Hellenic Journal of Surgery (2015) **87**:5, 407-414

The Effects of n-Acetylcysteine and Desferoxamine on IL-6, TNF-a, and oxLDL after Infrarenal Aortic Clamping

K. Katseni, A. Chalkias, G. Kaparos, N. Iacovidou, E. Logothetis, N. Dafnios, T. Kotsis, E. Karvouni, V. Arapoglou

Abstract

Aim-Background: Treatment of abdominal aortic aneurysms remains a challenging task. We examined the effects of n-acetylcysteine and desferoxamine on interleukin-6, tumour necrosis factor-a, and oxidized low density lipoprotein after infrarenal aortic clamping.

Methods: Fifteen piglets were assigned to three groups. The control group (Group C) received saline as placebo (10-mL dilution, bolus), whereas the Group of n-acetylcysteine (Group NAC) received 150mg/kg IV n-acetylcysteine 30 min prior to aortic clamping and 50mg/kg/h after its release (reperfusion). The desferoxamine group (Group D) received IM 100mg/kg/d desferoxamine for 3 days prior to the experiment.

Results: Interleukin-6 increased significantly one hour after aortic clamping, while administration of desferoxamine and n-acetylcysteine had no effect on its production. Tumour necrosis factor-a increased after aortic clamping and failed to normalise during the experiment in all groups. At 2 hours post-reperfusion, desferoxamine had a modest effect on tumour necrosis factor-a values, while in Group NAC, tumour necrosis factor-a had decreased by 50% at 10 min post-reperfusion. Oxidized low density lipoprotein in Group C did not increase significantly from baselines values. In Groups D and NAC, oxidized low density lipoprotein had increased at 10 min post-reperfusion, after which it progressively decreased until 2 hours post-reperfusion.

Conclusions: Interleukin-6 and tumour necrosis factor-a increased significantly after aortic clamping. Administration of desferoxamine and n-acetylcysteine had no effect on interleukin-6, while administration of n-acetylcysteine reduced tumour necrosis factor-a by 50% at 10 min post-reperfusion. Although in Groups D and NAC oxidized low density lipoprotein increased post-reperfusion, it progressively decreased with time.

Key words: *Infrarenal aortic clamping; n-acetylcysteine; desferoxamine; interleukin-6; TNF-a; oxidized low density lipoprotein*

Introduction-Aim

Treatment of abdominal aortic aneurysms (AAA) remains a challenging task despite improvements in perioperative care. The incidence of ruptured AAAs is 5.6-17.5 per 100,000 person-years in Western countries and the overall mortality rate of these patients is approximately 80-90% [1]. The high mortality rates constitute a significant medical problem and research has focused on the identification of key pathophysiological cascades of I/R after AAA surgery. To date, the consequences of AAA are known to be the result of a double physiological phenomenon that happens because of lower torso ischaemia and subsequent reperfusion, occurring in a more severe form in patients with ruptured AAAs.

The mechanisms of ischaemia/reperfusion (I/R) injury include various mediators which cause cellular dysfunction and postoperative organ injury failure. The inflammatory response involves the activation of immune system and the vascular endothelium, as well as a number of other pathways, including reactive oxygen species (ROS) mediators generated from components of blood or tissues. Previous studies have shown that ROS can participate in reduction-oxidation reactions, initiate lipid oxidation, and induce proinflammatory cytokines and chemokines [2] which in turn generate

K. Katseni MD, N. Dafnios MD, T. Kotsis, PhD, V. Arapoglou PhD
National and Kapodistrian University of Athens, Medical School, 2nd Surgical Department, Vascular Surgery Unit, Athens, Greece

A. Chalkias PhD
National and Kapodistrian University of Athens, Medical School, MSc "Cardiopulmonary Resuscitation", Hellenic Society of Cardiopulmonary Resuscitation, Athens, Greece, Tzaneio Hospital of Piraeus, Department of Anesthesiology, Piraeus, Greece

G. Kaparos MD, E. Logothetis MD, E. Karvouni PhD
National and Kapodistrian University of Athens, Medical School, Aretaion Hospital, Department of Biopathology, Athens, Greece

N. Iacovidou PhD
Hellenic Society of Cardiopulmonary Resuscitation, National and Kapodistrian University of Athens, Medical School, Aretaion Hospital, Department of Pediatrics, Neonatal Division, Athens, Greece

Corresponding author: Dr. Athanasios Chalkias
3 Ir. Politechniou Av, 18532, Piraeus, Greece
Tel.: +30 2104133992; Fax: +30 2110121758
e-mail: thanoschalkias@yahoo.gr

Received 21 June 2015; Accepted 30 July 2015

an increase in concentrations of acute-phase proteins, increasing postoperative complications and mortality rates.

Although the mechanisms underlying I/R are poorly understood, there may be an advantage associated with antioxidant treatment; antioxidant supplementation during AAA repair has been reported to be beneficial in reducing oxidative stress during the perioperative period [3]. In this study, we aimed to examine the effects of n-acetylcysteine and desferoxamine on the inflammatory cascade after I/R, with particular focus on interleukin-6 (IL-6), tumour necrosis factor-a (TNF-a), and oxidized low density lipoprotein (oxLDL).

Method

The experimental protocol was approved by the General Directorate of Veterinary Services (permit no. 416/14-01-2009) according to Greek legislation regarding ethical and experimental procedures. The experiment was carried out in the Department of Experimental Surgery in Aretaion Hospital, Athens, Greece. Fifteen healthy male Landrace/Large White piglets comprised the study population, all supplied by the same breeder (Validakis, Athens, Greece), aged 20-24 weeks, with an average weight of 30±2 kg. The animals were fasted overnight but had free access to water.

Animals were premedicated with an intramuscular injection of 11 mg/kg ketamine hydrochloride, 0.5 mg/kg midazolam, and 0.05 mg/kg atropine sulfate. The marginal auricular vein was catheterized, and anaesthesia was induced with an intravenous bolus dose of 6.6 mg/kg thiopental [4]. They were then intubated with a 5-mm endotracheal tube (Portex, 5 mm ID; Mallinckrodt Medical, Athlone, Ireland). Animals were immobilized in the supine position on a surgical table. Additional 2 mg/kg thiopental, 0.15 mg/kg cis-atracurium and 4μg/kg fentanyl were administered immediately before connecting the animals to a ventilator (Alpha Delta lung ventilator; Siare, Bologna, Italy) in 21% oxygen. Thiopental infusion of 5 mg/kg/hr and additional doses of cis-atracurium at 20 μg/kg/min and fentanyl at 0.6 μg/kg/min were given to maintain adequate anaesthetic depth. Fluid infusion of 2 ml/kg/h isotonic sodium chloride was started and remained constant throughout the experiment in order to prevent an artificial stress response [5].

All animals were volume controlled, ventilated with a total tidal volume of 15 mL/kg. End-tidal CO_2 pressure ($ETCO_2$) was monitored (Tonocap-TC200; Datex Engstrom, Helsinki, Finland), and the respiratory rate was adjusted to maintain an ETCO2 of 35 to 40 mm Hg. Non-invasive monitoring (Datascope Expert DS-5300 W ECG; Fukuda Denshi, Tokyo, Japan) also included electrocardiogram and pulse oximetry. For measurement of aortic pressures, an arterial catheter (model 6523, USCI CR; Bart Inc, Papapostolou, Athens, Greece) was inserted into the aorta via the right common carotid artery. The systolic (SAP) and diastolic (DAP) aortic pressures were recorded, while mean aortic pressure (MAP) was determined by the electronic integration of the aortic blood pressure waveform. The internal jugular vein was surgically prepared, and a Swan-Ganz catheter (Opticath 5.5 F, 75 cm; Abbott, Ladakis, Athens, Greece) was inserted into the right atrium for continuous measurement of right atrial pressure. Intravascular catheters were attached to pressure transducers that were aligned to the level of the right atrium and were calibrated prior to their use. CO was measured as the product of time-velocity integral of Doppler transaortic flow, the diameter of the aortic valve and the heart rate, as previously described [6]. Arterial blood gases were measured on a blood-gas analyzer (IRMA SL Blood Analysis System, Part 436301, Diametrics Medical Inc., USA). Body temperature was monitored by a rectal temperature probe and was maintained between 38.5 °C and 39.5 °C with a heating blanket.

Before the experimental procedure, the piglets were randomly assigned to three different groups, of five subjects each, according to the agents used. Randomization was achieved with sealed envelopes and only the investigator that administered the drug regimen was aware of the animal's allocation. This investigator was not involved in the data collection. The control group (Group C) received saline as placebo (10-mL dilution, bolus), whereas the n-acetylcysteine group (Group NAC) received 150mg/kg IV n-acetylcysteine 30 min prior to aortic clamping and 50mg/kg/h after its release. The desferoxamine group (Group D) received IM 100mg/kg/d desferoxamine for 3 days prior to the experiment.

The protocol was divided into three distinct phases: stabilization, laparotomy, maintenance, and the observational phase. The stabilization phase lasted for approximately 30 min after the instrumentation of the animals. Then, a laparotomy was performed and the abdominal aorta, inferior vena cava, renal veins, hepatic veins, and hepatic portal vein were surgically exposed and cannulated. The animals were then left to stabilize for another 30 min. During the maintenance phase, which lasted 60 min, the aorta was clamped inferiorly to the origination of renal arteries. During the observational phase, aortic clamping was progressively removed in order to minimize hypotension and stress response, and the animals were monitored for 120 min. Blood samples were collected in all phases for measurement of IL-6, TNF-a, and oxLDL.

All animals were humanely euthanatized with an IV bolus dose of propofol 40 mg, followed by 2 gr thiopental IV. Thoracic and abdominal organs were examined for gross evidence of traumatic injuries or other pathology.

Statistical analysis

Quantitative variables were expressed as mean values ±SD, while qualitative variables were expressed as absolute and relative frequencies. Student's t-tests and analysis of variance (ANOVA) were computed for the comparison of mean values. Bonferroni correction was used in order to control for type I error. All reported p-values are two-tailed. Statistical significance was set at $p<0.05$. The data analyses were performed with SPSS for Windows (version 19.0; SPSS, Chicago, IL) software.

Results

The haemodynamic and metabolic parameters in all groups are presented in Table 1. There was a significant difference between the three groups in heart rate, SAP, DAP, CPP and pH ($p=0.04$, 0.048, 0.046, 0.044, 0.048, respectively).

Interleukin-6 significantly increased one hour after aortic clamping in all groups and did not normalize during the experiment. After 2 hours post-reperfusion, the highest values were found in the portal vein and inferior vena cava samples in all groups, while administration of desferoxamine and NAC had no effect on IL-6 production (Table 2).

Tumour necrosis factor-a increased in all groups after aortic clamping and did not normalise during the experiment. In Group C, TNF-a progressively decreased with time, although it did not reach baseline levels. In Group D, TNF-a values rose and remained almost two-fold from baseline, while they were higher in portal vein and inferior vena cava at 2 hours post-reperfusion compared to other samples ($p<0.001$). At 2 hours post-reperfusion, desferoxamine had a modest effect on TNF-a values in all samples, while in Group NAC, TNF-a levels fell by 50% at 10 min post-reperfusion, maintaining a constant decreased rate until 2 hours post-reperfusion (Table 3).

Oxidized low density lipoprotein was not significantly increased compared to baselines values in Group C. In Groups D and NAC, oxLDL was increased at 10 min post-reperfusion, after which it was progressively decreased until 2 hours post-reperfusion (Table 4).

Discussion

Ischaemia-reperfusion contributes significantly to abdominal aortic aneurysm-related mortality and morbidity. The main effect of I/R is the activation of inflammatory pathways with oxidative stress occurring during aortic clamping and subsequent reperfusion [7]. The synthesis of cytokines during the inflammatory processes generates an increase in concentrations of acute-phase proteins as a response to tissue destruction [8], while high-grade oxidative stress during AAA repair operation has been related with a complicated postoperative course and transfer to intensive care unit [9].

In our study, IL-6 significantly increased one hour after aortic clamping in all groups and failed to normalise during the experiment. Interestingly, after 2 hours post-reperfusion, the highest values were found in portal vein and inferior vena cava samples in all groups, which is in line with the results of other authors reporting that I/R during AAA repair is associated with a marked increase in IL-6 concentration in the portal vein, suggesting that IL-6 is mainly produced by the gastrointestinal tract [10]. Circulating IL-6 levels have been reported to be higher in patients with AAA than those in subjects without AAA, showing a proportional relationship with AAA diameter [11]. In addition, IL-6 levels have been shown to increase further in plasma during AAA repair and they remain elevated in the postoperative period [12]. In a recent study, Vucevic et al. reported that the levels of IL-6 in supernatants of abdominal aortic aneurysm sample cultures were 73 times higher than their levels in plasma [13], thus reflecting the active inflammatory processes in the aortic lesions [14].

In our study, administration of desferoxamine and NAC had no effect on IL-6 levels. Similarly, in a prospective, randomized, double-blinded, placebo-controlled study that included critically ill patients with hypotension, Fraga et al. reported that the use of NAC plus desferoxamine decreased the oxidative damage parameters but not plasma IL-6 levels [15]. In an experimental pig model combining liver resection with prolonged ischaemia, administration of desferoxamine during reperfusion of the remnant liver was associated with an increase in serum IL-6, and was significantly higher after 12 h compared to controls [16]. Of note, increased doses of IL-6 during liver I/R have been reported to protect the liver from damage via its anti-inflammatory effects, as well as via TNF-a reduction [17]. In another murine study, IL-6 resulted in less intestinal injury and improved barrier function following I/R of the small bowel while inhibiting both constitutive and induced enterocyte cell death in vivo [18]. On the other hand, after intestinal I/R injury, NAC has been reported to protect healing of colonic anastomosis and may improve mesenteric blood flow and oxygen delivery [19].

After intestinal I/R, an inflammatory response and TNF-a increase may ensue [20]. In our study, TNF-a increased after aortic clamping in all groups and failed to normalise during the experiment, a finding which is in agreement with other studies [21], although some authors have reported no change in TNF-a levels in AAA repair or after infra-renal clamping [22]. Of note, TNF-a levels were higher in portal vein and inferior vena cava compared to other samples at 2 hours post-reperfusion, supporting recent evidence of TNF-a increase after intestinal I/R injury in humans [23]. Our

Table 1. *Haemodynamic variables of the groups.*

Variable	Group																		p value
	C						D						NAC						
	Ba	**Pc10m**	**R**	**R10m**	**R1h**	**R2h**	**Ba**	**Pc10m**	**R**	**R10m**	**R1h**	**R2h**	**Ba**	**Pc10m**	**R**	**R10m**	**R1h**	**R2h**	
HR	95.6±7.4	98.47±5.7	123.5±7.8	120.3±6.5	103.7±9.3	99.4±6.8	96.4±7.3	97.2±4.4	131.6±8.3	125.3±7.6	110.7±3.6	100.4±5.9	94.76±7.3	97.1±7.5	127.4±7.5	122.7±3.6	108.8±5.2	98.4±6.6	0.040
SAP	134.7±20.1	137.2±18.5	96.3±4.6	100.4±6.3	100.8±3.9	101.6±7.7	132.3±18.3	135.6±14.8	97.7±5.1	99.8±7.2	100.6±3.2	103.3±6.2	135.7±16.6	138.7±12.5	94.8±4.9	101.3±4.5	101.7±4.1	102.5±5.8	0.048
DAP	86.4±9.67	98.7±9.3	57.2±5.5	66.4±4.8	64.8±3.4	67.3±7.4	83.8±5.2	95.4±7.2	53.4±4.3	64.6±3.6	65.1±4.5	67.9±3.8	85.7±3.4	96.9±8.3	55.9±4.7	69.2±4.2	65.2±5.6	66.4±3.8	0.046
MAP	102.3±3.8	111.6±9.4	70.0±2.1	77.3±2.5	77.0±1.9	78.3±3.3	100.0±5.7	108.3±4.2	68.2±4.6	76.5±3.7	77.1±3.4	79.6±5.3	102.3±6.3	110.8±4.7	69.2±2.6	79.4±3.4	77.2±2.8	78.1±3.7	0.088
RASP	14.5±4.7	16.8±3.6	13.1±5.5	11.8±4.2	12.7±2.7	13.6±4.1	13.4±3.2	15.3±2.2	12.9±4.6	11.3±5.1	12.5±3.2	13.8±3.3	14.1±4.7	16.2±1.9	12.5±3.9	10.9±5.2	12.1±4.5	13.5±2.9	0.225
RADP	7.1±2.9	8.8±2.5	6.9±7.7	6.5±6.4	6.7±6.3	6.8±5.3	7.3±1.6	8.5±2.2	7.0±5.2	6.7±6.4	6.9±3.5	7.2±4.6	7.2±1.8	8.6±1.9	7.1±5.3	6.6±4.4	6.9±3.5	7.1±4.6	0.234
CPP	79.1±4.7	89.9±2.7	50.3±2.5	59.9±2.3	58.1±3.1	60.5±1.6	76.5±2.7	86.9±3.1	46.4±2.4	57.9±3.2	58.2±1.7	60.7±3.3	78.5±3.6	88.3±2.7	48.8±2.9	62.6±1.8	58.3±2.7	59.3±3.6	0.044
$ETCO_2$	38.6±3.6	39.8±4.3	36.7±5.2	33.4±4.3	36.8±2.7	37.9±7.8	39.4±4.6	41.3±2.6	35.1±6.1	34.1±6.2	37.6±3.1	38.6±5.8	36.4±4.6	40.3±2.6	34.2±6.1	33.1±6.2	35.6±3.1	38.6±5.8	0.062
CO	6.1±0.5	5.7±0.6	6.3±0.4	6.2±0.6	6.2±0.5	6.0±0.3	6.2±0.6	5.5±0.3	6.4±0.5	5.7±0.8	5.9±0.9	6.0±0.2	6.0±0.6	5.8±0.3	6.2±0.5	6.1±0.8	6.2±0.9	6.1±0.2	0.174
SPO_2	96.1±3.1	97.2±2.5	97.4±2.2	96.7±1.3	96.3±2.8	94.7±2.4	97.0±2.1	96.4±1.2	96.7±3.1	95.8±3.4	96.7±2.2	96.5±2.4	97.0±2.1	95.4±3.4	96.2±2.6	96.1±3.4	96.9±2.2	96.7±2.4	0.189
pH	7.41±0.04	7.36±0.03	7.37±0.04	7.36±0.02	7.37±0.03	7.38±0.02	7.40±0.04	7.37±0.03	7.36±0.01	7.36±0.02	7.37±0.04	7.37±0.08	7.43±0.02	7.36±0.03	7.36±0.02	7.36±0.03	7.36±0.03	7.37±0.04	0.048
PaO_2	91.5±5.1	83.4±4.8	82.4±5.2	83.5±3.2	89.2±4.4	92.1±5.3	92.2±3.6	84.4±6.8	85.5±5.5	85.3±5.4	89.4±3.4	92.3±4.6	90.3±4.1	85.1±4.3	84.4±3.5	85.6±4.1	90.5±2.2	92.7±3.6	0.137
$PaCO_2$	38.7±1.8	42.3±4.4	44.5±3.4	42.4±3.8	42.8±4.5	40.3±4.2	39.3±1.4	40.6±5.1	43.7±5.1	40.4±4.3	41.2±3.2	39.3±2.5	40.1±2.5	40.6±5.1	44.5±2.4	41.6±4.1	41.8±4.3	40.2±3.8	0.185
HCO_3	26.2±3.2	26.1±2.3	25.4±2.8	25.9±2.3	24.6±2.5	25.7±2.9	26.6±2.5	25.6±2.4	25.1±2.3	26.4±1.7	24.6±2.2	24.4±2.9	27.1±2.5	25.6±2.2	24.1±1.1	25.5±1.3	24.9±1.2	25.1±1.6	0.078
EB	3.7±2.3	-0.2±0.3	-0.2±0.4	-0.1±0.2	0.3±0.2	0.6±0.2	3.3±1.3	-0.1±0.2	-0.2±0.1	-0.1±0.5	0.4±0.3	0.5±0.4	3.3±1.3	-0.2±0.1	-0.2±0.2	-0.2±0.5	0.2±0.3	0.5±0.4	0.112
Lactate	0.7±0.3	3.1±0.5	3.2±0.4	3.1±0.5	2.8±0.4	2.4±0.3	0.4±0.2	2.9±0.6	3.1±0.4	3.0±0.3	2.6±0.4	2.3±0.4	0.4±0.2	2.6±0.7	3.3±0.4	3.2±0.3	2.7±0.4	2.5±0.4	0.055

Ba, Baseline; Pc10m, 10 min post-clamping; R, 1min after reperfusion; R5m, 10 min after reperfusion; R1h, 1 hour after reperfusion; R2h, 2 hours after reperfusion; HR, heart rate; SAP, aortic systolic pressure; DAP, aortic diastolic pressure; MAP, mean aortic pressure; RASP, right atrial systolic pressure; RADP, right atrial diastolic pressure; CCP, coronary perfusion pressure; $ETCO_2$, end-tidal carbon monoxide; CO, cardiac output; SpO_2, saturation of peripheral oxygenation; PaO_2, partial arterial oxygen pressure; $PaCO_2$, partial arterial carbon dioxide pressure; BE, base excess.

Table 2. *IL-6 values during the experiment.*

IL-6 (pg/ml)	Group			p value ANOVA
	C	D	NAC	
	Mean±SD	Mean±SD	Mean±SD	
Baseline	5.8±1	5.9±0.9	13.3±3.3	<0.001
Prior to clamping	7.2±1.1	16.7±7.4	20.1±9.2	0.339*
1 hour after clamping	122.7±38.6	177.2±45.3	297.3±275.9	0.151*
Immediately after reperfusion (inferior vena cava)	127.3±48.5	162.5±50.3	335.3±295.2	0.014
10 min after reperfusion	123.5±29.6	160.5±60.8	216.1±73.3	0.002
1 hour after reperfusion	158.4±40.3	242.8±109.3	278.5±54.4	0.324*
2 hours after reperfusion (arterial)	200.2±102.7	249.4±84.6	329.6±121.9	0.016
2 hours after reperfusion (internal jugular)	250.0±103.2	267.8±6.9	284.5±85.3	0.557*
2 hours after reperfusion (renal vein)	194.0±77.2	183.8±86.1	259.1±79.2	0.061
2 hours after reperfusion (hepatic vein)	191.5±89.6	214.3±47.8	304.9±87.2	0.002
2 hours after reperfusion (portal vein)	245.2±104.6	314.5±72.5	365.0±112.8	0.019
2 hours after reperfusion (inferior vena cava/ supra-coeliac blood drawn)	207.0±64.8	206.0±65.3	340.0±129.5	0.001
2 hours after reperfusion (inferior vena cava/infra-coeliac blood drawn)	222.7±82.6	242.7±43.5	324.4±93.7	0.006

**Student's t-test*

Table 3. *TNF-a values during the experiment.*

TNF-a (pg/ml)	Group			p value ANOVA
	C	D	NAC	
	Mean±SD	Mean±SD	Mean±SD	
Baseline	44.9±27.8	157.8±49.8	75±36.5	<0.001
Prior to clamping	55.6±22.7	212.7±93.4	126±104.3	0.043*
1 hour after clamping	191.5±90.2	262.3±61.1	326.3±310.2	0.550*
Immediately after reperfusion (inferior vena cava)	197.9±164.4	338.3±128.5	611±565.4	0.021
10 min after reperfusion	135.2±91.3	324.5±209.2	363.2±302.8	0.097
1 hour after reperfusion	110.7±87.6	345.5±118,8	295.6±279.7	0.575*
2 hours after reperfusion (arterial)	84.8±50.3	312.5±27.9	158.5±149.5	<0.001
2 hours after reperfusion (internal jugular)	99.5±52.8	312±81.5	167.7±114.6	<0.001*
2 hours after reperfusion (renal vein)	76.9±39.8	241.8±55.1	170±142.5	0.004
2 hours after reperfusion (hepatic vein)	97.8±46.2	291.8±85.1	168.2±120.9	0.001
2 hours after reperfusion (portal vein)	89.8±38.5	299.3±62.6	175.7±134.2	<0.001
2 hours after reperfusion (inferior vena cava/supra-coeliac blood drawn)	93.5±37.3	331.7±59.8	113.4±82.7	<0.001
2 hours after reperfusion (inferior vena cava/infra-coeliac blood drawn)	83.0±27.4	304.5±66.5	192.5±19.3	<0.001

**Student's t-test*

Table 4. *oxLDL values during the experiment.*

oxLDL	Group			p value ANOVA
	C	D	NAC	
	Mean±SD	Mean±SD	Mean±SD	
Baseline	21.9±12.7	53.9±36.1	24.4±8	0.002
Prior to clamping	23.5±7.8	32.7±9.5	22.2±5.5	0.003*
1 hour after clamping	25.2±3.9	33.5±13.5	18.2±5.3	<0.001
Immediately after reperfusion (inferior vena cava)	16.4±4.9	54.2±37.6	16.2±6	<0.001
10 min after reperfusion (inferior vena cava)	16.5±4.3	59.2±24.2	28±15.7	<0.001
1 hour after reperfusion (inferior vena cava)	22.8±4.1	34.2±6.1	24.1±11.6	0.015*
2 hours after reperfusion (arterial)	23.1±12.2	35.2±12	23±12.5	0.029
2 hours after reperfusion (internal jugular)	23.3±11.9	38.5±13.8	22.7±3.5	0.009*
2 hours after reperfusion (renal vein)	26.7±10.1	30.7±5.4	20.7±8	0.016
2 hours after reperfusion (hepatic vein)	21.1±12.3	32.8±8.1	19.2±6.4	0.002
2 hours after reperfusion (portal vein)	20.2±11.5	47.6±24.3	23.6±8.8	<0.001
2 hours after reperfusion (inferior vena cava/supra-coeliac blood drawn)	27.0±10.2	35.2±9.8	19.9±10	0.003
2 hours after reperfusion (inferior vena cava/infra-coeliac blood drawn)	16.0±1.7	31.0±9.1	24.8±6.8	<0.001

**Student's t-test*

results strengthen the available evidence and highlight the role of intestinal I/R injury in the inflammatory response during abdominal aortic aneurysm repair. Although administration of desferoxamine did not result in a significant TNF-a decrease after reperfusion, TNF-a levels fell by 50% in Group NAC at 10 min post-reperfusion, maintaining a constant decreased rate until 2 hours post-reperfusion. In a study investigating the effect of NAC preconditioning on the small bowel transplantation-I/R-induced inflammatory cascade, the authors reported that NAC may ameliorate the inflammatory cascade mediated by TNF-a, which was in line with the results of other authors [24]. Of note, several other studies have demonstrated an anti-inflammatory effect of NAC, confirming our results [25,26].

Although LDL particles pose a risk for cardiovascular disease when they invade the endothelium and become oxidized, oxLDL levels did not rise significantly in our study; only a mild increase was noticed at 10 min and 1 hour after reperfusion. Oxidized LDL accumulates in several tissues after intestinal I/R and acts synergistically with I/R to promote leukocyte recruitment, indicating that cellular oxidative stress is a critical step in I/R injury in both the intestine and end organs, mediating via endothelial activation and dysfunction and subendothelial migration of inflammatory cells [27]. In our study, all animals suffered a modest haemodynamic fluctuation during the peri-clamping period, which in turn affected CPP values. Myocardial I/R is known to upregulate lectin-like oxLDL receptors which have been associated with apoptosis, necrosis, and left ventricular functional deterioration [28], while activation of the lectin-like oxidized low-density lipoprotein receptor-1 pathway is involved in determining the extent of myocardial ischaemia-reperfusion injury [29]. Also, oxLDL may play a role in renal tissue damage after intestinal I/R [30]. Given the aforementioned significant evidence, as well as the fact that our animals were monitored for only 2 hours after reperfusion, further research is warranted to fully clarify the role of oxLDL after abdominal aortic clamping.

We recognize several limitations in this experimental study. Firstly, the sample size was small. Secondly, our experiment was conducted on apparently healthy pigs with no underlying disease. This is not the case in human patients who, most of the time, have various comorbidities. Another limitation is the relatively brief observation period of animals. However, this study protocol was designed to focus on the early effects of NAC and desferoxamine administration. Despite these limitations, it is hoped that this study will enlighten further clinical and experimental research in reducing I/R after AAA surgery.

Within these limitations, IL-6 and TNF-a levels sig-

nificantly increased after aortic clamping. Administration of desferoxamine and NAC had no effect on IL-6, while administration of NAC decreased TNF-a by 50% at 10 min post-reperfusion, maintaining a constant decreased rate until 2 hours post-reperfusion. Although in Groups D and NAC oxidized low density lipoprotein increased post-reperfusion, it progressively decreased with time.

Ethical Approval

The authors declare that the study has been approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

Conflict of Interest

All authors declare that they have no conflict of interest

References

1. Hoornweg LL, Storm-Versloot MN, Ubbink DT, Koelemay MJ, Legemate DA, Balm R. Meta analysis on mortality of ruptured abdominal aortic aneurysms. Eur J Vasc Endovasc Surg 2008;35:558-70.
2. Kono H, Asakawa M, Fujii H, et al. Edaravone a novel free radical scavenger, prevents liver injury and mortality In rats administered endotoxin. J Pharmacol Exp Ther 2003;307:74-82.
3. Wijnen MH, Roumen RM, Vader HL, Goris RJ. A multiantioxidant supplementation reduces damage from ischemia reperfusion in patients after lower torso ischemia. A randomized trial. Eur J Vasc Endovasc Surg 2002;23:486-90.
4. Swindle MM. Swine in the Laboratory: Surgery, Anesthesia, Imaging & Experimental Techniques. 2nd ed. Boca Raton: CRC Press; 2007.
5. Chalkias A, Spyropoulos V, Koutsovasilis A, Papalois A, Kouskouni E, Xanthos T. Cardiopulmonary Arrest and Resuscitation in Severe Sepsis and Septic Shock: A Research Model. Shock 2015;43:285-91.
6. Xanthos T, Bassiakou E, Koudouna E, et al. Combination pharmacotherapy in the treatment of experimental cardiac arrest. Am J Emerg Med 2009;27:651-9.
7. Aivatidi C, Vourliotakis G, Georgopoulos S, Sigala F, Bastounis E, Papalambros E. Oxidative stress during abdominal aortic aneurysm repair-biomarkers and antioxidant's protective effect: a review. Eur Rev Med Pharmacol Sci 2011;15:245-52.
8. Majewski W, Krzyminiewski R, Stanisić M, et al. Measurement of free radicals using electron paramagnetic resonance spectroscopy during open aorto-iliac arterial reconstruction. Med Sci Monit 2014;20:2453-60.
9. Papalambros E, Sigala F, Georgopoulos S, et al. Malondialdehyde as an indicator of oxidative stress during abdominal aortic aneurysm repair. Angiology 2007;58:477-82.
10. Norwood MG, Bown MJ, Sutton AJ, Nicholson ML, Sayers RD. Interleukin 6 production during abdominal aortic aneurysm repair arises from the gastrointestinal tract and not the legs. Br J Surg 2004;91:1153-6.
11. Takagi H, Watanabe T, Mizuno Y, Kawai N, Umemoto T; All-Literature Investigation of Cardiovascular Evidence Group. Circulating interleukin-6 levels are associated with abdominal aortic aneurysm presence: a meta-analysis and meta-regression of case-control studies. Ann Vasc Surg 2014;28:1913-22.
12. Norwood MG, Bown MJ, Sayers RD. Ischaemia-reperfusion injury and regional inflammatory responses in abdominal aortic aneurysm repair. Eur J Vasc Endovasc Surg 2004;28:234-45.
13. Vucevic D, Maravic-Stojkovic V, Vasilijic S, et al. Inverse production of IL-6 and IL-10 by abdominal aortic aneurysm explant tissues in culture. Cardiovasc Pathol 2012;21:482-9.
14. Harrison SC, Smith AJ, Jones GT, Swerdlow DI, Rampuri R, Bown MJ; Aneurysm Consortium, et al. Interleukin-6 receptor pathways in abdominal aortic aneurysm. Eur Heart J 2013;34:3707-16.
15. Fraga CM, Tomasi CD, Biff D, et al. The effects of N-acetylcysteine and deferoxamine on plasma cytokine and oxidative damage parameters in critically ill patients with prolonged hypotension: a randomized controlled trial. J Clin Pharmacol 2012;52:1365-72.
16. Kalimeris K, Nastos C, Papoutsidakis N, et al. Iron chelation prevents lung injury after major hepatectomy. Hepatol Res 2010;40:841-50.
17. Tiberio L, Tiberio GA, Bardella L, et al. Mechanisms of interleukin-6 protection against ischemia-reperfusion injury in rat liver. Cytokine 2006;34:131-42.
18. Jin X, Zimmers TA, Zhang Z, Pierce RH, Koniaris LG. Interleukin-6 is an important in vivo inhibitor of intestinal epithelial cell death in mice. Gut 2010;59:186-96.
19. Kabali B, Girgin S, Gedik E, Ozturk H, Kale E, Buyukbayram H. N-acetylcysteine prevents deleterious effects of ischemia/reperfusion injury on healing of colonic anastomosis in rats. Eur Surg Res 2009;43:8-12.
20. Gündüz D, Kasseckert SA, Härtel FV, et al. Accumulation of extracellular ATP protects against acute reperfusion injury in rat heart endothelial cells. Cardiovasc Res 2006;71:764-73.
21. Galle C, De Maertelaer V, Motte S, et al. Early inflammatory responses after elective abdominal aortic aneurysm repair: a comparison between endovascular procedure and conventional surgery. J Vasc Surg 2000;32:234-46.
22. Holzheimer RG, Gross J, Schein M. Pro- and anti-inflammatory cytokine-response in abdominal aortic aneurysm repair: a clinical model of ischaemia-reperfusion. Shock 1999;11:305-10.
23. Collange O, Tamion F, Meyer N, et al. Early detection of gut ischemia-reperfusion injury during aortic abdominal aneurysmectomy: a pilot, observational study. J Cardiothorac Vasc Anesth 2013;27:690-5.
24. Kostopanagiotou G, Avgerinos ED, Markidou E, et al. Protective effect of NAC preconditioning against ischemia-reperfusion injury in piglet small bowel transplantation: effects on plasma TNF, IL-8, hyaluronic acid, and NO. J Surg Res 2011;168:301-5.
25. Aksu V, Yüksel V, Chousein S, et al. The effects of sildenafil

and n-acetylcysteine on ischemia and reperfusion injury in gastrocnemius muscle and femoral artery endothelium. Vascular 2015;23:21-30.
26. Spapen H, Zhang H, Demanet C, Vleminckx W, Vincent JL, Huyghens L. Does N-acetyl-L-cysteine influence cytokine response during early human septic shock? Chest 1998;113:1616-34.
27. Tekin IO, Sipahi EY, Comert M, Acikgoz S, Yurdakan G. Low-density lipoproteins oxidized after intestinal ischemia/reperfusion in rats. J Surg Res 2009;157:e47-54.
28. Li D, Williams V, Liu L, et al. Expression of lectin-like oxidized low-density lipoprotein receptors during ischemia-reperfusion and its role in determination of apoptosis and left ventricular dysfunction. J Am Coll Cardiol 2003;41:1048-55.
29. Kataoka K, Hasegawa K, Sawamura T, et al. LOX-1 pathway affects the extent of myocardial ischemia-reperfusion injury. Biochem Biophys Res Commun 2003;300:656-60.
30. Yurdakan G, Tekin IO, Comert M, Acikgoz S, Sipahi EY. The presence of oxidized low-density lipoprotein and inducible nitric oxide synthase expression in renal damage after intestinal ischemia reperfusion. Kaohsiung J Med Sci 2012;28:16-22.