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Pre-operative delivery of a vitamin cocktail diminished oxidative stress after vascular surgery in PAD patients – a pilot investigation

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1. Introduction

Atherosclerosis is a generalized disease and becomes apparent among others as peripheral arterial disease (PAD). Although an array of protective enzymes (superoxide dismutase, catalase, glutathione peroxidase) as well as non-enzymatic antioxidants (e.g. glutathione, ascorbate, tocopherol) protect against the attack by reactive oxygen species (ROS), it has been reported previously that reperfusion is associated with augmented oxidative stress in the course of surgery, which might be counter-balanced through the application of a vitamin infusion [3].

In this pilot-investigation we examined the protective effect of an intravenous antioxidant supplementation just before reperfusion in the course of vascular surgery, which consisted of a mixture of ascorbate (500 mg) and a-tocopherol (45 mg). We applied several biochemical parameters to monitor the antioxidative potential and oxidative stress in these PAD subjects. We used a fluorimetric method for the specific determination of non-enzymatic water- and lipid-soluble antioxidants. The endogenous antioxidative capacity was measured by the serum total peroxidase-activity. Serum total peroxides and autoantibodies against oxidized LDL were applied as oxidative stress parameters to assess the protective and beneficial effects of this vitamin cocktail.

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2. Materials and methods

2.1. Subjects and samples

Seven patients undergoing vascular surgery (thrombo-endarteriectomy and bypass) were enrolled in this non-randomized pilot-investigation i.e. 4 patients without antioxidant supplementation hereafter referred to as control group (CG) and 3 patients with additional antioxidant supplementation (Mel-C® & Tocovenoes®; Fresenius-Kabi, Graz, Austria), hereafter referred to as vitamin group (VG).

Blood samples were taken at several time-points i.e. baseline (No. 1) at 30 minutes (No. 2), 60 minutes (No. 3), 120 minutes (No. 4), 24 hours (No.5) and 168 hours (No. 6) after reperfusion. Blood was centrifuged approximately 30 minutes after collection and serum samples were stored at $-70\,^{\circ}$C until use.

Vitamin cocktail: 1 ampoule Mel-C® (0.562 g sodium ascorbate) and 3 ampoules Tocovenoes ® (15 mg all-rac-a-tocopherolacetate) were injected into 500 ml Sodium Chloride (0.9%) and applied just before reperfusion within 30 minutes.

2.2. Lag-time measurement for water- and lipid-soluble antioxidants

The lag-time of *ex vivo* degradation of the fluorophores 1,6-diphenylhexatriene propionic acid (DPHPA) and 1-palmitoyl-2-((2-(4-(6-phenyl-trans-1,3,5-hexatrienyl)phenyl)ethyl)-carbonyl)-sn-glycero-3-phosphocholine (DPHPC), by ROS in serum was determined with fluorescent diagnostic assays (Protein-Ox® & Lipid-Ox®, Tatzber KEG, Austria) according to Mayer et al. [2] and Hofer et al. [1], with modifications to the oxidation part. Briefly, 100 μL serum were incubated with 2 nMol DPHPA or DPHPC and kept under argon at 37°C for 1 and 12 hours, respectively. Oxidation was started via a peroxide (0.004%) – peroxidase (10 U/ml) reaction and the time-dependent decrease in fluorescence intensity at 430 nm (excitation at 360 nm) was monitored on a FluoStar fluorometer (BMG Lab-Technologies; D-77656 Offenburg, Germany). Results were expressed as lag-time in minutes.

2.3. Serum total peroxidase-activity

Peroxidase activity in serum was determined by the reaction of endogenous peroxidases with hydrogen peroxide, using 3,5,3',5'-tetramethylbenzidine (TMB) as the chromogenic substrate (ARS®, Tatzber KEG, Klosterneuburg, Austria) as previously described [5]. Serum peroxidase-activity was calculated as the difference in the absorbance readings related to the horse-radish peroxidase standard curve. Results were expressed as milliunits per millilitre.

2.4. Serum total peroxides

Serum total peroxide concentrations were determined with a rapid enzymatic *in vitro* diagnostic assay (POX-Act®, Tatzber KEG, Klosterneuburg, Austria) as previously described [5]. The test system was based on a peroxide/peroxidase reaction using TMB as substrate. Peroxide levels were specified as "arbitrary units", because of the different contributions of various peroxides to the reaction.

2.5. Determination of autoantibodies against oxidized LDL (*oLAb*)

Titers of autoantibodies against oxidized LDL were measured in serum with a commercial enzyme immunoassay (oLAb, Tatzber KEG Klosterneuburg, Austria) according to the method of Tatzber and Esterbauer [6]. The assay is based on the binding reaction of the 1:50 diluted samples to the previously oxidized LDL (by cupric ions) bound to the microtiter wells. Detection was accomplished by binding a secondary, peroxidase-coupled anti-IgG antibody, which permitted colorimetric detection of this enzyme with tetramethylbenzidine as substrate. Results were expressed as mU/mL.

3. Results

Reperfusion-induced oxidative stress was accompanied by the consumption of water- and lipid-soluble antioxidants to about 50% of baseline levels in the CG, whereas the reduction was only marginal (less than 20%) in the VG (details are listed in Table 1). In the CG we observed a several-fold increase in endogenous peroxidase-activity at time-point No.2 in contrast to the VG, in which the endogenous peroxidase-activity was more or less unchanged. In both groups, total peroxide concentrations were reduced pronouncedly at time-point No. 2. In the VG, we observed a continuous increase in peroxide concentrations, which achieved baseline levels at time-point No. 6 whereas the peroxide level in the CG was overshooting up to 170% compared to baseline levels at this time-point. The antibody titer against oxidized LDL was decreased in both groups after reperfusion. Nevertheless, the oLAb titer in the VG already attained baseline titres at time-point No. 5, whereas the antibody titer in the CG remained depressed during the whole observation period.

4. Discussion

In this pilot-investigation we examined the effects of a combination of ascorbate and a-tocopherol, which represent the most prominent water- and lipid-soluble antioxidants, in PAD patients in the course of vascular surgery.

Reperfusion-induced oxidative stress was accompanied by the consumption of water- and lipid-soluble antioxidants, as indicated by markedly reduced lag-times in the CG as compared to the VG, who were better protected due to the antioxidant supplementation just before reperfusion. As a counterbalance to this deficiency, we observed a several-fold increase in endogenous peroxidase-activity in the CG, whereas the increase in the VG was only marginal, more or less to the same extent as antioxidants were consumed.

The reduction of peroxide concentrations after reperfusion indicates most probably an increase of uric acid. In the VG we observed baseline peroxide concentrations at time-point No. 6, whereas overshooting peroxide concentrations occurred in the CG, exceeding the baseline concentrations up to 170%.

Oxidative stress was further detected with respect to decreased oLAb titres, which were bound to epitopes, generated in the course of reperfusion induced radical attack as it was also shown in patients suffering from myocardial infarction [4]. In case of the VG we observed a continuous increase of these antibodies, which attained baseline levels at time-point No. 5, whereas the oLAb titer in the CG remained depressed during the whole observation period.

These results are in accordance with a previous report [7] that also indicated a beneficial effect of a multi-antioxidant supplementation after reperfusion.

Table 1
Summary of antioxidant and oxidative stress parameters. Data are presented as mean values ± standard deviation

	Standard Therapy ($n = 4$)						Standard Therapy with additional antioxidant supplementation ($n = 3$)					
Time-point	No.1	No.2	No.3	No.4	No.5	No.6	No.1	No.2	No.3	No.4	No.5	No.6
Protein-Ox (min)	40 ± 9	23 ± 8	28 ± 12	30 ± 9	42 ± 12	42 ± 9	48 ± 5	41 ± 4	42 ± 6	42 ± 7	45 ± 10	41 ± 15
Lipid-Ox (min)	44 ± 8	26 ± 8	32 ± 8	33 ± 8	47 ± 9	47 ± 8	52 ± 5	49 ± 6	51 ± 3	49 ± 2	50 ± 11	45 ± 14
ARS (mU/mL)	2 ± 1	8 ± 2	3 ± 1	3 ± 1	3 ± 1	5 ± 5	7 ± 2	10 ± 7	8 ± 6	10 ± 2	8 ± 0	8 ± 4
POX-Act (AU)	145 ± 141	47 ± 56	56 ± 66	48 ± 56	119 ± 118	246 ± 181	238 ± 231	95 ± 153	130 ± 193	158 ± 220	206 ± 259	249 ± 112
oLAb (mU/mL	275 ± 81	189 ± 43	191 ± 36	204 ± 52	226 ± 63	213 ± 48	307 ± 179	239 ± 152	251 ± 171	272 ± 186	318 ± 220	304 ± 179

5. Summary

These preliminary data indicated that the application of an ascorbate-a-tocopherol cocktail improved the antioxidant potential and diminished oxidative stress in PAD patients undergoing vascular surgery. Nevertheless, this beneficial effect needs to be corroborated in a large-scale study including clinical parameters.

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