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To cite this version:

A Baldi, V Bontempo, F Cheli, S Carli, C Sgoifo Rossi, et al.. Relative bioavailability of vitamin E in dairy cows following intraruminal administration of three different preparations of DL-α-tocopheryl acetate. Veterinary Research, BioMed Central, 1997, 28 (6), pp.517-524. <hal-00902499>

HAL Id: hal-00902499
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Submitted on 1 Jan 1997

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Relative bioavailability of vitamin E in dairy cows following intraruminal administration of three different preparations of DL-α-tocopheryl acetate

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(Received 16 January 1997; accepted 31 July 1997)

Summary – DL-α-tocopheryl acetate, a synthetic form of vitamin E, is routinely given as a dietary supplement to cattle. In this study we assessed the relative bioavailability of three formulations of DL-α-tocopheryl acetate in a kinetic study of plasma α-tocopherol in four Italian Friesian dairy cows, following intraruminal administration of a gelatin capsule containing 5 000 IU of DL-α-tocopheryl acetate. A Latin square design was used so that each animal received all formulations: (A) adsorbed on silica, (M) microencapsulated and (O) in oil form; 5 000 IU of DL-α-tocopheryl acetate was also administered intraperitoneally. The treatments were given following a 2-week period on a diet having no vitamin E supplementation with an interval of 8 days between each administration. Blood samples were collected at 0, 1, 10, 11, 21, 30, 48, 72, 96 and 168 h after each administration. The mean initial plasma α-tocopherol concentration (C₀) was 2.38 ± 0.57 µg/mL. Maximum plasma concentrations (Cₘₐₓ) of α-tocopherol, adjusted for pretreatment values, were 3.90 ± 0.13, 3.29 ± 0.13 and 4.07 ± 0.19 µg/mL, following administration of the A, M and O forms, respectively. The length of time required to obtain the maximum concentration (Tₘₐₓ) in plasma was 57.5 ± 7.8, 76.8 ± 8.9 and 73.1 ± 14.1 h, and the area under the curve (AUC) was 503.3 ± 63, 620.25 ± 108.5 and 465.4 ± 38.7 µg.h/mL for A, M and O forms, respectively. Administration significantly increased the plasma α-tocopherol levels in all cases; however the A and M formulations had a lower elimination rate than the O form.

vitamin E / pharmacokinetics / cow

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Résumé - Biodisponibilité relative de la vitamine E après administration par voie intraruminale de trois préparations différentes de DL-α-tocophéryl acétate chez la vache laitière. La supplémentation des aliments en vitamine E est réalisée communément chez la vache laitière par addition au régime alimentaire d’acétate de DL-α-tocopherol. Ce travail décrit la cinétique plasmatique de la vitamine E chez quatre vaches ayant reçu trois formes d’acétate de DL-α-tocophérol à la dose de 5 000 UI par voie intraruminale. Les traitements étaient : acétate de DL-α-tocophérol adsorbé sur silicate (A), microencapsulé (M) et sous forme huileuse (O). Les vaches ont reçu également 5 000 UI d’acétate de DL-α-tocophérol par voie intrapéritonéale. Les quatre vaches ont reçu chaque traitement (4 x 4). Des échantillons de sang ont été prélevés à la veine jugulaire à 0, 1, 10, 11, 21, 30, 48, 72, 96 et 168 h après administration des préparations. La concentration initiale moyenne (C₀) a été de 2,38 ± 0,57 ¡.¡g/mL. Les concentrations plasmatique maximales (Cmax) de α-tocopherol ajustées pour tenir compte des valeurs basales ont été : 3,90 ± 0,13 (A), 3,29 ± 0,13(M) et 4,07 ± 0,19(O) ¡.¡glmL. Les temps d’apparition du pic de concentration plasmatique ont été 57,5 ± 7,8, 76,8 ± 8,9 et 73,1 ± 14,1 h, et l’aire sous la courbe (AUC) 503,3 ± 63, 620,25 ± 108,5 et 465,4 ± 38,7 ¡.¡g.h/mL pour A, M et O respectivement. L’administration des trois formes de vitamine a augmenté les concentrations plasmatiques en vitamine E mais l’élimination a été plus rapide avec la formulation huileuse.

vitamine E / pharmacocinétique / vache

INTRODUCTION

In dairy cows there is evidence that vitamin E supplementation enhances the immune response (Politis et al, 1995) and plays an important role in the prevention of placental retention and other reproductive disorders (Miller et al, 1991). Decreased incidence of mastitis in the early post-partum period has been documented by several studies (Stowe et al, 1988; Weiss et al, 1990; Smith et al, 1995).

The dietary requirement of vitamin E in cattle can vary greatly depending on the feed composition, environmental conditions and the health of the animal (Herdt and Stowe, 1991). The vitamin E intake recommended by the US National Research Council (National Research Council, 1988) is 15 IU/kg dry matter, equivalent to about 150 and 300 IU/day for dry and lactating cows, respectively. However, these recommendations do not take into account the evidence that greater intake levels of vitamin E can reduce oxidative stress in tissues, promote the health of the mammary gland and improve reproductive efficiency (Smith et al, 1995).

It has been estimated that only about 30% of the oral intake level of vitamin E is absorbed in ruminants (National Research Council, 1988). Shin and Owens (1990) documented variable, but sometimes substantial, rumen degradation of liposoluble vitamins in steers, while Alderson et al (1971) reported ruminal destruction of about 40% of the ingested vitamin E in sheep fed a high grain diet.

On the other hand, Roquet et al (1992) found that plasma α-tocopherol in steers following rumen supplementation of vitamin E was higher than when they were dosed duodenally, and suggested that rumen activity helps the emulsification of liposoluble vitamins.

Since natural forms of vitamin E in feed are rapidly oxidized, and feed processing and storage further decrease dietary levels, adequate intake levels are achieved by dietary supplementation with a synthetic form of the vitamin, most commonly DL-α-tocopheryl acetate.

Hidiroglou and Charmely (1991) reported that the kind of α-tocopherol ester used as supplement (acetate or nicotinate) significantly influenced the plasma and tissue con-
centrations of α-tocopherol in sheep. In a recent study, Schelling et al (1995) showed that the physical form of the supplement can influence the amount of α-tocopherol absorption and hence its bioavailability, while Shin and Owens (1990) found that DL-α-tocopheryl acetate administered in a liquid form to adult steers had a higher rate of ruminal degradation than when it was adsorbed on silica or in spray form.

Since the rate of intestinal absorption is the first limiting step in the biological utilization of nutrients, it is important to formulate a vitamin preparation that is efficiently absorbed from the intestine. Coating techniques developed by the pharmaceutical industry are being adopted by the animal feed industry and are likely to lead to an increased variety of vitamin supplement formulations (Colombi, 1995). Consequently, it is important to evaluate the effects of different chemical and physical forms of vitamin E on its bioavailability. This is especially important for supplements intended for dairy cows, in view of the evidence that the bioavailability of this vitamin in ruminants differs from that in other species (Ingold et al, 1987).

Therefore in the present study we determined the relative bioavailability of different formulations of DL-α-tocopheryl acetate administered intraruminally to dairy cows and assessed the amount of transfer of the vitamin to the milk.

MATERIALS AND METHODS

Cows and diets

Four Italian Friesian multiparous dairy cows producing an average of 20 kg/day milk were fitted with a large rumen cannula (id 100 mm; Bar Diamond, Parma, USA). The animals were tethered in stalls and had free access to salt blocks and water. For 2 weeks before, and during the entire experimental period, they received a complete mixed diet consisting of 62% forage and 38% concentrate on a dry matter (DM) basis. The mineral and vitamin mixture (1.5% DM) supplied did not contain added vitamin E. The composition of the diet was determined on DM basis and is reported in table I. Individual feed intake was recorded.

Vitamin E and experimental design

Three different preparations of 5000 IU of DL-α-tocopheryl acetate were administered, via the rumen cannula (ir), to each cow, according to a replicated Latin square design. Preparation A was DL-α-tocopheryl acetate adsorbed onto a silica carrier (Coelho, 1991). Preparation M was DL-α-tocopheryl acetate microencapsulated in a lipido matrix (Colombi, 1995), which was a mixture of stearic and palmitic acid. Preparation O was DL-α-tocopheryl acetate dissolved in oil. Each preparation was administered as a single dose bolus in a gelatin capsule, with 8-day intervals between the administration of each preparation. Eight days after the last treatment, each animal was given a single intraperitoneal (ip) dose of 5000 IU of DL-α-tocopheryl acetate (Hurter, 1987) in 50 mL of a preparation containing 20% ethyl alcohol, 1% benzyl alcohol.

Table I. Diet: ingredients and chemical composition.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>% DM basis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haya</td>
<td>28.5</td>
</tr>
<tr>
<td>Maize silage</td>
<td>16.5</td>
</tr>
<tr>
<td>Alfalfa hay</td>
<td>16.4</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>16.4</td>
</tr>
<tr>
<td>Ground corn</td>
<td>12.4</td>
</tr>
<tr>
<td>Ground barley</td>
<td>8.3</td>
</tr>
<tr>
<td>Mineral vitamin mixture</td>
<td>1.5</td>
</tr>
</tbody>
</table>

Chemical composition

<table>
<thead>
<tr>
<th>Component</th>
<th>% DM basis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude protein (% DM)</td>
<td>14.36</td>
</tr>
<tr>
<td>Ether extract (% DM)</td>
<td>3.21</td>
</tr>
<tr>
<td>Neutral detergent Fiber (% DM)</td>
<td>43.14</td>
</tr>
<tr>
<td>Ash (% DM)</td>
<td>6.91</td>
</tr>
<tr>
<td>Vitamin E (IU/kg DM)</td>
<td>19.00</td>
</tr>
<tr>
<td>UFLb/kg DMc</td>
<td>0.88</td>
</tr>
</tbody>
</table>

*aMixed grass and leguminose; b unité foragère laitière; c according to Jarrige (1989).
and the emulsifying agent polyoxyethylene monooleate. The emulsion was prepared according to Hidiroglou (1989).

**Blood sampling and analysis**

Prior to the administration of the first vitamin E dose, the animals were fitted with a left jugular vein tygon catheter (id 1.0 mm, od 1.8 mm; Norcton Co, Akron, Ohio, USA) under local anaesthesia. Catheters were sutured to the skin and the external part taped around the neck; they were rinsed daily with saline containing heparin (250 IU/mL). Blood samples were drawn at 0, 1, 10, 11, 21, 30, 48, 72, 96 and 168 h after each administration of a vitamin E preparation. The samples were centrifuged, the plasma decanted immediately and stored at -20 °C pending analysis.

The animals were milked twice a day, and milk yield at 0, 24 and 48 h was recorded and samples taken for α-tocopherol determination. Alpha-tocopherol was determined in plasma and milk by high pressure liquid chromatography according to the methods of McMurray and Blanchflower (1979) and Hidiroglou (1989), respectively.

**Kinetic parameters and statistical analysis**

A least squares non-linear regression computer program (Easy Fit, Istituto Mario Negri, Milano, Italy) based on the Marquardt's algorithm (Marquardt, 1975) was used to fit pharmacokinetic parameters to the experimental data for each animal. The program provided estimates of the absorption ($K_a$) and elimination ($K_{el}$) rate constants, the area under the curve (AUC$_{0\rightarrow 168}$ h) and the elimination half-life ($t_{l/2(K_{el})}$). After ip or ir administration, tocopherol concentration profiles were fitted using the following equation:

$$C(t) = A (e^{-K_{el} t} - e^{-K_a t})$$

where $C(t)$ is the serum concentration at time $t$, $A$ is the intercept term, $K_a$ and $K_{el}$ are the apparent absorption and elimination rate constants following vitamin administration. Initial estimates of the parameters were obtained by the method of residuals (Gibaldi and Perrier, 1982). Observed peak plasma concentrations ($C_{max}$) were reported. Peak times ($T_{max}$) were calculated by $(\ln K_a - \ln K_{el})/(K_a - K_{el})$ (Wartak, 1983). The relative bioavailability ($F$) was calculated by dividing the AUC after ir administration by the AUC after ip injection. We chose the ip route of administration instead of the intravenous route to avoid possible shock, as suggested by Hidiroglou (1989), and because entry into the bloodstream via the lymphatic system is physiologically more similar to oral administration.

The data were analyzed by analysis of variance of main factors (vitamin treatment and period) plus their interactions using the general linear model of Statistical Analysis System Institute, Inc (1985). The initial plasma level of vitamin E ($C_0$) was included in the model as covariate if significant. Since ip administration was used to evaluate the relative bioavailability of the intraruminally administered vitamin, the pharmacokinetics of ip administration was not included in the statistical analysis.

**RESULTS**

Milk yield, feed intake (17.7 ± 1.1 kg/day) and health status of the animals did not differ between treatments throughout the experimental period.

Figure 1 shows the plasma concentration–time profiles of vitamin E following ip and ir administrations. The initial plasma α-tocopherol concentration ($C_0$) did not vary significantly with the ir preparation administered and the average value was 2.38 ± 0.57 μg/mL. After ip injection, plasma α-tocopherol concentration increased during the first 24 h to reach a maximum concentration of 9.6 ± 1.4 μg/mL, and then decreased slowly again to baseline values during the observation period. Following ir administration, plasma α-tocopherol levels increased more slowly and the maximum plasma concentration ($C_{max}$) was lower than that observed after ip injection.

Table II shows the mean values (±SEM) of the pharmacokinetic parameters obtained after ir administration of the vitamin. Since the covariance of $C_0$ values was significant
for $C_{\text{max}}$ and $C_{\text{end}}$, adjusted means for these parameters were reported in the table. A significant increase in plasma $\alpha$-tocopherol concentration was observed in all cases. However, $C_{\text{max}}$, adjusted for $C_0$, values, was significantly lower ($P < 0.01$) after the administration of vitamin E in microencapsulated form.

The rate of absorption ($K_a$) did not differ between preparations, while the rate of elimination ($K_{el}$) was significantly higher ($P < 0.05$) following treatment with prepa-

**Table II.** Pharmacokinetic parameters (mean ± SEM) of vitamin E after intraruminal administration of oil-based (O), adsorbed on silica (A) and microencapsulated (M) preparations.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>A</th>
<th>M</th>
<th>O</th>
<th>Level of significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{\text{max}}$ (µg/mL)$^a$</td>
<td>3.90 ± 0.14</td>
<td>3.29 ± 0.14</td>
<td>4.08 ± 0.21</td>
<td>$P &lt; 0.01$</td>
</tr>
<tr>
<td>$C_{\text{end}}$ (µg/mL)$^a$</td>
<td>2.55 ± 0.22</td>
<td>2.66 ± 0.22</td>
<td>2.58 ± 0.30</td>
<td>NS</td>
</tr>
<tr>
<td>$T_{\text{max}}$ (h)</td>
<td>57.5 ± 7.8</td>
<td>76.8 ± 8.9</td>
<td>73.1 ± 14.1</td>
<td>NS</td>
</tr>
<tr>
<td>$K_a$ (1/h)</td>
<td>0.038 ± 0.010</td>
<td>0.024 ± 0.010</td>
<td>0.026 ± 0.013</td>
<td>NS</td>
</tr>
<tr>
<td>$K_{el}$ (1/h)</td>
<td>0.005 ± 0.001</td>
<td>0.007 ± 0.001</td>
<td>0.008 ± 0.002</td>
<td>$P &lt; 0.05$</td>
</tr>
<tr>
<td>AUC (µg h/mL)</td>
<td>503.3 ± 63.0</td>
<td>620.25 ± 108.5</td>
<td>465.4 ± 38.7</td>
<td>NS</td>
</tr>
<tr>
<td>$F$ (%)$^b$</td>
<td>47.40 ± 4.46</td>
<td>58.40 ± 4.46</td>
<td>43.80 ± 9.11</td>
<td>NS</td>
</tr>
</tbody>
</table>

$^a$Least square means of observed data adjusted for pretreatment values; $^b$the relative bioavailability ($F$) was calculated dividing AUC after intraruminal treatment by AUC after intraperitoneal treatment (1061.35 µg.h/mL).
ration O. The different forms of α-tocopheryl acetate did not result in significantly different AUC values, although preparations A and M were characterized by a greater AUC than that of preparation O. Relative bioavailability (F) was lowest following administration of preparation O, although not significantly.

The baseline α-tocopherol concentration of milk was 0.25 ± 0.06 μg/mL. Administration of all three preparations of vitamin E significantly increased the concentration of the vitamin in milk (O = 0.33 ± 0.12 μg/mL; A = 0.44 ± 0.08 μg/mL; M = 0.48 ± 0.06 μg/mL).

DISCUSSION

The need to increase the stability of vitamin E supplements for cattle raises the problem of vitamin bioavailability following administration of these supplements. This problem is further complicated by indications that the optimum dietary level for this vitamin, in dairy cattle, may be higher than once thought and evidence that intestinal absorption differs in ruminants from that in many other species. There is therefore a need to establish methodologies for accurately establishing dietary need and absorption. Alpha-tocopherol concentrations in blood, tissues and milk are used as indicators of dietary supply in many animal species (Njeru et al, 1994). Toutain et al (1995) showed that plasma AUC is a reliable predictor of tocopherol levels in tissues. Initial α-tocopherol levels in the plasma of our animals were similar to those reported by Hidiroglou et al (1989) in lactating dairy cows and to those of Jensen and Nielsen (1996) for lactating cows given a diet deficient in vitamin E, but were lower than the 3.5 μg/mL, minimum value considered by Smith et al (1995) to indicate that supplementation is adequate for maintaining health and milk production in lactating dairy cows. This was expected since the animals used in our study received no vitamin supplement for the 2 weeks before the beginning of the experiment.

Intraperitoneal administration of DL-α-tocopheryl acetate has been shown to be an efficient method for introducing α-tocopherol into the bloodstream of ruminants (Hidiroglou, 1989; Toutain et al, 1995). The compound is readily deacetylated in the peritoneum prior to entering easily into the bloodstream via the lymphatic system. The plasma profile we observed following ip administration was similar to that observed by others using this route (Hidiroglou and Charmley, 1991).

Comparison of our experimental C_{max} values shows that the plasma peak was higher following administration of the oil-based preparation of vitamin E than with the microencapsulated preparation, whereas the peak times were comparable. However, with all the three preparations the plasma tocopherol concentration reached a plateau close to C_{max} after approximately 20 h, which persisted until approximately 90 h after administration, and slowly decreased again to the pretreatment values (fig 1). This behaviour, which has been reported by others (Hidiroglou and Singh, 1991), indicates that the absorption time for vitamin E in ruminants is much longer than in other species. Hidiroglou et al (1994) found a biphasic disappearance curve of tocopherol from the rumen, after ir vitamin E administration in sheep and suggested that vitamin E undergoes a mixing process in the rumen before reaching its site of absorption in the gut.

Roquet et al (1995) suggested that following intraruminal administration of the acetyl ester of α-tocopherol, the substance must be hydrolysed to the free alcohol by pancreatic lipases and emulsified before absorption can take place. However, Shin and Owens (1990) reported that vitamin E is partially destroyed in the rumen and that the extent of inactivation also depends on the
physical preparation. According to these authors, the oil-based vitamin E would be more susceptible to enzymatic digestion and breakdown in the rumen than the two other preparations we administered.

The AUC values we found were similar to those reported by Hidiroglou and Charmley (1991) following i.r administration of α-tocopheryl acetate to sheep, and showed that the relative bioavailability of all the preparations we tested was similar. The differences between the preparations point to a longer persistence of the vitamin in blood following administration of the adsorbed and microencapsulated preparations than occurs with the oil-based form. AUC and relative bioavailability were lower with the oil-based preparation, even if not significantly. Using a different experimental design, Shin and Owen (1990) found similar availability following administration of adsorbed α-tocopheryl acetate to steers.

Pumfrey et al (1993) estimated that vitamin E is inefficiently transferred to the milk of dairy cows; however little detailed information has been published on the relation between dietary and plasma vitamin E levels and levels in milk. The milk levels of the vitamin we found are similar to those observed by Hidiroglou (1989), and comparable to those recorded by Charmley et al (1993) using a higher supplementation of vitamin E. Administration of the adsorbed and microencapsulated preparations resulted in higher milk levels of the vitamin than administration of preparation O, but again the differences were not significant.

In conclusion, we assessed different vitamin E preparations by kinetic methods in dairy cows and found that DL-α-tocopheryl acetate adsorbed on the surface of silica (preparation A) or microencapsulated in a lipid matrix (preparation M) resulted in different pharmacokinetic profiles and greater, although not significant, relative bioavailability of α-tocopherol than an oil-based preparation (O) when administered intraruminally to lactating cows. Estimation of vitamin E bioavailability by studying plasma kinetics can be useful for predicting the efficiency of absorption of different preparations of the vitamin and can lead to increased understanding of the behaviour of different forms of a vitamin in relation to their storage characteristics.

ACKNOWLEDGMENTS

This research was supported by the grant MURST 60%. The authors are grateful to S Gianazza and Crippsar Italiana Srl.

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