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Hepatic processing determines dual activity of α-tocopheryl succinate: a novel paradigm for a shift in biological activity due to pro-vitamin-to-vitamin conversion☆

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Abstract

Redox-silent vitamin E analogues, represented by α-tocopheryl succinate, are potent anti-cancer drugs with potential secondary bioactivity due to their processing in vivo. Here we verified the hypothesis that hepatic processing of these agents determines the secondary effect. Mice were repeatedly injected with α-tocopheryl succinate, and their systemic and hepatic vein blood was assessed for α-tocopheryl succinate and its hydrolysis product, vitamin E (α-tocopherol). While levels of α-tocopherol doubled compared to control mice and α-tocopheryl succinate accumulated in the systemic blood, no α-tocopheryl succinate was detected in blood draining the liver. We conclude that hepatic processing endows compounds like α-tocopheryl succinate with a secondary, anti-oxidant/anti-inflammatory activity due to converting it to the redox-active α-tocopherol. Our finding epitomises a novel, general paradigm, according to which a drug can be converted in the liver into a product that has a different beneficial bioactivity.

Keywords: Vitamin E; α-Tocopheryl succinate; Hepatic processing; Dual activity

α-Tocopheryl succinate (α-TOS) is a potent selective anti-cancer agent [1,2] as shown in multiple pre-clinical models [3–7]. Importantly, numerous studies document its selectivity for malignant cells [3,8] due to several mechanisms, including its hydrolysis by normal cells [1], selective generation of reactive oxygen species [9–11], or facilitated uptake due to the low pH of the neoplastic as compared to normal tissue interstitium [12]. Probably the most intriguing aspect of the potential clinical use of α-TOS stems from its fate in vivo. It has been shown earlier that the pro-vitamin associates in the bloodstream with circulating lipoproteins [13] that have the propensity to carry it to the microvasculature of the neoplastic tissue, where it exerts anti-cancer activity [1].

Based on circumstantial evidence and several assumptions, we suggested that α-TOS might act via a dual mode [1], as a pro-vitamin, exerting anti-neoplastic activity [1], and as a vitamin, enhancing the antioxidant [14,15] and anti-inflammatory defences [16–18]. α-TOS is redox-silent, since the anti-oxidant hydroxyl group of α-tocopherol (α-TOH) is esterified. However, this modification endows it with a strong pro-apoptotic activity that translates into potent anti-neoplastic effect [1] (see Fig. 1 for structures of α-TOH and α-TOS). We hypothesised that upon clearance via the hepatic

☆ *Abbreviations:* NSE, non-specific esterase; α-TOH, α-tocopherol; α-TOS, α-tocopheryl succinate; α-TTP, α-tocopheryl transfer protein; VLDL, very low-density lipoprotein.

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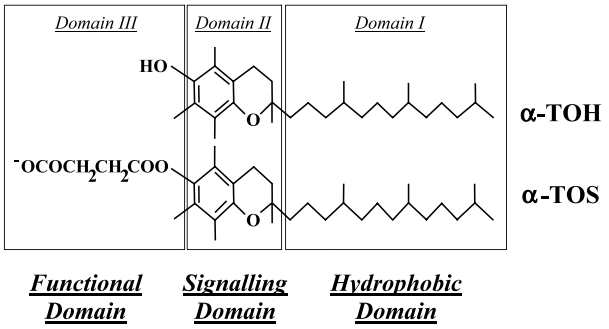

Fig. 1. Major domains in α-TOH and α-TOS. Domains I and II are identical in α-TOH and α-TOS, while Domain III differs. Domain I, also referred to as the *Hydrophobic Domain*, is responsible for docking the compound in biological membranes and circulating lipoproteins. Domain II, the *Signalling Domain*, is involved in regulation of certain signalling pathways, such as the protein phosphatase-2A/protein kinase C pathway. Domain II, the *Functional Domain*, makes α-TOH redox-active while it endows α-TOS with its apoptogenic activity.

system, α-TOS is hydrolysed and the resulting α-TOH is partially exerted in the bile, partially re-secreted into circulation. In this communication, we report that, indeed, this is the case, making α-TOS an intriguing anti-cancer agent with a second(ary) beneficial bioactivity.

Materials and methods

C57BL mice were used at 7–9 weeks of age. The animals (5 per group) were injected every third day with 100 μl of 100 mM α-TOS (*RRR*-α-TOS; Sigma) in DMSO or with 100 μl DMSO alone (control mice). Twenty-four hours following the last injection, hepatic and systemic blood was collected under a stereomicroscope as follows. A heparinised 1-ml syringe (26 g needle) was carefully inserted into the hepatic vein draining the left lobe in an anaesthetised mouse and ~200 μl of hepatic blood was withdrawn slowly. Cross-contamination from the inferior vena cava was prevented by clamping at its junction with the hepatic vein using a 10-mm mosquito clamp. Systemic blood was collected immediately following the completion of sample removal from the hepatic vein and occurred whilst cardiac contractions continued. To prevent retrograde flow from hepatic vein, the inferior vena cava was clamped immediately caudal to the junction of the hepatic vein and the sample was removed from the inferior vena cava proximal to the renal vein anastomosis. Care was taken during sample removal to minimise haemolysis of the sample. Blood was immediately centrifuged to remove blood cells, plasma was withdrawn and kept at −80 °C until analysed.

Analysis for α-TOH and α-TOS was performed by high-performance liquid chromatography as described elsewhere [15], and concentration of the two vitamin E analogues was expressed as μmol per ml of blood plasma.

Results and discussion

Previous circumstantial data suggested that α-TOS may be cleaved in vivo, presumably in the liver, whereby it is converted into the redox-active α-TOH. This is largely based on studies in which higher (2- to 3-fold) levels of α-TOH were observed in the circulation of mice subjected to chronic intraperitoneal administration of α-TOS at doses, at which the pro-vitamin strongly suppressed tumour growth in pre-clinical models [3,5]. Thus, it was apparent that disposition of α-TOS in vivo resulted in its hydrolysis, although the site of the hydrolysis has not been determined.

To resolve whether conversion of α-TOS to α-TOH occurs in the liver, we established a method that allowed us to discriminate between composition of systemic blood and blood drained from the liver via the hepatic vein (hepatic blood) before it combines with systemic blood. We then supplemented mice intraperitoneally with repeated doses of α-TOS at a level shown before to suppress experimental cancer [3,5]. After the last α-TOS administration or supplementation with the vehicle, mice were anaesthetised and the hepatic vein (hepatic blood) and inferior vena cava (systemic blood) were cannulated under a stereomicroscope. A procedure was established that allowed us to obtain at least 200 μl of blood with little or no appreciable haemolysis occurring. Most importantly, the protocol excluded cross-contamination of hepatic and systemic blood. This was essential since we were interested in assessing the levels of α-TOH and α-TOS in blood after it was processed in the liver and before it combined with systemic blood.

The results of this experiment are presented in Fig. 2. It shows that there was about 2- to 2.5-fold increase in α-TOH in both hepatic and systemic blood of the α-TOS-administered mice compared to that in the control mice. This is consistent with and complements our previous results [5]. In fact, it does suggest that levels of α-TOH are increased most likely due to hepatic processing of its precursor, α-TOS. This notion is strongly supported by the finding that while there was some 40–50 μM α-TOS in the systemic blood, no α-TOS was detected in the hepatic blood (Fig. 2).

Data provided in this report support our hypothesis according to which α-TOS and similar agents possess a primary activity, are associated with their pro-vitamin status, and are metabolised into their vitamin form, in which they exert a different beneficial bioactivity. The fact that there was no α-TOS detected in the hepatic blood and that its level was high in the systemic blood clearly indicates that it is primarily hydrolysed in the liver. Also, together with doubling the level of α-TOH in both systemic and hepatic blood when compared with its level in control mice, we may conclude that the additional α-TOH is derived from the endogenously administered α-TOS (see Fig. 2).

Based on the data presented here and on data of others, we now suggest that α-TOS associates with circulating lipoproteins [13,19], which carry it to the microvasculature of tumour tissue, where it is taken up by cancer cells, which then undergo apoptosis, a process leading to inhibiting tumour growth [1,3–8]. Because blood and peripheral tissues are a dynamic