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Original Article

Desferrioxamine attenuates minor lung injury following surgical acute liver failure

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Short title: Pulmonary injury during acute liver failure

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Abstract

Acute liver failure (ALF) can be complicated by lung dysfunction. The aim of this study is to test the hypothesis that inhibition of oxidative stress through iron chelation with desferrioxamine (DFX) attenuates pulmonary injury caused by ALF.

Fourteen adult female domestic pigs were subjected to surgical devascularization of the liver and were randomized to a study group (DFX group, *n*=7), which received postoperative intravenous infusion of desferrioxamine (14.5 mg/kg/h for the first 6h postoperatively and 2.4 mg/kg/h until completion of 24h), and a control group (*n*=7). Postoperative lung damage was evaluated by histological and bronchoalveolar lavage fluid (BALF) analysis.

Desferrioxamine resulted in reduced BALF protein levels and tissue phospholipase A_2 (PLA_2) activity. Plasma malondialdehyde and BALF nitrates + nitrites concentrations were lower, while catalase activity in the lung was higher after DFX treatment. Phospholipase A_2, platelet-activating factor acetylhydrolase and total cell counts in BALF did not differ between groups. Histological examination revealed reduced alveolar collapse, pneumonocytes necrosis and total lung injury in the DFX-treated animals.

Desferrioxamine reduced systemic and pulmonary oxidative stress during ALF. The limited activity of PLA_2 and the attenuation of pneumonocytes necrosis could represent beneficial mechanisms by which DFX improves alveolar-capillary membrane permeability and alveolar spaces collapse.

Keywords: alveolocapillary permeability, iron regulation, liver-lung interactions, lung injury, oxidants / antioxidants

Introduction

Acute liver failure (ALF) induces systemic derangements, including pulmonary edema [1, 2]. We previously showed that surgical devascularization of the liver produces inflammatory alterations in bronchoalveolar lavage fluid (BALF) and lung histology [3]. Numerous studies suggest that inflammation during lung injury necessitates that oxidative molecules production overwhelms the antioxidant defences of the lung [4, 5]. Since ALF and portocaval shunting induce oxidative stress not only in the liver, but also in systemic circulation and remote organs, we hypothesized that during ALF the lung could be exposed to increased oxidative stress [6-8].

Oxidative reactions are mediated by iron, which catalyzes hydroxyl radical production. Studies have shown that desferrioxamine (DFX), a potent iron chelator, improves experimental liver failure and liver transplantation by attenuating oxidative stress [9, 10]. Interestingly, the antioxidant effects of iron chelation were also proven beneficial in models of lung injury [11, 12].

Apart from liver transplantation, no therapeutic interventions are available today for lung injury during ALF [13]. The hypothesis behind our study is that oxidative stress could mediate remote lung injury during devascularization of the liver. In this respect, we aimed to investigate whether iron chelation with DFX can alleviate oxidative stress and improve lung damage in pigs with surgically induced, severe ALF.

Materials and Methods

The protocol was approved by the Animal Research Committee of the University of Athens. Care and handling of the animals was in accordance with National and European guidelines for ethical animal research.

Induction and Maintenance of Anesthesia

Fourteen young adult female domestic pigs (22-25 kg, 3-4 months old) were used. After 24h deprivation of food and unrestricted access to water, 4 mg/kg ketamine, 0.2 mg/kg midazolam and 0.5 mg atropine were administered intramuscularly. General anesthesia was induced with thiopental sodium 5 mg/kg and ketamine 2 mg/kg via an ear peripheral vein. The trachea was intubated with a cuffed tube of internal diameter of 5.5-6.0 mm and mechanical ventilation was initiated using a volume ventilator (Sulla 808 V, Dräger, Lübeck, Germany), with a fraction of inspired oxygen (FiO_2) of 0.6, tidal volume 10 mL/kg, no PEEP and respiratory frequency between 10 and 15 bpm, so that end-tidal CO_2 was 35-45 mmHg.

Following insertion of an orogastric tube, fentanyl 10-20 μg/kg and vecuronium bromide 0.5 mg/kg were given intravenously. Then, the right external jugular vein was exposed and a central vein catheter 6.5Fr (G986, Arrow International, Reading, PA, USA) was inserted to obtain central venous pressure measurements and blood samples. The right carotid artery was cannulated with a 20G catheter for arterial pressure monitoring. Blood samples were obtained at 0, 7 and 24h postoperatively. Intravenous cefuroxime 750 mg was given every 6h, beginning from incision. Anesthesia was maintained with continuous intravenous infusion of fentanyl 15-20 μg/kg/h, ketamine 5-8 mg/kg/h and vecuronium bromide 0.5 mg/kg/h. Dextrose 5%, normal saline, hydroxyethylstarch 6% and lactated Ringer's

solutions, 100-200 mL/h each, were given in order to maintain normoglycaemia, normal electrolytic values and central venous pressure between 4 and 9 mm Hg.

Surgical Procedure

Briefly, a midline abdominal incision was followed by an end-to-side portocaval anastomosis and transection of the hepatoduodenal ligament with its contents (hepatic artery, bile duct). To ensure interruption of collateral blood flow, all other ligamentous attachments of the liver were also transected. The procedure lasted approximately 60 minutes. At the end of the experiment animals were euthanized with KCl 2 gr, propofol 1% 20 mL and vecuronium bromide 20 mg. Autopsies verified absence of surgical complications and confirmed total liver necrosis in all animals. Biopsies were taken from the liver as well as the lower lobe of the left lung and were used for histological examination and tissue analysis.

Desferrioxamine protocol

In 7 randomly chosen animals desferrioxamine was given as follows: 2 gr of desferrioxamine in 250 mL Dextrose 5% were given during the first 6h (14.5 mg/kg/h), whereas for the next 18h animals received 1 gr of desferrioxamine in 250 mL Dextrose 5% (2.4 mg/kg/h).

BALF procedure

Bronchoalveolar lavage was performed with a fiberoptic bronchoscope (Fujinon BDR-YP 2, Fuji, Japan) after the insertion of central lines (baseline), immediately after completion of surgery (0h), and at 7h and 24h postoperatively. The bronchoscope was introduced through a swivel adaptor in the right middle lobe (recovery 50%-75%). The first aspirated fluid reflecting a bronchial sample was discarded. BALF was then filtered through sterile gauze,

collected and immediately centrifuged at 500*g* for 10 minutes at 4°C. The supernatant was stored at –80°C for biochemical analyses.

Tissue homogenization

Lung tissue samples were weighed and homogenized in cold phosphate buffer saline, pH=7.4, to produce a 1/10 (w/v) homogenate. Teflon pestle was used for tissue homogenization. The homogenate was sonicated and centrifuged at 12,000 rpm for 15 min. The supernatant was aliquoted and kept at -20º C until use.

Total protein, iron and cells in BALF

Total protein in BALF and tissue homogenates was measured according to Lowry et al [14]. Serum total iron was measured using a commercially available set (BioAssay Systems, Hayward, USA). Total BALF cells and differential counts were performed by counting at least 300 cells in cytocentrifuge preparations stained with hematoxylin-eosin.

Phospholipase A_2 and PAF-AcH assays

Phospholipase A_2 was measured fluorometrically with C_{12}-NBD-PC as substrate using either BALF or supernatant of the homogenate [15]. BALF PAF-AcH was determined after incubating BALF with [^{3}H]-PAF and measuring the radioactivity of the acetyl group, as previously described [15, 16].

Nitrites/Nitrates measurement

Nitrites/nitrates were quantified colorimetrically after their reaction with the Griess reagent. A nitrate standard solution (100 μl) was serially diluted (generally from 80-5 μM) in duplicate in a 96-well microtiter plate. Reduction of nitrate to nitrite with VCl_3 (100 μl) was

rapidly followed by addition of the Griess reagent, sulfanilamide (50 μl) and N-(1-Naphthyl)ethylenediamine dihydrochloride (50 μl). Nitrite was measured similarly except that samples and nitrite standards were exposed only to Griess reagent. In either case the absorbance was measured at 540 nm.

Malondialdehyde and Catalase measurement

Briefly, 0.65 ml of 10.3 mM N-methyl-2-phenyl-indole in acetonitrile was added to 0.2 ml of plasma. After vortexing for 3-4 s and adding 0.15 ml of HCl 37%, samples were incubated at 45°C for 60 min. The samples were ice-cooled and centrifuged and the absorbance was measured spectrophotometrically at 586 nm. A calibration curve of an accurately prepared standard MDA solution (2-20 nmol/ml) was run for quantitation. All measurements were performed in triplicate. Catalase activity was measured spectrophotometrically by a kit from Oxis Health Products Inc. (Oregon, USA).

Histological examination

Lung biopsies fixed with buffered formalin, cut at 3-5 μm sections and stained with hematoxylin-eosin, were blindly evaluated by two pathologists, using a previously described scoring system for acute lung injury [17]. Appropriate modifications to this system deemed essential in order to include all main histological findings, since no scoring system exists for this particular model of lung damage. Each section was given a score of 0 to 4 for alveolar collapse, hemorrhage, alveolar and interstitial edema, necrosis of alveolar epithelial cells (AECs), lymphocytes and leukocytes infiltration. For example, for alveolar collapse: 0, no alveolar collapse; 1, occasional fields with a low number of collapsed alveoli per field (minimal); 2, occasional fields with increased number (>3) of collapsed alveoli per viewed field (mild); 3, many, but not all, fields with alveolar collapse; 4, alveolar collapse in all

fields examined (severe). Total lung injury score of each section was calculated by adding the separate scores. Parafin-fixed sections of liver biopsies were stained with hematoxylin-eosin and evaluated by the same pathologists.

Statistics

Data are expressed as mean ± SD. Differences between different time-points inside each group were analyzed with one-way repeated measures analysis of variance followed by the Bonferroni post-hoc test for multiple comparisons. Differences between the two groups were analyzed with analysis of covariance, where baseline values, or values at 0h if baseline values were missing, were set as covariance. Parameters measured only once were compared with simple ANOVA. Statistical analysis was processed with SPSS v.15.0 software and was approved by a specialist in statistics. Level of statistical significance was defined as $p < 0.05$.

Results

Hemodynamics, Blood Gases, and Serum Markers

Mean arterial pressure decreased in controls at 24h, being lower compared to DFX group. No statistical differences were found between or inside groups regarding heart rate, PaO_2/FiO_2 ratio, $PaCO_2$, pH or temperature (Table 1). Hyperbilirubinemia developed gradually in both groups. Serum iron levels decreased in DFX group and were lower than in controls at 7h and 24h. Plasma MDA levels increased at 7 and 24 hours in controls, whereas in DFX group only at 7h. At 24h plasma MDA levels were lower in DFX compared to controls (Table 2).

BALF protein

Total protein in BALF increased in both groups compared to baseline values. In DFX group BALF total protein was statistically lower than in controls both at 7 and at 24h (Figure 1). In addition, the BALF to serum protein (B/S) ratio was also statistically lower in DFX group at 7h and 24h (Table 3).

Phospholipase-A_2 and PAF-AcH

In controls, BALF PLA_2 levels increased at 0h and subsequently decreased. In DFX group no significant changes from baseline values were observed. The ratio of PLA_2 activity to total protein in BALF did not change in either group but was higher in DFX group compared to controls at 24h (Table 3). However, when PLA_2 activity was measured in lung tissue (nmol FA/h/mg protein), it was significantly lower in DFX group (0.397 ± 0.251) compared to controls (0.824 ± 0.331) ($p = 0.026$).

BALF PAF-AcH levels in controls increased immediately after devascularization and then decreased. A similar pattern was observed in DFX group, but no statistical changes were found over time. The two groups did not differ in PAF-AcH levels or in the ratio of PAF-AcH to BALF protein (Table 3).

BALF NO· products and tissue catalase activity

BALF nitrates and nitrites (NO_2^- + NO_3^-) levels increased significantly 7h postoperatively in controls and then decreased towards initial values. In contrast, no change from initial levels was observed in DFX group (Table 3, Figure 2). Levels of nitrates/nitrites were significantly higher in controls at 7h compared to DFX group.

Catalase activity in lung tissue at 24h (u/ml) was significantly higher in group DFX (38.07 ± 22.88) compared to controls (11.23 ± 6.01) (p = 0.021).

BALF cells

In controls a significant increase was noted in BALF total cells and neutrophils counts at 7h. This was accompanied by decreased percentages of alveolar macrophages and increased percentages of neutrophils both at 7h and 24h. In contrast, no statistically significant changes were noted in total cells counts or percentages of alveolar macrophages or neutrophils in DFX group. However, increased alveolar macrophages counts were noted both at 0 and 7h compared to baseline values. The two groups did not differ between them at any time point (Table 4).

Histopathology

Histopathological scoring in the two groups is presented in Table 5. In DFX group alveolar collapse and necrotized AECs inside the alveoli were less compared to controls (Figure 3). Total lung injury score was also statistically less in the DFX group. Liver biopsies confirmed the presence of extensive centrolobular necrosis in all animals.

Discussion

The applied surgical model of ALF induction resulted in rapid loss of detoxifying liver function and complete, histologically-confirmed liver necrosis by the end of the experiment. The infusion of DFX resulted in a clear reduction of oxidative stress in plasma and BALF and enhanced catalase activity. BALF protein concentration, B/S ratio and tissue PLA_2 activity decreased also significantly. These results were accompanied by less degree of AECs necrosis, alveolar collapse, and total histological lung injury. The present data provide the

first experimental demonstration that lung injury due to ALF is, at least partially, mediated by oxidative molecules, and that inhibition of iron-dependent oxidative reactions alleviates lung injury during ongoing ALF.

Iron-catalyzed oxidative stress and disruption of iron homeostasis have been involved in many pulmonary diseases, including ARDS [11, 18]. Iron may contribute to alveolar-capillary membrane injury and lung inflammation in ARDS by yielding hydroxyl radicals through the Fenton-Haber-Weiss reaction [4, 11]. Iron chelation by DFX inhibits this reaction, thereby reducing the production of hydroxyl radicals. Additionally, DFX directly scavenges peroxyl radicals and inhibits nitrosative stress [10]. These antioxidative actions of DFX significantly improved lung function during lipopolysaccharide-induced lung injury [12].

In our experiment, DFX provided antioxidative protection both systematically and locally in the lung, as is shown by the decreased plasma MDA and BALF NO· levels and the increased catalase activity. Lipid peroxidation in the lung probably could contribute to plasma MDA, since plasma MDA levels were shown to depend on the degree of lung injury [19]. Nitric oxide is a well known oxidative molecule which oxidizes protein residues to nitrotyrosine, altering cellular signaling processes and inducing apoptosis [20]. In contrast, catalase converts hydrogen peroxide (H_2O_2) to molecular O_2 and H_2O, thereby augmenting antioxidant capacity [21]. Therefore, the reduction of oxidative products along with enhancement of antioxidant defences could account for less damage to the alveolar-capillary membrane proteins and cells. Additionally, inhibition of NO· production may have also contributed to the observed hemodynamic stability by preventing the ALF-induced vasodilatation [22].

The increase in BALF protein could result either from increased alveolar fluid volume recovery or from increased alveolar protein concentration. Although alveolar fluid increase could result from increased lavaged area, this distinction is not possible due to absence of reliable alveolar fluid volume markers [16]; however, the reproducibility of the results suggests that the increased protein occurs mainly due to increased alveolar protein concentration. The latter could ensue from disturbance and increased permeability of the alveolar-capillary membrane [23]. Therefore, the reduction of BALF protein suggests that DFX partially attenuated the alveolar-capillary barrier disruption. Antioxidant protection and preservation of the AECs could have contributed to this, by preserving the alveolar-capillary membrane integrity and preventing protein influx into the alveoli.

Phospholipase A_2 hydrolyzes surfactant phospholipids and induces the production of PAF, thus contributing to local inflammation [24]. Increased PLA_2 levels are assumed to derive either locally from inflammatory cells or from the circulation [25]. Although PLA_2 and PAF-AcH levels did not clearly differ between the two groups in BALF, tissue PLA_2 activity decreased in DFX group. This could be attributed to contamination of BALF by PLA_2 and PAF-AcH from plasma, where both enzymes exist in high concentrations, obliterating the differences. Nonetheless, the decreased pulmonary PLA_2 activity indicates an indirect protective effect of DFX on surfactant and a possible mechanism for the reduced alveolar collapse.

In contrast to controls, the composition of BALF cells in DFX group did not change significantly. Although the antioxidant and anti-inflammatory effects of DFX did not result in direct differences in immune cells counts, it could be possible that DFX altered immune

cells functions. It has been elsewhere shown that DFX inhibits neutrophils-induced endothelial cells dysfunction and death [26]. In addition, when Ritter et al administered DFX during lipopolysaccharide-induced lung injury, alveolar macrophages functions changed before changes in BALF cells counts could be seen. In that study, the effect of DFX on BALF cells counts became apparent only 48 hours after the initial insult [12]. A similar effect could be assumed also for our model, since the decreased PLA_2 activity could indicate a shift in immune cells functions. However, *in vitro* investigation would be more appropriate to clarify the effect of DFX on immune cells.

In microscopy two parameters of great importance to lung function were affected by the antioxidant treatment; AECs and alveolar collapse. AECs contribute to gas exchange and also protect the alveolar spaces against protein and plasma influx [27]. However, the large surface of the alveolar-capillary membrane renders it susceptible to systemic oxidative injury leading to protein and lipid oxidation, cell necrosis, and atelectasis due to surfactant depletion [4, 5, 20]. Scavenging of $NO\cdot$ products and H_2O_2, and inhibition of lipid peroxidation seems that efficiently inhibited alveolar cells necrosis. Since surfactant production by the AECs is crucial in preventing alveolar spaces collapse, it could be assumed that prevention of alveolar collapse was at least partially due to preservation of the alveolar cells.

Causes other than ALF could have contributed to the development of lung injury, such as intraoperative hypovolemic shock, septic shock and intestinal ischemia. However, none of these was observed. Theoretically, intracranial hypertension could induce neurogenic pulmonary edema, but this is inconsistent with the protein-rich BALF [28]. Another limitation includes the rapidity and severity of ALF, which both represent extreme situations

in clinical practice. It is therefore possible that the more prolonged clinical process of ALF allows other mechanisms to intervene. It is also notable that despite the histological alterations, PaO_2 remained unaffected. In this respect, it seems more appropriate to refer to this model as a model of minor lung injury. However, during the anhepatic phase of liver transplantation, total body oxygen consumption was shown to decrease over 35% [29]. Since our animals remained anhepatic, the PaO_2/FiO_2 ratio may not be accurate for estimating differences in gas-exchange. Nonetheless, experiments on oxygen metabolism and gas exchange in anhepatic animals would be more appropriate to clarify this issue.

Mechanical ventilation could contribute to pulmonary alterations, but as previously described [3], ALF can be considered as the major cause of lung injury in this model. Although we chose a relatively high FiO_2 to ensure adequate oxygenation throughout the 24h, the results show that this was unnecessary and can be avoided in future experiments. Another limitation is that the respiratory frequency was adjusted according to end-tidal CO_2, leading to slight hypocapnia. Although this allowed continuous maintenance of end-tidal CO_2 between 35-45 mmHg, following the $PaCO_2$ values perhaps would help optimize the present model. However, the hypotension observed in controls may have also contributed to hypocapnia.

Finally, although prolonged administration of DFX was reported to cause ARDS, this was attributed to iron-DFX-O_2 interaction [30]. Nonetheless, DFX is a powerful and indiscriminate iron chelator and its use for conditions without iron overload must be viewed cautiously.

Summary

Infusion of DFX during liver devascularization seems effective in attenuating systemic and pulmonary oxidative stress. Antioxidant therapy prevented AECs necrosis and alveolocapillary membrane disruption, which seem to be characteristics of the minor lung damage during ALF. In addition, downregulation of PLA_2 could limit surfactant hydrolysis, improving atelectasis. Our experimental study is the first to provide a possible treatment of pulmonary injury during ALF. Given the encouraging results and safety of the treatment, clinical studies should investigate its possible benefits in subjects with ALF presenting evidence of lung injury.

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Tables

Table 1

Hemodynamic parameters and blood gases in control and desferrioxamine groups

Parameter	**Group**	**Baseline (I)**	**0 h (II)**	**7h (III)**	**24h (IV)**
MAP (mm Hg)	Control	95 ± 23	99 ± 39	95 ± 22	51 ± 16 [(I)]
	DFX	89 ± 13	97 ± 34	107 ± 12	84 ± 27 [a]
HR (bpm)	Control	101 ± 21	148 ± 30	117 ± 25	120 ± 30
	DFX	112 ± 24	137 ± 31	118 ± 17	113 ± 11
PaO_2/FiO_2 (torr)	Control	461 ± 45	501 ± 171	625 ± 111	589 ± 78
	DFX	501 ± 137	622 ± 128	673 ± 99	578 ± 48
$PaCO_2$ (torr)	Control	35 ± 3	33 ± 9	32 ± 4	31 ± 10
	DFX	37 ± 4	37 ± 8	36 ± 10	36 ± 3
pH	Control	7.44 ± 0.08	7.51 ± 0.15	7.56 ± 0.09	7.55 ± 0.09
	DFX	7.45 ± 0.06	7.43 ± 0.06	7.50 ± 0.09	7.53 ± 0.06
T (^oC) *	Control	38 ± 1	38 ± 1	38 ± 1	38 ± 2
	DFX	38 ± 1	38 ± 1	39 ± 1	39 ± 1

HR = heart rate; MAP = mean arterial pressure; PaO_2/FiO_2 = ratio of partial arterial oxygen pressure to fraction of inspired oxygen; $PaCO_2$ = partial pressure of carbon dioxide in arterial blood; T = temperature. Ventilation settings: tidal volume 10mL/kg, no PEEP, FiO_2 = 0.60, respiratory rate adjustment between 10-15 bpm to maintain end-tidal CO_2 between 35-45 mmHg.

[a], $p < 0.01$ compared to control group; [(I)], $p < 0.05$ compared to baseline values in the same group.

*, normal temperature for pigs is $38.5 \pm 1^{o}C$

Table 2

Serum total protein, serum iron, plasma malondialdehyde and serum bilirubin concentrations in control and desferrioxamine groups

Parameter	**Group**	**Baseline (I)**	**0h (II)**	**7h (III)**	**24h (IV)**
TP (gr/dL)	Control	-	4.9 ± 0.7	4.1 ± 0.4 $^{(II)}$	2.2 ± 0.5 $^{(ii),(iii)}$
	DFX	-	5.7 ± 0.8	4.9 ± 0.8 $^{(II)}$	3.5 ± 0.4 $^{(ii),(III),a}$
Fe (ug/dL)	Control	-	95 ± 34	69 ± 26	108 ± 50
	DFX	-	105 ± 63	18 ± 28 $^{(II)\,a}$	43 ± 51 ^{b}
MDA (nmol/ml)	Control	1.7 ± 0.6	-	3.3 ± 0.3 $^{(I)}$	5.7 ± 1.8 $^{(I)}$
	DFX	1.4 ± 0.5	-	3.3 ± 0.3 $^{(i)}$	3.5 ± 1.5 ^{b}
TB (mg/dL)	Control	-	0.08 ± 0.08	0.89 ± 0.62 $^{(II)}$	1.31 ± 0.87 $^{(II)}$
	DFX	-	0.10 ± 0.11	0.74 ± 0.51 $^{(II)}$	1.31 ± 0.91 $^{(II)}$

Fe = serum iron levels, MDA = plasma malondialdehyde; TB, total serum bilirubin; TP = serum total protein.^{a}, $p < 0.01$ compared to control group; ^{b}, $p < 0.05$ compared to control group; $^{(I)}$, $p < 0.05$ compared to baseline values; $^{(II)}$, $p < 0.05$ compared to 0h (immediately after completion of surgery); $^{(III)}$, $p < 0.05$ compared to 7h (7h after completion of surgery) in the same group; $^{(i)}$, $p < 0.01$ compared to baseline values; $^{(ii)}$, $p < 0.01$ compared to 0h (immediately after completion of surgery); $^{(iii)}$, $p < 0.01$ compared to 7h (7h after completion of surgery) in the same group.

Table 3

Biochemical parameters in bronchoalveolar lavage fluid in control and desferrioxamine groups

Parameter	**Group**	**Baseline (I)**	**0 h (II)**	**7h (III)**	**24h (IV)**
Protein (μg/mL)	Control	48 ± 29	175 ± 134	398 ± 219 $^{(I)}$	261 ± 112 $^{(I)}$
	DFX	56 ± 27	193 ± 113	187 ± 67 $^{(I),\,b}$	162 ± 52 $^{(I),b}$
B/S ratio	Control	-	0.040 ± 0.029	0.104 ± 0.058	0.129 ± 0.069
	DFX	-	0.036 ± 0.014	0.037 ± 0.016 ^{b}	0.045 ± 0.015 ^{b}
PLA_2 (nmol C_{12}-NBD-FA/mL/h)	Control	1.27 ± 0.4	6.01 ± 1.5 $^{(I)}$	3.18 ± 1.7	3.07 ± 1.3
	DFX	1.33 ± 0.5	9.82 ± 9.1	3.77 ± 2.1	5.25 ± 1.1
PLA_2/mg protein (nmol C_{12}-NBD-FA//h/mg)	Control	38 ± 31	91 ± 99	8 ± 4	16 ± 13
	DFX	23 ± 7	62 ± 58	22 ± 13	35 ± 12 ^{b}
PAF-AcH (nmol-C_6-NBD-FA/mL/h)	Control	15.8 ± 5	65.2 ± 29 $^{(I)}$	26.5 ± 12 $^{(II)}$	10.4 ± 7 $^{(II)}$
	DFX	11.2 ± 4	45.4 ± 20	13.0 ± 7	10.1 ± 5
PAF-AcH/ mg protein (nmol-C_6-NBD-FA/h/mg)	Control	394 ± 250	503 ± 309	89 ± 45	52 ± 43
	DFX	196 ± 128	293 ± 181	75 ± 45	52 ± 14
NO_2^- + NO_3^- (μM)	Control	-	0.69 ± 0.52	4.59 ± 2.79 $^{(II)}$	0.51 ± 0.73 $^{(III)}$
	DFX	-	1.93 ± 3.36	0.70 ± 0.78 ^{b}	0.58 ± 0.52

B/S ratio = bronchoalveolar lavage to serum total protein ratio; NO_2^- + NO_3^- = nitrates and nitrites concentration; PAF-AcH = platelet-activating-factor acetylhydrolase; PLA_2 = phospholipase A_2. Ventilation settings: tidal volume 10mL/kg, no PEEP, FiO_2 = 0.60, respiratory rate adjustment between 10-15 bpm to maintain end-tidal CO_2 between 35-45 mmHg. ^{a}, $p < 0.01$ compared to control group; ^{b}, $p < 0.05$ compared to control group; $^{(I)}$, $p < 0.05$ compared to baseline values; $^{(II)}$, $p < 0.05$ compared to 0h (immediately after completion of surgery); $^{(III)}$, $p < 0.05$ compared to 7h (7h after completion of surgery) in the same group; $^{(i)}$, $p < 0.01$ compared to baseline values; $^{(ii)}$, $p < 0.01$ compared to 0h (immediately after

completion of surgery); [(iii)], $p < 0.01$ compared to 7h (7h after completion of surgery) in the same group

Table 4

Bronchoalveolar lavage fluid cell counts in control and desferrioxamine groups

Parameter	**Group**	**Baseline (I)**	**0 h (II)**	**7h (III)**	**24h (IV)**
Alveolar Macrophages (%)	Control	90 ± 8	88 ± 5	52 ± 19 $^{(II)}$	50 ± 15 $^{(I),(ii)}$
	DFX	91 ± 6	93 ± 4	61 ± 22	54 ± 22
Alveolar Macrophages (10^3 cells/mL)	Control	17 ± 1	299 ± 160	526 ± 321	522 ± 412
	DFX	17 ± 1	920 ± 180 $^{(I)}$	731 ± 130 $^{(I)}$	1004 ± 587
Neutrophils (%)	Control	7 ± 4	2 ± 3	43 ± 18 $^{(II)}$	42 ± 16 $^{(II)}$
	DFX	6 ± 3	1 ± 1	34 ± 21	34 ± 20
Neutrophils (10^3 cells/mL)	Control	2 ± 1	14 ± 11	356 ± 93 $^{(i),(ii)}$	397 ± 226
	DFX	2 ± 0	11 ± 11	575 ± 577	692 ± 619
Total cell counts (10^3 cells/mL)	Control	19 ± 4	448 ± 322	927 ± 318 $^{(i)}$	1014 ± 705
	DFX	18 ± 5	986 ± 434	1377 ± 688	1932 ± 1173

$^{(I)}$, $p < 0.05$ compared to baseline values; $^{(II)}$, $p < 0.05$ compared to 0h (immediately after completion of surgery); $^{(III)}$, $p < 0.05$ compared to 7h (7h after completion of surgery) in the same group; $^{(i)}$, $p < 0.01$ compared to baseline values; $^{(ii)}$, $p < 0.01$ compared to 0h (immediately after completion of surgery); $^{(iii)}$, $p < 0.01$ compared to 7h (7h after completion of surgery) in the same group. Ventilation settings: tidal volume 10mL/kg, no PEEP, FiO_2 0.60, respiratory rate adjustment between 10-15 bpm to maintain end-tidal CO_2 between 35-45 mmHg.

Table 5

Histological examination of lung tissue

Parameter	Group		p value
	Control	DFX	
Alveolar collapse	2.4 ± 1.1	1.3 ± 0.6	.043
Haemorrhage	1.2 ± 1.1	0.8 ± 0.7	.383
Edema	0.8 ± 0.8	1.2 ± 0.4	.270
AEC necrosis	2.0 ± 1.2	0.4 ± 0.5	.007
Lymphocytes	2.1 ± 0.9	1.1 ± 0.8	.071
Leukocytes	1.2 ± 1.3	0.4 ± 0.5	.131
Total lung injury score	9.7 ± 4.4	5.2 ± 2.6	.038

AEC = alveolar epithelial cells

Figure Legends

Figure 1. Time course of total protein levels (μg/mL) in bronchoalveolar lavage fluid from pigs subjected to liver devascularization (0h). Control group (control) did not receive antioxidant therapy, while pigs in the desferrioxamine group (DFX) received postoperative intravenous infusion of desferrioxamine for 24 hours. *, $p < 0.05$ between the two groups at 7h and 24h; #, $p < 0.05$ between the depicted time-points inside each group.

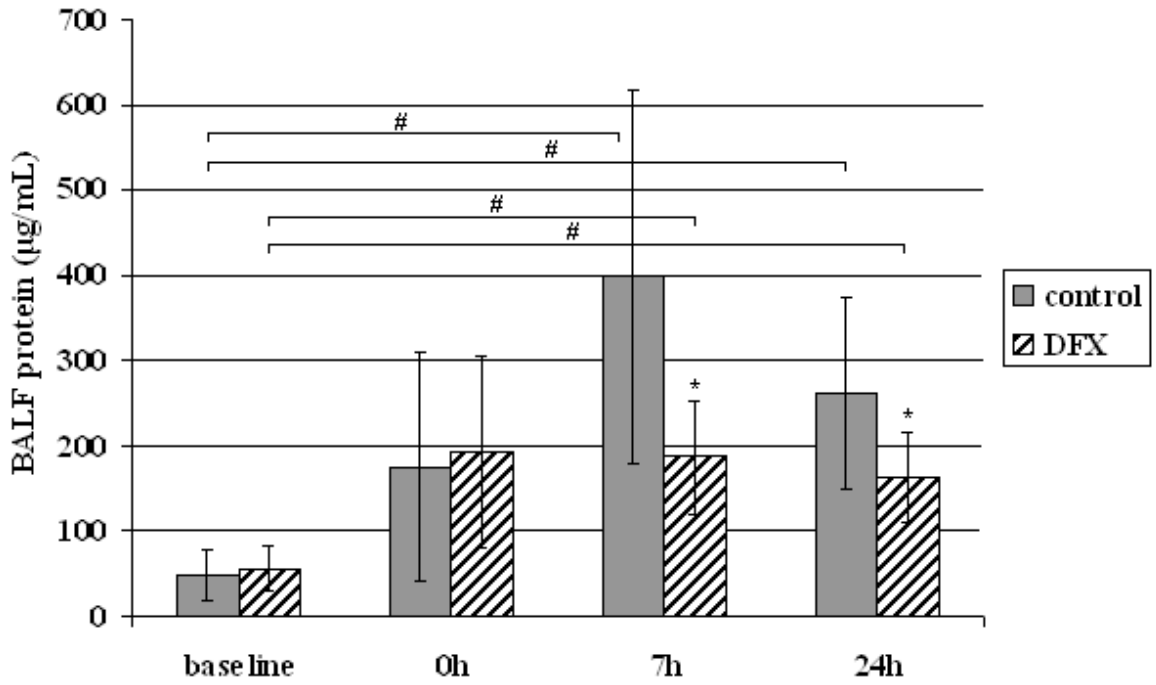


Figure 2. Time course of NO· products (NO_3^- + NO_2^-) (μM) in bronchoalveolar lavage fluid from pigs subjected to liver devascularization (0h). Pigs in control group (control) did not receive antioxidant therapy, while in the desferrioxamine group (DFX) they received postoperative intravenous infusion of desferrioxamine for 24 hours. *, $p < 0.05$ between the two groups at 7h; #, $p < 0.05$ between successive time-points in control group.

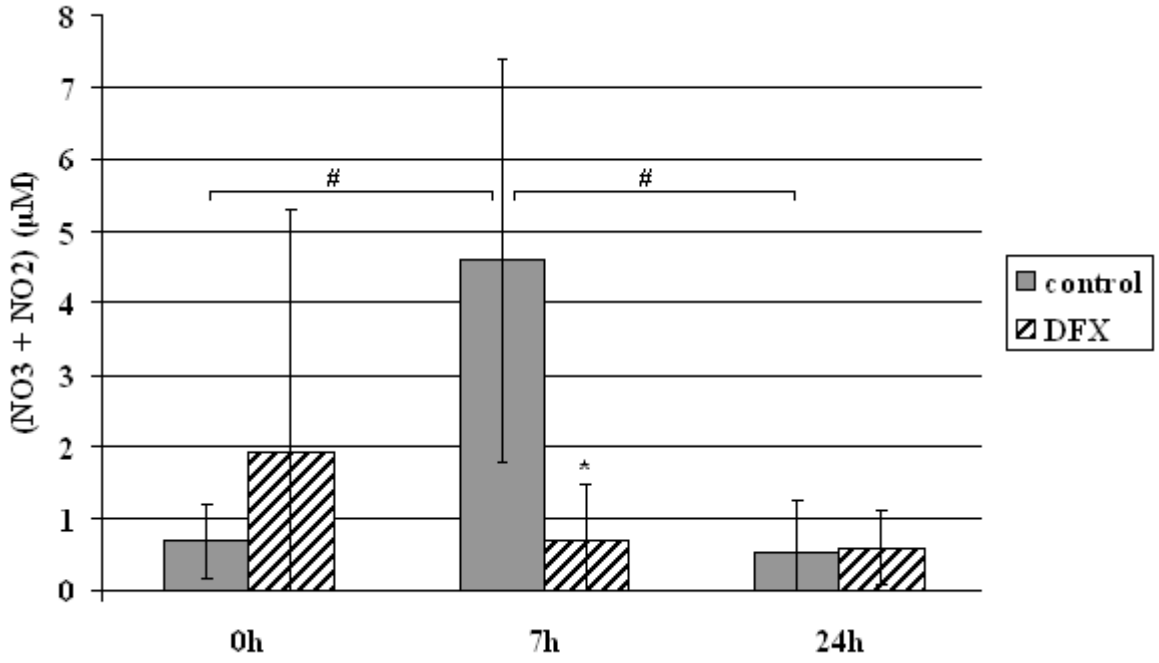
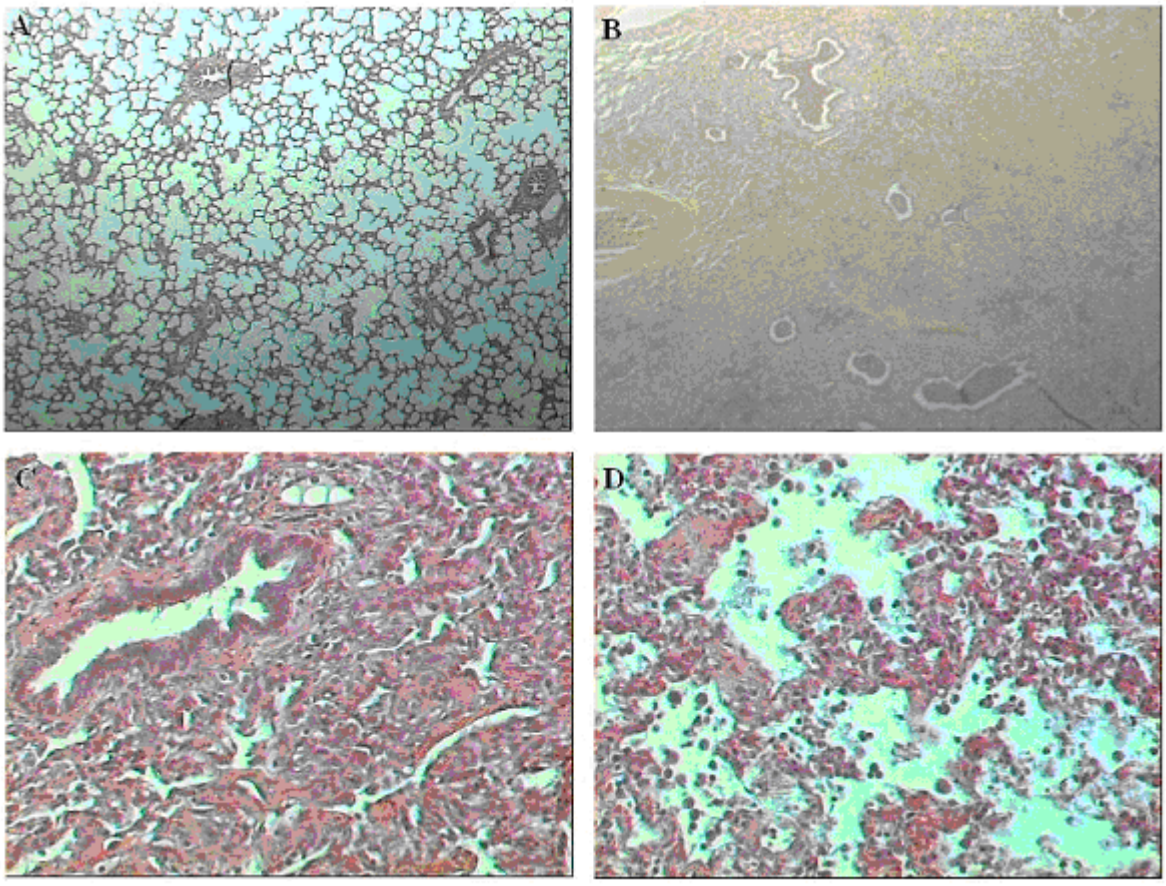


Figure 3. Hematoxylin-eosin [HE] sections of lungs from desferrioxamine (A) and control (B, C, D) groups. **A**, a normal region of the lung (HE, original magnification x 25); **B**, a region with extensive atelectatic changes (HE, original magnification x 25); **C**, larger magnification of a collapsed region (HE, original magnification x 100); **D**, intra-alveolar apoptotic alveolar epithelial cells detached from the alveolar wall (HE, original magnification x 100).


A
B
C
D