Free Radical Biology and Medicine 89 (2015) 952–962

Contents lists available at ScienceDirect

Free Radical Biology and Medicine

journal homepage: www.elsevier.com/locate/freeradbiomed

Original Contribution

Human serum determination and *in vitro* anti-inflammatory activity of the vitamin E metabolite α-(13'-hydroxy)-6-hydroxychroman

Silvia Ciffolilli [a,1], Maria Wallert [b,1], Desirée Bartolini [a], Verena Krauth [c], Oliver Werz [c], Marta Piroddi [a], Bartolomeo Sebastiani [d], Pierangelo Torquato [a,b], Stefan Lorkowski [b,2], Marc Birringer [e,2], Francesco Galli [a,2,*]

[a] Department of Pharmaceutical Sciences, University of Perugia, Italy
[b] Institute of Nutrition, Friedrich Schiller University Jena, Germany
[c] Institute of Pharmacy, Friedrich Schiller University Jena, Germany
[d] Department of Chemistry, Biology and Biotechnology, University of Perugia, Italy
[e] Department of Nutritional, Food and Consumer Studies, University of Applied Sciences Fulda, Germany

ARTICLE INFO

Article history:
Received 9 July 2015
Received in revised form
11 August 2015
Accepted 23 August 2015
Available online 8 October 2015

Keywords:
Vitamin E
Tocopherol
Long-chain metabolites
α-13'-OH
Cytochrome P-450
CYP4F2
Inflammation
Cytokines

ABSTRACT

Cytochrome *P*450-derived long-chain metabolites are gaining increasing interest as bioactive intermediates of vitamin E. In this study we first report on the HPLC-ECD and GC–MS analysis in human serum of the earliest metabolite of this vitamin, namely α-(13'-hydroxy)-6-hydroxychroman (α-13'-OH). The two chromatographic procedure are sensitive enough (LOQ of 10 nM) to measure α-13'-OH after hexane extraction of 1 ml of sample obtained from healthy volunteers supplemented for 1-week with 1000 IU/d (671 mg/d) *RRR*-α-tocopherol. The observed concentrations ranged between 15 and 50 nM, with minor differences between fasting and 4-hr post-meal state. Baseline (non-supplemented state) levels of 7.2 ± 1.6 nM were observed extracting higher volumes of serum. Biological effects of α-13'-OH investigated for the first time in RAW264.7 murine macrophages involved transcriptional control of inflammatory cytokines, and transcriptional and functional regulation of COX2 and iNOS enzymes in response to lipopolysaccharides. In conclusion, here we present the first quantitative evaluation of serum α-13'-OH also providing early evidence of the anti-inflammatory potential of this metabolite that is worth of further investigation in the area of functional and nutraceutical implications of vitamin E metabolism.

1. Introduction

The term “vitamin E” is used to describe a family of 8 fat-soluble molecules produced in plant organisms that show close structural homology in spite of a marked heterogeneity in terms of biological properties [1]. All the forms have in common a chromane structure with a hydroxyl group in position 6 while the degree of methylation differentiates 4 conformations identified with the Greek letters α, β, γ and δ. A 16-carbon atom branched (tocopherol subfamily, TOH) or isoprenoid (tocotrienol subfamily, *T*3) side chain is located in position 2 of the chromane ring. This confers marked lipophilic character to the vitamers acting as a docking domain into cell membranes and lipoproteins. These structural characteristics influence the antioxidant-dependent and -independent properties of these molecules, also influencing their metabolism and distribution in human tissues [1,2].

In fact, while all the vitamers have the same route of absorption together with dietary lipids, a tightly controlled mechanism of hepatic uptake regulates the fate of each vitamer in order to balance between the lipoprotein-dependent delivery to peripheral tissues and cytochrome *P*-450 metabolism or direct bile excretion. This selective liver uptake and lipoprotein assembly of the different forms of this vitamin are believed to depend on the binding and thus on the trafficking properties of α-tocopherol transfer protein (α-TTP). In fact, α-TTP has at least 10-fold higher affinity for the main form of vitamin E found in blood and several solid

Abbreviations: LCM, long-chain metabolite; TOH, tocopherol; T3, tocotrienol; TQ, tocopheryl quinone; α-13′-OH, α-13′-(6-hydroxy-2,5,7,8,-tetramethylchroman-2-yl)-2,6,10-trimethyltridecanol; α-13′-COOH, α-13′-(6-hydroxy-2,5,7,8, -tetramethylchroman-2-yl)-2,6,10-trimethyltridecanoicacid; CEHC, 2'-carboxyethyl)-6-hydroxychroman; α-TTP, α-tocopherol transfer protein; iNOS, inducible form of nitric oxide synthase; COX2, cyclooxygenase-2

* Corresponding author. Present address: Laboratory of Biochemistry and Nutrition, Department of Pharmaceutical Sciences, University of Perugia, Via del Giochetto, IT–06126 Perugia (Italy).
E-mail address: francesco.galli@unipg.it (F. Galli).
[1] These authors equally contributed to this research
[2] These authors equally contributed to this research.

http://dx.doi.org/10.1016/j.freeradbiomed.2015.08.019
0891-5849/

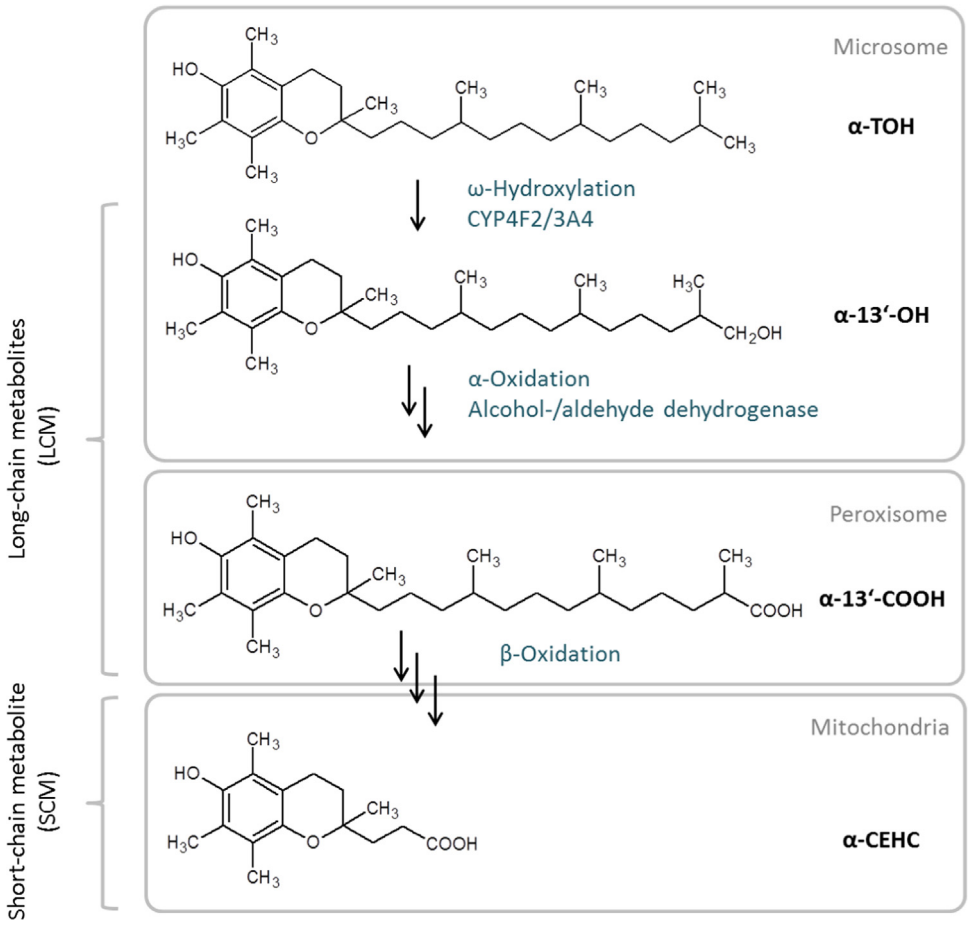

Fig. 1. Main steps in the hepatic metabolism of vitamin E. As shown here exemplarily for α-TOH, vitamin E is stepwise catabolized in the liver via subsequent steps of *ω*- and α-oxidation followed by several cycles of *β*-oxidation.

tissues, i.e. α-TOH in the RRR configuration, than for any other form [3]. Vitamers with lower affinity, such as desmethyl TOH and T3, are in fact scantily delivered to nascent VLDL. These forms are readily processed in the liver by cytochrome *P*-450 catabolism and/or excretion [4].

According to Mustacich and coworkers [5], in liver cells, vitamin E catabolism takes place in three different compartments. The initial CYP4F2-catalyzed ω-oxidation reaction at the endoplasmic reticulum produces the (13'-hydroxy)-6-hydroxychroman (13'-OH) derivative that is further oxidized in a second cycle of reaction that occurs in peroxisomes to the corresponding carboxylic acid (13'-COOH). Long-chain metabolites (LCMs) are very efficiently and rapidly processed in mitochondria through *β*-like oxidation degradation of the side-chain to form the end-product (2'-carboxyethyl)-6-hydroxychroman (CEHC) (Fig. 1) that is excreted under the form of sulfate and glucuronyl derivatives in the bile and urine. Emerging evidence suggests that vitamin *E* metabolites, and particularly LCMs, possess selected biological roles relevant to both the physiological and pharmacological functions of this vitamin (reviewed in [6–10]).

Analytical tools to investigate either LCMs or short-chain metabolites in cells, tissues and body fluids have been developed (reviewed in [6,8,11]). The analysis of CEHCs in serum and urine is a relatively straightforward and reliable procedure that has been widely employed to assess vitamin E metabolism in animal organisms. On the contrary, the analysis of LCMs is problematic due to the rapid metabolism that keeps their concentrations in biological fluids extremely low. The increasing interest in the biological functions of LCMs has stimulated our interest in developing unbiased analytical procedures to measure these metabolites in human blood. A former qualitative assessment of α-13'-COOH in human serum by LC-QTOF analysis was recently achieved [6], and further work is in progress to produce a quantitative estimation of this LCM in blood and solid tissues.

With this study we aim to define an assay procedure to measure α-13'-OH in human serum. Moreover, putative biological functions for this LCM have been investigated *in vitro* in the context of inflammatory homeostasis, which is one of the reported roles of vitamin *E* with possible impact for the prevention of most prevalent chronic diseases.

2. Materials and Methods

2.1. Chemicals and reagents

Synthesis and characterization of LCMs were described in [12–14]. Vitamins and metabolites were stored in sealed amber vials under nitrogen at −20 °C until use. Stock solutions of the analytical standards were prepared by dissolving them in absolute ethanol and before each session of analysis, purity and the actual concentration of the test molecules were verified by UV spectroscopy and HPLC analysis. Fine chemicals, generic reagents and solvents were of the highest grade available by commercial companies. If not indicated otherwise chemicals were obtained from Carl Roth, Sigma-Aldrich or Merck Millipore. For *in vitro* studies stock solutions of the LCMs were dissolved in DMSO, stored at −80 °C and concentrations were verified before each single