnutrition significantly altered the pharmacokinetics of doxorubicin and epirubicin, with a significant decrease in their elimination, and prolonged the exposure of the heart to the drugs. Histopathological examination and biochemical measurement of creatine kinase supported the cardiotoxicity findings. If similar alteration in anthracycline pharmacokinetics occurs in malnourished cancer patients, this phenomenon may contribute to increased risk of developing cardiotoxicity associated with anthracycline therapy. Further study is required to evaluate the clinical importance of these observations and to determine whether anthracycline dose adjustment is required in nutritionally deprived patients.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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