Antioxidant defence during cardiopulmonary bypass surgery

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Abstract

Objective: Cardiac surgery may lead to severe oxidative stress due to formation of oxidation products generated during ischemia and reperfusion. We investigated to which extent oxidative stress influences a number of endogenous antioxidants and markers of cellular activation.

Methods: At six time points blood was withdrawn from patients undergoing coronary artery bypass grafting, using the on-pump procedure.

Results: Both glutathione peroxidase and superoxide dismutase show a gradual and strong increase in activity during surgery (40 and 30%, respectively), returning to baseline values 24 h after surgery. The total antioxidant capacity has a maximum increase of 60%. Markers of cellular activation, such as eosinophil cationic protein and tryptase also increase during the procedure.

Conclusion: Cardiac surgery results in systemic inflammation accompanied or caused by severe oxidative stress. The human body has a strong innate oxidative defence screen, which is probably not sufficient to fully compensate for the total amount of oxidative damage.

Keywords: Bypass surgery; Antioxidants; Glutathione peroxidase; Superoxide dismutase; Oxidative stress

1. Introduction

Although open cardiac coronary artery by-pass grafting (CABG) surgery has become a routine procedure worldwide, patient morbidity and mortality due to adverse post-operative complications are still unacceptably high. The endothelial injury and/or cardiac, renal, hepatic or pulmonary dysfunction associated with CABG surgery have been linked to the inflammatory responses and systemic oxidative stress directly caused by this procedure but the underlying mechanisms have not been fully elucidated yet.

It has been suggested that in addition to the damage caused directly in the myocardium, a significant proportion of the adverse outcomes may also be caused by the systemic effects of cardiopulmonary bypass (CPB) [1]. It has moreover been demonstrated that on-pump procedure gives rise to a more pronounced systemic inflammation and oxidative stress than the off-pump procedure [2]. The mechanisms explaining these observations may be related to several deleterious events occurring during CPB [3] which are either material-dependent (caused by exposure of blood to non-physiologic surfaces and conditions during the extracorporal circulation, ECC) or material-independent (caused by surgical trauma, ischemia-reperfusion and changes in body temperature). One of the most damaging consequences of these events is the formation of reactive oxygen species (ROS) and radicals, which originate from various cellular and enzymatic sources such as myocardial cells, activated neutrophils [4] or endothelial xanthine oxidase. These are closely linked to inflammatory responses, including complement activation, release of cytokines and leukocyte activation, along with expression of adhesion molecules [5]. Many studies have described the nature of these ROS and the time course of their formation during CPB [6]. The nature of these oxidative events leads to depletion of plasma antioxidants, increased lipid peroxidation and formation of other damaging metabolites [7-9]. In order to counterbalance this sequence of events and to diminish oxidative injury, several studies have investigated the use of antioxidant supplements during ECC [10,11]. Less is known about the consequences of CPB on the endogenous antioxidant capacity that is derived from the activity of antioxidant enzymes such as glutathione peroxidase (GPx) and superoxide dismutase (SOD), responsible for the clearance of peroxides and superoxide, respectively.

In order to investigate this question, our study focused on the time course of innate antioxidant activity (antioxidant enzymes and global antioxidant capacity in plasma) in patients undergoing cardiopulmonary bypass surgery. In particular, we were interested in analysing to which extent...
antioxidants were being generated or activated during CPB. In order to elucidate the relationship between the changes in antioxidant status and the activation of inflammatory cells during CPB, we also monitored markers of leukocyte activation such as tryptase and eosinophil cationic protein.

2. Materials and methods

2.1. Study population

Ten patients (6 males, 4 females, mean age 68.4 yrs ± 7.7 SD) undergoing elective coronary artery bypass grafting were included in this study. Perioperative data are shown in Table 1. This study was in accordance to the principles outlined in the Declaration of Helsinki. Patients were informed of the procedure and the ethical committee of the University Hospital of Antwerp approved the study protocol.

2.2. Cardiopulmonary bypass

Anaesthesia with endotracheal intubation and balanced administration of premedication and transfusions was uniform in all cases. The CPB equipment consisted of a Bentley membrane oxygenator (Bentley Oxygenation system CM50, Baxter, Ivina CA, USA). The pump-oxygenator system was primed with 1800 ml crystalloid solution (Plasma-Lyte A, Baxter) and 6% HetaStarch. Anticoagulation was achieved by 300 U/kg heparin (Leo Pharma, Denmark). All patients were cooled to 28 °C. Surgery was performed using the intermittent cross-clamp technique. Fifteen minutes after decannulation, heparin was neutralised with protamine chloride (1:1 ratio; Roche, Belgium). Patients only received autologous transfusion with residual blood left in the pump after CPB.

2.3. Blood sampling

Blood samples were collected into sterile Lithium-heparin tubes and SST-tubes with clotting activator (Vacutainer, Becton Dickinson) at several time points during and after cardiac surgery: (a) 10 min after induction of anaesthesia (left radial artery), (b) 10 min after start of ECC (arterial side of pump), (c) at the end of ECC (arterial side of pump), (d) 10 min after protamine administration (left radial artery), (e) 4 h and (f) 24 h after surgery (left radial artery). Blood samples were processed within 10 min after sampling to avoid auto-oxidation of antioxidants. Hematocrit values were measured in every blood sample. In order to take dilution with the priming solution of the CBP system into account, the value of the first blood sample (pre-surgery) was set as a reference baseline value. All obtained data were then corrected for haemodilution using the hematocrit values to calculate the appropriate correction factor.

2.4. Antioxidant measurements

**Trolox equivalent antioxidant capacity (TEAC)** was measured in plasma according to the method of Rice-Evans and Miller. TEAC is a measure that is indicative for the whole pool of antioxidants in plasma and thus for the total antioxidant capacity of plasma. Trolox is a synthetic vitamin E analog with antioxidant activity, which is able to prevent radical generation by H2O2 in a reaction mixture containing metmyoglobin and Z, Z'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid (ABTS). When antioxidant activity in the milieu is depleted, ABTS forms stable coloured radicals that can be measured by spectrophotometry (absorption at 734 nm). Plasma antioxidant capacity is compared with a calibration curve of trolox concentration and thus trolox equivalent antioxidant capacity can be calculated. Intra and inter-assay CV was 5 and 14%.

**Glutathione peroxidase (GPx)** in full blood was measured with a Ransel Glutathione Peroxidase kit (Randox Laboratories Ltd), and is based on the method of Paglia and Valentine: GPx catalyses the oxidation of glutathione (GSH) by cumene hydroperoxide. In the presence of glutathione reductase and the oxidized glutathione (GSSG) is immediately converted to the reduced form with a concomitant oxidation of nicotinamide adenine dinucleotide phosphate, reduced form (NADPH) to NADP+. The decrease in absorbance of NADPH can be measured at 340 nm. Intra- and inter-assay CV was 2 and 6%.

**Superoxide dismutase (SOD)** was measured with a RanSOD superoxide dismutase kit (Randox Laboratories Ltd). The role of SOD is to accelerate the dismutation of the toxic superoxide radical O2·− to hydrogen peroxide and molecular oxygen. The Randox method uses xanthine and xanthine oxidase to generate superoxide radicals that react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium (I.N.T.) to form a red formazan dye. The SOD activity is then calculated from the degree of inhibition of this reaction compared to a standard curve of SOD. Intra- and inter-assay CV was 6 and 15%.

**Alpha-tocopherol and retinol** in serum were measured by High Performance Liquid Chromatography (Dionex, HPLC with a 100% methanol mobile phase) with detection at 292 and 325 nm, respectively. Intra- and inter-assay CV was 5 and 13%.

2.5. Markers of cellular activation

**Eosinophil cationic protein (ECP)** was measured in serum using a fluoroenzymeimmunoassay provided by Pharmacia (Uppsala, Sweden). Anti-ECP, covalently coupled to immunoCAP, reacts with the ECP in the patient’s serum specimen. After washing, enzyme-labelled antibodies against ECP are added to form a complex. After incubation, unbound enzyme-anti-ECP is washed away and the bound complex is
then incubated with a developing agent. After stopping the reaction, the fluorescence is measured. The fluorescence is inversely correlated with the concentration of ECP in the serum sample. Measuring range is 2–200 \( \mu \text{g/l} \). Within assay CV is 3.8%.

Tryptase is a serine protease released by mast cells upon activation. It can be measured in serum applying an immunoassay from Pharmacia, according to the same principle as described for ECP. Measuring range for undiluted sample is 1–200 \( \mu \text{g/l} \). Within assay CV is 3%.

2.6. Statistical analysis

Data are shown as mean \( \pm \) SEM. Analysis of variance for repeated measures was used to compare changes in time. Differences were considered significant at \( P \) value less than 0.05.

The power of the primary end-points (GPx, SOD and TEAC) was >0.7.

3. Results

Enzyme activity of the antioxidants GPx and SOD in full blood increased significantly in the initial stages of surgery. When looking at the changes within each individual (in-patient), GPx rose by an average of 20% at 10 min after starting the ECC compared to pre-surgery values, and reached a maximum average increase of 40% at the end of cross-clamp circulation (Fig. 1). SOD reached a 30% maximum rise in activity at the end of surgery, after the administration of protamine (Fig. 2).

Serum concentration of retinol and \( \alpha \)-tocopherol varied rather widely between patients in all measurements. We saw that both retinol (data not shown) and \( \alpha \)-tocopherol remained constant during CBP (Fig. 3). It is only from 4 h after surgery that serum concentration of these antioxidants decreased significantly.

Total antioxidant capacity of plasma (TEAC) (Fig. 4) increased from 0.9 mM Trolox equivalents to 1.45 mM at 10 min of ECC, corresponding to an increase of plasma antioxidant capacity of 60%.
Superoxide anion is formed during the operation. Our findings confirm the assumption that contact of blood with foreign substances, will induce systemic inflammatory responses associated with complement activation, cytokine release and cellular activation of neutrophils [1]. These are all sources of ROS production [12] which will ultimately lead to depletion of plasma antioxidants [8]. In a previous study we already described a decrease in plasma glutathione during CPB [9]. In the present study, we used TEAC as a marker for total antioxidant capacity in plasma. Unexpectedly, we did not document a decrease of TEAC value, or—in other words—a depletion of plasma antioxidants, but an almost twofold increase. The nature of this increase is not known. Two abundant plasma molecules with antioxidant activity, namely albumin and uric acid are the main determinants of the TEAC value. Albumin concentration in plasma, however, did not increase significantly during CPB (data not shown) and could therefore, not have contributed to the increase of TEAC value. The contribution of uric acid hereby could not be assessed, due to technical limitations. Another possible cause of increased TEAC value could have been haemolysis of red blood cells during or after sampling. Hemoglobin has been shown to interfere with the TEAC measurements. Thus the impact of free hemoglobin and uric acid in plasma should be taken into account when measuring global antioxidant capacity in future studies.

The activity of the enzymatic antioxidants glutathione peroxidase (GPx) and superoxide dismutase (SOD) increased significantly during ECC. This could explain the time course of depletion of GSH we observed previously, because GPx uses GSH as a cofactor [10]. Moreover, when comparing our time course of GPx activation and the time course of TBA-reactive peroxides as described by Davies [6], we see a close match. This parallelism suggests that glutathione peroxidase forms a first barrier against the reactive oxygen species being formed during the operation. Our findings confirm the results obtained by Arduini et al. [13], who also found an increase in GPx activity. Others studies, however, observed a decrease in GPx activity [14], or no change during CPB [15]. Most of these studies, however, were performed on animal models, which may explain part of the discrepancy between our study and the ones just mentioned.

SOD, the enzyme responsible for converting superoxide anion into hydrogen peroxide, was also strongly activated, a fairly acute response lasting from early CPB until after protamine administration. This can be explained by the fact that contact of blood with polymers of the CPB circuit releases cytokines and activates neutrophils [12], independent of heparin coating. Activated neutrophils will via the respiratory burst generate superoxide anion which is detoxified by SOD. These two findings illustrate that GPx and SOD form a strong first line of defence against reactive oxygen species. Moreover, GPx and SOD are enzymatic antioxidants which are not consumed during their detoxification activities. In contrast, antioxidants which act as free radical scavengers are consumed during oxidative processes and the time courses of depletion vary depending on the nature of the antioxidant.

Retinol is not considered to be a very potent antioxidant, although its precursors of the carotenoid group are. Alpha-tocopherol on the other hand is well known for its antioxidative capacity. However, its role is rather controversial, for some studies find supplementation of patients

**Fig. 5. Levels of serum tryptase before, during, and after cardiopulmonary bypass surgery. **P<0.05 when compared to the value before surgery.

**Fig. 6. Concentrations of eosinophil cationic protein (ECP) in serum before, during, and after cardiopulmonary bypass surgery. **P<0.05 when compared to the value before surgery.
with $\alpha$-tocopherol prior to CPB to be beneficial [16], while several big-scale studies such as the Heart Outcomes Prevention Evaluation (HOPE) study, clearly demonstrated that vitamin E supplementation, independently of surgery, had no apparent effect on cardiovascular outcomes [11]. Also, the role and consumption of $\alpha$-tocopherol during CPB are debatable. Some studies state that $\alpha$-tocopherol level decreases during and after CPB [17], while others find a slight or non-significant decrease in serum $\alpha$-tocopherol concentration [18]. Our results, after correction for haemodilution, are similar to the ones published by Barsacchi et al. [17] who found a decrease of $\alpha$-tocopherol concentration under conditions of ischemia and reoxygenation. However, the decrease observed in our study was only significant after surgery. This time course suggests that it can be the result of post-ischemic repair of (membrane) lipid peroxides. An added cause can be the depletion of vitamin C during CPB. Since vitamin C is able to regenerate vitamin E from its radical intermediate, consumption of $\alpha$-tocopherol is more likely if vitamin C is depleted [19]. Ballmer et al. have shown that vitamin C is strongly depleted after CPB [20]. These observations suggest that the oxidative burden during CPB would, in a first instance, consume vitamin C, together with reduced glutathione, which are the most effective water-soluble non-enzymatic antioxidants under conditions of oxidative stress. Their ensuing depletion and, as a consequence, that of $\alpha$-tocopherol, may seriously alter the rest of the anti-oxidative cascade and result in systemic whole-body inflammation which then increases the risk of post-ischemic damage.

In order to elucidate the relationship between the changes in antioxidant status and the activation of inflammatory cells during CABG, we also monitored markers of leukocyte activation. Evidence of neutrophil activation during ECC has been provided by other authors [4]. In addition, in a previous study performed at our institution by Jorens et al. [21], IL-8 response was monitored and found to be significantly increased during CPB, returning to normal values 20 h post-operative. In the present study we found a peri-operative increase in serum eosinophil cationic protein (ECP) and tryptase levels. This suggests that both eosinophils and mast cells were activated during or after CABG and points out to a possible anaphylactoid reaction. Nevertheless, ECP release is not only a sign of eosinophil activation as it is supposed to be transported by neutrophils [22]. Moreover, despite the fact that ECP is of eosinophilic origin, in some studies in asthma patients no relationship was found between eosinophil number and ECP levels in sputum [23]. This observation suggests that the ECP sputum concentrations are not merely a function of the eosinophil numbers but could also be an indirect marker of neutrophil activation.

With regard to the activation of mast cells during CABG, several underlying mechanisms could explain this observation. Mast cells are heavily granulated wandering cells found in connective tissue and are abundant beneath epithelial surfaces. The release of their granule content (heparin, histamine and many proteases such as tryptase) can be triggered by physical factors (mechanical trauma, changes in temperature), toxins, endogenous mediators (proteins, tissue proteases) and immune mechanisms (IgE dependent and independent). Also complement activation can cause mast cell degranulation (anaphylotoxins C5a, C3a and C4a are formed during complement activation) [24]. All these processes can occur during the ischemia-reperfusion and ECC of the CPB procedure and can, moreover, be linked to the oxidative activity. For example, activation of C5a receptors on mast cells during CPB may trigger degranulation. Furthermore, tryptase released from mast cells can further stimulate the release of IL-8 and up-regulate ICAM-1 on epithelial cells [24]. Based on these facts, the activation of mast cells during CPB is not necessarily a sign of allergy but could just be a consequence of complement activation. There is also a growing body of evidence that mast cells are activated during ischemia-reperfusion. The increased influx of oxidants occurring at the onset of reperfusion may thus also be responsible for mast cell activation [25]. It has already been reported that superoxide is known to activate these cells [26] which in turn play a role in neutrophil activation and infiltration into the lung [27], thus, in their turn, further aggravating the release of ROS.

In summary, this study, performed in a small number of selected patients, showed that there was a strong innate antioxidant response under the conditions of acute oxidative stress occurring during CABG. However, it is well documented that post-ischemic oxidative damage still occurs. This indicates that this antioxidant response is not sufficient to counteract the heavy oxidant burden of these surgical procedures. It would be interesting to investigate whether supplementation with antioxidant mixtures such as vitamin C plus glutathione could, in a first instance, prevent their depletion during CPB, and, in a second instance, suppress the post-ischemic inflammatory response.

This study also showed that there was a significant and parallel release of markers of anaphylaxis. The role played herein by mast cell and eosinophil activation needs further investigation. The information collected in such studies will aid in the search for effective systemic measures to prevent post-ischemic damage and thus ensure a better outcome for patients undergoing cardiac surgery.

References


