MEDICAL SCIENCE MONITOR

DIAGNOSTIC TECHNIQUES

e-ISSN 1643-3750
© Med Sci Monit, 2014; 20: 2453-2460
DOI: 10.12659/MSM.890774

Received: 2014.03.31
Accepted: 2014.06.30
Published: 2014.11.27

Measurement of Free Radicals Using Electron Paramagnetic Resonance Spectroscopy During Open Aorto-Iliac Arterial Reconstruction

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

ABCDEFG 1 Wacław Majewski
ABCDEF 2 Ryszard Krzyminiewski
ABCDEFG 1 Michał Stanisić
ACDEF 3 Maria Iskra
BDFG 1 Zbigniew Krasiński
BDF 1 Marek Nowak
CDF 2 Bernadeta Dobosz

1 Department of General and Vascular Surgery, Poznań University of Medical Sciences, Poznań, Poland
2 Institute of Physics, Adam Mickiewicz University, Division of Medical Physics, Poznań, Poland
3 Department of General Chemistry, Poznań University of Medical Sciences, Poznań, Poland

Corresponding Author: Michał Stanisić, e-mail: goranm@gazeta.pl
Source of support: Ministry of Science of Poland Grant NN 403 5877 38

Background: Aortic cross-clamping during abdominal aortic aneurysm (AAA) open repair leads to development of ischemia-reperfusion injury. Electron paramagnetic resonance spectroscopy (EPR) spin-trapping is a valuable method of direct measurement of free radicals.
The objective of the study was to evaluate the results of EPR as a direct method of free radical measurement and degree of inflammatory response in open operative treatment of patients with AAA and aorto-iliac occlusive disease (AIOD).

Material/Methods: The study was performed on a group of 32 patients with AAA and 25 patients with AIOD scheduled for open repair. Peripheral venous blood for EPR spectroscopy and for SOD, GPx, ox-LDL, Il-6, TNF-alfa, CRP, and HO-1 were harvested. Selected parameters were established accordingly to specified EPR and immunohistochemical methods and analyzed between groups by Mann-Whitney U test and Wilcoxon matched-pairs signed-ranks test with Bonferroni correction.

Results: Free radicals level was correlated with the time of the aortic cross-clamping after the reperfusion of he first and second leg in AAA (r=0.7; r=0.47). ox-LDL in AAA decreased 5 min after reperfusion of the first leg (32.99 U/L, range: 14.09–77.12) and 5 min after reperfusion of the second leg (26.75 U/L, range: 11.56–82.12) and 24 h after the operation (25.85 U/L, range: 14.29–49.70). HO-1concentration increased to above the level before intervention 24 h after surgery. The activities of GPx and SOD decreased 5 min after the first-leg reperfusion in AAA. Twenty-four hours after surgery, inflammatory markers increased in AAA to CRP was 14.76 ml/l (0.23–38.55), IL-6 was 141.22 pg/ml (84.3–591.03), TNF-alfa was 6.82 pg/ml (1.76–80.01) and AIOD: CRP was 18.44 mg/l (2.56–33.14), IL-6: 184.1 pg/ml (128.46–448.03), TNF-alfa was 7.74 pg/ml (1.74–74.74).

Conclusions: EPR spin-trapping demonstrates temporarily elevated level of free radicals in early phase of reperfusion, leading to decrease antioxidants in AAA. Elevated free radical levels decreased 24 h after surgery due to various endogenous antioxidants and therapies.

MeSH Keywords: Aortic Aneurysm, Abdominal • Aortic Diseases • Electron Spin Resonance Spectroscopy • Ischemic Preconditioning

Full-text PDF: http://www.medscimonit.com/abstract/index/idArt/890774

3094 4 1 43

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

Background

Aortic cross-clamping during bypass grafting in patients with abdominal aorta aneurysms (AAA) leads to development of ischemia-reperfusion injury. Previous studies have shown that increased production of reactive oxygen species (ROS), such as superoxide anions, hydroxyl radicals, or hydrogen peroxide, plays a major role in cellular damage during post-ischemic reperfusion [1,2]. Free radicals can participate in reduction-oxidation reactions, initiate lipid oxidation, degrade nucleic acids and membrane proteins, and increase endothelial adhesion molecule synthesis. However, free radicals as a result of the aortic cross-clamping during the surgical reconstruction, induce proinflammatory cytokines and chemokines [3]. The synthesis of cytokines during the inflammatory processes generates an increase in concentrations of acute-phase proteins as a response to the tissue destruction [4].

Endogenous antioxidant enzymes – superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), catalase (CAT), and heme oxygenase (HO) – have been shown to prevent skeletal muscle ischemia reperfusion injury by different mechanisms inhibiting ROS activity [5]. When a bypass graft patient develops imbalance between free radicals and antioxidants, it has been shown that it leads to oxidative tissue damage. In patients undergoing AAA bypass grafting, it is possible to reduce oxidative stress and cellular damage by introducing different types of antioxidant treatment such as vitamin E and C, statin, xanthine oxidase, or mannitol. Several reports have described cases of elective AAA patients in whom reperfusion during bypass grafting led to oxygen free radicals production resulting in lipid peroxidation, especially in rupture AAA [6–9]. However, there have been no comparative studies on production and inhibition of ROS in patients with AAA and aortoiliac occlusive disease (AIOD) with critical and moderate ischemia.

Identification of free radicals is commonly investigated by using indirect methods and measurements, such as conjugated dienes, hydroperoxides, and aldehydes [10]. Electron paramagnetic resonance spectroscopy (EPR) spin-trapping has been considered a valuable method because it directly measures free radicals production [11]. EPR is widely used in investigating the structure and dynamics of paramagnetic centers and free radicals that have 1 or more unpaired electrons. The main advantages of EPR spectroscopy are simple preparation of the sample, short time of measurement, and high sensitivity. These advantages are why EPR is becoming widely used in studies of biological samples. This method is based on interaction of an external magnetic field with magnetic moments of unpaired electrons in a sample, which leads to splitting the electron energy levels. An EPR signal is observed when the quantum of the electromagnetic wave energy (hv) incident on the sample is equal to the energy difference between the energy levels, which is described by the resonance condition:

$$h\nu = g\mu_B B_0 \quad (1)$$

where g is the so-called g-factor, μ_B is the Bohr magneton, and B_0 is the magnetic induction of an external magnetic field. The value of the g-factor can provide information about the kind of paramagnetic center, and the intensity of EPR signals is directly proportional to the free radical concentration in a sample. For this reason, many biological substances have been investigated by this method [12–14].

Therefore, in the present study the technique of electron paramagnetic resonance spectroscopy was used to examine the rate of free radicals generation in relation to the activity of antioxidant enzymes and proinflammatory cytokines production in AAA and AIOD patients treated surgically.

Material and Methods

The study was performed in a group of 32 AAA patients (mean age 66.78±7.64 years, 28 men and 4 women) and 25 AIOD patients (mean age 61.12±6.99 years, 19 men and 6 women) (Rutherford Stage 3). AAA and AIOD were diagnosed before surgery using Doppler ultrasonography, computed tomography, or arteriography. The mean internal diameter of the aneurysms was 6.58±1.28 cm. Our patients were examined so as to diagnose any concomitant diseases and provide proper application of treatment to prevent surgical complications. The perioperative data (particularly regarding cardiovascular risk factors), concomitant diseases, and medications are shown in Table 1. All patients on the day of surgery were on statins but only 16 AAA patients (50%) and 21 AIOD patients (84%) received statins for at least 3 months before surgical treatment. Additionally, administration of the indicated oral medications, particularly β blockers and ACE inhibitors, was continued until the reconstruction.

Preoperatively, an epidural catheter with bupivacaine was introduced and preoxygenation was performed prior to induction of general anesthesia. Propofol and sevoflurane were administered to all the patients to maintain anesthesia. In 5 AAA patients, simple prosthetic reconstruction of infrarenal aortic aneurysm was performed, and 27 patients who had additional iliac artery aneurysms underwent aortoiliac bifurcated graft implantation.

The treatment of choice in the AIOD patients was implantation of an aorto-bifemoral bypass graft. Before cross-clamping of the abdominal aorta, each patient received intravenously 5000 units of heparin and 0.5 g/kg mannitol. At the beginning

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

Table 1. Characteristics of the studied AAA and AIOD patients.

Parameters	AAA (32 patients) No. (%)	AIOD (25 patients) No. (%)
Age >70 years	11 (34)	3 (12)
Gender (male/female)	28/4	19/6
Current smoker	16 (50)	18 (72)
Hypertension	17 (53)	11 (44)
Hypercholesterolemia	12 (38)	12 (48)
Coronary artery disease	13 (41)	10 (40)
Previous myocardial infarction	8 (25)	5 (20)
Cerebrovascular accident	1 (3)	1 (4)
Type 2 diabetes	9 (28)	6 (24)
Renal insufficiency	2 (6)	1 (4)
Pulmonary disease	3 (9)	1 (4)
Medications		
Aspirin	32 (100)	25 (100)
β-blocker	16 (50)	11 (44)
ACE	17 (53)	10 (40)
Statins 3 month	16 (50)	21 (84)
Intraoperative heparin	32 (100)	25 (100)
Intraoperative mannitol	32 (100)	25 (100)
Mean cross-clamp time (min)		
a) Before first leg reperfusion	51.47±15.24	51.12±15.36
b) Before second leg reperfusion	62.50±17.80	62.92±18.84

of the reperfusion, brief clamping and declamping of the abdominal aorta was performed once or twice before starting the typical reperfusion. This procedure facilitates correction of blood pressure and proper fluid replacement before and during early-phase reperfusion.

Venous blood samples were collected before the induction of anesthesia, before the clamp removal, 5 min after the reperfusion of first and second leg, and 24 h after the operation. The mean intervals between the blood flow reconstruction in first and second legs were: AAA 11.03±6.53 and AIOD 11.80±6.23 min.

For EPR measurements, a sample of 0.5 ml of the patient's blood was mixed with 2 ml of nitrosobenzene solution immediately after the blood samples collection and divided into 3 identical samples immediately after collection. After 1 min, these samples were placed in a Dewar flask with liquid nitrogen (77 K) and stored. The nitrosobenzene spin trap (C_6H_5NO) (Aldrich Chemical Co.) was used in the experiments. We dissolved 107 mg of nitrosobenzene in 3 ml of methanol CH_3OH, and 97 ml of distilled water was added. The measurements were made with a Bruker EMX-10 X-band (9.4 GHz) spectrometer with magnetic field second modulation frequency of 100 kHz. The EPR spectra were recorded at 170 K temperature in a sweep width of 20 mT. Low temperatures were maintained by using an ER 4131VT Bruker temperature controller. The following time parameters were applied: a sweep time of 10.48 s and a time constant of 20.48 ms. The standard weak pitch sample with concentration of free radicals equal to 2×10^{13} spins was used to determine the concentration of free radicals in the samples of blood. The concentration of free radicals was calculated from integrated intensity of free radical signals. Customarily, the results are given in relative values of intensity changes because it has less error. The value of 10 000 a.u. corresponds to about 10^{15} spins in a sample.

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

Table 2. Measurement of free radicals by EPR in blood of AAA and AIOD patients treated surgically.

Blood sampling	Values of free radicals measurement by EPR (a. u.) ×10³			
	AAA (N=32) Median (range)		AIOD (N=25) Median (range)	
Before induction of anesthesia	34.0	(2.3–128.0)	30.0	(3.0–109.0)
Pre-release of aortic clamp	42.0	(6.0–144.0)	36.0	(10.0–118.0)
5 min after first leg reperfusion	54.5	(6.0–170.0)	55.0	(14.0–127.0)
5 min after second leg reperfusion of	50.0	(5.0–119.0)	43.0	(16.0–103.0)
24 hours after operation	35.0	(11.0–85.0)	39.0	(12.0–83.0)

AAA p-value <0.05 I/III; III/V. AIOD p-value<0.05 I/III; III/V.

Table 3. Median values of malondialdehyde (MDA) in sera of AAA and AIOD patients treated surgically.

Blood sampling	Values of MDA (µmol/L) during surgical treatment			
	AAA (N=32) Median (range)		AIOD (N=25) Median (range)	
Before induction of anesthesia	5.30	(4.29–9.77)	4.98	(3.67–8.06)
Pre-release of aortic clamp	5.36	(2.91–10.10)	5.18	(4.27–8.97)
5 min after first leg reperfusion	5.64	(3.32–10.42)	5.90	(3.60–9.45)
5 min after second leg reperfusion	5.58	(3.36–10.18)	5.48	(3.36–10.18)
24 hours after operation	5.49	(3.0–9.58)	5.26	(3.00–8.36)

GPx, SOD, CRP, IL-6, TNF-α, ox-LDL, HO-1, and TBARS assessment was conducted with use of standardized commercially available methods (Appendix 1).

Ethical approval

Institutional regulatory board approval 1021/08 16th October 2008.

Statistical analysis

The results were analyzed by a non-parametric Mann-Whitney U test. To compare the median values between the 2 dependent groups at different times before and following surgical treatment, the Wilcoxon matched-pairs signed-ranks test was applied with Bonferroni correction. Spearman's correlations were performed for correlation analyses. Statistical significance was accepted at the level of P<0.05.

Results

The highest radicals concentration was observed after aortic clamp removal. The measured median values of free radicals by EPR before induction of anesthesia, pre-release of aortic clamp, 5 min after aortic clamp removal, and reperfusion of first leg, 5 min after ipsilateral branch of prosthesis clamp removal, and reperfusion of second leg and 24 h after the operation are summarized in Table 2. Median values of the free radicals revealed a significant elevation after 5 min of the reperfusion of the first leg in surgical treatment of patients with AAA. In both groups of patients, 24 h after surgery, free radicals concentration decreased to a value similar to that before the operation. The values of MDA 5 min after aortic clamp removal and reperfusion of the first leg and also after the reperfusion of the second leg were increased significantly in AAA (Table 3). The changes in free radicals level significantly was correlated with the time of aortic cross-clamping after the reperfusion of the first and second leg in patients with AAA (r=0.7; r=0.47). Typical spectra of AAA samples are presented in Figure 1.

The median values of ox-LDL concentration in AAA patients were significantly decreased 5 min after reperfusion of the first leg (32.99 U/L, range: 14.09–77.12) and 5 min after reperfusion of the second leg (26.75 U/L, range: 11.56–82.12) as well as 24 h after the operation (25.85U/L, range; 14.29–49.70)

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

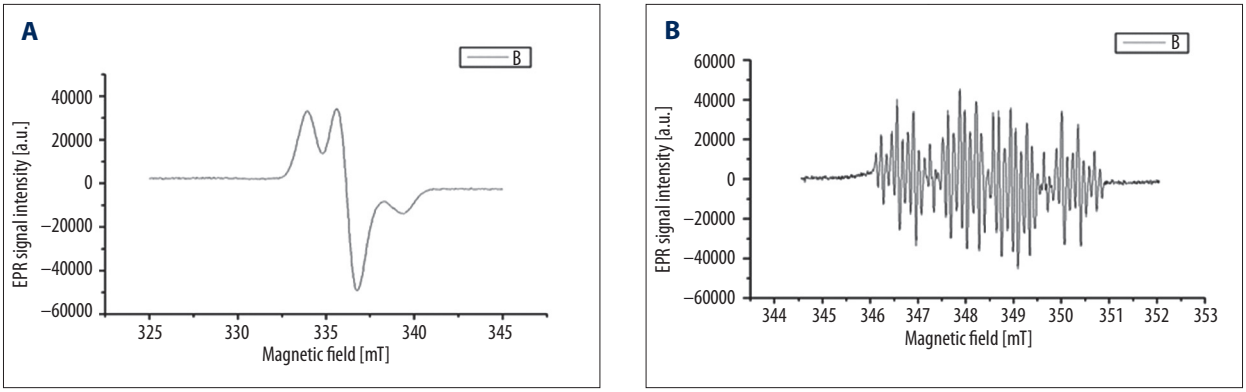

Figure 1. EPR spectra typical for the patient's blood mixed with nitrosobenzene: (**A**) recorded at 170K, (**B**) at room temperature.

Table 4. HO-1, GPx and SOD determination in patients with AAA and AIOD treated surgically.

	Values of antioxidants (HO-1, GPx, SOD)				
	I Before surgery Median (range)	**II Pre-release of aortic clamp Median (range)**	**III 5 min after first leg reperfusion Median (range)**	**IV 5 min after second leg reperfusion Median (range)**	**V 24 hours after operation Median (range)**
	AAA (N=32)				
HO-1 mg/ml	1.59 (1.04–3.25)	1.45 (0.71–2.58)	1.25 (0.73–2.68)	1.32 (0.83–3.15)	2.7 (1.19–4.62)
GPx U/L	3018 (1725–4587)	2156 (1207–3725)	2035 (1000–3552)	2311 (1265–4139)	2552 (862–4242)
SOD U/ml	154.24 (77.88–294.78)	127.96 (40.8–205.4)	109.16 (10.0–185.12)	155.44 (15.76–222.04)	138.9 (89.24–205.4)
	AIOD (N=25)				
HO-1 mg/ml	1.33 (0.86–2.5)	0.95 (0.55–1.95)	0.98 (0.04–2.07)	1.01 (0.65–2.01)	1.92 (1.19–6.79)
GPx U/L	2975 (1266–6967)	2759 (862–6001)	2587 (690–6036)	2500 (966–5967)	3018 (1552–7243)
SOD U/ml	157.28 (116.92–350.52)	147.98 (32.76–277.88)	98.14 (10.0–189.12)	119.48 (13.16–292.36)	128.72 (76.08–170.96)

AAA: p-value <0.05 HO-1 I/II; I/III; I/IV; I/V; III/V; IV/V, GPx I/III, SOD I/III. AIOD: p-value <0.05 HO-1 I/II; I/III; I/IV; III/V; IV/V, GPx I/III SOD I/III.

in comparison to the results obtained before the operation (42.27 U/L, range: 25.53–127.78). In the patients with AIOD, decreased values of ox-LDL concentration after reperfusion of the first leg: 30.13 U/L (12.05–116.19) and second leg: 27.16 U/L (9.92–55.33) and also 24 h after the operation: 27.18 U/L (9.38–57.30) were not significant as compared to the value observed before the operation 37.77 U/L (17.10–101.36).

The concentrations of HO-1, and the activity of GPx and SOD in AAA and AIOD before and during the surgical treatment are summarized in Table 4.

The following concentration medians of CRP, IL-6, and TNF-alfa before the operation were found in the AAA patients: 1.21 mg/l (0.2–3.24), 84.24 pg/ml (9.49–241.31), 3.77 pg/ml (0.44–58.45), respectively, and in the AIOD patients: 2.37 mg/l (1.53–6.84), 71.25 pg/ml (60.89–141.91), 3.13 pg/ml (0.39–24.85), respectively. Twenty-four hours after the surgery, inflammatory markers increased in the patients with AAA: CRP was 14.76 ml/l (0.23–38.55), IL-6 was 141.22 pg/ml (84.3–591.03), and TNF-alfa was 6.82 pg/ml (1.76–80.01). In the patients with AIOD, CRP was 18.44 mg/l (2.56–33.14), IL-6 was 184.1 pg/ml (128.46–448.03), and TNF-alfa was 7.74 pg/ml (1.74–74.74).

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

Discussion

Reperfusion of ischemic tissue due to cross-clamping of the aorta following AAA surgery induces an inflammatory response and free radicals production [15]. Using EPR and the nitrosobenzene spin trap, we found a significant increase in the AAA group in free radicals production during reperfusion of the first leg 5 min after the aortic clamp removal, but after reconstruction of blood flow to the second leg this elevation of free radicals was insignificant. Detected radicals are probably extracellular. In patient with AOID in which there was lack of ischemic period related to cross-clamping, no significant elevated free radicals level was observed in the reperfusion phase. Twenty-four hours after the operation, free radicals decreased to the level observed before surgery in both groups. In the first minutes of reperfusion and reoxygenation of hypoxic tissue, high formation of oxygen radicals such as superoxide radicals (O^{-}_{2}) hydroxyl radicals (.OH) and hydrogen peroxide (16) is observed. The superoxide radicals in the reperfusion phase react with nitric oxide (NO) to generate peroxynitrite anions,which leads to the peroxidation of lipid and membrane proteins [17]. Our data demonstrated significant increase in MDA concentration during reperfusion in the AAA group in comparison to the AOID group (Table 3). MDA is a final product of lipid peroxidation, derived from polyunsaturated fatty acids, and is used for estimation of free radicals produced by ischemic and reperfused legs [18].

Increased formation of reactive oxygen radicals during reperfusion is associated with different mechanisms of mitochondrial function, DNA damage, activation of apoptosis, destruction of cellular membranes, capillary perfusion failure, interstitial edema, and multiorgan dysfunction syndrome [19–21]. Severity of ischemia and reperfusion cellular injury is dependent on overproduction of free radicals, duration of ischemia, degree of surgical trauma, endogenous antioxidant capacity, and antioxidant therapy.

Our study demonstrated in the AAA group that among endogenous antioxidants HO-1 was significantly decreased 5 min after reperfusion of the first leg and the second leg. The beneficial role of HO-1 is related to protection of tissues against oxidative stress [22]. In addition, GPx and SOD were decreased 5 min after reperfusion of the first leg in patients with AAA, but SOD was also decreased in early reperfusion in patients with AOID [23]. SOD converts superoxide anions to hydrogen peroxide and then hydrogen peroxide is transformed to water and oxygen by glutathione peroxidase and catalase [24]. This activity of the GPx, SOD, and HO-1 levels was associated with an increase in production of free radicals during reperfusion of the first leg. To protect tissue against injury from peripheral ischemia and reperfusion, anesthetic drugs such as propofol and sevoflurane were administered to all the patients. Propofol is chemically similar to α-tocopherol and may decrease MDA level and inhibit lipid peroxidation [25–28]. Other drugs useful in attenuating the production of free radicals (e.g., statins, β-blockers, ACE inhibitors, heparin, and mannitol) were administered according to Table 1 [29–35]. Reperfusion was performed in 2 different periods and separately in both legs, containing a smaller area of ischemic tissue and creating better conditions for antioxidant activity to prevent excessive free radicals generation. There are several studies proving that ischemic post-conditioning decreases free radicals production by limiting glutathione oxidation, as well as reducing the incidence of postoperative complications such as myocardial infarction and renal failure [36–43].

A significant increase in CRP, IL-6, and TNF-α observed 24 h after the surgical treatment of patients with AAA and AIOD may not only cause tissue injury during surgery, but also cause muscle ischemia during temporary aortic clamping and reperfusion. In our study, we observed lack of significant correlation between inflammatory markers and production of free radicals during reperfusion. These markers decreased 6–8 days after the aortic reconstructive surgery in uncomplicated cases (54). The endogenous antioxidant enzymes, as well as antioxidant treatment, as shown in our study, are able to prevent increase of ROS during aortoiliac reconstruction of AIOD patients due to lower production of free radicals in comparison to the AAA group, probably as the result of already established collateral flow. Collaterals maintain sufficient blood supply in AOID (Rutherford 3) patients during aortic cross-clamping.

Conclusions

Using EPR spin-trapping, we demonstrated that in an early phase of reperfusion a temporarily elevated level of free radicals contributes to the reaction against ischemia of tissue, leading to a decrease in antioxidants such as HO-1, GPx, and SOD during surgical treatment of AAA. These elevated free radicals levels at the beginning of reperfusion decrease 24 h after surgery due to various endogenous antioxidants and antioxidant therapies. Surgical management including ischemic post-conditioning before starting reperfusion additionally make it possible to prevent development of irreversible tissue damage, morbidity, complications, and mortality in surgically treated patients.

Conflicts of interests

None.

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

Appendix 1

Methodology of GPx, SOD, CRP, IL-6, TNF-α, ox-LDL, HO-1 and TBARS assessment

The whole blood portion for the biochemical analysis of 100 µl was separated for the measurement of the GPx activity and 500 µl for the SOD activity. The plasma samples were prepared after centrifugation of the remaining whole blood at the speed 3000 rpm for 10 minutes at room temperature using a benchtop centrifuge (EBA20, Hettich, Gemany). Plasma samples were stored in a refrigerator at –20°C until assay.

C-Reactive Protein (CRP), interleukin-6 (Il-6) and TNF-α were measured by an immunometric assay based on a double-antibody sandwich technique (EIA kit, Cayman Chemical Company, Ann Arbor).

Samples for CRP were diluted (1:1000 and 1:16000) with ultra-pure water (Simplicity Millipore, 18.2 $M\Omega cm^{-1}$) both, and introduced to the well of the microwell plate coated with a monoclonal antibody specific for human CRP. After addition of a horseradish peroxidase (HRP) labeled CRP monoclonal antibody, a sandwich with CRP molecule was formed. Tetramethylbenzidine (TMB) was used as a chromogenic substrate and after 15 minute incubation at room temperature in the dark the reaction was stopped by addition of an acid, and the product was measured spectrophotometrically at 450 nm. CRP concentration was calculated from the standard curve and expressed in mg/L.

For Il-6 assay plasma samples were diluted 1: 2 with EIA Buffer. Each well of the microplate was filled with plasma samples and an acetylcholinesterase: Fab' conjugate (AChE: Fab') was added to the wells coated with a monoclonal antibody specific for Il-6. After overnight incubation at 4°C of the enzymatic activity of AChE and the addition of Ellman's reagent, the product was determined spectrophotometrically at 450 nm periodically over four hours. Il-6 concentration was calculated from the standard curve obtained at the development time of 120 minutes, and expressed in pg/mL.

For TNF-α assay a specific monoclonal antibody and an acetylcholinesterase: Fab' conjugate (AChE: Fab') were added to the wells. After overnight incubation at 4°C, TNF-α was determined by measuring the enzymatic activity of the AChE with Ellman's reagent. The concentration of TNF-α was measured spectrophotometrically at 450 nm, calculated from the equation of the standard curve and expressed in pg/ml.

Oxidized low density lipoproteins (ox-LDL) in plasma were determined by using the ELISA kit (Mercodia AB, Uppsala, Sweden). An anti-oxidized LDL antibodies coated on the microplate wells and a peroxidase conjugated anti-human apolipoprotein B antibody added to the wells recognized the ox-LDL. The conjugate was reacting with a substrate and the product was measured at 450 nm. The concentration of ox-LDL was calculated based on the standard curve and expressed in U/L.

Glutathione peroxidase (GPx) activity was measured in the whole blood by using RANSEL kit (RANDOX Lab., UK). The method is based on the catalysis by GPx of the oxidation of reduced glutathione by cumene hydroperoxide to oxidized form (GSSG). Glutathione reductase (GR) and NADPH convert GSSG to its reduced form and NADPH to $NADP^+$. GPx concentration was calculated based on the decrease in absorbance and expressed in U/L. Superoxide dismutase (SOD) activity was measured in whole blood by using RANSOD kit (RANDOX Lab., UK). The method employs xanthine and xanthine oxidase to generate superoxide anions and SOD activity is measured by the degree of inhibition of formazan dye formation. The activity of SOD was expressed in U/L of whole blood.

The HO-1 was quantitated with sandwich immunoassay EIA kit (Enzo Life Sciences). A mouse monoclonal antibody specific for HO-1 was pre-coated on the wells of the microplate. and HO-1 was captured by the immobilized antibody and detected with a HO-1 specific rabbit polyclonal antibody. The polyclonal antibody was subsequently bound by a horseradish peroxidase conjugated anti-rabbit IgG secondary antibody. The substrate added was TMB and the absorbance of the product was measured at 450 nm. HO-1 concentration was quantitated from a standard curve, and expressed in ng/mL.

Thiobarbituric Acid Reactive Substances (TBARS) concentration was measured by using a kit purchased at Cayman Chemical Company. The assay is based on the reaction of thiobarbituric acid with malondialdehyde (MDA) under high temperature (100°C) and in acidic solution. The product is measured spectrophotometrically at 530 nm, and the concentration of MDA was calculated from the standard curve and expressed in µM.

References:

1. Ozturk K, Ozyurt H, Somag A, Karaca C: The effects of nitric oxide donor molsidomine on skelet muscle damage In a rat hind limb model of ischemia-reperfusion. Eur Surg Res, 2009; 42: 71–77
2. Kutuya T, Hoshida S, Kim Y et al: Detection of oxygen-derived free radical generation In canine postischemic heart during late phase of reperfusion. Circ Res, 1990; 66: 1160–65

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

3. Kono H, Asakawa M, Fujii M 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
4. Violi F, Loffredo L: Peripheral arterial disease and oxidative stress. Vasc Dis Prevent, 2007; 4: 1–5
5. Dhalla NS, Elmoselhi AB, Hafa T, Mukino N: Status of myocardial antioxidants in ischemia-reperfusion injury. Cardiovasc Res, 2000; 47: 446–56
6. Westman B, Johansson G, Luo J-L et al: Effects on skeletal muscle glutatione status of ischemia and reperfusion following abdominal aortic aneurysm surgery. Ann Vasc Surg, 2006; 20: 99–105
7. Wijnen MHWA, Roumen RMH, Vader HL, Goris RJA: A multiantioxidant supplementation reduces damage from ischaemia reperfusion in patients after Lower torso ischaemia. A randomized trial. Eur J Vasc Endovasc Surg, 2002; 23: 486–90
8. Kertai MD, Boersma E, Westerhout CM et al: A combination of Statins and Beta-blockers is independently associated with a reduction in the incidence of perioperative mortality, and nonfatal myocardial infarction in patients undergoing abdominal aortic aneurysm surgery. Eur J Vasc Endovasc Surg, 2004; 28: 343–52
9. Mittal A, Philips ARJ, Loveday B, Windsor JA: The potential role for xanthine Oxidase inhibition in major intra-odbominal surgery, World J Surg, 2008; 32: 288–95
10. 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
11. Vergely C, Maupoil V, Clermont G et al: Identification and quantification of free radicals during myocardial ischemia and reperfusion using electron paramagnetic resonance spectroscopy. Arch Biochem Biophys, 2003; 420: 209–16
12. He G, Samouilor A, Kuppusamy P, Zweier JL: *In vivo* imaging of free radicals: applications from mouse to man. Mol Cell Biochem, 2002; 234/235: 359–67
13. Traverse JH, Nesmelov YE, Crapton M et al: Measurement of myocardial free radical production during exercise using EPR spectroscopy. Am J Physiol Heart Circ Physiol, 2006; 290: H2453–58
14. Mrakic-Sposta S, Gussoni M, Mantorosi M et al: Assessment of standardized ROS production profile In humans by electron paramagnetic resonance. Oxid Med Cell Longev, 2012; 2012: 1–10
15. Miller FJ, Sharp WJ, Fang X et al: Oxidative stress in human abdominal aortic aneurysms. A potential mediator of aneurismal remodeling. Arteriosder Thromb Vasc Biol, 2002; 22: 560–65
16. Irie H, Kato T, Ikebe K et al: Antioxidant effect of MCI-186, a New free- vadical scavenger, on ischemia-reperfusion injury In a rat hindhimb amputation model. J Surg Res, 2004; 120: 320–19
17. Crimi E, Ignarro LJ, Napoli C: Microcirculation and oxidative stress. Free Radic Res, 2007; 41: 1364–75
18. Ceconi C, Boraso A, Cargnoni A, Ferrari R: Oxidative stress in cardiovascular disease: myth or fact? Arch Biochem Biophys, 2003; 420: 217–21
19. Murphy MP: Review article. How mitochondria produce reactive oxygen pecies, Biochem J, 2009; 417: 1–13
20. Demirbag R, Yilmaz R, Sur M et al: DNA damage in metabolic sundrome and its association with antioxidative and oxidative measurements. Int J Clin Pract, 2006; 60: 1187–93
21. Kotani Y, Ishino K, Osaki S et al: Efficacy of MCI-186, a free-radical scavenger and antioxidant, for resuscitation of nonbeating donor he arts. J Thorac Cardiovasc Surg, 2007; 133: 1626–32
22. Abraham NG, Kappas A: Heme oxygenase and the cardiovascular-renal system free. Radic Biol Med, 2005; 39: 1–25
23. Johnson F, Giulivi C: Superoxide dismutases and their impact upon human health. Molecular Aspects of Medicine, 2005; 26: 340–52
24. Ergiin Y, Oksuz H, Atli Y et al: Ischemia- reperfusion injury in skeletal muscle: Comparison of the effects of subanesthetic doses of Ketamine, propofol, and etomidate. J Surg Res, 2010; 159: e1–e10
25. Arnaoutoglou H, Vretzakis G, Souliotis D et al: The effects of propofal or sevoflurane on free radical production after tourniquet induced ischaemia-reperfusion injury during knee arthroplasty. Acta Anaesth Belg, 2007; 58: 3–6
26. Vasiheiou I, Kalimeris K, Womikas T et al: Propofol prevents Lung injury following in testinal ischemio-reperfusion. J Surg Res, 2012; 172: 46–152
27. Wang H-h, Zhou H-y, Chen C-c et al: Propofol attenuation of renal ischemia/reperfusion injury involves heme oxygenase-1. Acta Pharmocal Sin, 2007; 28: 1175–80
28. Ozkan F, Senayli Y, Oxyurt H et al: Antioxidant effects of propofal on tourniquet-induced ischemia- reperfusion injury: an experimental study. J Surg Res, 2012; 176: 601–7
29. Velayutham P, Adhikary SD, Babu SK et al: Oxidative stress-associated hypertension In surgically induced brain jury patients: effects of β-blocker and angiotensin-converting enzyme inhibitor. J Surg Res, 2013; 179: 125–31
30. Aivatidi C, Vourliotakis G, Georgopoulos S et al: Oxidative stress during abdominal aortic aneurysm repair-Biomarkers and antioxidant's protective effect: a review. Eur Rer Med Pharmacol Sci, 2011; 15: 245–52
31. Paraskevas KI, Liapis CD, Hamilton G, Mikhailidis DP: Can statins reduce perioperative morbidity and mortality in patients undergoing non-cardiac vascular surgery? Eur J Vasc Endovasc Surg, 2006; 32: 286–93
32. Cowled PA, Khanna A, Laws PE et al: Statins inhibit neutrophil infiltration in skeletal muscle reperfusion injury. J Surg Res, 2007; 141: 267–76
33. Piecota-Polańczyk A, Goraca A, Demyanets S et al: Simvastatin decreases free radicals formation in the human abdominal aortic aneurysm Wall via NF-κB. Eur J Basc Endovasc Surg, 2012; 44: 133–37
34. Berg K, Haaverstad R, Astudillo R et al: Oxidative stress during coronary artery bypass operations: importance of surgical trauma and drug treatment. Scandinavian Cardiovasc J, 2006; 40: 291–97
35. Zoll J, Monassier L, Garnier A et al: ACE inhivition prevents myocardial infarction-induced skeletal muscle mitochondrial dysfunction. J Appl Physiol, 2006; 101: 385–91
36. Eberlin KR, McCormack MC, Nguyen JT et al: Sequential Limb Ischemia Demonstrates Remote Postconditioning Protection of Murine Skeletal Muscle. Plast Reconstr Surg, 2009; 123(2 Suppl.): 8S–16S
37. Park JW, Kang JW, Jeon WJ, Sik H: Postconditioning protects skeletal muscle from ischemia-reperfusion injury. Microsurgery, 2010; 30: 223–29
38. Gyurkovics E, Aranyi P, Stangl R et al: Posconditioning of the lower limb-protection against the reperfusion syndrome. J Surg Res, 2011; 169: 139–47
39. Mcalliser SE, Ashrafpour H, Cahoon N et al: Posconditioning of salvage of ischemia skeletal muscle from reperfusion injury: efficacy and mechanism. Am J Physiol regul Integr Comp Physiol, 2008; 295: R681–89
40. Majewski W, Laciak M, Kapcinska M et al: N-glicoforms of serum alpha-1-acid glikoprotein In patients with Lower limb chronic arterial ischaemia. Int Angiol, 2003; 12: 96–102
41. Majewska N, Stanisic MG, Blaszak MA et al: Clinical factors increasing radiation doses to patients undergoing long-lasting procedures: Abdominal stent-graft implantation, Med Sci Monit, 2011; 17(11): MT97–103
42. Xu T, Gong Z, Zhu WZ: Remote ischemic preconditioning protects neurocognitive function of rats following cerebral hypoperfusion, Med Sci Monit, 2011; 17(11): BR299–304
43. Weerateerangkul P, Chattipakorn S, Chattipakorn N: Roles of the nitric oxide signaling pathway in cardiac ischemic preconditioning against myocardial ischemia – reperfusion injury. Med Sci Monit, 2011; 17(2): RA44–52

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]