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Regulatory Mechanisms to Control Tissue α -Tocopherol

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Abstract

To test the hypothesis that hepatic regulation of α -tocopherol metabolism would be sufficient to prevent over-accumulation of α -tocopherol in extrahepatic tissues and that administration of high doses of α -tocopherol would up-regulate extrahepatic xenobiotic pathways, rats received daily subcutaneous injections of either vehicle or 0.5, 1, 2, or 10 mg α -tocopherol/100 g body wt for 9 days. Liver α -tocopherol increased 15-fold in rats given 10 mg α -tocopherol/100 g body weight (mg/100 g) compared with controls. Hepatic α -tocopherol metabolites increased with increasing α -tocopherol doses, reaching 40-fold in rats given the highest dose. In rats injected with 10 mg/100 g, lung and duodenum α -tocopherol concentrations increased 3-fold, while α -tocopherol concentrations of other extrahepatic tissues increased 2-fold or less. With the exception of muscle, daily administration of less than 2 mg/100 g failed to increase α -tocopherol concentrations in extrahepatic tissues. Lung cytochrome P450 3A and 1A levels were unchanged by administration of α -tocopherol at any dose. In contrast, lung P-glycoprotein (MDR-1) levels increased dose dependently and expression of this xenobiotic transport protein was correlated with lung α -tocopherol concentrations ($R^2 = 0.88$, $P < 0.05$). Increased lung MDR1 may provide protection from exposure to environmental toxins by increasing alveolar space α -tocopherol.

Keywords

α -tocopherol; cytochrome P450; xenobiotic metabolism; rat; MDR1; p-glycoprotein; CYP; lung

Introduction

Plants synthesize eight different molecules with vitamin E antioxidant activity, consisting of α -, β -, γ -, and δ -tocopherols and the corresponding four tocotrienols. Only α -tocopherol, not the others, is maintained in human plasma and tissues, as a result of the function of the hepatic α -tocopherol transfer protein (α -TTP) [1]. Unlike other fat-soluble vitamins, vitamin E is not accumulated in the body to toxic levels, suggesting that mechanisms, i.e., metabolism and excretion, prevent excess accumulation [2].

In studies using insect microsomes expressing recombinant human cytochrome P450 (CYP) enzymes, CYP4F2 metabolized γ -tocopherol, and to a much lesser extent α -tocopherol, to their respective 13'-OH-tocopherol metabolites [3]. The formation of 13'-OH- α -tocopherol is followed by several steps of β -oxidation leading to the formation of 5'- α -CMBHC (2,5,7,8-

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tetramethyl-2-(4'-carboxy-4'-methylbutyl)-6-hydroxychroman) and finally α -CEHC (2,5,7,8-tetramethyl-2-(2'-carboxyethyl)-6-hydroxychroman). In rats given daily subcutaneous α -tocopherol injections (10 mg/100 g body wt) metabolism of α -tocopherol increased significantly as evidenced by the 20-fold increase in 13'-OH- α -tocopherol levels [4]. Surprisingly, hepatic CYP4F protein levels were unchanged. However, these α -tocopherol injections increased hepatic protein levels of CYP3A, 2B, and 2C family members, as well as increased hepatic protein levels of MDR1 (multidrug resistance protein 1, ABCB1, or p-glycoprotein), one of the ABC transporter proteins located in the canalicular membranes of hepatocytes [4]. Other studies showed that hepatic Cyp3a protein levels increased in mice fed an α -tocopherol sufficient diet (31 mg/kg diet) as compared with mice fed an α -tocopherol deficient diet (<2mg/kg diet) and that hepatic Cyp3a protein levels correlate with hepatic α -tocopherol levels [5]. Similarly, hepatic Cyp3a11 mRNA expression was lowest in mice fed 2 mg α -tocopherol/kg diet, increased significantly in mice fed 20 mg α -tocopherol/kg diet and further increased in mice maintained for 9 months on a diet containing 200 mg α -tocopherol/kg [6].

CYP enzymes are involved in xenobiotic metabolism. Although the liver is the key organ responsible for the metabolism of most xenobiotics irrespective of the route of exposure, xenobiotic metabolism also occurs in the lung. Metabolism within the lung may be particularly important for inhaled compounds. Members of the CYP1A subfamily play a key role in the metabolism of environmental toxicants and are constitutively expressed at trace levels in the liver and lung [7]. However, exposure to environmental toxicants, i.e., polycyclic aromatic hydrocarbons and halogenated aromatic hydrocarbons, induces the protein expression of CYP1A enzymes in both the liver and lung, as reviewed in [7,8].

MDR1 is a member of the superfamily of ATP-binding cassette (ABC) transporters and has been shown to be localized to the apical (luminal) membranes of epithelial cells in organs that modulate drug distribution, i.e., canalicular membranes of hepatocytes, and the luminal surface of enterocytes and cells lining the proximal tubules of the kidney, as reviewed in [9,10]. In addition, MDR1 is localized to the apical membranes of epithelial cells lining the human bronchus [11], as well as the alveolar epithelium of human and rat lung [12]. MDR1 transports numerous structurally diverse therapeutic drugs, as well as insecticides [13]. The localization of MDR1 and outward vectorial transport of its substrates suggests a protective role against natural toxins and drugs, as well as environmental toxicants.

CYP3A proteins are also expressed in extrahepatic tissues, albeit at significantly lower concentrations than in the liver. Coordinate modulation of CYP3A and MDR1 in non-cancerous extrahepatic tissues has received very little attention and modulation by α -tocopherol of CYP1A, CYP3A, and MDR1 protein expression in extrahepatic tissues has not to our knowledge been previously reported.

We hypothesized that 1) regulation of α -tocopherol metabolism by the liver would be sufficient to prevent over-accumulation of α -tocopherol in extrahepatic tissues and 2) supplementation with high doses of α -tocopherol may up-regulate extrahepatic xenobiotic pathways, particularly in the lung and kidney, to further prevent over-accumulation of α -tocopherol. To test this hypothesis, using a subcutaneous vitamin E dosing regimen in rats, we measured tissue vitamin E increases, and the ability of low and high dose administration to alter the expression of extrahepatic CYP enzymes and MDR1.

Materials and methods

Reagents

Vital E-300 is a non-aqueous injectable form of *d*- α -tocopherol containing 300 IU *RRR*- α -tocopherol/ml compounded with 20% ethanol and 1% benzyl alcohol in an emulsified base (Schering-Plough Animal Health, Union, NJ). HPLC-grade methanol, hexane, ethanol, and glacial acetic acid were obtained from Fisher (Fair Lawn, NJ). Antibodies were obtained as follows: anti-rat CYP3A2 and anti-actin (Chemicon, Temecula, CA), anti-human MDR1 (C219, Signet, Dedham, MA), and anti-human CYP4F2 (a gift from J. M. Lasker). The CYP4F2 antibody has previously been shown to cross-react with rat and mouse hepatic CYP4F proteins [4,5,14]. The C219 antibody has been shown to cross-react with rat MDR1 [15]. Secondary antibodies were from Amersham Biosciences (Piscataway, NJ). All other chemicals were obtained at reagent grade quality from suppliers.

Animal Studies

Male Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA) 275-300 g, were maintained on a 12-h light/dark cycle; food (LabDiet 5001, Animal Specialties, Hubbard, OR) containing 49 IU vitamin E/kg, and water were given *ad libitum*. Rats were acclimated for 1 week prior to the start of injections. Just prior to injection, Vital E-300 was diluted to one of 4 α -tocopherol doses (0.5 mg, 1.0 mg, 2 mg, or 10 mg α -tocopherol/100 g body wt) with sterile saline (total volume = 0.4 ml/injection). Rats ($n = 4$ /group) received daily subcutaneous (SQ) injections of either vehicle (saline), or α -tocopherol (0.5 mg, 1.0 mg, 2 mg, or 10 mg α -tocopherol/100 g body weight) for 9 days. On day 10, following a 12 h fast and approximately 16 hours after their last injection of α -tocopherol, rats were euthanized, blood was collected in EDTA and tissues were perfused with 0.9% saline (containing 2 U/ml heparin) using a perfusion catheter inserted into the heart. Tissues were excised and aliquots frozen in liquid N_2 and stored at -80°C . Plasma was obtained by centrifugation and stored at -80°C . Animal protocols were approved by the Oregon State University Animal Care and Use Committee.

Microsome and Membrane Preparations

For membrane preparation, tissues (~200 mg) were homogenized on ice in ice-cold membrane Buffer A (0.25M sucrose, 10mM Tris-Cl (pH 7.4- 7.6)) using a Potter-Elvehjem-type homogenizer, filtered through cheese cloth, then centrifuged 60 min at $100,000 \times g$ (4°C). The resulting pellet was suspended in membrane buffer B (0.3 M sucrose, 10mM HEPES) and stored at -80°C for western blot analyses. Protease inhibitor cocktail (Santa Cruz Biotechnology, Santa Cruz, CA) was added to all buffers just prior to use.

Microsomes were isolated by ultracentrifugation, as described [4,16] and stored at -80°C until use for western analysis.

Western Blot Analyses

Total protein concentrations were determined by the method of Bradford, using Coomassie Plus reagent per the manufacturer (Pierce Biotechnology, Rockford, IL). Equal quantities of microsomal or membrane protein were resolved by SDS-Page electrophoresis (Bis-Tris gels, Invitrogen, Carlsbad, CA) and transferred to polyvinylidene fluoride (PVDF) membranes (Invitrogen). Protein transfer was confirmed by reversible staining of blots with 0.5% Ponceau-S red/1% acetic acid. Blots were blocked in Tris-buffered saline with 0.05% tween (TBST) and 3% non-fat milk (TBST/milk). Fresh TBST/milk containing primary antibody was added to the blots for overnight incubation at 4°C . Blots were washed with TBST, incubated with peroxidase-conjugated secondary antibody, washed with TBST and detected by enhanced chemiluminescence (Western Lightning, Perkin Elmer, Boston, MA). Blots were exposed to

film (VWR) and quantified by densitometry using an Alpha Innotech Photodoc system with FC8800 software (Alpha Innotech, San Leandro, CA).

CYP and MDR1 protein levels in each sample have been normalized to their respective actin protein concentrations based on densitometry data. Insect microsomes expressing the individual CYP proteins (BD Biosciences, San Jose, CA) and cell lines that have been documented for expression of the MDR1 transport protein (Santa Cruz Biotechnology, Santa Cruz, CA) were run on gels as positive controls.

Measurement of α -Tocopherol and α -CEHC

A modification of the method by Podda et al. [17] was used for the analysis of α -tocopherol, as previously described [18]. α -CEHC was extracted from liver following addition of an internal standard (trolox) and analyzed by LC/MS as previously described [18].

Statistical Analysis

Statistical analysis was performed using Prism version 4.0 (Graphpad Software, San Diego, CA). The mean of the normalized optical density measurements of vehicle (control) levels for a given protein have been assigned a value of 100% and normalized protein levels from rats injected daily with 0.5 mg, 1.0 mg, 2 mg, or 10 mg α -tocopherol/100 g body wt are expressed in the graphs as percent of the control mean value for that protein. Protein expression, as well as tissue and plasma α -tocopherol concentrations, were analyzed by one-way ANOVA and a value of $P < 0.05$ was considered statistically significant. Post hoc tests were performed using Dunnett's Multiple Comparisons when overall group effects were found to be significant. Data are expressed as mean $\pm$ SE.

Results

Tissue and Plasma α -Tocopherol Concentrations

α -Tocopherol (0.5 mg, 1 mg, 2 mg, or 10 mg α -tocopherol/100 g body wt), or vehicle (control), was administered daily to rats by SQ injection for 9 days, then rats were sacrificed on day 10, between 16 and 18 h following their last injection. Liver α -tocopherol concentrations from control rats were 36.7 ± 3.2 nmol/g (mean $\pm$ SE). Hepatic α -tocopherol levels increased 3-fold, 3.5-fold, 6-fold and 15-fold with the increasing daily dose injected in rats for 9 days, as compared with controls (Figure 1).

To evaluate the ability of SQ α -tocopherol injections to increase vitamin E concentrations throughout the body, α -tocopherol concentrations were determined in the plasma and extrahepatic tissues of vehicle- and α -tocopherol-injected rats (Figure 2). Plasma α -tocopherol concentrations approximately doubled following daily injections of either 2 or 10 mg/100 g body wt, but were unchanged by lower doses (Figure 2A). Similarly to liver, muscle α -tocopherol concentrations increased significantly at all dose levels of α -tocopherol injections (Figure 2B). Lung α -tocopherol concentrations doubled with 2 mg/100 g and nearly tripled with 10 mg/100 g body wt injected daily (Figure 2C). Kidney α -tocopherol levels were also significantly increased by daily α -tocopherol injections (Figure 2D), reaching a maximum concentration of 44.9 ± 5.6 nmol/g in rats injected with 10 mg/100 g body wt ($P < 0.01$) compared with control kidneys (25.1 ± 1.3 nmol/g). Heart α -tocopherol concentrations increased from 56 ± 9 nmol/g in control rats to 138 ± 8 nmol/g in rats receiving daily injections of 10 mg/100 g body wt ($P < 0.01$ compared with controls Figure 2E). Similar to the heart, α -tocopherol concentrations in the adipose tissue increased following daily injections of 10 mg/100 g body wt, but were unchanged by lower dose α -tocopherol injections (Figure 2F).

Within the intestinal tract, α -tocopherol concentrations in the duodenum, jejunum, and ileum of vehicle-injected rats were 42 ± 3 , 37 ± 4 , and 40 ± 4 , respectively (Figure 3). Daily injections of 10 mg/100 g body wt approximately tripled α -tocopherol concentrations in both the duodenum and jejunum and doubled ileum α -tocopherol concentrations. Lower injected doses were less effective at increasing intestinal α -tocopherol concentrations.

The vitamin E concentrations of various parts of the nervous system were also measured. Cerebrum, cerebellum, and sciatic nerve α -tocopherol concentrations were not significantly increased by daily α -tocopherol injections at any of the four dose levels (data not shown). However, α -tocopherol concentrations in the cervical, thoracic and lumbar regions of the spinal cord increased 1.6 to 2-fold ($P < 0.05$, compared with control tissues) following daily injections with 10 mg α -tocopherol/100 g body wt (Figure 4). Spinal cord α -tocopherol concentrations were unchanged by lower doses of daily α -tocopherol injections.

Tissue α -CEHC Concentrations

To evaluate the metabolism of α -tocopherol, as well as the possible accumulation of metabolites in tissues, hepatic and extrahepatic α -CEHC was determined in vehicle- and α -tocopherol-injected rats. In control rats, liver and kidney α -CEHC levels were 1.25 ± 0.48 and 0.27 ± 0.04 nmol/g, respectively, while lung α -CEHC levels were below levels of detection. α -CEHC levels increased with increasing dose of α -tocopherol, reaching 53.6 ± 8.5 , 17.8 ± 3.4 and 1.4 ± 0.2 nmol/g in liver, kidney and lung, respectively, in rats injected with the highest dose (Figure 5). Of note, both α -tocopherol and α -CEHC levels in all three tissues begin to plateau at SQ doses above 2 mg α -tocopherol/100 g body wt (Figure 5).

Cytochrome P450 Expression in Lung and Kidney in Response to α -Tocopherol Injections

Previously we demonstrated that daily SQ injections of 10 mg α -tocopherol/100 g body wt doubled hepatic CYP3A2 protein levels [4]. To evaluate the ability of the lung to increase CYP3A2 in response to increasing α -tocopherol concentrations, CYP3A protein levels were measured, but were found to be unchanged despite increased lung α -tocopherol concentrations (Figure 6A). In addition, CYP3A protein expression in the kidney was too low for quantification in vehicle- as well as α -tocopherol-injected rats. CYP1A1 protein levels in both the liver (not shown) and lung (Figure 6B) were unchanged at any dose of daily α -tocopherol injections.

CYP4F2 has been identified as the putative tocopherol ω -hydroxylase [3], and is expressed in the liver and kidney [19]. Human CYP4F2 and rat CYP4F1 have been shown to have similar catalytic specificities and are the most abundant 4F isoforms expressed in liver and kidney of humans and rats [20-22]. These CYP4F isoforms are both detected by the antibody used in this study. CYP4F protein levels were unchanged in the kidney at all dose levels of α -tocopherol injected (Figure 7); while CYP4F protein was not detected in the lung and did not become detectable at any dose of α -tocopherol used for this study (data not shown).

MDR Transporter Expression Modulated by α -Tocopherol Injections

MDR1 is responsible for the biliary excretion of numerous xenobiotics and hepatic MDR1 is often similarly regulated by compounds that induce hepatic CYP3A proteins [23]. However, coordinate regulation of these two proteins within the lung by α -tocopherol has not to our knowledge been previously reported. Therefore, we determined lung MDR1 concentrations in rats injected daily with vehicle or α -tocopherol. MDR1 protein in the lung increased in a dose-dependent manner reaching 1.5-times that found in control lungs ($P < 0.01$, Figure 6C). Importantly, lung MDR1 protein expression was correlated with lung α -tocopherol concentrations ($R^2 = 0.88$, $P < 0.001$) (Figure 6D).

Discussion

In the current study we have used various doses of α -tocopherol injected SQ in rats to increase plasma and tissue α -tocopherol levels and then determined α -CEHC, CYP and MDR1 expression in selected tissues. Importantly, our data shows that mechanisms are in place to prevent the excess accumulation of both α -tocopherol and α -CEHC in extrahepatic tissues.

In rats given daily SQ α -tocopherol injections (10 mg/100 g body wt) for 18 days, hepatic α -tocopherol and α -CEHC levels increased only up to about day 9, then began to decrease [4]. Interestingly, hepatic CYP3A almost immediately doubled, while the MDR1 proteins increased concurrently with decreasing α -tocopherol concentrations [4]. These results indicated that mechanisms that prevent the over-accumulation of α -tocopherol in liver are up-regulated in rats given pharmacologic doses of α -tocopherol and that administration of pharmacologic doses of α -tocopherol modulates hepatic expression of proteins involved in xenobiotic metabolism and excretion. Thus, we were interested to determine the extent to which extrahepatic tissues were protected from over-accumulation of α -tocopherol and to further elucidate the ability of α -tocopherol to modulate their xenobiotic metabolism and excretion pathways.

Interestingly, in contrast to hepatic α -tocopherol concentrations that increased 15-fold in rats given the highest α -tocopherol dose, only the α -tocopherol concentrations in lung, muscle, heart, jejunum and duodenum increased more than 2-fold, with the duodenum having the highest fold change (3.2-fold). The increase in the duodenum may be due in part to enterohepatic circulation of α -tocopherol excreted from the liver into the bile [24,25]. The 2-fold increase in plasma α -tocopherol concentration is consistent with studies where humans were given either 400 or 800 IU *RRR*- α -tocopherol/day for 8 weeks and plasma α -tocopherol concentrations were found to increase approximately 2-fold at both dose levels [26]. These data indicate that the body has the ability to eliminate “excess” α -tocopherol, even when pharmacologic doses are administered. Moreover, plasma and extrahepatic tissue α -tocopherol levels did not increase more than approximately 3-fold. The exception was the liver where excess α -tocopherol appears to be sequestered and then rapidly metabolized and/or excreted.

Systemic regulation of α -CEHC levels has not to our knowledge been previously reported. Our data shows that α -CEHC increased in liver, lung and kidney with increasing dose of α -tocopherol. However, even at the highest administered α -tocopherol dose, kidney α -CEHC levels were one-third and lung α -CEHC levels were one-fortieth of liver α -CEHC levels. In addition, α -CEHC levels in both lung and kidney began to plateau following administration of greater than 2 mg α -tocopherol/100 g body wt. These data indicate that, like α -tocopherol, mechanisms are in place to prevent the accumulation of α -CEHC in extrahepatic tissues.

Like CYP4F2 in humans, CYP4F1 is the most abundant CYP4F isoform found in the rat liver (80%) and kidney (95%), and has been shown to have similar substrate specificity to human CYP4F2, i.e., ω -hydroxylation of arachidonic acid and leukotriene B₄ [20,41,42]. CYP4F2 has been identified by in vitro studies as the putative tocopherol hydroxylase [3], and as such may be expected to increase under conditions of increased α -tocopherol metabolism. However, in our previous study hepatic α -CEHC concentrations increased more than 80-fold, while hepatic CYP4F protein expression remained unchanged [4]. In the current study, kidney CYP4F1 levels were unchanged and lung CYP4F1 remained undetectable despite significantly increased α -CEHC concentrations in both tissues. Thus, our data indicate that SQ administration of pharmacologic doses of α -tocopherol does not modulate CYP4F protein levels in rat liver, lung or kidney. Furthermore, CYP3A protein was not detected in kidney and was only expressed at very low levels in lung. Together, these data suggest the modest increase in lung α -CEHC levels is likely a result of plasma delivery and not metabolism of α -tocopherol

in situ. Presumably, the increase in kidney α -CEHC levels is due to its excretory function [2, 27]. However, since CYP4F1 is constitutively expressed in the kidney, α -tocopherol metabolism within the kidney may be an additional source of kidney α -CEHC.

MDR1 and CYP3A overlap with respect to substrate specificity and coordinate modulation of MDR1 and CYP3A protein expression in the liver has been demonstrated for several xenobiotic compounds [13,23]. In contrast to the numerous studies investigating the regulation of hepatic MDR1, regulation of lung MDR1 has received very little attention and, in a search of the literature, we were unable to find information with respect to coordinate regulation of MDR1 and CYP3A proteins in the lung. Interestingly, several agents, including clotrimazole (CLOT) and a mixture of DEX + pregnenolone 16 β -carbonitrile (PCN) in rats, and rifampicin exposure in rabbits [37], which increase hepatic CYP3A protein have failed to induce lung CYP3A protein or activity levels. Unfortunately, MDR1 protein expression in either the lung or liver was not studied [37].

Recently, we demonstrated in rats that supplementation with 10 mg α -tocopherol/100 g body wt increases the expression of hepatic MDR1 and CYP3A proteins [4]. To further elucidate the ability of α -tocopherol to modulate xenobiotic pathways in rat lung, we determined the ability of various α -tocopherol doses given daily by SQ injection to coordinately modulate CYP3A and MDR1. Importantly, lung MDR1 protein levels increased with increasing α -tocopherol concentrations, while lung CYP3A protein expression was unaltered. These data suggest that the mechanism(s) by which α -tocopherol increases CYP3A expression is: 1) tissue specific and 2) independent of the mechanism by which α -tocopherol increases lung MDR1 protein expression.

In addition to CYP3A, we determined the ability of α -tocopherol to alter the expression of CYP1A protein in the lung and liver of rats. The CYP1A subfamily are key enzymes in the metabolic activation of numerous environmental xenobiotics, including many that enter the body via the respiratory system. Metabolic activation by the CYP1A subfamily of enzymes results in the production of highly reactive intermediates that have been shown to damage DNA [39,40]. Thus induction of this enzyme in either the liver or lung may lead to increased tissue damage. Importantly, CYP1A protein was not changed by pharmacologic doses of α -tocopherol.

Hepatic expression of members of the CYP3A subfamily, as well as that of the MDR1 transporter, is regulated by nuclear receptors [15,43]. Specifically, both CAR (constitutive androstane receptor) and PXR (pregnane $\times$ receptor) have been shown to regulate CYP3A; PXR has been shown to regulate MDR1 expression [44,45]. Landes et al. [46] demonstrated that various forms of vitamin E activated PXR expressed in HepG2 cells. However, α -tocopherol was one of the least effective of the forms tested. Conversely, another study showed that only tocotrienols, and not tocopherols, activated PXR in both intestinal LS180 cells and primary human hepatocytes [47]. Chirulli et al. [37] have demonstrated a low level of constitutive expression of CAR mRNA, but no expression of PXR mRNA, in rat lung. These data indicate that mechanisms of xenobiotic pathway induction are tissue specific and suggest it is unlikely that α -tocopherol is modulating lung MDR1 via the PXR receptor. Therefore, further studies in vivo are needed to determine the mechanism(s) by which α -tocopherol modulates lung MDR1 expression, as well as the mechanism(s) for modulation of hepatic CYP and MDR1 expression.

In conclusion, α -tocopherol concentrations increased 2-fold or less in most extrahepatic tissues and did not exceed 3.5-fold in any tissue, except the liver, even at the highest levels of α -tocopherol administered. In addition, our results indicate that unlike hepatic CYP3A expression, lung and kidney CYP3A expression is not altered by excess α -tocopherol.

Furthermore, no α -tocopherol dose altered either hepatic or extrahepatic expression of either CYP1A or CYP4F proteins. Thus, induction of CYP enzymes by α -tocopherol is both tissue and CYP-subfamily specific. Importantly, lung MDR1 expression increased with α -tocopherol concentrations. Because MDR1 is responsible for the elimination of numerous toxic xenobiotics and based on its location in the alveolar membranes of both the rat and human respiratory system, we propose that induction of this transporter in the lung may represent an important mechanism for protection of the lung from exposure to xenobiotics and their metabolites, as well as a mechanism for delivery of α -tocopherol to the alveolar space.

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Abbreviations

α-CEHC

2,5,7,8-tetramethyl-2-(2'-carboxyethyl)-6-hydroxychroman

CYP

	cytochrome P450
αTTP	α -tocopherol transfer protein
MDR	multi-drug resistance
SQ	subcutaneous
TBS	tris-buffered saline
ABC transport protein	ATP-binding cassette transport protein
PCN	pregnenolone 16 β -carbonitrile
DEX	dexamethazone
CLOT	clotrimazole
CAR	constitutive androstane receptor
PXR	pregnane $\times$ receptor

Fig. 1.

Hepatic α -tocopherol concentrations in response to subcutaneous (SQ) vehicle or α -tocopherol injections. Vital E-300 was diluted to one of 4 α -tocopherol doses (0.5 mg, 1.0 mg, 2 mg, or 10 mg α -tocopherol/100 g body wt) with sterile saline. Rats ($n = 4$ /group) received daily SQ injections of either vehicle (saline), or one of the four α -tocopherol doses for 9 days. On day 10, following a 12 h fast, rats were killed, blood collected and tissues were perfused with 0.9% saline (containing 2 U/ml heparin) using a perfusion catheter inserted into the heart. Tissues were excised and aliquots frozen in liquid N₂ and stored at -80°C. α -Tocopherol concentrations were determined from SQ vehicle- and α -tocopherol-injected rats, as described in the methods. All values are expressed as mean $\pm$ SE, $n = 4$, with * = $p < 0.01$ as compared with vehicle-injected rats (see methods).

Figure 2a

Figure 2b

Figure 2c

Figure 2d

Figure 2e

Figure 2f

Fig. 2.

Plasma and tissue α -tocopherol concentrations in response to SQ vehicle or α -tocopherol injections. α -Tocopherol concentrations in (A) Plasma, (B) Muscle, (C) Lung, (D) Kidney, (E) Heart and (F) Adipose tissue were determined from SQ vehicle- and α -tocopherol-injected rats, as described in Fig 1 and the methods.

Figure 3a

Figure 3b

Figure 3c

Fig. 3. Intestinal tissue α -tocopherol concentrations in response to SQ vehicle and α -tocopherol injections. α -Tocopherol concentrations in (A) Duodenum, (B) Jejunum and (C) Ileum were determined from SQ vehicle- and α -tocopherol-injected rats, as described in Fig 1 and the methods.

Figure 4a

Figure 4b

Figure 4c

Fig. 4. Spinal cord α -tocopherol concentrations in response to SQ vehicle and α -tocopherol injections. α -Tocopherol concentrations in (A) Cervical, (B) Thoracic and (C) Lumbar regions of the spinal cord were determined from SQ vehicle- and α -tocopherol-injected rats, as described in Fig 1 and the methods.

Figure 5a

Figure 5b

B.

Figure 5c

Fig. 5. α -Tocopherol and α -CEHC concentrations in response to SQ vehicle or α -tocopherol injections. α -Tocopherol and α -CEHC concentrations in (A) Liver, (B) Lung and (C) Kidney were determined from SQ vehicle- and α -tocopherol-injected rats, as described in Fig 1 and the methods. Points represent α -tocopherol ($\blacktriangledown$) and α -CEHC ($\bullet$) concentrations for each individual animal. Data were analyzed and the curves generated by non-linear regression.

Figure 6a

Figure 6b

Figure 6c

Figure 6d

Fig. 6.

Lung immunoreactive protein concentrations in response to SQ vehicle and α -tocopherol injections. Lung microsomes and membrane fractions were prepared as described in the methods from SQ vehicle- and α -tocopherol-injected rats. Lung microsomal protein (50 μ g) and membrane protein (75 μ g) were used for determination of CYPs and MDR1 protein expression, respectively (see Methods). Actin was detected on each blot for use as a loading control as described in the methods. The actin-normalized protein levels of vehicle-injected rats (controls) were set to 100% and protein levels from SQ α -tocopherol-injected rats are expressed as percent of control. Quantification of the indicated protein levels by densitometry: (A) CYP3A, (B) CYP1A and (C) MDR1. Each time point represents mean $\pm$ SE of 4 rats (error bars may be smaller than the symbols). Insect microsomes expressing the individual rat CYP protein of interest were utilized as controls to confirm the identification of bands on each CYP blot. MES-SA/D $\times$ 5 cell lysates (Santa Cruz) that express MDR1 were used as a control on MDR1 blots. A representative western blot of lung MDR1 protein expression is inset in (C) with V = vehicle, 0.5, 1, 2, and 10 = α -tocopherol dose, and C = MES-SA/D $\times$ 5. (D) Correlation of MDR1 protein levels with lung vitamin E concentrations ($R^2 = 0.88$, $P < 0.001$).

Fig. 7.

Kidney CYP4F immunoreactive protein concentrations in response to SQ vehicle and α -tocopherol injections. Kidney microsomes were prepared from SQ vehicle- and α -tocopherol-injected rats, as in the methods. Kidney microsomal protein samples were analyzed by western blot, quantified by densitometry, normalized to actin expression (see methods) and are expressed as percent of control as described in figure 6. Each time point represents mean $\pm$ SE of 4 rats (error bars may be smaller than symbols). Insect microsomes expressing CYP4F protein were utilized to confirm the identification of bands.