Graphical Review

Regulatory metabolites of vitamin E and their putative relevance for atherogenesis

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\begin{abstract}
Vitamin E is likely the most important antioxidant in the human diet and \alpha-tocopherol is the most active isomer. \alpha-Tocopherol exhibits anti-oxidative capacity \textit{in vitro}, and inhibits oxidation of LDL. Beside this, \alpha-tocopherol shows anti-inflammatory activity and modulates expression of proteins involved in uptake, transport and degradation of tocopherols, as well as the uptake, storage and export of lipids such as cholesterol. Despite promising anti-atherogenic features \textit{in vitro}, vitamin E failed to be atheroprotective in clinical trials in humans. Recent studies highlight the importance of long-chain metabolites of \alpha-tocopherol, which are formed as catabolic intermediate products in the liver and occur in human plasma. These metabolites modulate inflammatory processes and macrophage foam cell formation via mechanisms different than that of their metabolic precursor \alpha-tocopherol and at lower concentrations. Here we summarize the controversial role of vitamin E as a preventive agent against atherosclerosis and point the attention to recent findings that highlight a role of these long-chain metabolites of vitamin E as a proposed new class of regulatory metabolites. We speculate that the metabolites contribute to physiological as well as pathophysiological processes.
\end{abstract}

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of the arterial wall was due to ‘excessive nourishment’ from the blood. Many decades later da Vinci’s observation was studied in more detail by Carl von Rokitansky (1852) and Rudolf L. K. Virchow (1821–1902). In 1856 Virchow proposed that injury of the endothelium may initiate the disease process of atherosclerosis. Based on this idea, Russell Ross (1929–1999) and John A. Glomset came up in 1973 with the ‘response-to-injury’ hypothesis which is still generally accepted today in the form of the more generalized concept of endothelial dysfunction as the initial cause of atherosclerosis.

The pioneering work of Virchow and Nikolai N. Anitschkow (1885–1964) provided first evidence for the importance of the deposition of lipids from the blood, in particular cholesterol, in the arterial wall. Their findings formed the basis for the lipid hypothesis which connects plasma cholesterol levels to the development of the disease. In 1951, G. Lyman Duff (1904–1956) and Gardner C. McMillan (1918–2004) formulated the lipid hypothesis in its modern form, which is, despite controversial discussions, still widely accepted today. Since the discovery of the importance of the cholesterol contained in low-density lipoprotein (LDL) particles for the pathogenesis of atherosclerosis, the concept of endothelial dysfunction has become tightly linked to the lipid hypothesis.

Almost 30 years ago the concept originated from work by Daniel Steinberg and Joseph L. Witzum that oxidative stress and the oxidation of LDL particles might contribute to atherosclerosis. The idea came up from the observation that the incubation of macrophages with oxidized LDL (oxLDL) but not with native LDL led to the intracellular accumulation of cholesterol esters. The idea that oxidative stress is involved in atherogenesis gained much attention and created tremendous excitement to look for oxLDL in vivo as well as for different kinds of oxidized lipid species within the particle. Since oxLDL appears in human plasma as well as within the arterial wall it was even a small step to the idea that supplementation with antioxidants may prevent atherosclerosis by inhibiting the formation of oxLDL. This hypothesis appeared to be on solid ground due to epidemiological evidence and the success in several animal studies using a variety of antioxidants. The euphoria of initial success led to clinical trials to validate the hypothesis and natural antioxidants were of particular interest as the expectation was that these natural compounds would have less undesirable effects. Accordingly a number of clinical trials were performed using, for example, vitamin E, which surprisingly have not been overwhelmingly supportive of the hypothesis. An overview on the controversial findings for vitamin E obtained from clinical trials is given in Fig. 1.

In this review, we want to summarize the controversial role of vitamin E as a preventive agent against atherosclerosis and to point the attention to recent findings by our group that highlight a role of long-chain metabolites of vitamin E as a proposed new class of regulatory metabolites and to their potential contribution to atherosclerotic processes.

### Pathogenesis of atherosclerosis

The endothelium covering the arterial walls comprises a physiological and selective barrier, the so-called intima, between blood and the inner layer of the arterial wall. This so-called media is comprised by contractile smooth muscle cells. Pathophysiological stimuli cause endothelial dysfunction triggering inflammatory processes in the vascular wall which result under chronic conditions in extensive morphological changes characterized by intimal thickening, deposition of cholesterol and fibrotic material, loss of elasticity, reduction of vascular lumen, and widening of the vessel diameter [1]. Endothelial dysfunction is thought to be caused by exogenous stimuli, such as environmental factors (e.g., toxicants such as dioxins, PCBs, and pesticides), unhealthy lifestyle (e.g., smoking and physical inactivity) and dietary habits (e.g., high intake of saturated fat). The impact of exogenous factors depends on endogenous local and systemic conditions. Local factors are vessel-associated junctions, bifurcations and curvatures which are responsible for increased shear stress caused by turbulences of the blood stream in these areas, which are thus predestinated for the formation of atherosclerotic lesions [2]. Proatherogenic systemic factors are determined either genetically or pathophysiologically, for example, in case of increased LDL and triglyceride plasma levels [3,4] as well as inflammatory conditions [5]. The process of atherosclerosis is outlined and explained in more detail in Fig. 2.

A key event of atherogenesis is the loss of the selective endothelial barrier by endothelial dysfunction which allows, for example, LDL to enter the arterial wall. Once inside the vessel wall, LDL particles become prone to oxidation. The oxidized particles cause damage to the tissue thus triggering a cascade of immune and inflammatory responses. In addition, macrophages, the phagocytic cells of the immune system, are recruited to the affected tissue sites to clear the oxLDL particles. As a consequence oxidized lipids and particularly cholesterol accumulate within the macrophages as these cells are not able to process the oxLDL completely. This causes transformation of the cells into so-called foam cells and ultimately cell death as the excessive accumulation of intracellular lipids is cytotoxic. Death of macrophage foam cells results over time in the extracellular deposition of cholesterol in the arterial wall and the formation of an atheroma. The process of atherosclerosis is outlined and explained in more detail in Fig. 2.

Thus, vitamin E was considered as an anti-atherogenic agent for a long time as prevention of LDL oxidation by providing increased levels of antioxidants would prevent the formation of macrophage foam cells and atheroma, and would dampen the immune and inflammatory response.

### Effects of α-Tocopherol on atherogenic processes

Vitamin E is likely the most important lipid antioxidant in the human diet. The term vitamin E comprises a group of eight abundant isomers (α-, β-, γ-, δ-tocopherol and tocotrienol), that differ by their methylation patterns of the hydroxycromanol ring and saturation of the side-chain. Many in vitro studies have been performed with

| Nomenclature |  |
|--------------|-------------------------|
| α-13′-OH     | α-13′-(6-hydroxy-2,5,7,8-tetramethylchroman-2-y1)-2,6,10-trimethyl-tridecanol |
| α-13′-COOH   | α-13′-(6-hydroxy-2,5,7,8-tetramethylchroman-2-y1)-2,6,10-trimethyl-tridecanoic acid |
| α-CEHC       | α-carboxyethyl-hydroxychroman |
| α-LCM        | α-tocopherol long-chain metabolites |
| α-SCM        | α-tocopherol short-chain metabolites |
| α-TOH        | α-tocopherol |
| COX          | cyclooxygenase |
| CYP3A4       | cytochrome P450, subfamily IIIA, polypeptide 4 |
| CYP4F2       | cytochrome P450, subfamily IVF, polypeptide 2 |
| LDL          | low density lipoprotein |
| oxLDL        | oxidized low density lipoprotein |
α-tocopherol (α-TOH) is the most active isomer within the group of vitamin E [6]. α-Tocopherol exhibits anti-oxidative capacity in vitro [7], and it has been shown to particularly inhibit, for example, the oxidation of LDL. Besides this, α-TOH shows anti-inflammatory features by, for example, inhibiting cyclooxygenase (COX) 2. Next to its anti-inflammatory and anti-oxidative properties, the vitamin E

α-TOH, α-tocopherol.

COX, cyclooxygenase.
isomers may have a variety of further independent properties, namely the modulation of gene expression, particularly that of genes encoding proteins involved in signaling but also the uptake, transport and degradation of tocopherols, as well as the uptake of lipoproteins and the storage and export of lipids such as cholesterol. The \( \textit{in vitro} \) and \textit{ex vivo} effects of \( \alpha \)-tocopherol on cellular processes are depicted in Fig. 3.

\textbf{\( \alpha \)-Tocopherol metabolites and their bioactivity}

Metabolic degradation of \( \alpha \)-TOH takes place almost exclusively in the liver. Beside metabolites resulting from oxidation of the chroman moiety, hepatic metabolism of \( \alpha \)-TOH involves CYP3A4-dependent \( \alpha \)-hydroxylation and \( \alpha \)-oxidation, which results in the formation of \( \alpha \)-tocopherol long-chain metabolites (\( \alpha \)-LCM) \( \alpha \)-13-OH\( \) (13’-\( \alpha \)-hydroxy-2,5,7,8-tetramethylchroman-2-yl), 2,6,10-trimethyl-tridecanol) and \( \alpha \)-13’-COOH\( \) (13’-\( \alpha \)-hydroxy-2,5,7,8-tetramethylchroman-2-yl), 2,6,10-trimethyl-tridecanoic acid), and further steps of \( \beta \)-oxidation, which results in the formation of middle- and short-chain metabolites (\( \alpha \)-SCM)10 with the catabolic end-product \( \alpha \)-carboxyethyl-hydroxychroman (\( \alpha \)-CEHC11), respectively [8–10]. The short-chain metabolites are excreted via urine and are often used as a marker for \( \alpha \)-TOH supply [11]. Other tocopherols, such as \( \gamma \)- and \( \delta \)-tocopherol, are almost quantitatively degraded and excreted via the urine as the corresponding \( \gamma \)- and \( \delta \)-CEHCs. The hepatic metabolism of \( \alpha \)-TOH is illustrated in Fig. 4.

Regulatory activity is not restricted to \( \alpha \)-TOH as its short-chain metabolite \( \alpha \)-CEHC also exhibits bioactivity. It has been shown that \( \alpha \)-CEHC is anti-proliferative [12], anti-inflammatory [13], and antioxidative [14], and inhibits oxLDL formation [15] and protein kinase C (PKC)12 signaling [16]. Recently, researchers focused also on investigating the cellular effects of the \( \alpha \)-LCM as \( \alpha \)-13’-COOH was detected in human serum, a finding providing clear evidence for its systemic bioavailability. Until now, only a few cellular effects of the \( \alpha \)-LCM have been described, such as pro-apoptotic, anti-proliferative and anti-inflammatory features [17–20], which are highlighted in Fig. 4.
A recent study by our group showed that α-LCM also affect macrophage foam cell formation by regulating uptake of oxLDL by macrophages via down-regulation of its phagocytic uptake (Fig. 4) [17]. A key finding of our study was that bioactivity of the α-LCMs occurs at much lower concentrations and with mechanisms distinct from those of their metabolic precursor α-TOH.

Perspective
The findings obtained from clinical trials with humans raise the question whether vitamin E in vivo exhibits modes of action different from those found in vitro. Recent studies shed new light on mechanistic aspects of α-TOH function, which appear to be complicated by...
Although hepatic metabolism of α-tocopherol quinone results from oxidation of the chroman moiety. The major oxidation product in the liver was described as α-tocopherol radical when α-T0H has exerted its antioxidant activity. The Simon metabolites were therefore considered as urinary indicators that α-T0H had reacted as an antioxidant [11]. Today some researcher raise the question whether Simon metabolites are artefacts produced during sample preparation as α-CEHC is easily converted to α-tocopheronolactone by oxygenation [10,12]. α-Tocopherol is physiologically catabolized in the liver via the xenobiotic detoxification system involving CYP3A4 [8] and CYP4F2 [133]. Cytochrome-dependent α-hydroxylation results in the formation of the long-chain alcohol 13-OH, 13-(6-hydroxy-2,5,7,8-tetramethylchroman-2-yl)-2,6,10-trimethyl-tricarboxylic acid. Subsequent α-oxidation leads to α-13-COOH, 13-(6-hydroxy-2,5,7,8-tetramethylchroman-2-yl)-2,6,10-trimethyl-tricarboxylic acid. The following β-oxidation steps in peroxisomes and mitochondria form the α-SCM, α-carboxyethyl-hydroxychroman (α-CEHC) [6]. This end-product of α-T0H metabolism can be conjugated and is excreted via urine [154]. The intact chroman structure indicates that α-CEHC is derived from α-T0H that has not reacted as an antioxidant. As α-CEHC excretion increases when certain plasma levels of α-LCM are exceeded, excretion of α-LCM is considered as an indicator of adequate or excessive α-T0H supply. Although hepatic metabolism of α-T0H and the formation of the metabolite long- and short-chain intermediates are known for several years [8–10], the physiological function of the α-LCM α-13-OH and α-13-COOH is still unknown. Due to a lack of the pure compounds α-13-OH and α-13-COOH, only a few studies on the function of these α-LCM have been performed. Work so far focused on anti-proliferative effects, modulation of inflammatory processes and modulation of lipid homeostasis. Our group described anti-proliferative effects of the α-LCM due to pro-apoptotic action [20]. In HepG2 cells, the α-LCM induced cleavage of caspases 3, 7 and 9 as well as PARP-1 and induced mitochondrial dysfunction as characterized by reduced mitochondrial membrane potential and induced intra-mitochondrial ROS formation. The anti-proliferative effect of α-13-COOH was shown also in murine glioma C6 cancer cells [19]. Others have reported that the α-LCM interfere with inflammatory processes by modulating activity of COX1 and COX2 and consequently by blocking production of PG E2 [18]. Recent work by our group focused on the effects of the α-LCM on macrophage foam cell formation [17]. We have shown that α-LCM induce expression of the scavenger receptor CD36, the major receptor responsible for oxLDL uptake, in human macrophages, in contrast to the inhibiting actions of α-T0H [17]. Despite up-regulation of CD36, uptake of oxLDL and oxLDL-induced lipid accumulation was reduced in human macrophages, similar to the effects of α-T0H on oxLDL-mediated foam cell formation. An important finding of this recent study was that the metabolite α-13-COOH was detected in serum providing for the first time evidence for the bioavailability of the α-LCM outside the liver. Another key finding of the study was that bioactivity of the α-LCM occur at lower concentrations and with mechanisms distinct from those of α-T0H. Taken together, these recent studies provide evidence for a role of the α-LCM as signaling molecules derived metabolically from α-T0H.

α-LCM circulating in the blood. We speculate that the α-LCM represent a new class of regulatory metabolites and propose that unraveling the molecular modes of action of the α-LCM and identifying the key players involved in their signaling may provide new fundamental insights into the biology and mode of function of vitamin E. Further studies are therefore required to elucidate the physiological role of the α-LCM and their contribution to disease processes, such as atherosclerosis. We also hypothesize that the discrepancy between the results obtained in vitro and in vivo in humans may be due to the physiologic metabolism of α-T0H and the formation of α-LCM in the liver and their release into circulation.

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Disclosures

None.

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