Deferiprone Does Not Protect against Chronic Anthracycline Cardiotoxicity in Vivo

Olga Popelová, Martin Štěrba, Tomáš Šimůnek, Yvona Mazurová, Ivana Gunčová, Miloš Hroch, Michaela Adamcová, and Vladimír Geršl

Department of Pharmacology (O.P., M.S., M.H., V.G.), Department of Histology and Embryology (Y.M., I.G.), Department of Physiology (M.A.), Faculty of Medicine in Hradec Králové, and Department of Biochemical Sciences, Faculty of Pharmacy in Hradec Králové (T.Š.), Charles University in Prague, Hradec Králové, Czech Republic

Received February 5, 2008; accepted April 22, 2008

ABSTRACT

Anthracycline cardiotoxicity ranks among the most severe complications of cancer chemotherapy. Although its pathogenesis is only incompletely understood, “reactive oxygen species (ROS) and iron” hypothesis has gained the widest acceptance. Besides dexrazoxane, novel oral iron chelator deferiprone has been recently reported to afford significant cardioprotection in both in vitro and ex vivo conditions. Therefore, the aim of this study was to assess whether deferiprone 1) has any effect on the anticancer action of daunorubicin and 2) whether it can overcome or significantly reduce the chronic anthracycline cardiotoxicity in the in vivo rabbit model (daunorubicin, 3 mg/kg i.v., weekly for 10 weeks). First, using the leukemic cell line, deferiprone (1–300 μM) was shown not to blunt the antiproliferative effect of daunorubicin. Instead, in clinically relevant concentrations (>10 μM), deferiprone augmented the antiproliferative action of daunorubicin. However, deferiprone (10 or 50 mg/kg administered p.o. before each daunorubicin dose) failed to afford significant protection against daunorubicin-induced mortality, left ventricular lipoperoxidation, cardiac dysfunction, and morphological cardiac deteriorations, as well as an increase in plasma cardiac troponin T. Hence, this first in vivo study changes the current view on deferiprone as a potential cardioprotectant against anthracycline cardiotoxicity. In addition, these results, together with our previous findings, further suggest that the role of iron and its chelation in anthracycline cardiotoxicity is not as trivial as originally believed and/or other mechanisms unrelated to iron-catalyzed ROS production are involved.

Anthracyclines [e.g., doxorubicin, daunorubicin (DAU), and epirubicin] rank among the most effective antineoplastic agents ever developed. In clinical practice today, they are widely indicated in the treatment of a number of hematological and solid malignancies (Jones et al., 2006). However, their clinical potential is considerably hampered by the risk of severe dose-dependent cardiotoxicity, which manifests months or years after the completion of chemotherapy (Minotti et al., 2004). These chronic and delayed types of cardiotoxicity are characterized by continuous and irreversible myofibrillar loss, cytoplasmic vacuolization, and degeneration of cardiomyocytes that can ultimately result into dilated cardiomyopathy and congestive heart failure (Herman et al., 1997). Despite a number of theories proposed (Minotti et al., 2004; Chen et al., 2007), the precise molecular basis of this phenomenon still remains elusive. The prevailing hypothesis emphasizes the iron-catalyzed formation of reactive oxygen species (ROS) (Keizer et al., 1990). It is known that anthracyclines chelate free or loosely bound iron within the cardiomyocytes to form anthracycline-Fe\(^{3+}\) complexes, which can undergo cascade of reactions resulting in a production of extremely reactive and toxic hydroxyl radicals. In addition, anthracycline molecule can induce the production of superoxide radicals via redox cycling of the quinone-semiquinone ring of its aglycone. The superoxide produced through this route can dismutate to hydrogen peroxide, which may in turn enter the iron-catalyzed Haber-Weiss reaction, resulting again in an overproduction of hydroxyl radicals (Keizer et al., 1990). These can attack and damage all biomolecules (lipids, proteins, and nucleic acids) within their vicinity and thus promote the death of cardiac cells either by apoptosis or necrosis. A number of interventions have been proposed to prevent the anthracycline-induced ROS formation (Saad et al., 2001;
Oliveira et al., 2004; Bast et al., 2007). Nevertheless, so far, dexrazoxane (ICRF-187) is the only agent, which is clearly able to protect the myocardium from anthracycline-induced toxicity both in experimental and clinical settings (Wouters et al., 2005). Dexrazoxane is a prodrug, which is enzymatically hydrolyzed (inside the cardiomyocytes) to its active metal-chelating metabolite, ADR-925. This metabolite is believed to be responsible for the cardioprotective effects by displacing iron from its complex with anthracyclines and/or via chelation of intracellular labile iron pool (Hasinoff et al., 1998). However, dexrazoxane may potentiate the myelotoxicity of anthracyclines, which together with its high cost limit its wider use in clinical practice (van Dalen et al., 2006).

Deferiprone (Fig. 1, L1) is the first orally active iron chelator introduced into the clinical practice for the treatment of iron-overloaded patients (Kontoghiorghes et al., 2004). It is a synthetic bidentate chelator with a small molecular weight, binding specifically ferric iron in a 3:1 ratio. Under physiological conditions, it has a favorable lipophilicity and thus can readily enter the cardiomyocytes to reach therapeutic levels (Glickstein et al., 2006). It has been also shown to efficiently bind labile cellular iron, both free as well as accumulated, within mitochondria and lysosomes (Glickstein et al., 2006). Using an in vitro model of iron overload, L1 has been reported to significantly attenuate ROS formation within mitochondria and restore contractility impaired by iron loading (Link et al., 1996). L1 given orally on a daily basis has been also shown to effectively reduce myocardial iron burden and improve ventricular function in iron-overloaded patients with β-thalassemia (Anderson et al., 2002). With respect to anthracycline cardiotoxicity prevention, L1 has been recently shown as capable to protect isolated ventricular cardiomyocytes from doxorubicin-induced cellular toxicity (Barnabé et al., 2002). This effect has been attributed to its iron-chelating properties, which allow displacing the iron from its complexes with anthracyclines and/or reduce the availability of free iron for catalysis of the Haber-Weiss reaction. Moreover, a recent study performed on the isolated spontaneously beating atria further confirmed the cardioprotective potential of L1 against acute anthracycline cardiotoxicity (Xu et al., 2006).

To summarize, several lines of evidence indicated that L1, already a clinically approved drug, might be a promising and readily available option to increase the cardiac safety of cancer patients undergoing treatment with anthracyclines. The present investigation was undertaken to further the development of this drug as a potential cardioprotectant. Two main aims of this study were 1) to preliminarily assess whether deferiprone has any potential to blunt the anticancer efficacy of anthracyclines (as this could preclude practical utility of this approach) and 2) to examine the cardioprotective potential of this drug using a clinically relevant model of chronic anthracycline cardiotoxicity, which was previously validated with dexrazoxane.

**Materials and Methods**

**Proliferation Studies with HL-60 Cells**

HL-60 human acute promyelocytic leukemia cell line was obtained from American Type Culture Collection (Manassas, VA). Cells were maintained in RPMI-1640 medium (Sigma-Aldrich, Prague, Czech Republic) supplemented with 10% heat-inactivated fetal bovine serum (Sigma-Aldrich) and 1% penicillin/streptomycin (PAA Laboratories, Pasching, Austria) and grown in humidified atmosphere at 37°C in 5% CO₂. Medium was renewed every 2 to 3 days. For proliferation studies, the cells were seeded at a density of 10⁵ cells/ml. Tested substances (L1 and/or DAU) had been added, and cells were allowed to proliferate under standard conditions. The tested concentrations of L1 ranged from 1 to 300 μM. For combination assays, 12 nM DAU was used, which was previously shown to induce 50% growth inhibition. To quantify the number of viable cells after each treatment, suspension samples were taken and mixed 1:1 with 0.4% trypsin blue solution (Sigma-Aldrich), and the living ( unstained) cells were counted using a Bürker’s hemocytometer under a light microscope.

**Cardioprotection Studies in Rabbits**

**Animals.** Forty-two adult Chinchilla male rabbits of an average body weight of 3.44 ± 0.03 kg at the beginning of the experiment were housed under a 12-h light cycle and constant temperature and humidity. The animals had free access to tap water and a standard laboratory pellet diet. Before experimental procedures, the animals were fasted overnight. Administration of drugs, blood sampling, and noninvasive measurements during the study were carried out under ketamine (50 mg/kg, i.m.; Narkeetan inj., Vetoquinol AG, Switzerland) and midazolam (1.25 mg/kg, i.m.; Midazolam Torrex; Torrex Chiesi Pharma GmbH, Vienna, Austria) anesthesia. Pentobarbital (30 mg/kg i.v.; Sigma-Aldrich) was used for anesthesia during final invasive hemodynamic measurements and for an overdose of animals at the end of the experiment. All experiments were approved and supervised by the Ethical Committee of Charles University (Prague, Czech Republic) and the Faculty of Medicine (Hradec Králové, Czech Republic) and were in accordance Institute of Laboratory Animal Resources (1996).

**Experimental Design.** All substances were administered once a week for 10 weeks. The study was carried out with five groups of animals: 1) control group—animals were receiving saline (1 mL/kg i.v., n = 8; Natrium Chloratum, Biotika, Slovakia); 2) DAU group—animals were injected with DAU (3 mg/kg i.v., n = 11; Daunoblastina; Pharmacia Italia S.p.A., Nerviano, Italy) in a validated schedule for induction of chronic anthracycline cardiomyopathy (Simunek et al., 2004); 3) L1 group—animals received L1 (50 mg/kg p.o., in 0.5% carboxymethylcellulose, n = 6; kindly provided by ApoPharma Inc.); 4) L1 10 + DAU group (n = 8)—this group received L1 (10 mg/kg p.o., in 0.5% carboxymethylcellulose) 45 min before each DAU administration (3 mg/kg i.v.); and 5) L1 50 + DAU group (n = 9)—L1 (50 mg/kg p.o.) was administered before DAU in the same schematic as in the previous combination group.

Body weight was recorded weekly, whereas mortality, general appearance, and behavior were observed daily. Noninvasive echocardiographic measurements were performed at the beginning of the study and later in weeks 8, 9, and 10 and, finally, at the end of the study and later in weeks 8, 9, and 10 and, finally, at the end of the study.
experiment (5-7 days after the last administration of drugs). Plasma for cardiac troponin T (cTnT) determination was sampled before the first, fifth, eighth, and tenth administration and at the end of the study. Standard biochemical and hematological parameters were determined from blood sampled from the ear artery before the first and fifth administrations and at the end of the study. The experiment was terminated 5 to 7 days after the last administration when final invasive hemodynamic measurements were performed. Thereafter, the animals had been overdosed with pentobarbital, and an autopsy had followed. The heart of each animal was rapidly excised, washed in ice-cold saline, and briefly retrogradely perfused with cold saline through the aorta. Tissue blocks of the transversely sectioned left and right cardiac ventricles underwent histological examination. The rest of the left ventricle was snap-frozen in liquid nitrogen and kept frozen at −80°C until further analyzed.

**Noninvasive Cardiac Function Measurements.** Echocardiographic examination of the LV systolic function was carried out using a Vivid 4 echocardiograph (GE Medical Systems Ultrasound; GE Healthcare, Chalfont St. Giles, UK) equipped with a 10-MHz probe. The LV long axis view was obtained through the left parasternal approach, and a guided M-mode measurement at the tips of the mitral valve was performed. The LV fraction shortening (LVFS) was calculated from the LV end-diastolic (LVED) and end-systolic diameters (LVES) determined from at least four heart cycles in each M-mode examination as follows:

\[
\text{LVFS} (\%) = \frac{\text{LVED} - \text{LVES}}{\text{LVED}} \times 100
\]

At least three independent examinations were used to determine the individual LVFS values.

**Invasive Hemodynamic Measurements.** In pentobarbital anesthesia, the left carotid artery was prepared, and a Micro-Tip Pressure Catheter (2.3F Nylon; ADInstruments Pty Ltd., Castle Hill, Australia) was introduced into the left heart ventricle. After a 10-min equilibration period, the measurement of the following parameters was performed; the maximum of the first derivative of the LV pressure rise in the isovolumic phase of the systole (dP/dt\_max) and the minimum of the first derivative of the LV pressure decline in the isovolumic phase of diastole (dP/dt\_min) as well as the heart rate. For the arterial blood pressure measurements, a polyethylene cannula, filled in with heparinized (10 IU/ml) saline, was inserted into the right femoral artery. The ADI PowerLab/8SP (ADInstruments Pty Ltd., Castle Hill, Australia) with appropriate transducers and the Chart software 5.4.2 were used for pressure measurements, obtaining from derivatives and recordings.

**Cardiac Troponin T Determination.** cTnT, as a selective and sensitive marker of heart injury, was determined in heparinized plasma using an Elcysys Troponin T STAT Immunoassay (Roche Diagnostics, Basel, Switzerland) and an Elcysys 2010 (Roche Diagnostics) immunosassay analyzer with the detection limit of 0.010 ng/ml. The values below this detection limit were considered to be zero.

**Determination of Total Malondialdehyde in Myocardial Samples.** The samples of the LV myocardium were pulverized under liquid nitrogen. Radioimmunoprecipitation assay buffer (500 ng/ml) was added to the myocardial samples (~70 mg), and the mixture was homogenized and vortexed. After centrifugation (3000 rpm, 10 min, 4°C), the supernatant was removed, and 250 μl of supernatant was taken and analyzed according to the Piţa et al. (2000) with minor modifications. In brief, 50 μl of NaOH (6 M) were added to the taken amount of supernatant, and after vortexing, the solution was kept at 60°C for 30 min. The samples were then cooled on ice, and 125 μl of perchloric acid (35% (v/v)) was added. After centrifugation (13,000 rpm, 10 min, 4°C), 250 μl of supernatant was taken, and derivatization was performed using 25 μl of 5 mM 2,4-dinitrophenyhydrazine. After 10 min in the dark, the solution (30 μl) was analyzed using an high-performance liquid chromatography system (Shimadzu, Kyoto, Japan) with UV detection: column—EC Nucleosil 100-5 C18, 4.6 × 125 mm heated at 30°C; mobile phase—acetonitrile/water/acetic acid: 380/620/2 (v/v/v), pH 3.4; flow rate—1.0 ml/min, UV detector set on 310 nm.

**Histological Examination.** Tissue blocks of the transversely sectioned left and right cardiac ventricles were fixed for 3 days by immersion in 4% neutral formaldehyde. Paraffin sections (6 μm thick) were stained with hematoxylin-eosin and Mason’s blue trichrome. Photomicrographs were made with a Cybernetics software version 4.51 (Laboratory Imaging, Prague, Czech Republic).

**Biochemical and Hematological Analyses.** Standard biochemical parameters were determined in plasma/serum using an automatic analyzer (Hitachi 737; Hitachi, Tokyo, Japan) at the Institute of Clinical Biochemistry and Diagnostics, University Teaching Hospital (Hradec Králové, Czech Republic); hematological parameters were measured using an automatic analyzer Coulter T890 (Beckman Coulter, Fullerton, CA) at the Institute of Clinical Hematology, University Teaching Hospital. Content of iron in the left ventricular myocardium samples was determined at the Institute of Clinical Biochemistry and Diagnostics using graphite furnace atomic absorption spectrometry (Solaar 959; Thermo Fisher Scientific, Waltham, MA) as described previously (Simůnek et al., 2005b). The results are expressed as micromole per gram of dry tissue.

**Data Analysis.** Statistical software SigmaStat 3.5 (SPSS Inc., Chicago, IL) and STATISTICA Cz (StatSoft, Tulsa, OK) were used in this study. All data are expressed as mean ± S.E.M. Significances of the differences were determined using one-way ANOVA unpaired test (comparison between groups) or paired t test (comparison with the initial value within each group). For exploratory data analysis (Principal Component Analysis, Hierarchical Tree Clustering), data from all parameters (general toxicity, cardiovascular, biochemical, and hematological parameters) were employed.

**Results**

**Proliferation Studies with HL-60 Cells**

As seen in Fig. 2A, whereas the lower concentrations (1–30 μM) of L1 had no significant effect on the proliferation of HL-60 cells, its higher concentrations (>100 μM) were able to significantly decrease the tumor cell growth. The experiments examining a 72-h coincubation of L1 with DAU revealed that, within the broad and clinically relevant concentration range (1–300 μM), the chelator did not have any negative effect on the antitumor efficacy of DAU. Moreover, at the higher L1 concentrations (>10 μM), the augmentation of DAU-antiproliferative effects was observed (Fig. 2B).

**Cardioprotection Studies in Rabbits**

**General Observations.** No premature deaths and no changes in appearance or behavior were observed throughout the experiment in the control and L1 groups. In contrast, chronic DAU treatment resulted in 18% mortality. Coadministration of L1 (10 mg/kg) together with DAU was lethal for two of eight animals (25% mortality). A further (5-fold) rise of the L1 dose led to an even more pronounced and also earlier mortality. In both the DAU and L1 10+DAU groups, the mortality occurred only between the 9th and last weeks of the study, whereas in the group cotreated with the higher L1 dose, the first premature deaths occurred much earlier, between weeks 3 and 5 (Fig. 3). Before the deaths, the animals lost appetite; their body weight tended to stagnate or decrease and/or exhibited signs of lethargy. The body weight changes in all groups are shown in Fig. 4. There were no significant differences between the control and L1 groups. The body weights of animals in the DAU and L1 50+DAU groups were lower than in the control and L1 groups. “Heart
weight/body weight” ratio, determined at the end of experiment, was significantly higher in the DAU group (2.82 ± 0.10 g/kg) compared with both the control (2.14 ± 0.10 g/kg) and L1 (2.06 ± 0.14 g/kg) groups. In the L1+DAU combination groups, no significant difference was found in comparison with either the DAU or control groups (L1 10 DAU: 2.51 ± 0.14 g/kg; and L1 50 DAU: 2.70 ± 0.19 g/kg).

**Determination of Malondialdehyde in Myocardial Samples.** Malondialdehyde (MDA) was used as a marker of lipid peroxidation in the LV myocardium. As shown in Fig. 5, the MDA content was significantly increased in the DAU group. Although the MDA concentration was somewhat lower when L1 (10 mg/kg) was coadministered with DAU, the change did not reach the statistical significance. The escalation of the chelator dose did not improve the results, and MDA levels remained significantly higher than in controls and close to the DAU group.

**Echocardiography.** Left ventricular fractional shortening (FS) was gradually decreasing in the DAU group from 42.9 ± 0.6% at the beginning to 31.6 ± 2.3% at the end of the experiment; the decline was significant in comparison with the initial values as well as with the control group (Fig. 6). In both doses of L1 coadministered with DAU, the LV fractional shortening values did not differ significantly from the DAU group. Although the combination with the lower L1 dose tended to have somewhat better results than the combination with the higher dose, no significant difference between these values was detected. In addition, in both groups, the decline in the fractional shortening was significant compared with the initial and the control values.
Invasive Hemodynamic Measurements. Invasively determined indexes of both the LV contractility (dP/dt max) and relaxation (dP/dt min) were significantly lower in the DAU than in the control group (Fig. 7). Concurrent administration of L1 in both doses resulted in similar outcomes with regard to both LV contractility and relaxation; no statistical differences were observed between these groups. A similar trend was determined in arterial blood pressure and heart rate values (Table 1).

Cardiac Troponin T Determination. Ten weeks of repeated administration of DAU led to progressive and significant elevation of cardiac troponin T levels, starting with the 8th week (Fig. 8). Correspondingly, cotreatment with either dose of L1 induced very similar changes in plasma concentrations of cTnT as in the DAU group.

Histological Examination. Histological examination (Fig. 9) of myocardial samples obtained from both the control and the L1 group revealed a comparable morphological picture, which comprised mainly less intensive, acute, and scattered changes (some groups of cardiac cells exhibited increased eosinophilia of the cytoplasm, or pyknotic nuclei, which is likely to be associated with protracted anesthesia performed at the end of experiment). On the other hand, DAU treatment induced a typical massive toxic focal damage predominantly in the LV myocardium. A large number of cardiomyocytes revealed a different level of degeneration resulting in necrosis, whereas most of others have at least intensely eosinophilic cytoplasm due to the myofibrillar damage. Necrotic cardiomyocytes were gradually replaced by the granulation tissue, and the interstitial myofibrosis subsequently developed. In both groups cotreated with L1, a similar pattern of myocardial changes as in the DAU group was observed. The toxic damage tended to be somewhat less pronounced in the group cotreated with the lower chelator dose, whereas the changes in the myocardium of animals cotreated with the higher chelator dose closely resembled those induced by DAU alone.

Biochemical and Hematological Analyses. Repeated DAU administration caused significant changes in the plasma biochemical parameters, mainly related to the renal damage (e.g., increase in creatinine, decrease in total plasma protein) and impairment in lipid metabolism (Table 2). Similar results were also observed in the groups cotreated with L1. As seen in Table 3, the DAU administration caused also a significant decrease in erythrocyte and thrombocyte counts, a concentration of hemoglobin and in hematocrit. Most of the hematological parameters under study tend to change similarly in the groups cotreated with either dose of deferiprone. L1 alone did not induce any abnormalities in biochemical as well as hematological parameters.

With regard to myocardial iron content, no significant changes between the daunorubicin and control group were detected (2.2 ± 0.2 and 2.5 ± 0.4 μmol/g, respectively). However, significantly higher values were found in the L1-treated (50 mg/kg) and in the L1- (50 mg/kg) and DAU-cotreated animals (4.2 ± 0.7 and 4.9 ± 0.5 μmol/g, respectively, p ≤ 0.05), whereas only insignificant difference was determined in the group cotreated with the lower dose of the chelator (3.4 ± 0.3 μmol/g).

Exploratory Data Analysis. Hierarchical tree clustering (Fig. 10A) and principal component analysis (Fig. 10B) are two independent exploratory multivariate statistical methods, which were used to identify the natural grouping of 42 individual animals in this study with respect to all evaluated parameters [general toxicity, cardiovascular, biochemical, and hematological parameters (25 variables)]. Both exploratory data analyses grouped all examined objects into two well separated clusters. In the first cluster (I), all animals from the control and L1 groups were found, whereas the
individuals from the other groups (DAU, L1 10+DAU, and L1 50+DAU) were arranged into the second cluster (II).

Discussion

Theoretical assumptions as well as recent in vitro/ex vivo results (Barnabé et al., 2002; Xu et al., 2006) have strongly suggested that L1, a novel oral iron chelator, could be a rational and promising alternative to dexrazoxane in cardioprotective indications. To further explore therapeutic potential of L1, there was a need to eliminate a possibility that it can attenuate the antiproliferative efficacy of anthracyclines. Hence, using a leukemic cell line, we have demonstrated that this does not take place. In contrast, L1 alone was capable of significantly reducing proliferation of leukemic cells in the concentrations, which might be achieved in vivo. Furthermore, when HL-60 cells were treated with DAU (IC50) together with different concentrations of L1, it was obvious that the chelator did not possess any potential to blunt the antiproliferative effect. Instead, in higher doses of L1, the summation of antiproliferative effects of both compounds was observed. These outcomes are in good agreement with other reports describing antiproliferative effects of L1 (Yasumoto et al., 2004) and other iron-chelating compounds (Yu et al., 2006). Several lines of evidences suggest that inhibition of ribonucleotide reductase can be involved in this matter (Green et al., 2001), although other mechanisms have been also proposed. Different iron chelators have been shown to cause cell cycle arrest and apoptosis and antimetastatic and antiangiogenic effects (Richardson, 2005). Using L1 and HL-60 leukemic cell line, it has been shown that chelator treatment induces apoptosis via both intrinsic (mitochon-

dria-mediated) and extrinsic pathways (Yasumoto et al., 2004). Furthermore, recently, it has been reported that L1 anticancer effects can be related to the deregulation of the polyamine metabolism. It has been suggested L1 anticancer effects are associated with induction of spermidine/spermine N1-acetyltransferase pathway leading to higher putrescine levels, resulting in the cell cycle arrest in G2-M phase (Lescoat et al., 2007).

In the major part of the study, we aimed to assess whether L1 cotreatment can overcome or significantly diminish chronic anthracycline cardiotoxicity. For this purpose, a clinically relevant dexrazoxane-validated rabbit model of chronic anthracycline cardiotoxicity was used (Simunek et al., 2004). L1 was administered orally in two doses of 10 and 50 mg/kg, 45 min before each DAU injection. It is surprising that, in both doses, L1 was unable to reduce anthracycline-induced mortality. Moreover, the higher L1 dose (50 mg/kg) actually led to earlier deaths, which can probably be explained by the occur-

<table>
<thead>
<tr>
<th>Group</th>
<th>BP</th>
<th>HR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>101.0 ± 3.9</td>
<td>312.3 ± 9.6</td>
</tr>
<tr>
<td>L1 (50 mg/kg)</td>
<td>107.3 ± 2.5</td>
<td>335.8 ± 10.4</td>
</tr>
<tr>
<td>DAU</td>
<td>88.9 ± 3.8d</td>
<td>284.0 ± 12.3d</td>
</tr>
<tr>
<td>L1 (10 mg/kg) + DAU</td>
<td>91.3 ± 2.4d</td>
<td>275.3 ± 12.9d</td>
</tr>
<tr>
<td>L1 (50 mg/kg) + DAU</td>
<td>86.4 ± 6.1d</td>
<td>283.9 ± 3.2d</td>
</tr>
</tbody>
</table>

BP, blood pressure; HR, heart rate.

FIG. 7. Index of systolic (dP/dtmax) and diastolic (dP/dtmin) function of the left ventricle at the end of the experiment. Data presented as mean ± S.E.M. Statistical significances (ANOVA, P < 0.05) in comparison with control (c) group and deferiprone (d) group are given.

FIG. 8. cTnT plasma concentrations during the experiment. Statistical significances (ANOVA, P < 0.05) in comparison with control (c) and deferiprone (d) group are given.
merous reports published so far (Lebrecht et al., 2007). How-

tion) following DAU administration, which is in line with nu-

clearly demonstrated that L1 is not able to protect the myocar-

dience of extracardiac toxicity, the nature of which could not be

iene. Most importantly, we have also clearly demonstrated that L1 is not able to protect the myocardium against either anthracycline-induced oxidative stress or LV cardiac damage and heart failure. Using a validated high-

performance liquid chromatography method, we have found marked elevation of MDA (a standard marker of lipoperoxida-

tion) following DAU administration, which is in line with nu-

merous reports published so far (Lebrecht et al., 2007). How-

ever, we have surprisingly failed to detect a significant impact of L1 on this parameter in either dose. This evidently con-

dicts the results provided in previous reports (Barnabé et al., 2002; Xu et al., 2006). Nevertheless, both the latter studies could have certain limitations with respect to translatability of their results to the clinically relevant situation. In the first study, although L1 has been shown to reduce the anthracycline-

induced ROS production using electron paramagnetic reso-

nance spectroscopy, this was not followed within the biological system and particularly not in the chronic setting. In second case, myocardial injury was induced with an acute exposure of the isolated atria (which are not the main target for anthracyclines) to relative high and clinically unachievable doxorubicin concentration (30 μM).

The inability of L1 to overcome DAU-induced oxidative stress was further supported by the outcomes of functional LV examination, performed using both echocardiography and LV catheterization. Furthermore, L1 exerted no effect on DAU-induced plasma concentrations cTnT, which is a very sensitive and selective biochemical marker of cardiac injury (Sterba et al., 2007b). The good agreement of these results was further supported by similar findings from the histopathological examination of the myocardium. On the other hand, repeated administration of L1 alone (in the higher dose of 50 mg/kg) was well tolerated, and no abnormalities were found in the most of the parameters evaluated, which corre-

sponds with a preclinical investigation of L1 performed on a

number of species (Porter et al., 1990). The empiric assump-

tions given above were further supported by two independent exploratory data analyses. These have clearly revealed that L1 had shown no clear tendency to affect the general toxicity, cardiovascular, hematological, and plasma biochemistry pa-

rameters. The only exception was found in the case of the higher myocardial iron content in the animals treated or cotreated with the higher dose of the chelator. This finding was rather surprising in the light of the proved efficacy of this compound to mobilize iron from the myocardium of iron-overloaded animals and patients upon regular daily treat-

ment (Anderson et al., 2002; Wood et al., 2006). Furthermore, the biological half-life (approximately 2 h) of L1 excludes any potential direct effect of the compound on this parameter a week after its last dose. Hence, one can only reasonably explain these findings like a certain adaptive reaction of myocardium on the repeated intermittent iron chelation. Most importantly, these changes have not been associated with increased oxidative stress or other correlating myocardial disturbances, which indicates that this compensatory reaction was instead within a physiological range.

The results of this study strongly suggest that oral treat-

ment with L1 has no beneficial effect on chronic anthracy-

cline cardiotoxicity, which certainly raises several questions. One may speculate that the failure of L1 could have been

associated with its poor or erratic bioavailability. However, it

should be noted that L1 is characterized with very good oral bioavailability; it is fairly well absorbed from the gastroin-

testinal tract in humans as well as in rabbits (Fredenburg et

al., 1993). Furthermore, the doses and timing of administra-

tion were designed according to the pharmacokinetic study

performed on a species (Porter et al., 1990). The empiric assump-
tions given above were further supported by two independent exploratory data analyses. These have clearly revealed that L1 had shown no clear tendency to affect the general toxicity, cardiovascular, hematological, and plasma biochemistry parameters. The only exception was found in the case of the higher myocardial iron content in the animals treated or cotreated with the higher dose of the chelator. This finding was rather surprising in the light of the proved efficacy of this compound to mobilize iron from the myocardium of iron-overloaded animals and patients upon regular daily treat-

ment (Anderson et al., 2002; Wood et al., 2006). Furthermore, the biological half-life (approximately 2 h) of L1 excludes any potential direct effect of the compound on this parameter a week after its last dose. Hence, one can only reasonably explain these findings like a certain adaptive reaction of myocardium on the repeated intermittent iron chelation. Most importantly, these changes have not been associated with increased oxidative stress or other correlating myocardial disturbances, which indicates that this compensatory reaction was instead within a physiological range.

The results of this study strongly suggest that oral treat-

ment with L1 has no beneficial effect on chronic anthracy-

cline cardiotoxicity, which certainly raises several questions. One may speculate that the failure of L1 could have been

associated with its poor or erratic bioavailability. However, it

should be noted that L1 is characterized with very good oral bioavailability; it is fairly well absorbed from the gastroin-

testinal tract in humans as well as in rabbits (Fredenburg et

al., 1993). Furthermore, the doses and timing of administra-

tion were designed according to the pharmacokinetic study

performed on a species (Porter et al., 1990). The empiric assump-
tions given above were further supported by two independent exploratory data analyses. These have clearly revealed that L1 had shown no clear tendency to affect the general toxicity, cardiovascular, hematological, and plasma biochemistry parameters. The only exception was found in the case of the higher myocardial iron content in the animals treated or cotreated with the higher dose of the chelator. This finding was rather surprising in the light of the proved efficacy of this compound to mobilize iron from the myocardium of iron-overloaded animals and patients upon regular daily treat-

ment (Anderson et al., 2002; Wood et al., 2006). Furthermore, the biological half-life (approximately 2 h) of L1 excludes any potential direct effect of the compound on this parameter a week after its last dose. Hence, one can only reasonably explain these findings like a certain adaptive reaction of myocardium on the repeated intermittent iron chelation. Most importantly, these changes have not been associated with increased oxidative stress or other correlating myocardial disturbances, which indicates that this compensatory reaction was instead within a physiological range.

The results of this study strongly suggest that oral treat-

ment with L1 has no beneficial effect on chronic anthracy-

cline cardiotoxicity, which certainly raises several questions. One may speculate that the failure of L1 could have been

associated with its poor or erratic bioavailability. However, it

should be noted that L1 is characterized with very good oral bioavailability; it is fairly well absorbed from the gastroin-

testinal tract in humans as well as in rabbits (Fredenburg et

al., 1993). Furthermore, the doses and timing of administra-

tion were designed according to the pharmacokinetic study

performed on a species (Porter et al., 1990). The empiric assump-
tions given above were further supported by two independent exploratory data analyses. These have clearly revealed that L1 had shown no clear tendency to affect the general toxicity, cardiovascular, hematological, and plasma biochemistry parameters. The only exception was found in the case of the higher myocardial iron content in the animals treated or cotreated with the higher dose of the chelator. This finding was rather surprising in the light of the proved efficacy of this compound to mobilize iron from the myocardium of iron-overloaded animals and patients upon regular daily treat-

ment (Anderson et al., 2002; Wood et al., 2006). Furthermore, the biological half-life (approximately 2 h) of L1 excludes any potential direct effect of the compound on this parameter a week after its last dose. Hence, one can only reasonably explain these findings like a certain adaptive reaction of myocardium on the repeated intermittent iron chelation. Most importantly, these changes have not been associated with increased oxidative stress or other correlating myocardial disturbances, which indicates that this compensatory reaction was instead within a physiological range.

The results of this study strongly suggest that oral treat-

ment with L1 has no beneficial effect on chronic anthracy-

cline cardiotoxicity, which certainly raises several questions. One may speculate that the failure of L1 could have been

associated with its poor or erratic bioavailability. However, it

should be noted that L1 is characterized with very good oral bioavailability; it is fairly well absorbed from the gastroin-

testinal tract in humans as well as in rabbits (Fredenburg et

al., 1993). Furthermore, the doses and timing of administra-

tion were designed according to the pharmacokinetic study

performed on a species (Porter et al., 1990). The empiric assump-
tions given above were further supported by two independent exploratory data analyses. These have clearly revealed that L1 had shown no clear tendency to affect the general toxicity, cardiovascular, hematological, and plasma biochemistry parameters. The only exception was found in the case of the higher myocardial iron content in the animals treated or cotreated with the higher dose of the chelator. This finding was rather surprising in the light of the proved efficacy of this compound to mobilize iron from the myocardium of iron-overloaded animals and patients upon regular daily treat-

ment (Anderson et al., 2002; Wood et al., 2006). Furthermore, the biological half-life (approximately 2 h) of L1 excludes any potential direct effect of the compound on this parameter a week after its last dose. Hence, one can only reasonably explain these findings like a certain adaptive reaction of myocardium on the repeated intermittent iron chelation. Most importantly, these changes have not been associated with increased oxidative stress or other correlating myocardial disturbances, which indicates that this compensatory reaction was instead within a physiological range.

The results of this study strongly suggest that oral treat-

ment with L1 has no beneficial effect on chronic anthracy-

cline cardiotoxicity, which certainly raises several questions. One may speculate that the failure of L1 could have been

associated with its poor or erratic bioavailability. However, it

should be noted that L1 is characterized with very good oral bioavailability; it is fairly well absorbed from the gastroin-

testinal tract in humans as well as in rabbits (Fredenburg et

al., 1993). Furthermore, the doses and timing of administra-

tion were designed according to the pharmacokinetic study

performed on a species (Porter et al., 1990). The empiric assump-
tions given above were further supported by two independent exploratory data analyses. These have clearly revealed that L1 had shown no clear tendency to affect the general toxicity, cardiovascular, hematological, and plasma biochemistry parameters. The only exception was found in the case of the higher myocardial iron content in the animals treated or cotreated with the higher dose of the chelator. This finding was rather surprising in the light of the proved efficacy of this compound to mobilize iron from the myocardium of iron-overloaded animals and patients upon regular daily treat-

ment (Anderson et al., 2002; Wood et al., 2006). Furthermore, the biological half-life (approximately 2 h) of L1 excludes any potential direct effect of the compound on this parameter a week after its last dose. Hence, one can only reasonably explain these findings like a certain adaptive reaction of myocardium on the repeated intermittent iron chelation. Most importantly, these changes have not been associated with increased oxidative stress or other correlating myocardial disturbances, which indicates that this compensatory reaction was instead within a physiological range.
TABLE 2
Selected biochemical parameters
Statistical significance (P < 0.05, ANOVA) in comparison with control (c), deferiprone (d), and daumorubicin (a) group is given.

<table>
<thead>
<tr>
<th>Parameter/Group</th>
<th>Beginning of the Study</th>
<th>Before the Fifth Administration</th>
<th>End of the Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum iron (µl)</td>
<td>Control 31.8 ± 3.5</td>
<td>36.2 ± 2.8</td>
<td>26.3 ± 1.4</td>
</tr>
<tr>
<td></td>
<td>L1 (50 mg/kg) 36.0 ± 5.7</td>
<td>33.2 ± 2.7</td>
<td>29.7 ± 3.6</td>
</tr>
<tr>
<td></td>
<td>DAU 35.7 ± 2.6</td>
<td>19.5 ± 2.9*cd</td>
<td>19.7 ± 4.1*</td>
</tr>
<tr>
<td></td>
<td>L1 (10 mg/kg) + DAU 35.3 ± 2.7</td>
<td>25.5 ± 2.3*c</td>
<td>23.4 ± 4.6</td>
</tr>
<tr>
<td></td>
<td>L1 (50 mg/kg) + DAU 45.0 ± 2.3</td>
<td>18.7 ± 3.7*cd</td>
<td>35.9 ± 12.0</td>
</tr>
<tr>
<td>Creatinine (µl)</td>
<td>Control 82.8 ± 4.2</td>
<td>95.1 ± 4.2*</td>
<td>89.0 ± 4.1</td>
</tr>
<tr>
<td></td>
<td>L1 (50 mg/kg) 83.4 ± 3.7</td>
<td>89.2 ± 5.4</td>
<td>77.0 ± 5.2</td>
</tr>
<tr>
<td></td>
<td>DAU 91.1 ± 8.0</td>
<td>127.1 ± 11.1*cd</td>
<td>262.0 ± 112.9*cd</td>
</tr>
<tr>
<td></td>
<td>L1 (10 mg/kg) + DAU 88.5 ± 4.7</td>
<td>102.0 ± 3.5*</td>
<td>152.3 ± 19.5*cd</td>
</tr>
<tr>
<td></td>
<td>L1 (50 mg/kg) + DAU 85.4 ± 4.1</td>
<td>108.3 ± 5.0*</td>
<td>162.8 ± 30.1cd</td>
</tr>
<tr>
<td>Urea (ml)</td>
<td>Control 5.83 ± 0.51</td>
<td>7.58 ± 0.31*</td>
<td>6.65 ± 0.25</td>
</tr>
<tr>
<td></td>
<td>L1 (50 mg/kg) 6.02 ± 0.80</td>
<td>6.88 ± 0.48</td>
<td>6.78 ± 0.42</td>
</tr>
<tr>
<td></td>
<td>DAU 7.11 ± 0.92</td>
<td>7.25 ± 0.40</td>
<td>12.70 ± 4.26</td>
</tr>
<tr>
<td></td>
<td>L1 (10 mg/kg) + DAU 6.50 ± 0.35</td>
<td>7.39 ± 0.49</td>
<td>8.27 ± 0.51*</td>
</tr>
<tr>
<td></td>
<td>L1 (50 mg/kg) + DAU 7.06 ± 0.39</td>
<td>6.78 ± 0.38</td>
<td>8.84 ± 2.29</td>
</tr>
<tr>
<td>Protein (g/l)</td>
<td>Control 59.8 ± 0.7</td>
<td>65.7 ± 1.6*</td>
<td>60.9 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>L1 (50 mg/kg) 60.8 ± 0.3</td>
<td>65.4 ± 1.0</td>
<td>63.8 ± 0.9*</td>
</tr>
<tr>
<td></td>
<td>DAU 61.9 ± 1.4</td>
<td>60.6 ± 1.3</td>
<td>49.1 ± 1.6*cd</td>
</tr>
<tr>
<td></td>
<td>L1 (10 mg/kg) + DAU 64.7 ± 0.9c</td>
<td>62.8 ± 1.4</td>
<td>48.6 ± 1.4*cd</td>
</tr>
<tr>
<td></td>
<td>L1 (50 mg/kg) + DAU 65.2 ± 1.0c</td>
<td>55.9 ± 6.5</td>
<td>49.3 ± 0.8*cd</td>
</tr>
<tr>
<td>Cholesterol (µl)</td>
<td>Control 1.47 ± 0.14</td>
<td>1.75 ± 0.23</td>
<td>1.49 ± 0.13</td>
</tr>
<tr>
<td></td>
<td>L1 (50 mg/kg) 1.82 ± 0.52</td>
<td>1.89 ± 0.51</td>
<td>1.48 ± 0.28</td>
</tr>
<tr>
<td></td>
<td>DAU 1.43 ± 0.16</td>
<td>2.52 ± 0.52</td>
<td>3.65 ± 0.44*cd</td>
</tr>
<tr>
<td></td>
<td>L1 (10 mg/kg) + DAU 1.36 ± 0.13</td>
<td>2.76 ± 0.26*</td>
<td>5.81 ± 0.95*cd</td>
</tr>
<tr>
<td></td>
<td>L1 (50 mg/kg) + DAU 1.27 ± 0.12</td>
<td>3.23 ± 0.89*</td>
<td>4.24 ± 0.89*cd</td>
</tr>
<tr>
<td>TAG (ml)</td>
<td>Control 0.86 ± 0.06</td>
<td>0.62 ± 0.09*</td>
<td>0.82 ± 0.08</td>
</tr>
<tr>
<td></td>
<td>L1 (50 mg/kg) 1.16 ± 0.16</td>
<td>1.01 ± 0.33</td>
<td>1.02 ± 0.13</td>
</tr>
<tr>
<td></td>
<td>DAU 1.19 ± 0.17</td>
<td>1.10 ± 0.14</td>
<td>3.73 ± 0.94*cd</td>
</tr>
<tr>
<td></td>
<td>L1 (10 mg/kg) + DAU 0.87 ± 0.07</td>
<td>1.38 ± 0.21*c</td>
<td>5.00 ± 1.71cd</td>
</tr>
<tr>
<td></td>
<td>L1 (50 mg/kg) + DAU 1.13 ± 0.16</td>
<td>2.22 ± 0.77c</td>
<td>2.54 ± 0.46c</td>
</tr>
<tr>
<td>ALT (µkat/l)</td>
<td>Control 0.96 ± 0.20</td>
<td>0.77 ± 0.05</td>
<td>0.76 ± 0.07</td>
</tr>
<tr>
<td></td>
<td>L1 (50 mg/kg) 0.80 ± 0.16</td>
<td>0.95 ± 0.14</td>
<td>0.73 ± 0.09</td>
</tr>
<tr>
<td></td>
<td>DAU 1.00 ± 0.10</td>
<td>0.73 ± 0.05*</td>
<td>0.72 ± 0.15</td>
</tr>
<tr>
<td></td>
<td>L1 (10 mg/kg) + DAU 1.07 ± 0.11</td>
<td>1.14 ± 0.18a</td>
<td>0.66 ± 0.10*</td>
</tr>
<tr>
<td></td>
<td>L1 (50 mg/kg) + DAU 1.50 ± 0.28</td>
<td>0.91 ± 0.12*</td>
<td>0.45 ± 0.07</td>
</tr>
<tr>
<td>AST (µkat/l)</td>
<td>Control 0.64 ± 0.09</td>
<td>0.57 ± 0.05</td>
<td>0.50 ± 0.06</td>
</tr>
<tr>
<td></td>
<td>L1 (50 mg/kg) 1.04 ± 0.23</td>
<td>1.34 ± 0.52</td>
<td>0.62 ± 0.09</td>
</tr>
<tr>
<td></td>
<td>DAU 0.71 ± 0.15</td>
<td>0.40 ± 0.05d</td>
<td>0.51 ± 0.18</td>
</tr>
<tr>
<td></td>
<td>L1 (10 mg/kg) + DAU 0.64 ± 0.06</td>
<td>1.38 ± 0.67</td>
<td>0.45 ± 0.07</td>
</tr>
<tr>
<td></td>
<td>L1 (50 mg/kg) + DAU 1.26 ± 0.31</td>
<td>1.47 ± 0.93</td>
<td>0.16 ± 0.02</td>
</tr>
</tbody>
</table>

TAG, triacylglycerides.
* Statistical significance (P < 0.05, paired t-test) paired comparison with the initial values.
a bidentate iron chelator, which forms a complex with iron in a ratio of 3:1. This might be a limitation from the viewpoint of protection, because the probability of insufficient occupation of all sites of ferric ions is higher. Furthermore, we cannot rule out that, although L1 entered the cardiac cells, it did not reach the desirable intracellular compartment and remained there for a sufficient time to induce effective cardioprotection.

Previously, the well known iron chelator deferoxamine had been shown to be ineffective against chronic anthracycline toxicity in spontaneously hypertensive rats (Herman et al., 1994). Nevertheless, this was well attributable to the hydrophilic nature of this drug, which hinders its penetration into the cardiomyocytes. On the other hand, using our in vivo rabbit model, we have recently shown that lipophilic arylhydrazone iron chelators are able to protect against anthracycline cardiotoxicity in terms of overall mortality, functional parameters, and histopathology (Simunek et al., 2005a; Sterba et al., 2006, 2007b). However, at the same time, it was revealed that dexrazoxane is superior to arylhydrazones, as the cardioprotection of the latter compounds—although significant—was always only partial. Moreover, dose escalations of all arylhydrazones surprisingly resulted in the disappearance of protection, which was puzzling and unexpected. These results together with the findings from the present study indicate that the role of iron in anthracycline cardiotoxicity is not as trivial as originally supposed. For instance, a number of studies provided evidence for anthracycline-induced dysregulation of iron homeostasis with potentially serious consequences (Xu et al., 2005). Anthracyclines can perturb iron metabolism by interacting with various molecular targets, including iron regulatory proteins, ferritin, or transferrin receptor (Kwok and Richardson, 2003; Xu et al., 2008).

The observed lack of cardioprotective effect of L1 can also mean that iron-catalyzed formation of ROS is not the pivotal and ultimate executioner responsible for chronic anthracycline cardiotoxicity. ROS can also be produced without presence of iron, and they might be rather important as the triggering factor for a number of successive molecular and cellular events. In addition, anthracyclines and their metabolites have been reported to induce a number of distinct cellular effects, which do not appear to be ROS-mediated (Menna et al., 2007). For example, anthracyclines have been demonstrated to induce a number of perturbations in cellular calcium homeostasis (Simunek et al., 2005b; Wallace, 2007). Unlike in the case of selective iron chelator L1, it can not be excluded that dexrazoxane may also chelate calcium, which can potentially account for the difference in cardioprotective effects of both chelators.

This study also points out on difficulties with the translation of in vitro and ex vivo cardioprotection results into the chronic in vivo settings, reflecting more closely the anthracycline cardiotoxicity seen in clinics. It is interesting that a similar scenario took place with a number of antioxidants (e.g., vitamins A and E and acetylcysteine) (Dresdale et al., 1982; Legha et al., 1982; Myers et al., 1983).

In conclusion, this study revealed that, despite the promising results obtained previously in vitro/ex vivo, iron chelation with L1 was unable to protect the myocardium against lipoperoxidation, cardiomyopathy, and heart failure induced by repeated administration of DAU to rabbits. Together with our previous findings, this study strongly suggests that the role of iron and its chelation in anthracycline cardiotoxicity is
probably not as trivial as originally believed and/or other mechanisms unrelated to iron-catalyzed ROS production are involved in this pathology.

Acknowledgments

We thank Dr. Magdaléna Holečková for myocardial iron content determination, Ludmila Latýnová for skillful technical assistance, and Dr. John Connelly (ApoPharma Inc.) for kindly providing a drug substance of deferiprone.

References


Herman EH, Zhang J, Hasinoff BB, Chadwick DP, Clark JR Jr., and Ferrans VJ (1997) Comparison of the protective effects against chronic doxorubicin cardiotoxicity...


Address correspondence to: Dr. Olga Popelová, Department of Pharmacology, Charles University in Prague, Faculty of Medicine in Hradec Králové, Šimkova 870, 500 38 Hradec Králové, Czech Republic. E-mail: popelova@lfhk.cuni.cz