BIOCHEMICAL FUNCTIONS, CLINICAL IMPLICATION AND ANALYSIS OF α-TOCOPHEROL

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ABSTRACT

α-Tocopherol (vitamin E) is a lipid-soluble antioxidant. Its deficiency symptoms depend on α-tocopherol content, uptake and utilization. Vitamin E concentrations are dependents on its unoxidised state in the body. Vitamin E deficiency has been related with cognitive impairment and Alzheimer’s disease. The low concentration of serum α-tocopherol and ascorbic acid may lead to inflammatory and cardiovascular diseases. The determination of α-tocopherol in clinical samples has largely been carried out by UV spectrophotometry and high-performance liquid chromatography.

BIOCHEMICAL FUNCTIONS OF α-TOCOPHEROL

α-Tocopherol (vitamin E) is a lipid-soluble antioxidant. α-Tocopherol transfer protein (α-TTP) is a cytosolic protein and is present in the liver of rats, humans, mice, dogs, chickens and other species. The gene is localized in the human 8q 13.1-13.3 region of chromosome 81,2. It has been reported that rat brain, spleen, lung, kidney and human brain are the sites of α-TTP in RNA3,4. α-TTP has also been located in pregnant mouse uterus in human placenta5,6. It has been suggested that the function of α-TTP is to maintain an adequate level of α-tocopherol during pregnancy. α-TTP has been found to preferentially transfer α-tocopherol in the system, compared with the dietary vitamin E form7. The mechanism of α-TTP involvement in the α-tocopherol secretion into plasma is not clearly known. However, it is suggested that α-TTP translocates from the cytosol to endosomes to obtain α-tocopherol. Later, α-TTP- α-tocopherol moves to the plasma membrane to release α-tocopherol8. α-TTP is a pocket shaped protein that specifically binds α-tocopherol. The phytyl tail is bent to fit the pocket and 2-position is critical for this conformation9.

α-tocopherol is not accumulated in the liver10 and is metabolized to α-CEH (2,5,7,8-tetramethyl-2-(2′-carboxyethyl)-β-hydroxychroman). The reaction involves the hydroxylation of the tail followed by α-oxidation of the molecule (→CH3→CH2OH→-COOH)11,12.

The administration of equimolar amount of labeled tocopherols (~50 mg each d6-α-tocopherol acetate and d2-γ-tocopheryl acetate) plasma concentrations of d6-α-CHHC (carboxyethyl hydroxychroman) are undetectable, however, the rates of disappearance of α-CECH and α-tocopherol from plasma have been found to be similar13. The dietary α-tocopherol is metabolised in 9-12 hr by humans.

The major biochemical role of α-tocopherol is to modulate some cellular functions. It inhibits protein kinase smooth muscle cell proliferation14 as well as platelet aggregation and adhesion15. Supplementation of α-tocopherol to humans (1200 IU or 900 mg/day) has been found to decrease monocyte superoxide production via inhibition of protein kinase C16,17, and decrease IL-1β release from monocytes by inhibiting 5-lipoxygenase18.

CLINICAL IMPLICATIONS

The influence of vitamin E on the immune system is an important clinical markers of its bioactivity. A relatively simple normal phase HPLC method has been used for the analysis of individual stereoisomers of α-tocopherol. The stereoisomers are a measure of their bioavailability and are influenced by factors such
as age, dietary levels, and time after dosage\(^{10}\). The α-tocopherol concentration and stereoisomer composition in plasma and milk of dairy cow few natural or synthetic vitamin E around calving have been studied. Analysis of the distribution of the individual stereoisomers of α-tocopherol indicate that the bioavailability of RRR-alpha- tocopherol relative to synthetic stereoisomers in cattle is considerably higher\(^{20}\).

Lipid peroxidation in high lipid: low dextrose (HL:LD) parenteral admixtures is common. This has been measured by the formation of hydroperoxides in the admixtures to determine the optimal dose of d-a-tocopherol to minimize peroxidation during a 24 hr period. These results have clinical implications for parenteral feeding in critically ill patients\(^{21}\). The effects of α-tocopherol and ascorbic acid on Helicobacter pylori colonization and severity to gastric inflammation has been studied. Tissue samples of the patients have been used for the histopathological examination and determination of the vitamins. The patients were given vitamin E 200 IU BID and vitamin C 500 mg BID for 4 weeks orally. The mean vitamin E and C concentrations in gastric mucosa at the end of the 4\(^{th}\) week were higher than those at the beginning (p=0.006 and p=0.000) and showed increased eradication of the bacteria\(^{22}\). All damage (α-tocopherylquinone, 5-nitro-g-tocopherol) have been examined in relation to mild cognitive impairment (MCI) and Alzheimer’s disease (AD). Compared with cognitively normal subjects, MCI and AD showed lower levels of total tocopherols, total tocotrienols and total vitamin E. In both disorders tocotrienols levels have been associated with increased cases of MCI and AD\(^{23}\).

The low concentrations of serum α-tocopherol and ascorbic acid are involved with higher risk of many inflammatory diseases related to oral heal. Poisson regression analysis has been conducted to assess predictors of periodontal disease, with serum α-tocopherol and ascorbic acid as the primary predictors. The multivariate adjusted relative risk (95% confidence intervals) in the highest, middle and lowest tertiles are 1.00 (reference), 1.09 and 1.15 for α-tocopherol and 1.00 (reference), 1.12 and 1.30 for ascorbic acid. The findings support the hypothesis that low serum levels of these vitamins may be a risk factor for periodontal disease in elderly\(^{24}\). Tocopherols and tocotrienols, parent congeners in the vitamin E family are effective in decreasing mortality due to cardiovascular diseases.

Tocotrienols have several cardioprotective effects, including antagonizing the oxidation of low density lipoproteins, antitherosclerotic, inhibiting platelet aggregation and monocyte adhesion, preventing smooth muscle proliferation and various other cardiovascular disorders\(^{25}\). High-dose vitamin E may be associated with increased mortality in some populations. Vitamin E may increase the production of CYP3A4 in the liver resulting in an increase in drug metabolism, potentially lowering the efficacy of therapeutic drugs such as midazolam\(^{26}\). Oxidative stress plays a significant role in allergic airway inflammation. Supplementation with α-tocopherol cause intervention in this disease. Enhancing levels of antioxidants with oral supplements has been suggested as an intervention to protect individuals from the effect of inhaled oxidants\(^{27}\). Cardiopulmonary bypass (CPB) is associated with oxidative stress. Antioxidant levels in patients undergoing CPB surgery has been examined and correlated with clinical variables.

Plasma α-tocopherol (HPLC) levels reduced along CPB with post-operative values approximately 25% lower than baseline. A correlation existed for α-tocopherol depletion versus maximal PaO\(_2\) throughout CPB\(^{28}\).

**CLINICAL ANALYSIS**

A novel, simple and fast reversed-phase HPLC/UV method has been developed and validated for simultaneous determination of α-tocopherol and all-tretins- retinol in human serum using retinyl acetate as internal standard (0.5 mg/ml). Aliquid-phase extraction is applied to 250 ml of serum with n-hexanec dichloromethane mixture (70:30, v/v). The analysis
has been carried out on Kromasil 100c18 column (150 mm x 4.6 mm, 5 mm) using 292 nm as the detection wavelength. Complete separation has been achieved in 3 and 6 mm and 5 mm Columns, respectively, by using 20 ml sample.

Serum α-tocopherol has been determined by HPLC with electrochemical detection. Serum a-tocopherol is increased by 100% (P=0.002) and its urinary metabolite excretion is increased 20-fold in a treatment group versus placebo (P=0.001).

An online method has been developed to determine the antioxidant capacity of vitamin E-modified dialyzer membranes during circulation. This method is based on a spectrophotometric assay known as the ferric reducing/antioxidant power assay (FRAP) and involves the iron-catalyzed oxidation of vitamin E to its quinone derivative. The method shows good reproducibility and intra- and inter-assay precision.

A HPLC method for the assay of α-tocopherol and carotenoids in blood samples has been reported. A simple and rapid HPLC method for selective and sensitive determination of α-tocopherol, retinal and retinyl esters (palmitate and stearate) in blood serum using a diode-array detector (DAD) at 295, 325 and 330 nm, respectively, has been reported. The separation is achieved on a Chromolith Performance RP-18e 100x4.6 nm monolithic column using methanol-water (95:5, v/v) as mobile phase. The method may represent a valuable aid in the laboratory monitoring of the toxicity of anticancer therapy.

CONCLUSION
α-Tocopherol plays important biochemical role in the physiological system. The α-tocopherol transfer protein is involved in the release of α-tocopherol in plasma. The metabolism of the vitamin is carried out by hydrolysis and β-oxidation of the molecule. α-Tocopherol has important clinical implications and its deficiency may lead to different diseases. Low concentrations of vitamin E and C are involved with high risk of inflammatory disease. Vitamin E also has a cardioprotective effect. The clinical analysis of the vitamin is carried out by UV spectrophotometry and HPLC methods.

REFERENCES


23. Mangialasche, F., Xu, W., Kivipelto, M., Costanzi, E., Erocolani, S., Pigliautile, M., Cecchetti, R., Baglioni, M., Simmons, A., Soininen, H., Tsolaki, M., Kloszewska, I., Vellas, B. and Lovestone, S., Tocopherols and tocotrienols plasma levels are associated with cognitive impairment, Neurobiol Aging., 2011; Dec20. [Epub ahead of print].


