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Massive and long-lasting decrease in vitamin C plasma levels as a consequence of extracorporeal circulation

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ABSTRACT

Objective: The use of cardiopulmonary bypass (CPB) is suggested to induce oxidative stress, reflected by an imbalance between prooxidant and antioxidant substances. The majority of studies published have either focused on only one aspect (prooxidant or antioxidant side) or covered only a short observation period. Therefore, the aim of this study was to investigate the long-term effects of CPB on the balance of prooxidative markers and antioxidant substances in one single group of patients, being able to estimate the degree of oxidative stress.
Methods: Blood samples were taken from 29 patients undergoing cardiovascular surgery beginning the day before surgery through postoperative day 6 (discharge). Plasma concentrations of vitamins C (total ascorbic acid) and E and malondialdehyde were measured by high-performance liquid chromatography. Plasma levels of ascorbyl free radical were determined using electron paramagnetic resonance spectroscopy.
Results: The study showed a significant decrease in vitamin C plasma levels during CPB without any recovery of vitamin C up to the time of discharge. Furthermore, CPB induced a significant increase in malondialdehyde plasma concentrations immediately after unclamping, accompanied by a significant increase in the ascorbyl free radical to total ascorbic acid ratio. The latter stayed elevated until the end of observation.
Conclusions: Our findings indicate that the oxidative stress event after CPB can be divided into two phases: Immediately after reperfusion, a massive oxidative stress occurs, reflected by the increase in malondialdehyde. During convalescence, there must be an ongoing situation of oxidative stress, especially in the water-soluble compartment, leading to the consumption of vitamin C. Because the main antioxidant substance, vitamin C, did not increase again over the entire observation period, supplementation should be given consideration.

Introduction

Cardiovascular surgery usually is attended by extracorporeal circulation using cardiopulmonary bypass (CPB). Despite advances in technology, a number of pathophysiological and immunologic events induced by the use of CPB remain [1–3]. These events postoperatively often result in the development of various comorbidities, e.g., atrial fibrillation or the so-called systemic inflammatory response syndrome followed by sepsis or multiple organ failure [1,2,4,5] that impair convalescence and lead to higher costs and longer hospital length of stay. Those comorbidities are most likely to be related to an increase in oxidative stress during and after CPB [6–8].

As a result of aortic cross-clamping, the application of CPB always involves a situation of ischemia reperfusion, leading to the production of reactive oxygen species by activation of xanthine oxidase and nicotinamide adenine dinucleotide phosphate oxidase, as well as uncoupling of nitric oxide synthases in the ischemic tissue and activation of leukocytes as systemic response [1,9,10]. Studies comparing CPB-attended cardiac

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surgery with off-pump techniques revealed CPB to be the major cause of those inflammatory and oxidative events [11,12]. The resulting situation of oxidative stress is thought to cause a depletion of plasma antioxidants that, in consequence, might lead to higher morbidity and mortality [2,13–16].

The subsequent oxidative stress is reflected by several plasma and tissue parameters, e.g., lowering of plasma antioxidant capacity, increase of thiobarbituric acid reactive substances and phospholipid-esterified dienes, as well as an increase of myocardial phosphocreatine kinase and decrease of cardiac glutathione levels [17–20]. Also, several proinflammatory mediators are released mainly during reperfusion, suggesting a possible reason for pathophysiological changes [21]. A strong increase of endogenous antioxidants during CPB with a return to baseline 24 h after surgery also has been observed, indicating a higher need of those enzymes to combat the oxidative events [22].

Unfortunately, most of the studies mentioned were focusing at only one aspect, either the prooxidant or the antioxidant side. Additionally, the observation period mostly was restricted to the perioperative and early postoperative phase until 24 h post surgery.

For a better understanding and a more comprehensive view, we investigated both prooxidant as well as antioxidant plasma parameters in one single group of patients considering a longer observation period until the sixth day after surgery to elucidate whether the effect of CPB may have long-term consequences. To be able to explore the possible correlations within the cascade of oxidative stress and antioxidant defense in both compartments, we examined the antioxidant plasma parameters of vitamin C (total ascorbic acid [TAA]) and vitamin E (α-tocopherol) as well as the prooxidative markers ascorbyl free radical (AFR) and malondialdehyde (MDA), representing the water- and lipid-soluble compartments, respectively.

Material and methods

Patients

Twenty nine patients (21 men, 8 women) ages 32 to 75 y (mean 64.2 ± 8.9 y) undergoing cardiac surgery (coronary artery bypass grafting: 51.7%, heart valve replacement: 27.6%, or a combination of both: 20.7%) with CPB were included in the study. Study protocol was approved by the local Ethic's Committee of the University of Tuebingen. All study participants provided informed written consent before inclusion in the study.

Material

Perchloric acid (PCA), butylated hydroxytoluene (BHT), and sodium hydroxide were from Merck (Darmstadt, Germany). Tris(2-carboxyethyl) phosphine hydrochloride (TCEP) was purchased from ABCR (Karlsruhe, Germany). Ascorbic acid, methanol, pure ethanol, butanol, methyl tertiary butyl ether, and acetonitrile were from Roth (Karlsruhe, Germany). 3-Carboxy-PROXYL, thiobarbituric acid, MDA-bis(diethyl acetal) and ethyl beta-apo-8'-carotenoate were purchased from Sigma-Aldrich (Steinheim, Germany). Tocol was purchased from Matreya LLC (Pleasant Gap, PA, USA).

Blood sampling and storage

Venous blood samples were collected in EDTA-tubes 1 d before surgery (baseline), after induction of anesthesia (T0), immediately before CPB (T1), 30 min after the end of CPB (T2), in the evening at the day of surgery (T3), and on each of the six postoperative days (D1–D6).

Immediately after drawing, plasma was separated by centrifugation at 3000 *g* and +4°C for 5 min. Aliquots (200 μL each) of plasma were filled into cryo conservation tubes (prefilled with 200 μL PCA [5%] for vitamin C measurement, with 9 μL BHT [5% in pure ethanol] for MDA determination, and without any further additives for AFR and lipid-soluble vitamin analysis), instantly snap frozen in liquid nitrogen, and stored at −80°C until analysis. Time span between blood withdrawal and snap freezing did not exceed 20 min.

Measurement of vitamin C

Samples were thawed and centrifuged at 13 000 *g* for 5 min at +4°C. Supernatant was split into two fractions for measuring ascorbic acid (AA) and TAA, respectively. Dehydroascorbic acid (DHAA) content of the samples then was calculated by subtracting the concentration of AA from TAA concentration.

For determination of AA, the supernatant was mixed with ultrapure water (v/v:2/1), centrifuged at 13 000 *g* for 5 min at +4°C and transferred into light-protected microvials for chromatographic analysis. For reduction of DHAA and determination of TAA, the same procedure was performed, but dilution of the samples was done with 0.15 M aqueous TCEP solution (v/v:2/1). Calibration was performed using external AA standards dissolved in PCA (2.5%) and treated the same way as TAA samples. All analytical procedures were performed on ice and under dimmed light to avoid AA degradation.

The high-performance liquid chromatography (HPLC) system consisted of a cooled autosampler, a C18 reversed-phase column (Trentec, Gerlingen, Germany), an electrochemical detector (ESA Detektor Coulochem II, Chelmsford, United Kingdom), and a Model 5011 high-sensitivity analytical cell (ESA) at −300 mV (E1, upstream) and +300 mV (E2, downstream), and the mobile phase consisting of 5 mM aqueous sodium phosphate buffer, pH 2.5 at a flow rate of 1 mL/min. Chromatograms were recorded and analyzed using Star chromatography workstation software version 5.31 (Varian, Darmstadt, Germany).

Measurement of AFR by electron paramagnetic resonance spectroscopy

Immediately before analysis, samples were thawed in a water bath at 30°C for 1 min, transferred into a quartz flat cell and measured in a Miniscope MS200 x-band spectrometer. Instrument settings were B(0)-field: 3340 ± 27.5 G, sweep time: 100 sec, modulation: 1 G, microwave frequency: 9.43 GHz, microwave intensity: 10 mW.

Calculation of AFR concentration was performed by using 3-Carboxy-PROXYL as a stable external standard.

Measurement of malondialdehyde

MDA analysis was performed based on the thiobarbituric acid method [23]. Briefly, after thawing, samples were centrifuged at 13 000 *g* and 4°C for 5 min. Supernatant was mixed with phosphoric acid (0.44 M) and thiobarbituric acid solution (42 mM), kept at 95°C in a water bath for 1 h, and then instantly put on ice. Before application to the HPLC system, the mixture was neutralized with an equal amount of NaOH-solution (0.1 M in 90% methanol), centrifuged, and transferred into light-protected vials.

The HPLC system consisted of a cooled autosampler, a C18 reversed-phase column (Trentec, Gerlingen, Germany), a Jasco FP_920 fluorescence detector (Jasco, Gross-Umstadt, Germany) set at 525 nm for excitation and 550 nm for emission, and the mobile phase consisting of 50 mM aqueous potassium phosphate buffer, pH 6.8 mixed with pure methanol (v/v:60/40) at a flow rate of 1 mL/min. Chromatograms were recorded and analyzed as for AA analysis.

For calibration, a standard curve was performed with each run using different concentrations of a MDA-bis(diethyl acetal) solution treated the same way as the samples.

Measurement of lipid-soluble vitamins

Samples were thawed and lipid-soluble vitamins were extracted by mixing with the fivefold amount of extraction solution (50% ethanol, 50% butanol, containing 0.5% BHT, 8 μM tocol, 0.1% ethyl beta-apo-8'-carotenoate, the last two serving as internal standards). Mixture was centrifuged at 13 000 *g* and 4°C for 5 min and supernatant was transferred into light-protected vials for HPLC analysis.

The HPLC system consisted of a cooled autosampler, a Spherisorb column (Trentec, Gerlingen, Germany), a Waters 474 fluorescence detector for detection of α-tocopherol and tocol set at 298 nm for excitation and 328 nm for emission, a Waters 2487 dual-absorbance UV detector (Waters, Arcade, NY, USA) for detection of β-carotene and ethyl beta-apo-8'-carotenoate set at changing wavelengths (0–3 min: 325 nm, 3–11 min: 450 nm, then again 325 nm) and the mobile phase consisting of a mixture of acetonitrile, methyl tertiary butyl ether, and methanol (v/v/v:87/10/3) at a flow rate of 1.6 mL/min. Chromatograms were recorded and analyzed using galaxy chromatography workstation software version 1.9.3.2 (Varian, Darmstadt, Germany).

Concentrations were calculated using a National Institute of Standards and Technology (NIST)-calibrated plasma pool and corrected for loss due to extraction with internal standards. Furthermore, α-tocopherol and β-carotene plasma levels were corrected to plasma cholesterol levels determined with an Olympus AT200 lab analyzer.