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Research Article

Effects of Vitamin A and β-Carotene Injection on Levels of Vitamin E and on Glutathione Peroxidase Activity in Pregnant Tuj Sheep

Nadide Nabil KAMİLOĞLU*, Ebru BEYTUT
Department of Physiology, Faculty of Veterinary Medicine, Kafkas University, Kars - TURKEY
Kutlay GÜRBULAK
Department of Obstetric and Gynecology, Faculty of Veterinary Medicine, Kafkas University, Kars - TURKEY
Metin ÖĞÜN
Department of Biochemistry, Faculty of Veterinary Medicine, Kafkas University, Kars - TURKEY

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Abstract: The objective of research was to study the effect of vitamin A and β-carotene injections, before breeding and during pregnancy, on the levels of vitamin E in plasma and on the activity of erythrocyte glutathione peroxidase (GSH-Px) in sheep. Thirty-two healthy female and four healthy male Tuj sheep, 4-5 years old and weighing approximately 57 kg each, were used. The female animals were divided into 4 groups with 8 sheep in each. The oestrus cycles of the ewes were synchronised by the application of sponges containing medroxyprogesterone acetate (MPA) and by the intramuscular administration of 500 IU of PMSG. Twenty-four hours after the injection of PMSG, a ram was added to each group and allowed to mate with the sheep. The first group was used as the control. 8 mg/kg of β-carotene (Group II), 200,000 IU of vitamin A (Group III) and a combination of 100,000 IU of vitamin A and 4 mg/kg of β-carotene (Group IV) were administered intramuscularly at 30-day intervals, 15 days before breeding and during pregnancy. To measure the levels of vitamin E in plasma and the activity of erythrocyte GSH-Px, blood samples were taken from the jugular vein on days 1 and 15 after injection and after the birth.

A statistically significant difference ($P < 0.001$) was observed during pregnancy in the levels of vitamin E in the plasma of Groups II and IV following injection in comparison with those of the control group. It was also observed that vitamin E levels changed in the treated groups in relation to the time of injection. We further determined that the levels of vitamin E in plasma were little affected by injections of vitamin A. Erythrocyte GSH-Px activity increased in Groups II, III and IV compared with that of the control group ($P < 0.001$). This increase was higher in the β-carotene group than in the other groups.

It was concluded that supporting aspects of the antioxidant system such as vitamin E levels and GSH-Px activity with vitamin A and β-carotene injections may prevent the undesired effects of the free radicals associated with pregnancy.

Key Words: Vitamin A, β-carotene, vitamin E, glutathione peroxidase (GSH-Px), pregnancy

Vitamin A ve β-Karoten Enjeksiyonunun Gebe Tuj Koyunlarında Vitamin E Düzeyleri ve Glutatyon Peroksidaz Aktivitesi Üzerine Etkileri

Özet: Bu araştırmada, çiftleşme öncesinde ve gebelik süresince koyunlara vitamin A ve β-karoten enjeksiyonu yapılmasının plazma vitamin E düzeyleri ile birlikte ertrosit glutatyon peraksidaz (GSH-Px) aktiviteleri üzerine etkilerinin belirlenmesi amaçlanmıştır. Bu amaçla sağlıklı 4-5 yaşlarında ve ortalama 57 kg ağırlığında 32 Tuj ırkı koyun ile 4 adet koç kullanıldı. Tüm koyunların östrusu sinkronize edildi. Bu amaçla medroksiprogesteron asetat (MPA) içeren süngerlerin uygulanmasını takiben 14 gün sonra, süngerler çıkarılarak her bir koyuna 500 IU PMSG intra muskular (i.m.) olarak enjekte edildi. PMSG enjeksiyonundan 24 saat sonra her gruba bir koç katılarak koyunlarla çiftleşitirildi. İlk grup kontrol grubu olarak değerlendirildi ve Freund's adjuvant incomplete i.m. enjekte edildi. 8 mg/kg β-karoten (II. Grup), 200,000 IU vitamin A (III. Grup) ve 100,000 IU vitamin A ve 4 mg/kg β-karoten (IV. Grup) kombinasyonu 30 gün aralıklarla çiftleşmeden 15 gün önce ve gebelik süresince i.m. olarak enjekte edildi. Plasma vitamin E düzeylerini ve eritrosit GSH-Px aktivitelerini belirlemek için kan numuneleri enjeksiyonların 1. ve 15. günleri ile doğumdan sonra V. jugularis'ten alındı.

Bu çalışmada, enjeksiyonu takiben 2. ve 4. Grupların plazma vitamin E düzeylerinde gebelik süresince kontrol grubu ile karşılaştırıldığında istatistisel olarak önemli bir farklılık ($P < 0,001$) gözlendi. Aynı zamanda vitamin E düzeylerinin enjeksiyon zamanlarına bağlı olarak değiştiğini belirledik. Ayrıca plazma vitamin E düzeylerinin vitamin A enjeksiyonları ile çok az etkilendiğini saptadık. Eritrosit GSH-Px aktivitesi II., III., ve IV. Gruplarda kontrol grubuna göre yüksek olarak belirlendi ($P < 0,001$). Bu değerler β-karoten grubunda diğer gruplardan daha yüksekti.

Sonuç olarak, vitamin A ve β-karoten enjeksiyonları ile vitamin E ve GSH-Px gibi antioksidanların desteklenmesi gebeliğe bağlı olarak oluşan serbest radikallerin istenmeyen etkilerini önleyebilir.

Anahtar Sözcükler: Vitamin A, β-karoten, vitamin E, glutatyon peroksidaz (GSH-Px), gebelik

* E-mail: nkamiloglu@hotmail.com

Introduction

Several studies (1-3) have indicated that vitamins such as vitamin A and β-carotene may potentially enhance reproduction. Hence, supplementation of these may be of importance during breeding and gestation to support reproductive performance and fertility and also to reduce the increase in physiological free radical generation which occurs with the progression of pregnancy (4). Physiological free radical generation can originate from normal metabolic reactions. Under normal conditions a balance exists between lipid peroxidation (LPO) and the antioxidant system. When cellular defence system deficiencies occur due to pregnancy, peroxides stimulate prostaglandin H synthase and this may lead to further increases in LPO (5). Therefore, increased LPO causes distribution of membrane lipids and other cell components in pregnancy. The first step in endogenous cellular defence against LPO is initiated by antioxidant enzymes, superoxide dismutase, catalase and glutathione peroxidase (GSH-Px; EC: 1.11.1.9). GSH-Px reduces hydrogen peroxide and organic hydroperoxides using glutathione (GSH) as an electron donor (6). There are also indicators that LPO may be controlled by an adequate antioxidative response in pregnancy (7). On the other hand, researchers have reported both increases and decreases in GSH-Px activity against the oxidative stress which occurs during pregnancy. Although Behne and Wolters (8) reported low erythrocyte GSH-Px activity throughout pregnancy, another study (9) found no significant difference in the GSH-Px activity of erythrocytes during pregnancy. Furthermore, little information is available as to the association between vitamin A and β-carotene administration and endogenous cellular defence mechanisms.

However, it has been reported that the bioavailability of certain lipid-soluble vitamins such as vitamin E, the best known biological chain-breaking antioxidant (6), may be affected by other lipid-soluble vitamins like vitamin A and β-carotene, a quencher of singlet oxygen and a radical-trapping antioxidant (6,10). Likewise, vitamin A metabolism has been connected with vitamin E in relation to antioxidative defence mechanisms against LPO and also to the stability of membranes (11-13). Furthermore, β-carotene, which is thought to be embedded deeply in the membrane, may enhance the antioxidant activity of vitamin E (14). It has been reported that administration of β-carotene and of a large dose of vitamin A reduces levels of vitamin E in the plasma of rats (13). Literature regarding the relationship between the administration of vitamin A, β-carotene and levels of vitamin E is rather limited. The interaction between vitamin A and β-carotene injections and vitamin E levels in pregnant sheep has not been previously documented.

Little is known about the influence of vitamin A and β-carotene injections, especially on levels of vitamin E in plasma and on the activity of erythrocyte GSH-Px during the oxidative stress induced by pregnancy. Therefore, the present study was undertaken to assess the effects of vitamin A and β-carotene injections on vitamin E levels and on GSH-Px activity in fat-tailed sheep during pregnancy.

Materials and Methods

Animals and Treatments

The experiment was carried out on 4 male and 32 female clinically healthy Tuj sheep (a fat-tailed sheep) weighing an average of approximately 57 kg (50-65) and aged 4-5. During a 14-day adaptation period, the animals were given a basic diet (Table 1) and tap water ad libitum. At the end of this period, the ewes were divided into 4 groups (8 ewes per group). However, because of health and insemination problems the number of animals in the groups was changed during the study. The oestrus cycle of the ewes in all groups was synchronised by the application of sponges containing medroxyprogesterone

Table 1. % diet compound of feed given experimental animals.

Feed	(%)
Sugar beet pulp	65.0
Dried clover	16.2
Hay	16.1
Starch	1.0
Urea	1.0
Mineral compound*	0.1
Vitamin compound**	0.5
Sodium sulphate	0.1

* in 100 kg of feed: 0.0161 g CaI_2, 1.492 g ZnO, 0.625 g CuO, 0.0217 g $CuCO_3$, 2.272 g $MnCO_3$, 225 g $FeSO_4$.

** in 100 kg of feed: 5 g AD_3E, 0.416 g B_1, 1.25 g B_2, 0.625 g B_6, 1.5 g calpan, 2 g niacin, 0.2 g B_{12}, 0.25 g biotin.

acetate (MPA), which were inserted into the vagina. The sponges were removed 14 days later and 500 IU of PMSG was intramuscularly administered to all sheep. Twenty-four hours after the PMSG injection, a ram was added to each group and allowed to mate with the sheep. The first group was used as the control. At 30-day intervals, 15 days before breeding and during pregnancy, 8 mg/kg of β-carotene (Sigma, C9750, Germany) in 2 ml of Freund's adjuvant incomplete (Sigma, F5506, Germany), 200,000 IU of (63.16 mg of all-trans-retinol (Sigma, R7632, Germany) in 2 ml of Freund's adjuvant incomplete) vitamin A and a combination of 100,000 IU of vitamin A and 4 mg/kg of β-carotene in 2 ml of Freund's adjuvant incomplete were intramuscularly administered to Groups II, III and IV, respectively. The control group was given 2 ml of Freund's adjuvant incomplete as a placebo.

Collection of Samples

To measure the levels of vitamin E in plasma and activity of erythrocyte GSH-Px, blood samples were obtained from the jugular vein on days 1 and 15 after the injections during pregnancy and after birth.

Blood was collected using heparinised vacutainer tubes. The plasma and the red blood cells (RBC's) were separated by centrifugation (2500 g, for 15 min at 4 ºC). The plasma was frozen (-20 ºC) until further analysis. The RBC's samples were washed 3 times with 0.9% sodium chloride and then haemolysed by exposure to a 9-fold volume of redistilled water followed by freezing (-20 ºC for 18 h) and thawing before analysis. Plasma specimens were used for the determination of vitamin E and the RBC's samples were used for the determination of GSH-Px.

Analytical Procedures

The tocopherol content of plasma was measured spectrophotometrically according to Kayden et al. (15). GSH-Px (EC 1.11.1.9) activity was determined using cumene hydroperoxide and reduced GSH as co-substrates and the loss of GSH following enzymic reaction was measured spectrophotometrically with Ellman's reagent at 37 ºC and 412 nm according to Lawrence and Burk (16). The haemoglobin concentration in lysed erythrocytes was determined by the cyanmethemoglobin method (17).

Statistical Analysis

Data were analysed by analysis of variance (ANOVA), using the general linear model procedure of the Statistical Analysis System (SAS Institute Inc., Cary, NC USA.) with Duncan's multiple-range test. All results were expressed as the mean ± standard deviation (SD). A P value < 0.05 was considered statistically significant.

Results

Table 2 shows the effects of vitamin A and β-carotene injections on plasma vitamin E levels. In the β-carotene and combination groups the plasma vitamin E levels of the ewes showed a significant increase on day 1 after injection ($P < 0.001$), recovering almost to previous levels on day 15. However, in the vitamin A group vitamin E levels decreased on day 1 after injection, showed less recovery on day 15 after injection and began to decrease on day 75 of pregnancy. In the β-carotene group, the levels of vitamin E were 1.75 times higher on day 15 of pregnancy, but in the combination group the levels of vitamin E were 1.57 times higher on day 45 of pregnancy. Plasma vitamin E in the control group decreased with progression of pregnancy compared to the other groups. However, vitamin E levels significantly decreased after parturition in all groups. Nonetheless, these levels remained higher in the β-carotene and the combination groups than in the control group ($P < 0.001$).

The effects of vitamin A and β-carotene injections on erythrocyte GSH-Px activity are shown in Table 3. The activity of the enzyme in all groups started to increase on day 30 of pregnancy. GSH-Px activity was higher in the β-carotene and combination groups than in the control group and remained high after day 60 of pregnancy. On the other hand, GSH-Px activity showed little increase in the vitamin A group against the controls when compared to the other groups. While erythrocyte GSH-Px activity showed no increase on day 1 after injection in the experimental groups, a significant increase was determined on day 15 after injection ($P < 0.001$). Although erythrocyte GSH-Px activity increased in the control group with the progression of pregnancy, it remained significantly higher in the experimental groups than in the control group. GSH-Px activity reached its highest value on day 120 of pregnancy in all groups. After parturition, GSH-Px activity in all groups decreased significantly, but this decrease was most apparent in the controls ($P < 0.001$).

Table 2. Levels of plasma vitamin E before pregnancy, during pregnancy and after parturition in Tuj sheep injected with vitamin A and β-carotene (μg/ml).

DAYS		GROUPS			
		Control n = 7	Vitamin A[1] n = 5	β-Carotene[2] n = 8	Combination[3] n = 5
B.G.	day 0	1.09 ± 0.04	1.08 ± 0.05	1.07 ±0.06	1.08 ± 0.05
	day 2*	1.05 ± 0.07	1.05 ± 0.06	1.36 ± 0.04**	1.25 ± 0.03**
D. G.	day 1*	1.08 ± 0.14	1.09 ± 0.06	1.17 ± 0.04	1.12 ± 0.02
	day 15*	1.03 ± 0.07	0.98 ± 0.08	1.87 ± 0.13**	1.67 ± 0.12**
	day 30	0.99 ± 0.10	1.05 ± 0.07	1.62 ± 0.13**	1.51 ± 0.02**
	day 45*	0.95 ± 0.06	0.98 ± 0.06	1.85 ± 0.13**	1.70 ± 0.04**
	day 60	0.92 ± 0.06	1.03 ± 0.06	1.50 ± 0.15**	1.58 ± 0.04**
	day 75*	0.91 ± 0.06	0.89 ± 0.05	1.83 ± 0.10**	1.49 ± 0.04**
	day 90	0.84 ± 0.06	0.90 ± 0.04	1.53 ± 0.09**	1.45 ± 0.02**
	day 105*	0.85 ± 0.04	0.85 ± 0.04	1.65 ± 0.04**	1.38 ± 0.04**
	day 120	0.78 ± 0.04	0.88 ± 0.02	1.42 ± 0.13**	1.38 ± 0.02**
	day 135*	0.69 ± 0.04	0.72 ± 0.05	1.29 ± 0.03**	1.18 ± 0.02**
A.P.		0.60 ± 0.06	0.67 ± 0.05	1.03 ± 0.04**	0.90 ± 0.02**

** : P < 0.001 B.G.: Before gestation D.G.: During gestation A.P.: 12 h after parturition, *: 1 day after injection,
[1]Sheep received an intramuscular injection of 200,000 IU of all-trans-retinol in 2 ml of Freund's adjuvant,
[2]Sheep received an intramuscular injection of 8 mg/kg of BW β-carotene in 2 ml of Freund's adjuvant,
[3]Sheep received an intramuscular injection of 4 mg/kg of BW β-carotene and 100,000 IU all-trans-retinol in 2 ml of Freund's adjuvant.

Table 3. Levels of erythrocyte $GSHP_X$ before pregnancy, during pregnancy and after parturition in Tuj Sheeps injected with vitamin A and β-carotene (U/g Hb).

DAYS		GROUPS			
		Control n = 7	Vitamin A[1] n = 5	β-Carotene[2] n = 8	Combination[3] n = 5
B.G.	day 0	16.71 ± 2.37	16.06 ± 2.35	16.51 ± 1.74	17.03 ± 6.84
	day 2*	17.51 ± 2.40	14.78 ± 1.93	16.01 ± 2.41	16.56 ± 2.79
D. G.	day 1*	19.45 ± 2.25	16.35 ± 1.75	18.39 ± 2.50	18.81 ± 2.38
	day 15*	17.34 ± 2.91	14.90 ± 2.82	14.18 ± 2.48	14.60 ± 1.30
	day 30	30.34 ± 3.76	33.66 ± 4.07	34.39 ± 4.14	35.44 ± 1.87
	day 45*	34.90 ± 1.74	38.86 ± 1.96*	39.76 ± 4.53*	38.76 ± 2.75*
	day 60	35.21 ± 4.49	37.04 ± 3.69	45.64 ± 3.92**	45.44 ± 1.87**
	day 75*	43.26 ± 2.04	43.61 ± 2.74	57.76 ± 1.72**	48.14 ± 2.60**
	day 90	46.51 ± 1.80	53.61 ± 2.74**	62.26 ± 2.36**	55.76 ± 2.50**
	day 105*	48.08 ± 2.90	52.11 ± 2.38**	67.76 ± 1.72**	56.01 ± 2.86**
	day 120	52.01 ± 2.13	56.61 ± 2.56**	68.89 ± 2.77**	58.45 ± 1.44**
	day 135*	45.51 ± 3.34	47.74 ± 3.31	55.14 ± 3.35**	51.23 ± 3.53**
A.P.		29.56 ± 3.07	32.45 ± 1.94	41.56 ± 3.54**	35.90 ± 3.47**

*: P < 0.01, **: P < 0.001 B.G.: Before gestation D.G.: During gestation A.P.: 12 h after parturition, *: 1 day after injection,
[1]Sheep received an intramuscular injection of 200,000 IU of all-trans-retinol in 2 ml of Freund's adjuvant,
[2]Sheep received an intramuscular injection of 8 mg/kg of BW β-carotene in 2 ml of Freund's adjuvant,
[3]Sheep received an intramuscular injection of 4 mg/kg of BW β-carotene and 100,000 IU all-trans-retinol in 2 ml of Freund's adjuvant.

Discussion

It has been suggested that the administration or supplementation of antioxidant vitamins is necessary to support fertility and to prevent increased oxidative stress in pregnancy (5,18). Nevertheless, it has been indicated that cells use enzymes such as GSH-Px, catalase and superoxide dismutase against the oxidative damage which occurs in pregnancy (8,9). A study by Uotila et al. (7) reported that the activity of GSH-Px increased by about 23% until the end of pregnancy. In addition, Zamora et al. (19) have shown that β-carotene may function as an antioxidant in RBCs and may increase the activity of GSH-Px. In the present study, we observed that injections of β-carotene and of a combination of vitamin A and β-carotene before breeding and during pregnancy supported the antioxidant activity of GSH-Px in sheep. The mechanism behind the increase in the activity of erythrocyte GSH-Px is not known; however, increased oxidative stress or administration of antioxidant vitamins may cause induction of the enzyme. In contrast, some research determined that GSH-Px activity did not change or was low during pregnancy (8,9). These different findings may reflect insufficient induction of the reduced GSH which has a function in the regeneration of GSH-Px activity.

β-carotene and vitamins A and E have recently attracted considerable attention in relation to the protection of cells against the free radical activity which occurs in pregnancy. In addition, our previous unpublished data show that increased oxidative stress due to pregnancy may be attenuated by vitamin A and β-carotene injections (20). These injections may also support vitamin E levels that protect the cells against oxidative damage during pregnancy. However, studies on the possible role of injected vitamin A and/or β-carotene on vitamin E levels in different species are controversial. Researchs suggest an interaction between β-carotene and vitamin E and between vitamins A and E. While the mechanism of the interaction between the lipid-soluble vitamins is not well understood, some reports indicate that vitamin A antagonises vitamin E (12,21). In addition, it is indicated that there is a significant vitamin A-β-carotene interactive effect involved in the lowering of vitamin E levels (22). Moreover, the antagonism which exists between retinol and β-carotene may produce different effects on vitamin E levels dependent upon differences in the β-carotene-retinol ratio. Differences in species, dosage levels and trial length may affect the interaction between vitamins A and E and β-carotene. Likewise, Kormann and Schlachter have shown that supplementation of a rabbit diet with β-carotene increased vitamin E levels 2-fold above those of the controls. Postaire et al. (24) have also shown that daily vitamin E and β-carotene intake increased plasma vitamin E levels. In our study, following intramuscular injection, increased levels of β-carotene in the plasma of sheep during pregnancy (20) improved the levels of vitamin E in plasma. However, supplementation with β-carotene in humans has been shown to have no effect on plasma vitamin E levels (25). The results of research into plasma vitamin E levels in pregnancy are controversial. While Aksakal et al. (26) showed that vitamin E levels decreased with the progression of pregnancy in sheep, Uotila et al. (7) observed that plasma vitamin E levels increased with the progression of pregnancy in humans. Jendryczko and Drozdz (27), however, indicated that vitamin E levels did not show any significant change with the progression of pregnancy. We observed that injections of β-carotene and of a combination of vitamin A and β-carotene significantly supported vitamin E levels in pregnancy whereas vitamin A injection had no effect on plasma vitamin E levels. These findings in relation to the interaction between vitamin A and/or β-carotene and levels of vitamin E are similar to those of previous studies (12,15).

It is concluded in this study that the injection of β-carotene and of a combination of β-carotene and vitamin A, which prolong the existence of β-carotene and vitamin A in peripheral circulation compared to dietary intake, supports vitamin E levels in plasma and GSH-Px activity in erythrocytes, whereas vitamin A injections have no effect on vitamin E levels or on GSH-Px activity.

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