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Short-term Parenteral Application of α-Tocopherol Leads to Increased Concentration in Plasma and Tissues of the Rat

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Numerous studies suggest that supplemental vitamin E prior to or during vast surgeries might diminish or even prevent ischemia/reperfusion-induced injuries. In the present placebo-controlled study male Sprague-Dawley rats were supplemented parenterally or orally with α-tocopherol for three consecutive days. The applied amount of α-tocopherol was 2.3 μmol per day for oral and 1.2 μmol per day for parenteral supplementation. The enrichment of vitamin E concentrations in plasma and tissue samples (aortic endothelium, liver, and lung) was determined by HPLC. The vitamin E level was elevated following intravenous supplementation in plasma (21.4 ± 1.9 μmol/L vs. 10.2 ± 1.7 μmol/L in parenteral control group), in aortic endothelium (1.1 ± 0.2 pmol/mm^2 vs. 0.5 ± 0.1 pmol/mm^2) and in liver and lung (41.3 ± 7.5 pmol/mg vs. 22.9 ± 6.5 pmol/mg and 75.6 ± 13.6 pmol/mg vs. 51.7 ± 5.9 pmol/mg, respectively). Oral supplementation for three days also led to an increased level in liver (38.2 ± 7.7 pmol/mg vs. 22.9 ± 6.6 pmol/mg in oral control group) and in lung (67.8 ± 5.7 pmol/mg vs. 51.7 ± 9.3 pmol/mg) but not in aortic endothelium or plasma (0.8 ± 0.3 pmol/mm^2 vs. 0.6 ± 0.3 pmol/mm^2 and 12.0 ± 2.2 μmol/L vs. 10.7 ± 2.6 μmol/L).

Keywords: Vitamin E, α-tocopherol, infusion, parenteral, oral, oxidative stress

INTRODUCTION

Oxidative stress is believed to be involved in acute and/or chronic postoperative complications, such as lung distress syndrome, inadequate wound healing, sepsis, and multiple organ failure.[1,2] Oxidative stress is defined as an imbalance between the extent of reactive oxygen species and a comparable low antioxidative capacity of a biological system.[3] A major component of this antioxidative network is vitamin E, the most important lipid-soluble antioxidant. It helps to prevent membrane-destabilizing lipid peroxidation by breaking lipid radical chain reactions.[4–6] Increased lipid peroxidation occurs during ischemia/reperfusion due to increased formation of superoxide anion and consecutive production of hydroxyl radicals due to iron-catalyzed Fenton reaction. Sources of superoxide anion formation during ischemia/reperfusion are conversion of xanthine dehydrogenase to xanthine oxidase[7]

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and activation of polymorphonuclear cells.[8] Combined oral supplementation with α-tocopherol and ascorbic acid – the water-soluble counterpart of vitamin E – prevented ischemia/reperfusion-induced damage in experimental studies.[9,10]

Indeed, primary prevention during ischemia/reperfusion injury might be achieved by using oral supplementation. Yet, this approach is apparently not practicable during acute episodes of oxidative stress. In these situations parenteral provision of vitamin E might be a more appropriate approach. The availability of vitamin E for endothelial cells depends on the concentration of the vitamin in VLDL and LDL.[11,12] The uptake into the cells occurs *via* lipoprotein lipase activity or *via* receptor-mediated endocytosis.[13] The question thus should be raised whether and to what extent vitamin E is taken up into main target cells when the normal absorption route is bypassed by parenteral supply.

The present experimental investigation aims to assess the degree of α-tocopherol enrichment in plasma, aortic endothelium, liver and lung tissues following parenteral supply of this vitamin compared to oral application.

MATERIALS AND METHODS

Animals and Experiment

Twenty-four male Sprague-Dawley rats (Interfauna, Tuttlingen, Germany) weighing about 180 g were used. The animals were housed in a temperature-controlled room with a 12 h light–dark cycle. Four days prior to the experiment they were accommodated individually in a metabolic cage and received standard laboratory diet (Altromin, Altromin International, Lage, Germany) and water *ad libitum*. The animals were allocated into four groups of six each: (I) parenteral application of the vitamin E-containing solution, (II) parenteral application of the placebo solution, (III) oral application of the vitamin E-containing solution, and (IV) oral application of the placebo solution. The placebo solution (Elolipid 10%) consists of 10 g soybean oil, 1.2 g egg lecithin, 2.5 g glycerol, 30 mg oleic acid and 3 mg NaOH per 100 mL. The vitamin E content of this solution was 4.4 μmol/L. The same solution was used for the vitamin E-containing solution with all-rac-α-tocopherol added to a final concentration of 12.5 mmol/L (i.e. 9.3 mmol/L RRR-α-tocopherol equivalent). Both solutions were kindly provided by Fresenius Pharma Austria GmbH, Graz, Austria, and used for both, parenteral and oral supplementation study.

Intravenous α-tocopherol was infused by using a central venous catheter.[14] In brief, the rats were anesthetized intraperitoneally with 1 mL/kg Narketan 10% (Chassot, Ravensburg, Germany) and 0.8 mL/kg Xylazin 2% (Medistar, Holzwickede, Germany). Under anesthesia the right jugular vein was cannulated with a small sterile silicone tube. The cannula was passed subcutanously to a small incision in the midscapular region and further passed through a flexible stainless steel spring which was connected to a leather cuirass. The cannula was fixed at the end of the spring. Cannula and spring were mounted on a swivel apparatus fixed to the top of the metabolic cage to ensure free mobility of the rats. Throughout the experiments, the rats were given free access to standard diet and water.

The animals of group I and II received parenteral supply of either vitamin E or placebo with an infusion pump (Braun, Melsungen, Germany). The α-tocopherol-containing infusion was diluted with isotonic saline to achieve a concentration of 0.58 mmol/L α-tocopherol equivalent. Two milliliters (1.16 μmol) were infused over 1 h on three consecutive days. The placebo infusion was diluted and infused in the same manner. On the third day, 3 h after the last administration the animals were killed in anesthesia by exsanguination from the abdominal aorta and EDTA plasma was obtained. Tissue samples from liver and lung and the aorta were harvested, washed in isotonic saline and immediately frozen in liquid

nitrogen. The specimens were stored at −80°C until analyzed.

For oral application the α-tocopherol solution was diluted with saline to a concentration of 2.32 mmol/L α-tocopherol equivalent and the animals were ingested 1 mL (2.32 μmol) with an esophageal tube on three consecutive days (group III). The placebo solution was diluted and supplied in the same manner (group IV). Throughout the experiment, the rats were given free access to standard diet and water. Twenty-four hours after the last supply the rats were killed in anesthesia by exsanguination from abdominal aorta and plasma and tissue samples were collected (cf. above). Since it is known that α-tocopherol absorption varies between 57–69%,[13,15] double the amount of vitamin E was supplied orally compared to the parenteral supply in group I. Orally administered vitamin E has to be incorporated into VLDL in the liver before being accessible for tissues, therefore this longer period between last administration of vitamin E and collection of the samples was chosen.[16]

The study was conducted in accordance with the National Research Council's guide for the care and use of laboratory animals.

Analysis

Plasma and tissue concentrations of α-tocopherol were analyzed using a modification of a previously described high-performance liquid chromatography (HPLC) method.[17] In brief, plasma samples (200 μL) were mixed with 200 μL isopropanol for precipitation of the proteins and extracted twice with each 500 μL n-hexane. The hexane extracts were dried under a continuous stream of nitrogen and redissolved in 200 μL of n-hexane. Tissue samples (approx. 200 mg wet weight) were weighed exactly and homogenized in 50 mmol/L Tris buffer pH 7.4 containing 1.15% KCl, 1.0 mM ethylenediamine tetraacetate, disodium salt, and 0.02 mM butylated hydroxytoluene. The homogenates were extracted as described for the plasma samples. For determination of vitamin E in the aortic endothelium the aorta was cut lengthwise and washed thoroughly with isotonic saline. The endothelial cells were harvested by scraping the inner surface of the aorta and the scraped endothelium was collected in 200 μL isotonic saline containing 1 mg/mL collagenase (Boehringer Mannheim, Mannheim, Germany). After incubation for 5 min at 37°C the cells were extracted as described for the plasma samples. We used the area of the scraped aorta as reference for the measured tocopherol content in the probe. Preliminary investigations have shown that determination of protein or DNA in such small amount of cell material resulted in a relatively high bias. Since the membrane of the endothelial cells is the target of vitamin E the tocopherol level in the endothelium is given as pmol/mm^2.

α-Tocopherol was then separated on a 250 × 4 mm LiChrosphere CN column (E. Merck, Darmstadt, Germany) using n-hexane as eluent and detected with UV (293 nm) and fluorescence (exc. 292 nm, em. 320 nm). The limit of detection was 0.5 μmol/L and 40 pmol/L using UV and fluorescence detection, respectively. The coefficient of variance for intra- and interassay precision was less than 5%.

Statistics

All results are expressed as mean ± SD. Data were analyzed with Prism 2.0 (GraphPad, Inc.) software. Student's unpaired t-test was used to assess statistical differences. Results with $p < 0.05$ were considered significant.

RESULTS

Three days parenteral provision of α-tocopherol (1.16 μmol/day) leads to significant increase in aortic endothelial cell-associated vitamin E level being twice of that in the placebo-treated animals (1.1 ± 0.2 vs. 0.5 ± 0.1 pmol/mm^2; $p < 0.05$). In contrast, daily oral administration of 2.32 μmol

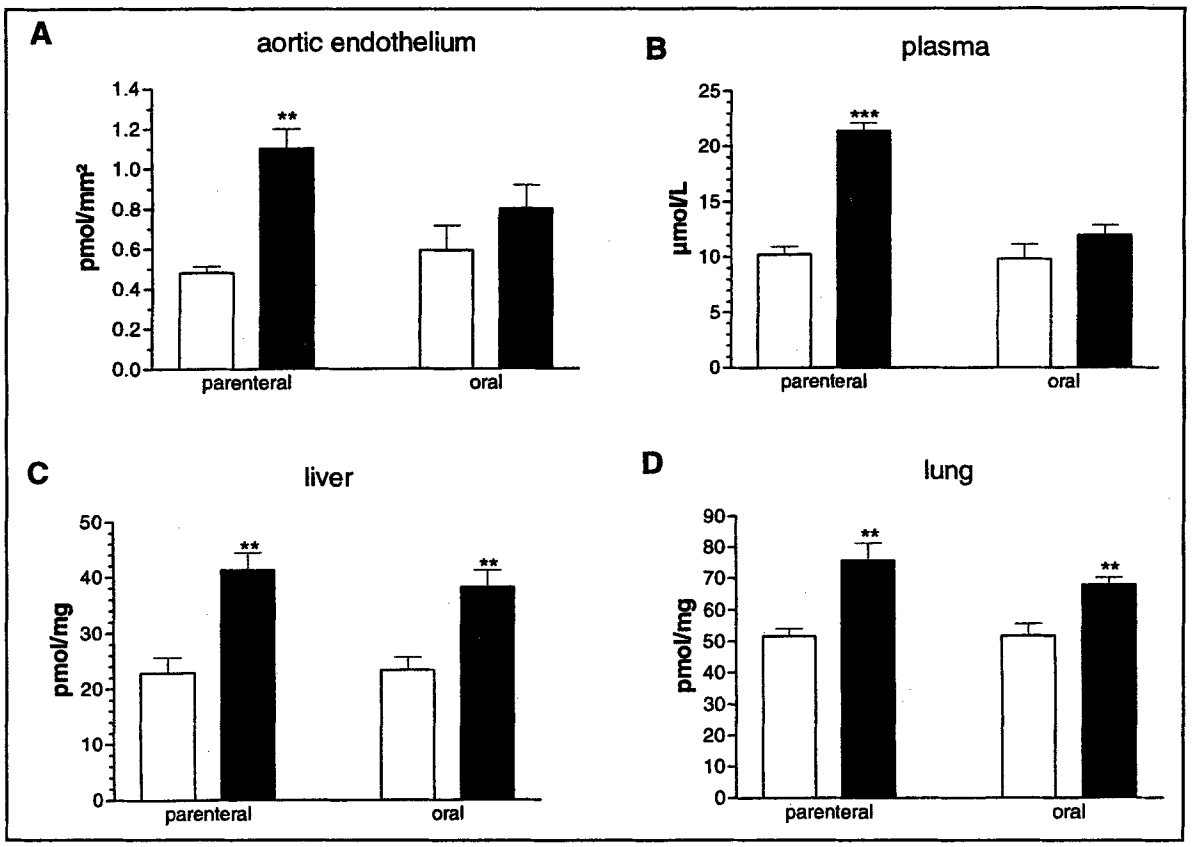

FIGURE 1 α-Tocopherol levels in aortic endothelium (A), plasma (B), liver (C), and lung (D) after parenteral and oral supplementation with α-tocopherol for three days (1.2 and 2.3 µmol/day respectively). Placebo, open bars; vitamin E, solid bars. The statistically significant difference between vitamin E levels in the groups is marked with ** $p \leq 0.01$, *** $p < 0.001$.

vitamin E was not associated with enrichment in the aortic endothelium in comparison to the placebo group (Figure 1A). Plasma vitamin E concentration was increased considerably after parenteral supplementation compared to that in the placebo group (21.4 ± 1.9 vs. 10.2 ± 1.7 µmol/L; $p < 0.001$). Oral supplementation of vitamin E leads to a 12% increase in plasma concentration compared to placebo (12.0 ± 2.2 vs. 10.7 ± 2.6 µmol/L) (Figure 1B). Yet, it did not reach significance level ($p = 0.1$). The α-tocopherol levels in liver and lung tissues were markedly elevated after parenteral supplementation compared to those observed with placebo (41.3 ± 7.5 vs. 22.9 ± 6.6 pmol/mg; $p < 0.005$ and 75.6 ± 13.6 vs. 51.7 ± 5.9; $p < 0.005$, respectively) as shown in Figure 1C and D. The enrichment in these tissues was similar after oral ingestion; liver 38.2 ± 7.7 pmol/mg vs. 22.9 ± 6.6 pmol/mg ($p < 0.005$) and lung 51.7 ± 9.3 pmol/mg vs. 67.8 ± 5.7 pmol/mg ($p \leq 0.005$).

DISCUSSION

In the present study we show that parenteral provision of vitamin E is a rapid and efficient approach to enrich endothelial cells with this antioxidative vitamin. The striking difference in endothelial cell-associated vitamin E level following oral or parenteral administration might be due to the distinct differences of vitamin E delivery to the cells. Subsequent to oral uptake and intestinal absorption of vitamin E it is incorporated into

chylomicrons and is mainly transported to the liver. From there it is released *via* VLDL and finally reaches cells and tissues after receptor-mediated uptake of LDL. The relatively low increase in plasma vitamin E concentration (12%) after oral intake in this study might be explained by the liver-controlled pathway of vitamin E distribution following oral intake. Even in studies with high dose supplementation over a long period in man (400–1600 IU/day, 21 days) plasma concentration rose only 60–230%.[18] The significant increase in liver and lung α-tocopherol levels after oral ingestion, however, indicates that α-tocopherol was absorbed, incorporated into lipoproteins, and transported to the peripheral tissues. The aortic endothelium might not be a primary target for vitamin E delivered in LDL. The liver as the major control organ and the lung with its permanent oxidative stress may be more important targets.

In contrast, parenteral vitamin E might be more or less directly available for endothelial cells. Although we cannot exclude completely that the vitamin E measured in the endothelium was adhered in part and not completely incorporated into the cell, this possibility seemed not to be likely. Incubation of a human endothelial cell line *in vitro* with media enriched with the α-tocopherol-containing infusion in different concentrations resulted in a time- and dose-dependent enrichment of the cells between 0 and 24 h (data not shown). Chan and Tran[19] also showed a linear time and dose-dependent enrichment of endothelial cells with α-tocopherol out of culture media within 8 h. This time course makes a remarkable adherence of vitamin E at the cell surface less likely, since a rapid early increase and not a time- and dose-dependent pattern would have been expected. Once in close contact with the cell surface vitamin E will either leave the polar surface of the membrane or penetrate into the lipophilic compartments of the cell. This notion is supported by a current investigation showing that intravenously administered emulsion droplets were immediately available for removal from the circulation.[20] Less than 40% of labelled emulsion droplets were found in blood and liver 20 min after intravenous administration, indicating that most of the droplets must have located in extrahepatic tissues.[21] Indeed, this route is strikingly different from the usual chylomicron metabolism. In chylomicrons the rate of disappearance for core lipids has been found to be very slow initially.[20] We propose that only a minor part of vitamin E is available for aortic endothelial cells following oral intake and during subsequent transport in chylomicrons. The extent of increase in the level of vitamin E in liver and lung following either enteral or parenteral nutrition was similar. This might be explained by the predominating LDL receptor-mediated pathway following hepatic transfer of the vitamin.

The endothelium is one of the primary targets of oxidative stress during ischemia/reperfusion. Consequently, a high content of vitamin E in the endothelium might help to prevent reperfusion-induced lipid peroxidation and subsequent membrane damage. Keaney *et al.*[22] have demonstrated that the content of vitamin E in blood vessels is correlated with vessel resistance to endothelial dysfunction after incubation with oxidized LDL in hypercholesterolemic rats.

At present parenteral vitamin E preparations including vitamin E in small amounts are not available for clinical practice, due to problems of solubilisation. Therefore, only a few studies are available concerning the effects of parenteral antioxidants during surgery. In patients undergoing kidney transplantation the transient increase of lipid peroxidation in plasma after 1 h of reperfusion could be abolished with an intravenous infusion of a multi-vitamin mixture containing antioxidants including vitamin E.[23] Similar prevention of lipid peroxidation after intravenous antioxidative therapy have been shown in patients undergoing limb revascularization.[24]

Our study shows that short-term parenteral supplementation with α-tocopherol leads to its enrichment in plasma and subsequently in endothelium and organ tissues. We propose that the

predominant enrichment of the endothelium is due to direct transfer of the vitamin from the infusion to the endothelial cells. Therefore, parenteral infusion of α-tocopherol during episodes of acute oxidative stress might serve as suitable tool for short-term enrichment of endothelial cells and tissues. Whether this is of clinical significance in ischemia/reperfusion-induced oxidative stress is currently under investigation. In addition it should be elucidated whether short-term application (e.g. prior to surgery) would be an efficient and consequently a preventive approach.

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