A Multiantioxidant Supplementation Reduces Damage from Ischaemia Reperfusion in Patients after Lower Torso Ischaemia. A Randomised Trial

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Background: open repair of intra-abdominal aortic aneurysm (AAA) is associated with lower torso ischaemia and reperfusion

Objective: to examine the effect of antioxidants on the activation and sequestration of white blood cells and muscle injury during AAA repair.

Method: forty-two patients undergoing elective infrarenal aneurysm repair, were randomised to either standard therapy (22 patients) or standard therapy with additional multiantioxidant supplementation (20 patients). Vitamin E and C, Allopurinol, N-acetylcysteine and mannitol was administered perioperatively. White blood cell count (WBC), serum creatine kinase, aspartateaminotransferase, lactate and lipofuscine were measured.

Results: WBC remained higher after reperfusion in the antioxidant group (\(p = 0.008\)). CK, ASAT and lipofuscine levels were significantly lower after reperfusion in the antioxidant group (\(p = 0.02\), \(p = 0.018\), \(p = 0.017\)).

Conclusion: multi-antioxidant supplementation was associated with a reduction in serum CK and ASAT after AAA repair. This is likely due to a reduction in oxidative stress and a decreased leucocyte sequestration and activation.

Key Words: Ischaemia; Reperfusion; Aneurysm; Leucocytes; Antioxidants; Lipofuscine.

Introduction

Polymorphonuclear adhesion, activation, migration indicates ischaemia reperfusion injury\(^1\)-\(^12\) and lead to\(^1\)-\(^12\) remote organ injury torso I-R\(^2\),\(^13\)-\(^21\)

This damage is partly caused by oxygen-derived free radicals (ODFR).\(^22\)-\(^24\) Several studies have shown that I-R damage can be ameliorated by antioxidants and scavengers in both direct and indirect I-R models.\(^25\)-\(^34\)

The aim of the study was as same as indicated.

Patients and Methods

The protocol was approved by the local ethics committee. A power calculated was not performed and the study was not blinded. After informed consent, forty-two consecutive patients needing elective open transperitoneal surgery for an infrarenal abdominal aneurysm were included over 36 months. Excluded were patients with renal failure, needing artificial support and those classified as A.S.A. 3 and higher. Patients were randomised, by envelope to either standard therapy or standard therapy supplemented with antioxidants. The multi-antioxidant therapy consisted of vitamin E, 5 days prior to the operation, 200 mg daily orally; vitamin C, on the morning of operation, 2000 mg orally; Allopurinol, 1 day before surgery 300 mg orally, and 300 mg intravenously before the start of the operation; N-acetylcysteine, 150 mg/kg before the start of the operation; N-acetylcysteine, 150 mg/kg before the start of the operation and subsequently 200 mg/kg in a drip over 12 hours; mannitol, 10% 500 ml, in 12 hours starting at the beginning of surgery. Blood samples were taken before the start of the antioxidant therapy (T Pre-op), before clamping (T1), before release of the clamp (T2), 5 minutes after release of the first leg (T3), during closure of the skin (T4), 6 hours after release of the first clamp (T6) and 24 (Day 1) and 48 hrs (Day 2) after surgery. Preoperatively an epidural catheter with bupivacaine and sufentanyl was introduced and continued for three days postoperatively.

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Anaesthesia was administered using propofol, vecuronium bromide and sufentanyl for induction, followed by continuous infusion of propofol till the end of surgery. A urinary catheter was placed after induction of anaesthesia.

Serum total antioxidant capacity was measured using a Randox antioxidant status kit (Randox Laboratories Ltd, Diamond Rd., Crumlin, Co. Antrim, United Kingdom). Serum lactate was measured on a Vitros 950 analyser using standard Ektachem Slide technology.

A white blood-cell count (WBC) was performed using a Coulter GEN.S haematology analyser (Coulter Corporation, Miami, Florida, U.S.A.). Serum CK and ASAT activities were measured on a Vitros 950 analyser using standard Ektachem Slide technology. Lipofuscin was measured with the method of Tsuchida et al.\textsuperscript{38} using the fluorescence capacity of lipofuscin relative to quinine sulphate at excitation and emission wavelengths of 345 and 430 nm.

Statistical analysis

To compare the two groups we used a non-parametric test for unpaired samples (Mann–Whitney U test). To compare the values at different points in time, we used a non-parametric test for paired samples (Wilcoxon signed rank test). Statistical significance was accepted if $p < 0.05$.

Results

Patient characteristics are shown in Table 1 and operative data are shown in Table 2.

No significant differences between groups were found as to ischaemia time ($p = 0.67)$, blood loss ($p = 0.78$), time of hypotension ($p = 0.51$) and serum lactate values ($p = 0.43$). One patient received a second operation because of rebleeding and subsequently died due to multiple organ failure.

Serum total antioxidant capacity was significantly higher in the group receiving antioxidants at T1 ($p < 0.001$), T2 ($p = 0.049$), T3 ($p = 0.016$) and T4 ($p = 0.050$). No statistical significant difference was found after T4 (Fig. 1A).

In order to assess the effect of the multitioxidant supplementation it is necessary to compare the changes within both groups, not the differences between the groups.

WBC was decreased significantly in both groups at T1 (median 5.9 and $6.5 \times 10^9$) and T2 (median 6.3 and $6.4 \times 10^9$) when compared to pre-operative values (median 8.1 and $7.7 \times 10^9$) ($p < 0.02$). After T3, WBC was increased in the group receiving antioxidants (median 7.8, 9.2 and $12.2 \times 10^9$). However, in the standard treatment group WBC remained significantly lower than pre-operative values until T6 (median 6.3, 7.2 and $10.05 \times 10^9$) (see Fig. 2). WBC was significantly lower in the control group at T3 ($p = 0.027$), T4 ($p = 0.008$) and T6 ($p = 0.046$). After this time no differences were found (Fig. 1 B).

CK values were significantly lower in the antioxidant group at day 1 (median 311 and 910 U/l) compared to preoperative values (median 38 and 39 U/l) ($p = 0.02$) (Fig. 1C).

ASAT values were significantly lower in the antioxidant group at day 1 (median 18 and 28 U/l) compared to preoperative values (median 16 and 15 U/l) ($p = 0.018$) (Fig. 1D).

Serum lipofuscin levels were significantly lower in the antioxidant group at day 1 (median 0.14 and 0.18) compared to preoperative values (median 0.30 and 0.33) ($p = 0.017$) (Fig. 1E).

Discussion

Our results show that in the group receiving the antioxidant supplementation more leucocytes remain in
circulation after reperfusion. After an initial drop in leucocytes at the start of the operation, explained by the preoperative haemodilution and early sequestration, WBC increases much faster in the antioxidant group. This suggests that more leucocytes are sequestered in the non-antioxidant group, possibly because of the antioxidants reducing the activation of neutrophils. Once activated, neutrophils start producing not only ODFR but also several granule associated proteins such as elastase. This can cause local

Fig. 1. (a) Serum total antioxidant capacity (mmol/l), (b) White blood-cell count (10⁹/l), (c) Serum creatine kinase (U/l), (d) Aspartate aminotransferase (U/l) and (e) Serum lipofuscine (µg/l) in patients undergoing elective open repair of an infrarenal abdominal aneurysm receiving either standard therapy (N=22) or standard therapy with multiantioxidant supplementation (N=20). Preoperative values (Pre-op) and values before ischaemia (T1), before reperfusion (T2), 5 min. after reperfusion (T3), at closure of the skin (T4), 6 (T6) hrs after reperfusion and 1 and 2 days after reperfusion are shown. Values as median and interquartile range. * indicates statistical significant difference p < 0.05, ▲ = with antioxidants, — = without antioxidants.

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muscle damage as shown by the rise of enzymes such as CK and ASAT. The fact that the rise in both enzymes is less in the antioxidant group combined with the faster rise in the leucocyte count in this group may indicate that there is less sequestration of leucocytes in the ischaemic lower torso in the antioxidant group. This may be caused by the reduced oxidative stress in the treatment group. Oxidative stress is hard to measure and quantify in vivo. We can only measure the results of damage that is thought to be caused by oxidative stress. In anticipation of the development of more accurate markers for oxidative stress caused by ischaemia reperfusion we use end-products of lipid peroxidation such as malondialdehyde and lipofuscin as indicators for the amount of oxidative stress. Lipofuscin is a pigment that is formed as an end-result of in vivo lipid peroxidase in organelles and is being used as a parameter of lipid peroxidation.

Peroxidation organelles and is being used as a parameter of lipid peroxidation. Lipofuscin is a pigment that is expected about 24 hours after the ischaemic event. We have found a difference in serum lipofuscin levels between the study groups indicating less lipid peroxidase in the antioxidant group. In order to sort maximum antioxidative effect during reperfusion we chose to administer large doses of several antioxidants and scavengers. Not much is known about the possible cumulative effect of antioxidants and scavengers in vivo. It is very well possible that the damage during I-R occurs due to several different pathways and can be blocked by several agents. This means that in a situation where only one pathway is blocked the antioxidant effect may be invisible, whereas where more pathways are blocked this can result in a clinically detectable effect. We therefore chose mannitol as a free radical scavenger of superoxide and hydrogen peroxide, N-acetylcyesteine as a glutathione agonist, Allopurinol as a free radical production inhibitor and the antioxidative vitamins E and C, both decreasing lipid peroxidation. All these substances have shown to ameliorate I-R damage. The dose chosen was the maximum daily dose allowed by the manufacturers.

Even though the serum total antioxidative capacity in the treatment group was significantly increased compared to the non-treatment group, the absolute increase was not spectacular. This may be due to the fact that very little is known about increase in total antioxidative capacity by multiantioxidant supplementation. Thus, there may be still room for improvement for the antioxidant supplementation. Also the aortic aneurysm model is very difficult to standardise and is subject to many variables and better models are needed.

**Conclusion**

In our study we have shown a reduction in serum CK and ASAT after lower torso ischaemia–reperfusion, most likely caused by a reduction in oxidative stress and a decreased leucocyte sequestration and activation by administering a multi-antioxidant supplementation to patients undergoing an infrarenal aneurysm repair. Future studies will have to determine the impact on clinical outcome of multiantioxidant supplementation after lower torso ischaemia.

**References**


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