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High-dose supplementation with natural α-tocopherol does neither alter the pharmacodynamics of atorvastatin nor its phase I metabolism in guinea pigs

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ABSTRACT

It has been hypothesized in the literature that intake of high-dosage vitamin E supplements might alter the expression of cytochrome P_{450} enzymes (CYP), particularly CYP3A4, which may lead to adverse nutrient–drug interactions. Because previously published studies reported conflicting findings, we investigated the pharmacodynamics of the lipid-lowering drug atorvastatin (ATV), a CYP3A4 substrate, in response to high-dose α-tocopherol (αT) feeding and determined protein expression and activities of relevant CYP. Groups of ten female Dunkin–Hartley guinea pigs were fed a control (5% fat) or a high-fat control diet (HFC; 21% fat, 0.15% cholesterol) or the HFC diet fortified with αT (250 mg/kg diet), ATV (300 mg/kg diet) or both ATV + αT for 6 weeks. Relative to control, HFC animals had increased serum cholesterol concentrations, which were significantly reduced by ATV. High-dose αT feeding in combination with ATV (ATV + αT), albeit not αT feeding alone (αT), significantly lowered serum cholesterol relative to HFC, but did not alter the cholesterol-lowering activity of the drug compared to the ATV treated guinea pigs. Protein expression of CYP3A4, CYP4F2, CYP20A1 and OATP C was similar in all groups. Accordingly, no differences in plasma concentrations of phase I metabolites of ATV were observed between the ATV and ATV + αT groups. In conclusion, feeding guinea pigs high-doses of αT for 6 weeks did neither alter the hepatic expression of CYP, nor the pharmacodynamics and metabolism of ATV. High-dose αT intake is thus unlikely to change the efficacy of drugs metabolized by CYP enzymes, particularly by CYP3A4.

Introduction

Even 90 years after the discovery of α-tocopherol (αT), the predominant form of vitamin E (α-, β-, γ-, δ-tocopherol and -tocotrienol) in the body, as a dietary factor required for fertility in rats (Evans and Bishop, 1922), the biological function underlying its essentiality remains unclear. Hoping to improve their health, millions of people take vitamin E supplements (Balluz et al., 2000) that often contain 10–50 times more vitamin E than the recommended daily allowance (adult women 15; adult men 12 mg/day) (Institute of Medicine, 2000). Initiated by a meta-analysis that postulated increased all cause-mortality with intake of high-dosage vitamin E supplements (≥400 I.U./day), the safety of vitamin E was questioned (Miller et al., 2005), although it had been previously documented in numerous intervention trials (reviewed in Hathcock et al. (2005)). Furthermore, most of the vitamin E-supplementation trials included in the meta-analysis were conducted in high-risk patients taking multiple drugs (Miller et al., 2005), who may thus have been at risk for adverse nutrient–drug interactions (Brigelius-Flohe, 2007).

The majority of prescription drugs are converted by phase I enzymes, such as cytochrome P_{450} (CYP); particularly the CYP isoform 3A4 is involved in the metabolism of ca. 50% of all prescription drugs (Guengerich, 1999). Depending on the character of the drug, whether it is a pro-drug or an active drug, phase I metabolism may convert the ingested drug to biologically active or inactive metabolites, respectively. Altering the expression or activity of CYP may thus change the pharmacokinetics, pharmacodynamics, and the toxicity of drugs.

In reporter gene assays, tocotrienols (5- to 10-fold), but not – or only to a very limited extent – αT (up to 2-fold), activated the pregnane X receptor, a transcription factor regulating CYP expression (Landes et al., 2003; Zhou et al., 2004). Mice fed natural RRR-αT (Kluth et al., 2005) did not, while mice fed very high concentrations of synthetic all rac-αT did have an increased hepatic mRNA expression of cyp3a11, the murine homolog of the human CYP3A4 (Mustacich et al., 2009). On the other hand, incubation of HepG2 cells with increasing concentrations (0–300 μmol/L) of either RRR- or all rac-αT for 7 days did only change the expression of the orphan CYP20A1, but none of the

Abbreviations: ATV, atorvastatin; CEHC, carboxyethyl hydroxychromanol; CYP, cytochrome P_{450}; HFC, high-fat control; OATP C, organic anion-transporting peptide C; T, tocopherol.

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other 13 CYP (including CYP3A4) expressed in these cells (Hundhausen et al., 2006). Accordingly, the hepatic mRNA of 33 CYP expressed in rats that were fed diets containing either <2 or 60 mg RRR-αT for up to 9 month did not vary with the ingested dose (Hundhausen et al., 2006).

Atorvastatin (ATV), the most commonly prescribed cholesterol-lowering agent, undergoes extensive intestinal and hepatic first pass metabolism and is converted by CYP3A4 to its two active metabolites ortho-hydroxy- and para-hydroxy-atorvastatin (Lennernas, 2003); both are in equilibrium with their inactive lactone forms (Jacobsen et al., 2000). The hydroxy-metabolites are responsible for up to 70% of the cholesterol-lowering activity of the drug, which functions by inhibiting 3-hydroxy-3-methylglutaryl-coenzyme A reductase, the rate-limiting enzyme in cholesterol biosynthesis (Lea and McTavish, 1997). The consumption of grapefruit juice, known for its high content of CYP-inhibitors, reduces the conversion of ATV to its active metabolites and thus enhances the accumulation of the parent drug, which in turn increases its toxicity (Lilja et al., 1999). Because ATV is metabolized specifically by CYP3A4, it is a good probe for investigating drug-interactions mediated by inhibition or induction of this enzyme (Feidt et al., 2010).

Previous experiments yielded inconsistent results with respect to the induction of hepatic CYP by high doses of dietary αT and none of the previous experiments quantified the activity of the putatively altered CYP. We therefore studied the pharmacodynamics and toxicity of ATV in the absence or presence of high doses of αT in a model of diet-induced hypercholesterolemia in guinea pigs. In order to appreciate if alterations in pharmacodynamics may have been caused by changes in CYP expression or activity and subsequent conversion of the drug to its active metabolites, CYP protein concentrations in the liver and intestine as well as metabolites of atorvastatin and vitamin E were quantified.

Methods

Experimental animals and diets. All animal procedures were carried out in accordance with the FELASA guidelines for the care and use of laboratory animals and approved by the regional council Stuttgart (trial no. V 279/10 BC). Fifty 3-week old female Dunkin–Hartley guinea pigs weighing 163–202 g were purchased from Harlan Laboratories GmbH (Eystrup, Germany) and housed in groups of five in rabbit cages ($W \times H \times D$, 90×80×70 cm) in a controlled environment (22 °C, 50% humidity, 12 h light/dark cycle). The cages were equipped with two water bottles, a feeding tray, a house and two table tennis balls each. The animals were given fumigated hay (4 h, 80 °C) to aid digestion and for nest-building every day except Saturday.

The animals were randomly divided into five groups of 10 animals and fed a purified control diet (protein, 18.4%) with 5% fat (3.33% milk fat, 1.67% coconut fat), or a high-fat control (HFC; protein, 18.4%) diet with 21% fat (14% milk fat, 7% coconut fat) and 0.15% cholesterol, or the HFC diet supplemented with 300 mg/kg atorvastatin (ATV), or 250 mg/kg RRR-α-tocopherol (αT), or 300 mg/kg atorvastatin plus 250 mg/kg RRR-α-tocopherol (ATV + αT; Altromin Spezialfutter GmbH + Co.KG, Lage, Germany). The dietary fat was chosen for its rich content of myristic and lauric acids, which induce atherosclerosis in the guinea pig (Conde et al., 1996). The mineral and vitamin composition of the diet was formulated to meet the nutritional requirements of guinea pigs (National Research Council, 1995). All diets contained 2500 mg vitamin C (to prevent deficiency induced reduction in CYP expression (Rikans et al., 1977)), 20 mg α-tocopherol, and 20 mg γ-tocopherol per kg before addition of the respective test compounds. The animals were ad libitum-fed the control diet for 1 week and thereafter the respective experimental diets for 6 weeks and had free access to water at all times. Body weights and food consumption were determined weekly. At the end of the experimental period, animals were anesthetized with carbon dioxide and killed by decapitation. Blood was collected into test tubes, centrifuged at 2000×*g* for 10 min to obtain lithium heparin plasma and serum or at 2500×*g* for 10 min to obtain EDTA plasma, and stored at −80 °C. Liver and muscle tissue was excised, weighed, and cut into segments that were snap-frozen in liquid nitrogen, and kept at −80 °C until further analyses.

Quantification of plasma, liver, and muscle tocopherols. The quantification of αT and γT was carried out as described before (Augustin et al., 2008) with minor modifications. Briefly, 100 μL EDTA plasma, 2 mL 1% ascorbic acid in ethanol, 900 μL water, and 25 μL BHT (1 mg/mL ethanol) were added to a glass tube and vortex-mixed. For analysis of tissue tocopherols, approximately 200 mg of liver or 50 mg of muscle was weighed into a test tube and 2 mL 1% ascorbic acid in ethanol, 900 μL water, and 300 μL saturated KOH were added. The mixture was incubated for 30 min at 70 °C, cooled on ice, 25 μL BHT (1 mg/mL ethanol), 1 mL water added and acidified with 300 μL glacial acetic acid. Next, both plasma and tissue samples were extracted by hand-inversion with 2 mL n-hexane and centrifuged (1000 rpm, 3 min) and 1 mL of the supernatant was transferred to a 2 mL reaction tube. The extraction was repeated once and the combined supernatants were evaporated until dryness in a Savant SpeedVac®. The dried extracts from plasma and muscle were resuspended in 100 μL and those from liver in 200 μL methanol:ethanol (80:20, v:v). Forty microliters were injected into a Jasco HPLC system (Jasco, Gross Umstadt, Germany) with fluorescence detection (excitation, 296 nm; emission, 325 nm). The column was a ODS-2 Spherisorb (100×4.6 mm; 3 μm) and the mobile phase was a mixture of methanol:water (90:10, v:v) delivered at a flow rate of 1.2 mL/min. The column oven was maintained at 40 °C and the autosampler cooled to 8 °C. All samples were analyzed in duplicate.

Quantification of liver carboxyethyl hydroxychromanols. For the analysis of α- and γ-carboxyethyl hydroxychromanols (CEHC; extraction modified from (Freiser and Jiang, 2009)), liver samples (ca. 500 mg) were homogenized in 1 mL PBS and 8 μL aqueous ascorbic acid (100 mg/mL), and then 2 mL methanol and 5 mL n-hexane were added. Samples were centrifuged at 3000 rpm for 3 min and the hexane fraction was discarded. A volume of the methanol–water phase (1.8 mL) was transferred to a clean tube and evaporated to dryness. Dried samples were resuspended in 100 μL water and 125 μL β-glucuronidase (3 mg/100 μL in 0.1 M acetate buffer), and incubated for 8 h at 37 °C at 300 rpm. Samples were acidified with 15 μL glacial acetic acid, vortexed, and extracted twice with 1 mL ethyl acetate, and then centrifuged (5000 rpm, 3 min). The total volume of the combined supernatants was 1.9 mL and was dried in a Savant SpeedVac®. Dried samples were resuspended in 100 μL mobile phase and 40 μL were injected into a Jasco HPLC System (Jasco, Gross Umstadt) connected to an ESA Model 5600A CoulArray Detector (ESA Inc., Chelmsford, MA, USA). The electrode potentials were set to 0, 200, 400, and 600 mV. The mobile phase consisted of 50 mM ammonium acetate and acetonitrile (78:22) and the flow rate was set to 1 mL/min. A Trentec C18 column (250×4.6 mm, 5 μm) was used for separation of analytes. The column and electrodes were maintained at 40 °C and the autosampler cooled to 8 °C. All analyses were carried out in duplicate.

Quantification of serum total and LDL cholesterol and creatine kinase. Total and LDL cholesterol and creatine kinase were measured using diagnostic kits (OSR6183, OSR6116, and OSR6179 respectively; Beckman Coulter) adapted for the Olympus AT200 auto-analyzer.

Western blot analysis. Liver and intestinal tissue (ca. 20 mg) was homogenized in RIPA buffer (Tris, 50 mM; NaCl, 150 mM; SDS, 0.1%; sodium deoxycholate, 0.5%; Triton X 100, 1%; EDTA, 20 mM (pH 7.2); dithiothreitol, 1 mM; protease inhibitor cocktail for liver and 10 mM for intestine) and the amount of protein in the supernatant was quantified according to the Bradford method (Bradford,