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ORIGINAL ARTICLE

Antioxidant preconditioning attenuates liver ischemia/reperfusion injury after hepatectomy in swine

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Summary

Purpose: *The purpose of this study was to evaluate whether antioxidant preconditioning with Deferoxamine can attenuate liver ischemia reperfusion injury associated with extended hepatectomy in swine.*

Methods: *Eighteen swine were randomly assigned to two groups: Deferoxamine (DFO) and Surgery Only (SO). The animals in both groups were subjected to laparotomy, prolonged temporary occlusion of the right and middle hepatic pedicles and subsequent left hepatectomy. The DFO group received IV deferoxamine prior to induction of liver ischemia. Monitoring was performed for 6 h and samples (Protein carbonyls, Thiobarbituric acid reactive substances, Histology, ALT, AST, Lactic acid and WBC) were drawn at 0, 60 and 360 min.*

Results: *Protein carbonyls and Thiobarbituric acid reactive substances had significantly lower concentration and higher reduction rates in serum and liver tissue of the DFO group. The histological examination of liver tissue showed less inflammation and necrosis in the DFO group. Hepatic enzymes and lactic acid measurements showed higher reduction rate in the DFO group by the end of the experiment.*

Conclusions: *This experimental study documents an early protective effect of deferoxamine administration in major hepatectomies against liver ischemia/reperfusion injury.*

Key words: *deferoxamine, ischemia, reperfusion, hepatectomy, swine*

Introduction

Ischemia/reperfusion (I/R) injury appears in major hepatectomies, where the period of ischemia of the organ is often more than 15 min. This may not only be harmful to the liver, but may affect other organs as well (myocardium, lung, kidney, adrenals, pancreas & intestine) [1,2]. Reactive oxygen species (ROS) are substances which mediate liver damage during the hypoxic phase of liver injury associated with I/R in liver surgery. Antioxidants can limit hepatocellular death which is caused by ROS during hypoxia and reperfusion [3].

ROS half-lives are short and they are difficult to evaluate. Instead, several products of the oxidative stress damage can be measured [4].

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To evaluate the effect of antioxidants we measured the levels of oxidative stress markers Protein carbonyls (PCs) and Thiobarbituric acid reactive substances (TBARS) and examined liver tissue for necrosis and proliferation.

Protein carbonylation is protein oxidation which is caused by ROS. Assessment of protein carbonylation as a marker for oxidative damage stems from the studies on bacterial glutamine synthetase, which is targeted for proteolytic degradation upon oxidation of its amino acid chains [5].

TBARS are by-products of lipid peroxidation that can be measured by the TBARS assay. TBARS can also be increased by myocardial ischemia [6].

Various factors have been studied that could possibly prevent or minimize both local and systemic damage from I/R by intervening in the mechanisms of oxidation and cell apoptosis [7,8]. The purpose of our experimental study described below was to assess whether antioxidant preconditioning prior to major liver surgery can be beneficial.

Methods

Animals

The experiment was approved by the Hellenic Veterinary Services (2046/5-04-2017) and adhered to the ARRIVE guidelines and EU Directive 2010/63/EU for animal experiments and by the Institution Review Board (IRB) of 'Attikon' University Hospital with associated number (EBD2328/05-05-2017). Eighteen male swine (Landrace/Large White) weighing 30±5 kg were obtained from the same farm (Validakis, Koropi, Greece). The animals were kept for 7 days before the experiment at the Research and Experimental Center Elpen (EL 09 BIO 03) in a controlled environment. One day before the experiment, the animals were not fed, and given only water.

The animals were randomly separated into two groups according to the research protocol: Deferoxamin (DFO) group - administration of antioxidants before the surgical procedure and Surgery Only (SO) group - surgical procedure without deferoxamine administration. The procedure consisted of laparotomy, cystostomy placement, right hepatic artery clamping for 60 min and left hepatectomy after reperfusion. All laparotomies were performed under general anesthesia and continuous monitoring.

Anesthesia

The animals were prepared with IM administration of 0.5 mg/kg midazolam (DORMIXAL, Demo S.A., Hellas). An ear vein was catheterized and the induction into anesthesia was performed with IV administration of 3 mg/kg propofol (PROPOFOL, Fresenius Kabi, Austria) and 0,012 mg/kg fentanyl (FENTANYL, Janssen Cillag, Belgium). Next, 0,5 mg/kg cis atracurium (NIMBEX, GlaxoSmith, Italy) was administered bolus IV and endotracheal intubation was performed. The swine were mechanically ventilated with controlled volume (Ventilator parameters: tidal volume 10±2 ml/kg & respiratory rate 12-14/min). Anesthesia maintenance was achieved with IV administration of propofol, fentanyl and cis atracurium at adjusted rates. During this period, the swine received 20 mL/kg/h N/S 0.9% IV to maintain MAP±20% of baseline without vasoactive drugs.

Surgical protocol

The swine were positioned in dorsal supine position and fore and hind legs were retracted laterally cranially and laterally caudally respectively with elastic bands. Temperature was measured per rectum and was controlled throughout the experiment with a heating pad.

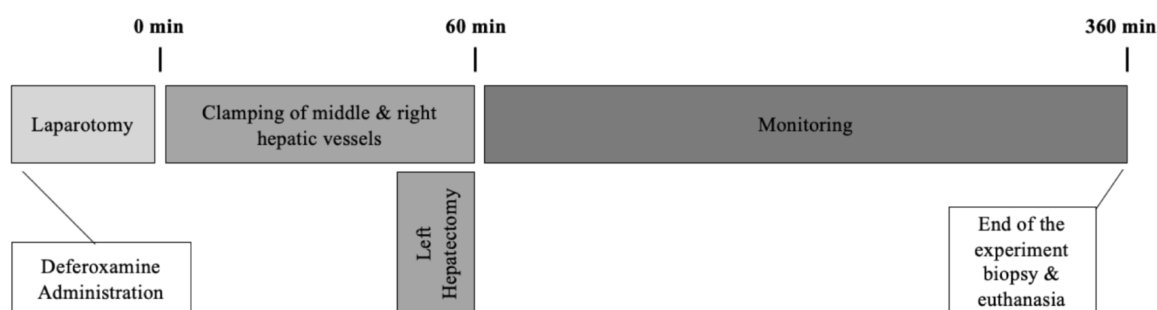

Figure 1. Time line of event and stages of the experiment.

Table 1. Histologic Scoring System

	Periportal fibrosis	*Hepatic vein inflammation*	*Capillary sinus inflammation*	*Hepatocellular degeneration*
0	-	-	-	-
1	+	+	+	-
2	++	++	+	+
3	+++	+++	++	+

(+) mild, (++) moderate, (+++) severe injury.

The internal jugular vein and carotid artery were cannulated proximally, using dual lumen venous catheters. Monitoring of MAP and CVP was performed with intravascular sensors and of HR, RR, SpO2 with percutaneous sensors.

The animals were subjected to midline laparotomy. A cystostomy was performed and a No 16 Foley catheter was inserted for hourly urine output measurement. Liver hilar components were identified and prepared. Ischemia was induced in most of the liver parenchyma (approximately 60% of total organ volume) by blocking right and middle hepatic pedicles (hepatic artery, portal vein and hepatic duct) for 1 h. The left liver lobe remained functional in order to act as an intestinal shunt.

At the end of the first hour the left liver lobe (approx. 40% of total organ volume) was removed, the cut liver surface was sutured with Prolene 2-0 and the middle and right pedicles were declamped. With this surgical protocol the liver remnant has been subjected to extensive I/R. The abdominal wall was then closed in one layer using a No1 Prolene suture in a continuous fashion (Figure 1).

Deferoxamine

Deferoxamine (DESFERAL, Novartis, Greece) was administered immediately after the induction of anesthesia and before the liver ischemia in an IV dose of 100 mg/kg diluted in 250 ml D/W 5%.

Administration of deferoxamine refers to DFO group of animals. In SO group the same volume of 250 ml D/W 5% was infused.

Blood and tissue samples

Arterial and venous blood was sampled immediately after laparotomy (0 min), at the end of the hepatectomy (60 min) and at the end of the protocol (360 min). Samples were collected for CBC and lactic acid (LAC). The blood serum (after centrifugation at 4000 rpm/ 10 min) was used for the measurement of antioxidant markers and liver enzymes.

Biopsies of the liver were taken at the end of the experiment for oxidative stress markers evaluation and histological assessment. Part of the liver tissue was frozen and then used for lipid peroxides estimation by the TBARS assays and PCs determination, after elimination of nucleic acids [9].

The other part was fixed in 4% formaldehyde solution at 25°C and then encapsulated into paraffin cubes. Hematoxylin-eosin stained slices of the paraffin cubes were assessed under 100x magnification Zeiss microscope for periportal fibrosis, hepatic vein inflammation, capillary sinus inflammation and hepatocellular degeneration using a three-point scoring system (Table 1). Each animal was classified according to highest scoring category.

Statistics

Data was expressed with mean value or median±standard deviation. The one-way ANOVA model was used to compare the variables at 0, 60, 360 min between DFO and SO groups and for the comparison of the percentage of values. The groups were compared pairwise with the Bonferroni test. Statistical significance was set at $P<0.05$. SPSS v. 21.00 (IBM Corporation, Somers, NY, USA) was used for statistical analyses.

Results

Hemodynamic and descriptive variables

All groups were matched for weight (DFO group: 28.9±2.4 kg and SO group: 29.7±2.4 kg (p=0.457)). Excised liver mass was comparable between groups (DFO group: 110±9.7 g and SO group: 115±11.6 g (p=0.422)). Baseline MAP, heart rate, CVP and temperature were similar in both groups. Urine output per hour remained stable in both

Table 2. WBC, Lactic acid levels and Hepatic transaminases (mean values±SD)

	0 min	*60 min*	*360 min*	*p value*
WBC (10^3/μL)				
DFO	-	-	13.12±8.79	0.008
SO	-	-	21.70±7.71	
LAC (mmol/L)				
DFO	-	-	0.59±0.18	0.033
SO	-	-	1.18±0.42	
ALT (U/L)				
DFO	37.9±3.4	35.4±2.3	29.4±4.6	0.018
SO	39.4±4.2	38.9±4.1	35.8±2.5	0.032
p.v. 0-360		DFO -22.55±10.42% vs SO -8.47±8.8%		0.024
AST (U/L)				
DFO	57.9±18.2	110.5±40.9	123.9±16.6	0.024
SO	44.4±8.1	100.6±38.7	96.5±24.2	0.035
p.v. 0-360		DFO 129.6±60.8% vs SO 117.8±44.3%		0.406

DFO: deferoxamine; SO: surgery only; WBC: white blood cells; LAC: lactic acid; ALT: alanine aminotransferase; AST: aspartate aminotransferase; SD: standard deviation; p.v. 0-360 = percentage variation 0 to 360 min.

groups (80±11 ml/h). All animals survived the procedures and were humanely terminated 360 min after initial incision.

CBC, lactic acid and liver biochemistry

WBC count in the SO group was elevated compared to the DFO group with statistically significant values (DFO 13.12±8.79×10^3/μL vs SO 21.70±7.71×10^3/μL; p=0.008) at the end of the experiment (Table 2).

LAC was measured before liver specimens were gathered at 360 min from laparotomy and was statistically significantly higher in the SO group vs the DFO group (DFO 0.59±0.18 mmol/L vs SO 1.18±0.42 mmol/L; p=0.033) (Table 2).

Alanine aminotransferase (ALT) was significantly lower at 360 min, in comparison to baseline values, in both groups (Table 2). ALT decrease from 0 to 360 min was significantly more pronounced in the DFO group compared to the SO group (-22.55% vs -8.47%; p=0.024) (Table 2).

Aspartate aminotransferase (AST) was elevated at 360 min, in both groups, compared to baseline values (Table 2). The percentage variation in the DFO group was 129.6% vs the SO group 117.8%, with no statistically significant value (p=0.406) (Table 2).

Oxidative stress markers

Animals treated with the antioxidant factor deferoxamine (DFO group), were found to have significantly reduced levels of PCs and TBARS values in serum and liver tissue, compared to SO group, at the end of the experiment, as shown in Table 3. Percentage variation of serum PCs in the DFO group vs the SO group was significant (-28.63% vs 25.75%; p=0.025)) and of serum TBARS in the DFO group vs the SO group was also significant (10.31% vs 0.64%; p=0.005; Table 3).

Table 3. Oxidative stress markers in serum and liver tissue (mean values±SD)

	Group DFO	*Group SO*	*p value*
PCs (nmole/mg)			
0 (min)	2.4±1.4	2.6±1.3	0.056
60 (min)	2.3±0.9	2.5±1.2	0.086
360 (min)	1.6±0.6	3.0±0.9	0.041
p.v. 0-360	DFO -28.63±14.81% vs SO 25.75±34.92%		0.025
TBARS (μM), min			
0	0.79±0.11	0.79±0.15	0.041
60	0.87±0.23	0.89±0.14	0.070
360	0.72±0.22	0.79±0.38	0.083
p.v. 0-360	DFO -10.31±18.64% vs SO 0.64±49.04%		0.005
Liver tissue			
PCs (nmol/mg prot)	10.2±0.7	16±2.9	0.043
TBARS (nmol/mg prot)	81±12	101±26	0.011

DFO: deferoxamine; SO: surgery only; PCs: protein carbonyls; TBARS: thiobarbituric acid reactive substances; SD: standard deviation; p.v. 0-360 = percentage variation 0 to 360 min.

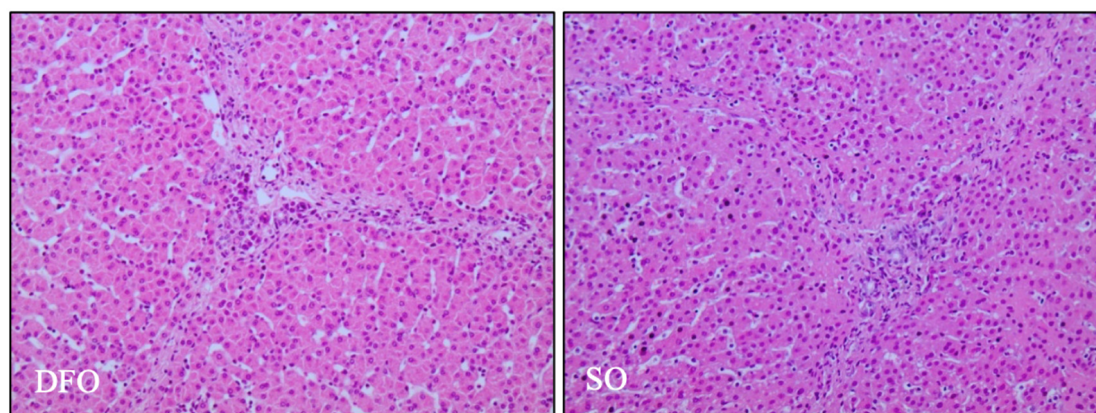

Figure 2. Hematoxylin-eosin stained sections of hepatic parenchyma. Signs of milder inflammation and necrosis in the DFO group compared to the SO group (magnification ×100).

Hepatocellular pathology

Liver tissue damage due to I/R was assessed with hematoxylin-eosin tissue staining. This revealed milder inflammation and necrosis in the DFO group compared to the SO group (Figure 2). Hepatocellular injury, as measured with our scoring system, was attenuated in the DFO group compared to the SO group, however, this trend was not statistically significant. (0.83±0.75 vs 1.33±1.03; p=0.346).

Discussion

The Pringle maneuver, first described by Pringle for liver trauma [10], is a widely used maneuver in liver surgery, nevertheless a main disadvantage is the ensuing I/R injury.

Several pathways are activated during I/R, resulting in I/R injury. Blood supply deprivation leads to hypoxia which affects mitochondrial function causing tissue degeneration [11]. This tissue damage is aggravated by reperfusion which causes production of ROS, activation of anti-inflammatory mediators and upregulating of cell adhesion molecules [12,13].

Several ways to avoid the above effects have been discussed. There are various surgical and pharmaceutical approaches and preconditioning with antioxidants is one of them [14,20].

The present study aimed to evaluate the effect of DFO, an antioxidant agent, in a setting of major hepatectomy with prolonged (60 min) I/R time in a large animal model. DFO is an iron chelator which neutralizes iron thus limiting lipid peroxidation.
Omar et al have studied DFO administration (60 mg/kg) in rats undergoing total liver I/R and have reported lower MDA levels and hepatocellular damage showing an improvement of I/R injury [21]. In our experiment, preconditioning swine with 100 mg/kg DFO led to reduced lipid peroxidation. This benefit was evidenced by liver histopathological findings as well as the levels of oxidative stress markers.

Tokyol et al also investigated preconditioning with DFO and Quercetin before total hepatic I/R in rodents. They evaluated liver tissue samples and MDA levels and documented improvement in both DFO and Quercetin groups [22]. Our study provides similar results in a larger porcine model that seems to be comparable to human physiology [23].

Arkadopoulos et al reported an improvement of I/R injury during extended hepatectomies in swine with the use of DFO. They performed a porto-caval bypass before the operation to avoid portal occlusion disadvantages during the prolonged Pringle maneuver. Deferoxamine-treated animals had reduced liver tissue damage [24]. In our experiment we avoided the porto-caval shunt and its complications using the left hepatic vessels as a portal venous bypass. Our main findings were lower levels of oxidative stress markers (PCs and TBARS) and serum markers associated with hepatocellular injury (ALT and AST) in DFO preconditioned animals. Additionally, we documented amelioration of hepatic tissue damage in the DFO group expressed by histopathological findings.

The main limitation of the study is the relatively short follow-up period. Although I/R injury is present at the moment of reperfusion of the liver parenchyma, it is our belief that a longer follow-up period would likely clarify if our observed early effects of deferoxamine preconditioning provide a lasting benefit.

In conclusion, the present study shows that DFO preconditioning in major hepatectomies with prolonged I/R attenuates liver damage. Our results demonstrated that DFO is associated with an antioxidant protective effect on I/R injury to the liver. This effect may translate into clinical benefit in human studies. With several surgical procedures still requiring intraoperative Pringle maneuver, more studies need to be conducted in order to standardize the deferoxamine administration protocol in the clinical setting and to elucidate the mechanisms behind our observations.

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Conflict of interests

The authors report no conflicts of interest and no financial disclosures. The authors alone are responsible for the content and writing of the paper.

The authors report no proprietary or commercial interest in any product mentioned or concept discussed in this article.

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