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Short Communication

Regulation of sheep α-TTP by dietary vitamin E and preparation of monoclonal antibody for sheep α-TTP

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ABSTRACT

α-Tocopherol transfer protein (α-TTP) is a cytosolic protein that plays an important role in regulating concentrations of plasma α-tocopherol (the most bio-active form of vitamin E). Despite the central roles that α-TTP plays in maintaining vitamin E adequacy, we have only recently proved the existence of the α-TTP gene in sheep and, for the first time, cloned its full-length cDNA. However, the study of sheep α-TTP is still in its infancy. In the present study, thirty-five local male lambs of Tan sheep with similar initial body weight were randomly divided into five groups and fed with diets supplemented with 0 (control group), 20, 100, 200, 2000 IU·sheep^{-1}·d^{-1} vitamin E for 120 days. At the end of the experiment, the plasma and liver vitamin E contents were analyzed first and then α-TTP mRNA and protein expression levels were determined by quantitative real-time PCR (qRT-PCR) and Western-blot analysis, respectively. In addition, as no sheep α-TTP antibody was available, a specific monoclonal antibody (McAb) against the ovine α-TTP protein was prepared. The effect of vitamin E supplementation was confirmed by the significant changes in the concentrations of vitamin E in the plasma and liver. As shown by qRT-PCR and Western-blot analysis, dietary vitamin E does not affect sheep α-TTP gene expression, except for high levels of vitamin E supplementation, which significantly increased expression at the protein level. Importantly, the specific sheep anti-α-TTP McAb we generated could provide optimal recognition in ELISA, Western-blot and immunohistochemistry assays, which will be a powerful tool in future studies of the biological functions of sheep α-TTP.

1. Introduction

Vitamin E was first discovered as a dietary factor necessary for reproduction in rats (Evans and Bishop, 1922). The vitamin E family is now well-known for its potent radical trapping (i.e., antioxidant) activity both in vitro and in vivo (Morley et al., 2004). In addition to such antioxidant effects, vitamin E also exerts non-antioxidant cellular activities, suggesting alternative molecular pathways for disease prevention. Indeed, the ability of vitamin E to modulate signal transduction and gene expression has been observed in numerous studies (Zingg, 2007). Our previous research revealed that supplementing 80 and 320 IU·kid^{-1}·d^{-1} vitamin E in diets can significantly stimulate the development of reproductive organs in Boer goats while no significantly improved effects were found when supplementing 880 IU·kid^{-1}·d^{-1} vitamin E (Hong et al., 2009). Vitamin E supplementation also can protect testis from damage by preoxidation in Boer goats, especially when 80 and 320 IU·kid^{-1}·d^{-1} vitamin E are supplemented in diets (Hong et al., 2010). By supplementing Aohan fine-wool sheep with 0, 20, 200, 1000 or 2400 IU·sheep^{-1}·d^{-1} vitamin E for 12 months, we found that supplementing vitamin E in concentrate can improve semen quality and ameliorate testicular cell membranal and mitochondrial antioxidant abilities, especially at 200 IU·sheep^{-1}·d^{-1} supplement concentration (Yue et al., 2010). In addition, supplementing vitamin E can have a positive role in improving testicular marker enzyme activity of Aohan fine-wool sheep, the optimum dose range being 100 to 200 IU·sheep^{-1}·d^{-1} (Yan et al., 2010). All of these findings suggest that the effects of vitamin E are not proportional to its supplemented levels, which might be due to some changes of its transport pathway. So, it is of great interest to explore how vitamin E transport is modulated in response to different dietary vitamin E supplements.

Knowledge on vitamin E absorption, intracellular trafficking, and interorgan distribution is still fragmentary, which markedly impedes our understanding of the molecular mechanisms by which vitamin E status is regulated. It is now generally accepted that all forms of vitamin E are first taken up with micelles without discrimination in the intestine and then released from enterocytes with chylomicrons into the circulation. In the plasma, triglycerides in chylomicrons are cleaved by the

Abbreviations: α-TTP, α-tocopherol transfer protein; qRT-PCR, quantitative real-time polymerase chain reaction; McAb, monoclonal antibody; AVED, ataxia with vitamin E deficiency; NRC, National Research Council; HRP, horseradish peroxidase; KLH, keyhole limpet hemocyanin; NPC1, Niemann–Pick disease type C class 1.

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endothelial cell-bound lipoprotein lipase resulting in chylomicron remnant formation. After chylomicron remnant-bound vitamin E enters the liver cells, a special protein called α-tocopherol transfer protein (α-TTP) binds α-tocopherol with high affinity and selectivity and then releases it into the plasma to maintain α-tocopherol levels in the peripheral circulation (Brigelius-Flohe, 2009).

α-TTP was first described in rat liver cytosol because it exhibited preferential binding to α-tocopherol over γ-tocopherol (Catignani, 1975). Sato et al. purified α-TTP to homogeneity from rat liver cytosol (Sato et al., 1991), and later cloned the α-TTP transcript from the same source (Sato et al., 1993). The human transcript encoding α-TTP was then cloned and mapped to the q13 region of human chromosome 8 (Arita et al., 1995). The identification of hepatic α-TTP as a critical mediator of vitamin E function came about from the fact that it binds α-tocopherol with high affinity and catalyzes its transfer between lipid membranes. On the other hand, heritable mutations in the human α-TTP gene caused ataxia with vitamin E deficiency (AVED), which shows autosomal recessive inheritance of progressive neurodegenerative symptoms (e.g., ataxia, dysarthria, loss of deep tendon reflexes), coupled with low plasma vitamin E levels (≤3 mM) (Manor and Morley, 2007).

Despite the well-documented regulatory role of α-TTP in maintaining vitamin E levels, it remains uncertain if the expression of α-TTP is affected by vitamin E intake. Kim et al. (1998) reported that increased vitamin E intake could lower α-TTP mRNA levels in rat liver while in rats that were fed vitamin E-deficient diets, α-TTP mRNA levels were higher. On the other hand, Fechner et al. (1998) found that refeeding α-tocopherol to vitamin E-depleted rats increased the expression of α-TTP about 7 fold and rat hepatic α-TTP mRNA levels were unaffected by dietary vitamin E depletion. Ulatowski et al. (2012) also showed that treatment with vitamin E caused a marked increase in the level of α-TTP mRNA. However, some other authors suggested that dietary vitamin E did not affect α-TTP gene expression (Barella et al., 2004; Shaw and Huang, 1998; Thakur et al., 2010). As to α-TTP protein regulation, the conclusions were also inconsistent. Kim et al. (1998) found that the α-TTP protein levels were lower when the vitamin E intake increased while protein levels did not change when the animals were fed a vitamin E-deficient diet. In contrast, Shaw and Huang (1998) showed that control and vitamin E-supplemented rats had similar α-TTP protein levels, but when fed vitamin E-deficient diet, the animals had lower hepatic α-TTP protein levels compared with rats fed with control diets. Different from these, Bella et al. (2006) and Mustacich et al. (2006) both reported that changes in hepatic α-TTP concentrations were minimal in response to dietary vitamin E levels and assumed that hepatic α-TTP concentrations may be at sufficient levels so that they are unaffected by modulations in dietary vitamin E. However, by using human hepatocytes, Thakur et al. (2010) suggested that vitamin E imparted a distinct conformation on α-TTP so that the protein is less susceptible to proteasomal degradation, leading to a time- and dose-dependent increase in the steady-state levels of α-TTP following treatment with vitamin E. In summary, available studies do not provide a thorough and consistent conclusion regarding the regulation of α-TTP by vitamin E supplementation.

The majority of studies that address the regulation of α-TTP levels have focused on humans and rodents, while very little is known regarding the sheep α-TTP. In our previous research we have proved the existence of the α-TTP gene in sheep and for the first time, cloned the full-length cDNA of the ovine α-TTP gene using Aohan fine-wool sheep and Tan sheep, respectively (Jia et al., 2012; Liu et al., 2012). In the present study, we report our findings regarding the regulation of sheep α-TTP by dietary vitamin E using quantitative real-time PCR (qRT-PCR) and Western-blot analysis. Monoclonal antibodies (McAb) against ovine α-TTP were also produced and used in immunohistochemical studies to analyze the sheep α-TTP expression levels under different vitamin E supplementations and to more precisely clarify its biological function.

2. Materials and methods

2.1. Animals and management

Thirty-five local male lambs of Tan sheep, 20 to 30 days after weaning with similar body weight (16.20 ± 1.65 kg), were purchased from the Nuanquan farm, Ningxia, China. They were housed individually in pens (1.1 m long, 1.0 m wide), allowed visual contacts and constant water availability. The sheep were randomly divided into five groups (n = 7) and fed with diets supplemented with 0 (control group), 20, 100, 200, 2000 IU·sheep^{-1}·d^{-1} vitamin E (treatments denoted as E0, E20, E100, E200, and E2000) for 120 days. These dose levels were 0, 1, 5, 10, and 100 times the National Research Council (NRC) feeding standard (NRC, 1985) and based on our previous research finding (Liu et al., 2012). The composition of the basic ration for this study is given in Table 1.

2.2. Tissue sampling and preparation

At the end of the experiment, blood was first collected from the jugular vein into a heparinized tube, mixed, separated by centrifugation, and the plasma was stored at −20 °C. Food was then withheld overnight and the sheep were slaughtered. The liver was immediately collected, excised, either stored at −20 °C or quick-frozen in liquid nitrogen and stored at −80 °C.

In this study, all procedures involving animals were approved by the China Agricultural University Animal Care and Use Committee.

2.3. Biochemical analysis

Vitamin E was extracted from the plasma and liver and analyzed following the instructions of a commercially available vitamin E assay kit (Institute of Biological Engineering of Nanjing Jiancheng, Nanjing, China). Briefly, in the presence of phenanthroline, Fe^{3+} is reduced to Fe^{2+} by vitamin E. Phenanthroline then forms a complex with Fe^{2+} forming a colored adduct. Vitamin E content can be calculated using the following equation:

$$\text{Vitamin E concentration} = \frac{OD_U - OD_B}{OD_S - OD_B} \times C_S \times N,$$

where ODU = absorbance of the assay tube, ODS = absorbance of the standard tube, ODB = absorbance of the blank tube, CS = standard concentration, and N = dilution ratio.

Table 1
Ingredients and nutritional composition of the experimental diet.

Diet ingredients, % (DM basis)	Ratio
Corn silage	50.00
Corn	27.81
Soybean meal	13.00
Wheat bran	4.93
Shelled sunflower meal	2.11
Sodium chloride	0.68
Calcium carbonate	0.23
Calcium hydrophosphate	0.11
Premix[a]	1.13
Nutrient composition (DM basis)	
Metabolizable energy, MJ/kg	8.95
Crude protein, %	12.30
Ether extract, %	5.08
Neutral detergent fiber, %	48.77
Acid detergent fiber, %	33.89
Calcium, %	0.51
Phosphorus, %	0.36

[a] Per kilogram of premix: 100,000 IU vitamin A, 20,000 IU vitamin D_3, 60 IU vitamin E, 1 g Fe, 1 g Mn, 0.78 g Zn, 0.27 g Cu, 0.012 g Se and 0.01 g I.