Abstract. – Background and Objectives: Oxidative stress during abdominal aortic aneurysm (AAA) repair is likely to result as a response to an ischemia-reperfusion injury (IRI) to the lower limbs and gastrointestinal tract. This paper reviews the oxidative stress during AAA repair, with specific reference to biological markers and the potential antioxidant’s protective effect.

Evidence and Information Sources: The current literature (1966 to July 2010) was reviewed specifically for all articles describing human studies relevant with the particular subject: oxidative stress in patients with AAA repair. Keywords used as single or combined searches included “abdominal aortic aneurysm”, “open repair”, “EVAR”, “oxidative stress”, “oxidation” and “antioxidant”.

Results: A total of 14 relevant human studies were identified. In the majority of studies all samples (blood samples or/and muscle biopsies) were obtained from the patients using regional sampling techniques before or after anaesthesia, during aortic clamping or balloon occlusion (ischemic time) and after aortic clamp removal (reperfusion time) in different time intervals up to 24 or 48 hours. The oxidative status during AAA repair operation was evaluated by measuring quantitative changes of different substances including mainly vascular endothelial adhesion molecules, lipid peroxidation by-products or reactive oxygen species (ROS) and their metabolites. Two studies compared two groups of patients with AAA treated either by open or endovascular repair (EVAR), while four studies used different types of antioxidant supplementation in order to correlate it with a reduction in oxidative stress and damage in the antioxidant group of patients.

Perspectives and Conclusions: Current evidence suggests that there is a high-grade oxidative stress during AAA repair operation. This was higher in cases of open repair beside EVAR and in cases with ruptured AAAs beside elective cases. The beneficial effect of an antioxidant supplementation in reducing the oxidative stress during AAA repair was also demonstrated. The use of a biological marker as a predictor of the development of systemic complications could also give a therapeutic advantage.

Key Words: Abdominal aortic aneurysm, Oxidative stress, Open repair, Antioxidant.

Introduction

Despite the grade improvements in surgical technique, grafts and perioperative care, abdominal aortic aneurysm (AAA) repair especially in cases of rupture carries a considerable risk of morbidity and death with mild improvements of results during the last decades\(^2\).

Ischemia and reperfusion during an AAA open repair is a double physiological phenomenon that happens because of the ischemia during aortic cross clamping and reperfusion, which follows after the aortic clamp removal. That occurs in a more extensive degree in patients with ruptured AAAs (RAAs) as a result of the additional hypovolaemic shock secondary to hemorrhage and lower torso ischemia. This “two-hit” ischemia/reperfusion injury (IRI)\(^3\) is the main reason for the extensive systemic inflammatory response and the grader incidence of the post-operative multi organ failure in patients with RAA. Tissue ischemia results in anaerobic cellular metabolism, leading to localized acidosis, decreased

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cellular glycogen and ATP levels. This reduces cell membrane function, causes vascular endothelial adhesion molecule and enzyme xanthine oxidase (XO) upregulation and activation of kinins, complement and clotting pathways. 

After tissue reperfusion occurs, re-oxygenation leads to oxygen free radicals (OFR) production. Oxygen free radicals as superoxide, hydroxyl radical, hydrogen peroxide and hypochloric acid attack to the biomembrane of polyunsaturated fatty acids resulting in lipid peroxidation, complement activation, arachidonic acid release and an increased endothelial adhesion molecule synthesis.

Tissues, which are more at risk of oxidative damage in patients with AAA during aortic clamping and reperfusion, are the lower limbs and gastrointestinal tract.

Oxygen free radicals are produced as a response to IRI attack lipid cell membranes, leading to the creation of lipid peroxidation by-products. Such products like F(2)-isoprostane, malondialdehyde (MDA), lipid hydroperoxides (LH) and lipofuscin can be used as biological markers of lipid peroxidation.

This article will provide an overview of the oxidative stress involved in AAA repair in human studies and a brief summary of the ways were used in order to prove that in each study.

Methods

Articles and original papers, all describing human studies were examined for their relevance to the particular question: oxidative stress in patients with AAA repair. A systematic literature search was conducted using PubMed (1966-Jule 2010) search engine. Keywords used as single or combined searches included “abdominal aortic aneurysm”, “open repair”, “EVAR”, “oxidative stress”, “oxidation” and “antioxidant”. Articles were examined for their relevance to the particular subject. Articles in languages other than English were excluded.

Results

A total of 14 human studies were found describing different techniques of sample collection and combinations of measured substances (Table I). Blood samples were obtained from various veins like a peripheral or a central vein as the inferior mesenteric vein, the inferior vena cava or the common femoral vein. In three studies were also used arterial blood samples. In four studies muscle biopsy was used (quadriceps) in order that ischemic and reperfused muscle tissue to be evaluated for changes in structure, morphology and neutrophil infiltration. In the majority of studies all samples were obtained from the patients using regional sampling techniques before or after anaesthesia, during aortic clamping or balloon occlusion (ischemic time) in different time intervals and after aortic clamp removal (reperfusion time) in different time intervals up to 24 or 48 hours.

The oxidative stress was evaluated by measuring quantitative changes of different substances including vascular endothelial adhesion molecule like E-selectin (muscle expression) or intercellular adhesion molecule 1 (ICAM-1), lipid peroxidation by-products like MDA, lipofuscin, F(2)-isoprostanes and aldehydes [diene conjugates (DC)], cytokines like interleukin-1β (IL-1β), interleukin-6 (IL-6), interleukin-8 (IL-8), interleukin-10 (IL-10) and tumor necrosis factor-α (TNF-α). Oxygen free radicals production was directly or indirectly measured by quantifiable oxidation of immunoglobulin G (IgG) in plasma, or by measuring reactive oxygen species (ROS) and their metabolites. When human IgG is exposed to free radical generating systems such as peroxidizing lipids, or activated human neutrophils, characteristic auto-fluorescent monomeric and polymeric IgG is formed. The fluorescent IgG complexes, when produced in vitro, can stimulate the release of superoxide from normal human neutrophils. In the presence of excess unaltered IgG, further fluorescent damage to IgG occurs. Measurement and isolation of fluorescent monomeric and polymeric IgG is performed by high performance liquid chromatography. Reactive oxygen species (ROS) and their metabolites were also used like thiobarbituric acid reactive substances (TBARS), the total antioxidative capacity (TAC), glutathione redox ratio (the ratio of oxidized to reduced glutathione or GSSG/GSH), total (tGSH) or reduced glutathione (GSH-Px), superoxide dismutase (SOD) and catalase (CAT). Likewise other authors measured factors like creatine kinase (CK), lactate dehydrogenase (LD), white blood cell count (WBC), Aspartateaminotransferase (ASAT) and changes in complement factors like C3 and C4.
### Table I. Oxidative stress during AAA repair

<table>
<thead>
<tr>
<th>Author</th>
<th>Patients with AAA</th>
<th>Region of sample</th>
<th>Time intervals</th>
<th>Substance measured (OFR)/antioxidants/lipid peroxidation products</th>
<th>Results</th>
</tr>
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<tbody>
<tr>
<td>Formigli et al(^7)</td>
<td>9 elective open repair</td>
<td>FV, SV, Muscle</td>
<td>Pre-clamp Before Clamp removal Post-clamp 30 min</td>
<td>Complement (C3, C4), Neutrophils Superoxide Muscle neutrophil infiltration</td>
<td>FV &lt; SV No difference Increase during IR Altered muscle morphology</td>
</tr>
<tr>
<td>Formigli et al(^9)</td>
<td>12 elective open repair</td>
<td>Muscle biopsy</td>
<td>Pre-clamp Before clamp removal after clamp 30 min</td>
<td>Muscle E-selectin expression</td>
<td>Increase of E-selectin muscle expression and neutrophil infiltration during IR</td>
</tr>
<tr>
<td>Khaira et al(^9)</td>
<td>21 elective open repair</td>
<td>PV, CV, IMV</td>
<td>Before anaesthesia Before, during, after clamp removal, 2,6,24 h</td>
<td>Consumption of endogenous scavenging antioxidants</td>
<td>Levels decreased significantly in central and inferior mesenteric blood during and after clamping, but returned to normal by 24 h</td>
</tr>
<tr>
<td>Thompson et al(^10)</td>
<td>Elective 6 open repair 6 EVAR</td>
<td>FV</td>
<td>Pre-clamp, During proximal, distal anastomosis Post-clamp 5, 30 min</td>
<td>Quantifiable oxidation of IgG in plasma IL-1β, IL-6, TNF-α</td>
<td>Significant reduction of OFR, IL-1β, TNF-α with EVAR</td>
</tr>
<tr>
<td>Chello et al(^11)</td>
<td>Co Q10 pretreatment 2 groups (AAA or OAID)</td>
<td>IVC, arterial</td>
<td>After anaesthesia Clamp 5, 30 min Post-clamp 5, 30 min</td>
<td>MDA, DC, CK, LD</td>
<td>Significant decrease of all substances in pretreatment group</td>
</tr>
<tr>
<td>Novelli et al(^12)</td>
<td>Vitamin E pretreatment 2 groups</td>
<td>Muscle biopsy</td>
<td>Pre-clamp Before Clamp removal Post-clamp</td>
<td>MDA Muscle morphology Neutrophil infiltration</td>
<td>In vitamin E patients: No changes during ischemia Significant reduced changes during reperfusion Decreased expression of E-selectin and ICAM-1, decreased accumulation of neutrophils within the ischemic and reperfused muscle</td>
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<td>Muscle E-selectin, ICAM-1 expression Neutrophil infiltration</td>
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</tr>
<tr>
<td>Lindsay et al(^13)</td>
<td>22 elective 14 ruptured</td>
<td>RA, CV, PV</td>
<td>Before anaesthesia, Pre-clamp Before Clamp removal Post-clamp 15, 60, 240 min daily</td>
<td>F(2)-isoprostanes</td>
<td>Significant increase in ruptured AAAs starting from hospital admission</td>
</tr>
<tr>
<td>Rowlands and Homer-Vanniasinkam(^14)</td>
<td>Elective 16 open repair 16 EVAR</td>
<td>1. SV, 2. FV</td>
<td>Before anaesthesia, Before clamp removal Post-clamp 4, 24, 72, 144 h Post-clamp 0, 4, 24 h</td>
<td>IL-6, IL-8, IL-10</td>
<td>Significant increase of IL-6 in open repair. Similar but smaller response in EVAR FV&gt;SV IL-6</td>
</tr>
</tbody>
</table>

Continued
Formigli et al. suggested that E-selectin expression on the vascular endothelium of human skeletal muscle may represent a key regulatory point in the process of neutrophil tissue accumulation and indicated an active role for the venular endothelium in the development of human ischemia-reperfusion syndrome. This phenomenon was dependent upon a complex series of events that included neutrophil adhesion to the inner surface of the postcapillary venules, passage through endothelial intercellular junctions, and migration distally into the interstitial spaces of the skeletal muscle tissue. Neutrophil tissue infiltration was also associated with ultrastructural signs of tissue damage at reperfusion. This is in agreement with accumulating evidence indicating a role for tissue infiltrating neutrophils in the genesis of toxic oxygen free radicals (OFRs).

Glutathione as antioxidant is an important endogenous scavenger against ROS, which is found almost exclusively in its reduced form (GSH), since the enzyme that reverts it from its oxidized form, glutathione reductase, is constitutively active and inducible upon oxidative stress. In fact, the ratio of oxidized glutathione to reduced glutathione within cells is often used as a measure of cellular toxicity. Elective abdominal surgery without ischemia and reperfusion leads to decreased muscle GSH concentrations 4-72 hr postoperatively without altering GSH redox status. Westman B et al. investigated to what extent muscle GSH status was affected during and fol-
lowing elective AAA repair in 10 patients. Thigh muscle specimens were taken preoperatively, at maximal ischemia and after reperfusion. The antioxidative capacity in terms of muscle levels of GSH was decreased. However, the oxidative stress during reperfusion did not change GSH status more than what has been reported following abdominal surgery without ischemia and reperfusion. Those results indicated that the oxidative stress elicited by elective AAA repair is outbalanced by a compensated GSH metabolism not giving rise to an increased amount of oxidized GSH or an altered GSH redox status. Significant increase of CAT and GSHPx in combination with decrease of TAC during reperfusion was also demonstrated from another Author.16

Lipid peroxidation by-products like: MDA, lipofuscine, F(2)-isoprostanes and aldehydes (DC) were used as specific biologic markers of lipid peroxidation in human studies. One of the most frequently used biomarkers providing an indication of the overall lipid peroxidation level is the plasma concentration of MDA.19,20 The MDA was used in three human studies as biological marker of oxidative status in patients with AAA repair.11,12,18 Blood samples were used in two cases and muscle tissue expression in one study. The MDA was reduced significantly during reperfusion or ischemia/reperfusion in patients with pretreatment antioxidant supplementation. In the third and more recent study the MDA was significantly increased in symptomatic patients, in patients who had been transferred to the intensive care unit (ICU) and during the reperfusion (15,60 min).

Lipofuscine, F(2)-isoprostanes and aldehydes (DC) as other lipid peroxidation by-products were used as single or in combination with other indirect oxidative markers. Lindsay et al.3 using the F(2)-isoprostane levels, as specific markers of lipid peroxidation, showed that patients with RAAAs had two phases of oxidative injury: before arrival at hospital and after surgery. The significant relationship between the postoperative increase in F(2)-isoprostane levels and the neutrophil oxidant production implicates neutrophils in the oxidative injury that occurs during reperfusion and may increase organ failure and ultimately mortality in patients with RAAAs.

Two studies also attempted to compare two groups of patients with AAA treated either by open or endovascular repair (EVAR). Thompson et al.10 demonstrated the production of OFRs in patients with AAAs undergoing both conventional repair and EVAR. The OFR generation was maximal after 5 min of lower limb reperfusion, declining after 30 min of reperfusion. OFRs production was lower in the EVAR group, although that did not reach statistical significance. Rowland et al.14 measuring pro- and anti-inflammatory release during AAA repair came to a conclusion that venous pro-inflammatory cytokine changes (IL-6) were consistent with significantly greater lower-torso reperfusion injury in patients undergoing open repair. Smaller responses were seen after EVAR (IL-6 and IL-8), although both groups showed a similar anti-inflammatory response (IL-10). This pro- and anti-inflammatory imbalance may account for the increased morbidity associated with open repair.

Some studies used different type of antioxidant supplementation in order to correlate it with a reduction in oxidative stress and damage in the antioxidant group of patients. Novelli et al.12 and Formigli et al.13 using different types of oxidative markers showed the protective effect of vitamin E. The MDA content, the ultrastructural injuries and neutrophil infiltration were significantly reduced by vitamin E administration. This treatment was able to prevent the accumulation of neutrophils within the ischemic and reperfused muscle. The beneficial effect of Vitamin E was due to its ability to hinder the expression of E-selectin and ICAM-1, molecules known to increase the adhesiveness of endothelium to circulating neutrophils. After treatment with Vitamin E a marked attenuation of the reperfusion injury was also evident. Both Authors concluded that Vitamin E treatment might be considered a valuable tool for protection against the ischemia reperfusion injury (IRI) of human skeletal muscle.

Chello et al.13 found that the concentrations of MDA, DC, CK and LD in patients who received coenzyme Q10 (CoQ10) were significantly lower than in the placebo group and they concluded that pre-treatment with CoQ10 may play a protective role during routine vascular procedures requiring abdominal aortic cross clamping by attenuating the degree of peroxidative damage.

Wijnen et al.15 in a small randomized trial (42 patients), concluded that there was a reduction in serum CK, ASAT and leukocyte sequestration after lower torso-ischemia-reperfusion in patients with infrarenal AAA open repair by administering a multi-antioxidant supplementation (vitamin E and C, allopurinol, N-acetylcysteine and mannitol).
Discussion

Aortic cross-clamping is a significant cause of ischemia-reperfusion injury (IRI) and activation of inflammatory pathways\textsuperscript{21,22}. Oxidative products are also recruited to these sites of injury by damaged native cells. Several mechanisms and mediators (eg, neutrophils, the xanthine/xanthine oxidase enzyme system, and mitochondrial electron transport leakage) cause oxidant-mediated tissue injury after IRI\textsuperscript{23-25}. Additionally, a lower-torso IRI results in a diffuse inflammatory response leading to a sequence of potentially life-threatening events\textsuperscript{26}.

There is also evidence for a strong association between aneurysmal formation and inflammation, including infiltration of leucocytes, production of ROS, and activation of matrix metalloproteinase-9\textsuperscript{27-31}.

Measurement of OFRs by direct methods using electron spin resonance spectroscopy is limited because of high cost and lack of data regarding electron spin resonance spectroscopy spectra and in vivo biological systems\textsuperscript{32}. Alternatively, OFRs can be indirectly detected by measuring their oxidative attack on lipids and proteins resulting in products such as DC, hydroperoxides, and aldehydes\textsuperscript{33,34}.

All human studies, which were described, utilized different combinations of measured substances in blood samples mainly or muscle biopsies. However, all measured markers were parts from a number of almost all pathways influenced by the double phenomenon of IRI during the AAA repair. ROS and their metabolites, lipid peroxidation by-products, inflammatory cytokines, vascular endothelial adhesion molecules, complement factors, neutrophil tissue infiltration and ultrastructural signs of tissue damage at reperfusion were examined in muscle tissue samples. Finally some investigators used the beneficial effect of some antioxidants in protection human tissues during AAA repair in order to prove the oxidative stress and damage that takes place because of the operation.

Whether or not, the alteration of the oxidative status during AAA repair was well documented in every study. These data suggest that an oxidative stress occurred during aortic clamping and also during subsequent reperfusion.

The time intervals when the oxidative stress reached the peak, in most of the studies were during ischemic time (15-60 min of clamping) while muscle neutrophil infiltration was maximal during reperfusion. Almost all changes returned to normal by 24 h. Oxidative stress as it was predictable was higher in cases of open repair beside EVAR and in cases with RAAAs beside elective cases.

However, the clinical significance of these changes has not been fully elucidated. Papalambros et al.\textsuperscript{18} correlated the oxidative stress with the patient clinical outcome. They studied the degree of the oxidative stress in patients with AAA using as biological marker the MDA, and concluded that there was a high-grade oxidative stress during AAA repair operation. A relationship between plasma MDA levels and the need for transfer to ICU was also demonstrated giving a possible additional value to that marker in predicting which patients were at risk of having a complicated postoperative course. The Authors proposed that preoperative statin (3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors) therapy could be initiated in all AAA patients to obtain optimal perioperative and postoperative results. Their antioxidant properties are already known\textsuperscript{35} when their utility to improve perioperative, as well as long-term, morbidity and mortality rates in patients undergoing AAA operations has been proven\textsuperscript{36-39}.

The beneficial effect of an antioxidant supplementation in reducing the oxidative stress during AAA repair was also demonstrated from four other studies\textsuperscript{11-13,15}.

Thus, there may be a therapeutic advantage associated with antioxidant supplementation in these patients, especially if there was a biological marker as a predictor of the development of systemic complications. A standard antioxidant administration in form of a multivitamin and statin combination (multidrugs) could be initiated in all patients who will be operated for AAA with open repair particularly for symptomatic and ruptured aneurysms even though prior an emergent EVAR. This treatment may be considered a valuable tool for protection against the ischemia-reperfusion damage and its systemic complications.

References

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