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Pl Toutain, Mp Laurentie, N Hidiroglou, M Hidiroglou. Vitamin E kinetics after intraperitoneal administration of DL- α -tocopherol and DL- α -tocopherol acetate in sheep. *Annales de Recherches Vétérinaires*, 1992, 23 (2), pp.117-130. hal-00902054

HAL Id: hal-00902054

<https://hal.science/hal-00902054v1>

Submitted on 11 May 2020

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Vitamin E kinetics after intraperitoneal administration of DL- α -tocopherol and DL- α -tocopherol acetate in sheep

PL Toutain 1*, MP Laurentie 1, N Hidiroglou 2, M Hidiroglou 3

¹ *École Nationale Vétérinaire de Toulouse, Département de Physiopathologie, F 31076 Toulouse, France;*

² *East Tennessee University, Department of Surgery, Johnson City, TN, USA;*

³ *Animal Research Centre, Agriculture Canada, Ottawa, Ontario, K1A0C6, Canada*

(Received 15 October 1991; accepted 17 January 1992)

Summary — The disposition of radiotocopherol after intraperitoneal administration of ¹⁴C-labelled-DL- α -tocopherol (1 μ Ci/kg bw, dose 1) and ³H-labelled-DL- α -tocopherol (4 μ Ci/kg bw dose 4) and of D- α -tocopherol, after intraperitoneal administration of DL- α -tocopherol acetate (100 mg/kg bw) was investigated in sheep. Plasma samples were taken at regular intervals after dosing and assayed for radioactivity and D- α -tocopherol. Plasma profiles were modeled using a compartmental approach and the input entry rate (for radioactivity) was identified using a numerical deconvolution method. Based on plasma specific activity (dose 1 and dose 4) and D- α -tocopherol plasma concentration (unlabelled α -tocopherol acetate), half-lives were not significantly different (99.6 ± 28.3 h, 121.3 ± 38.5 h and 75.0 ± 49.75 h after dose 1, dose 4 and unlabelled α -tocopherol respectively). The times to reach maximal plasma concentrations were similar for the 3 test articles (mean values ranging from 21.5 to 26.7 h). The only significant difference was observed for the apparent volume of distribution (with respect to bioavailability) which was much larger for the unlabelled test article. Deconvolution study of plasma specific activity showed: i), that the maximal input rate was reached only after a short delay (3 to 12 h); ii), that half of the activity was absorbed after a delay of 38.13 ± 16.76 h (dose 1) and 44.3 ± 6.50 h (dose 4); and iii), that 90% of the activity was absorbed after 151.2 ± 27.3 h (dose 1) and 162.3 ± 11.2 (dose 4). It is concluded that the intraperitoneal route is of interest for α -tocopherol administration, but that more information is required to determine the exact process of absorption.

α -tocopherol / pharmacokinetics / deconvolution / intraperitoneal route / sheep

Résumé — Pharmacocinétique de la vitamine E après une administration de DL- α -tocophérol et d'acétate de DL- α -tocophérol chez le mouton. La disposition du tocophérol marquée après une administration intrapéritonéale du ¹⁴C-DL- α -tocophérol (1 μ Ci/kg, dose 1) et de ³H-DL- α -tocophérol (4 μ Ci/kg, dose 4) avec du D- α -tocophérol et d'acétate de DL- α -tocophérol (100 mg/kg) a été étudiée chez le mouton; des prélèvements sanguins ont été régulièrement obtenus et analysés pour mesurer leur radioactivité et les concentrations de D- α -tocophérol. Les cinétiques plasmatiques ont été modélisées avec une approche compartimentale et la vitesse d'entrée du tocophérol (radioactivité) identifiée avec une méthode de déconvolution numérique. En se basant sur l'activité spécifique du plasma (doses 1 à 4) et les concentrations en D- α -tocophérol froid, les temps de demi-vie n'ont pas

* Correspondence and reprints

été significativement différents ($99,6 \pm 28,3$ h, $121,3 \pm 38,5$ h et $75,0 \pm 49,75$ h après respectivement les doses 1, 4 et le tocophérol froid). Le temps d'occurrence des concentrations plasmatiques maximales a été similaire pour les 3 formulations (de 21,5 à 26,7 h). La seule différence significative a été le volume de distribution (compte non tenu de la biodisponibilité) qui a été beaucoup plus large pour le tocophérol froid. L'étude par déconvolution de l'activité spécifique du plasma a montré (i) que la vitesse maximale d'arrivée de la molécule dans le plasma était rapidement atteinte (3-12 h) (ii) que la moitié de l'activité était absorbée après un délai de $38,13 \pm 16,76$ h (dose 1) et $44,3 \pm 6,5$ h (dose 4) et (iii) que 90% de l'activité était absorbée après un délai de $151,2 \pm 27,3$ h (dose 1) et $162,3 \pm 11,2$ (dose 4). Il est conclu que la voie intrapéritonéale mérite d'être prise en considération pour l'administration de l' α -tocophérol.

α -tocophérol / administration intrapéritonéale / pharmacocinétique / déconvolution

INTRODUCTION

Vitamin E is largely used to prevent muscular dystrophy in different animal species including ruminants (Hidiroglou and Karpinski, 1987). Vitamin E can be administered in the form of D or DL- α -tocopherol, or as the respective acetates or acid succinates: D- α -tocopherol is the active form but the acetate or succinate esters are generally preferred in therapeutics due to their greater stability to oxidation. The *in vivo* performance of a formulation is related to its ability to release the active moiety (*ie* D- α -tocopherol) in the systemic circulation. In this regard the administration route can influence both absorption and hydrolysis rates of an ester prodrug. In sheep, after administration of [3 H]-labelled D- α -tocopherol, the absolute availabilities were only 27.7, 21.0 and 51.0% for the oral, intraruminal and intramuscular (IM) routes respectively (Hidiroglou and Karpinski, 1987). In veterinary medicine, the IM route is the most often used for drug administration. After IM administration of an aqueous solution of α -tocopherol to sheep, the plasma peak of α -tocopherol occurred within 24 h; then elimination occurred relatively rapidly over the following days (Hidiroglou *et al*, 1970). In contrast, after IM administration of an oily formulation (2 000 IU), the peak plasma concentration was observed

after more than 24 h and concentrations of therapeutic value were maintained for several weeks (Doncon and Steele, 1988). However, oily formulations have the disadvantage of causing fibrotic lesions at the injection site and high concentrations of tocopherol were present in the local lymph glands after administration (Dickson *et al*, 1986). As for all oily formulations, sequestration of oily particulates in the lung must be expected after administration of vitamin E in an oily vehicle.

In an effort to select both an efficient and a safe means of vitamin E administration, the intraperitoneal (IP) route was tested. In sheep, after IP administration of DL- α -tocopherol acetate (100 mg/kg), D- α -tocopherol concentration in plasma increased rapidly and peaked at 32 h post-dosing (Hidiroglou and Charmley, 1990). In this assay, the relative availability of tocopherol was shown to be 1.2 times higher for the IP than for the intraruminal route. In the cow, it has been shown that IP administration of DL- α -tocopherol is an effective way to increase both plasma and milk vitamin E concentrations (Hidiroglou and Atwal, 1989). After IP drug administration, the process of absorption can be rather complex with lymphatic drainage, direct absorption through capillaries, or portal system drainage. The purpose of the present investigation was to qualify the

pharmacokinetic profile of plasma vitamin E following IP administration of DL- α -tocopherol in sheep, with special emphasis on the process of absorption.

MATERIALS AND METHODS

Animals

Fifteen crossbred (Finnish Landrace x Shropshire x Suffolk) wethers weighing about 45 kg were used. All animals originated from the same flock and were born and raised in confinement at the Animal Research Centre (Canaja). Before and during the experiments, the sheep were fed on a diet *ad libitum* consisting of (g/kg): grass silage, 400; hay, 400; maize silage, 200. About 10 days before dosing, they were placed in metabolism cages. Four or 5 animals were randomly assigned to each of the 3 groups.

Administration and sampling

Every week 2 sheep from each group were assigned at random to the experiment, which consisted of the intraperitoneal (IP) administration of [^{14}C]-DL- α -tocopherol (1 $\mu\text{Ci}/\text{kg}$ bw, dose 1) and [^3H]-DL- α -tocopherol (4 $\mu\text{Ci}/\text{kg}$ bw, dose 4) and unlabelled DL- α -tocopherol acetate (100 mg/kg bw) prepared according to a previously described technique (Hurter, 1987). Labelled tocopherol was administered in emulsion (Hidioglou and Karpinski, 1988). Blood samples (10 ml) were taken from the left jugular vein at 1, 3, 6, 10 and 30 min, and 1, 2, 3, 4, 5, 6, 7, 24, 32, 48 h and then regularly up until 232 h post-injection (labelled tocopherol) or 320 h (unlabelled tocopherol acetate). Blood was collected in heparinized tubes and centrifuged. The plasma was removed and stored at -20°C until analysed.

Test article

[^{14}C]-DL- α -tocopherol (specific activity 93.7 $\mu\text{Ci}/\text{mg}$) was obtained from Hoffman-La-Roche, Basel, Switzerland. [^3H]-DL- α -tocopherol (specific

activity 92.5 mCi/mg or 39.8 Ci/mmol) was purchased from Amersham International plc, Amersham, Bucks; its purity as determined by thin layer chromatography on silica gel in chloroform (95%), hexane-chloroform-acetic acid: 70-30-0.5 (94%), benzene (94%), was not less than 95%. Unlabelled DL- α -tocopherol acetate was purchased from Sigma, St Louis, MO, USA.

Analytical methods

D- α -tocopherol plasma concentrations were determined by high performance liquid chromatography (HPLC) (McMurray and Blanchflower, 1979) using a Perkin-Elmer 650-105 fluorescent detector. Identification and quantitative determination of α -tocopherol was made by comparison of retention times and peak areas with standard D- α -tocopherol. All samples were assayed in duplicate.

Radioactivity analysis

Triplicate samples (0.5 ml) of plasma were assayed for total radioactivity in 10 ml of a phase combined system (Beckman 3801) with aquasol 2 scintillation fluid (New England Nuclear, Boston, MA, USA). All samples were counted in a Beckman LS 280 liquid scintillation spectrometer (Beckman Instruments Ltd, Fullerton, Ca, USA) using an external standard. The counting efficiency was $40 \pm 3\%$ (SD) for [^3H] and 95 ± 2 for [^{14}C] respectively. The counts were corrected for background and quenching effects. All results were converted into disintegrations per min (dpm).

Non-compartmental and compartmental analyses

The mean residence time (*MRT*), *ie* the time for a molecule to transit in the system was calculated by a log-linear trapezoidal method (Proost, 1985) with extrapolation to infinite time. The terminal rate constant necessary for an accurate calculation of different moments was obtained by linear regression on the last points.

Data (specific activity) were analysed according to 3 different models: i), a monocompartmental model with a first-order rate constant of absorption (model I); ii), a monocompartmental model with a discontinuous first-order rate constant of absorption (model II); iii), a monocompartmental model with a discontinuous zero-order rate constant of absorption (model III).

For model I, equation 1 was used to fit the data:

$$C = \frac{BoK_a}{(K_a - K_{10})} [e^{-k_{10}t} - e^{-k_a t}] \quad [1]$$

Where C is DL- α -tocopherol plasma specific activity (dpm/ μ g) at time t ; K_{10} (h^{-1}) is the apparent first-order rate constant of elimination; K_a (h^{-1}) is the apparent first-order rate constant of absorption; $BoK_a/K_a - K_{10}$ (dpm/ μ g) is the intercept and the base of natural logarithm. The parameters identified were Bo , K_a and K_{10} .

For model II, equation 1 was used to fit the data up until a time (t^*) at which absorption ceased. After this delay, equation 2 was used to fit the data:

$$C = \frac{BoK_a}{K_a - K_{10}} [e^{-k_{10}t^*} - e^{-k_a t^*}] \cdot e^{-k_{10}(t-t^*)} \quad [2]$$

with $Bo - K_a/K_a - K_{10}$, K_a and K_{10} as previously defined. Parameters which were identified were Bo , K_a , K_{10} and t^* .

For model III, equations 3 and 4 were used to fit the data before and after the time (t^*) at which the zero-order absorption ceased respectively:

$$C = \frac{Bo}{K_{10}t} [1 - e^{-k_{10}t}] \quad [3]$$

$$C = \frac{Bo}{K_{10}t^*} [1 - e^{-k_{10}t^*}] \cdot e^{-k_{10}(t-t^*)} \quad [4]$$

Where Bo (dpm/ μ g) is the available dose (dpm/kg) divided by the pool (μ g/kg) of unlabelled α -tocopherol and K_{10} is as previously defined. The parameters identified were Bo , K_{10} and t^* .

Initial estimates of the different parameters were obtained by the method of residuals (Gibaldi and Perrier, 1982). The same initial esti-

mates were used for models II and III (K_{10}); t^* was estimated graphically. The initial estimates were refined using an extended non-linear least-square regression using a program adapted from Multi (Yamaoka *et al*, 1981). In this method, data points were weighed by their variance. The variance was modeled according to a power model with the exponent being estimated simultaneously with the structure parameters of the model (Peck *et al*, 1984).

Comparisons of models were based upon Akaike's information criterion (Yamaoka *et al*, 1978a). Model I was selected based on this criterion. Accordingly, in the *Results* section only parameters derived from model I are presented.

The time (T_{max}) to reach maximum plasma concentration (C_{max}) after administration was calculated by equation 5:

$$T_{max} = \text{Ln} (K_a/K_{10})/K_a - K_{10} \quad [5]$$

C_{max} was calculated by replacing t by T_{max} in equation 1.

The terminal half-life ($t_{1/2}$ K_{10}) was calculated by equation 6:

$$t_{1/2} K_{10} = 0.693/K_{10} \quad [6]$$

The apparent volume of the central compartment (Vc/F) was obtained with equation 7:

$$Vc/F = Bo/D\text{-}\alpha\text{-tocopherol level} \quad [7]$$

Where Vc is the volume of the central compartment; F is the unknown bioavailability; the mean control D- α -tocopherol plasma level was estimated by the trapezoidal rule over the entire sampling session.

The time-course of D- α -tocopherol plasma concentrations following DL- α -tocopherol acetate administration were analysed with the same model as that for specific activity. To take into account the presence of plasma DL- α -tocopherol before administration, a supplementary parameter (C_0) corresponding to the D- α -tocopherol plasma concentration control value was incorporated in equations 1-4 and estimated. Due to the relatively low plasma concentration values achieved after the IP administration, the estimate of the baseline value may have a profound effect on the concurrent estimate of the slope of the last phase. In order to avoid a bias, a Bayesian constraint was used to estimate the base-

line. The mean and SD of the baseline ($1.139 \pm 0.406 \mu\text{g/ml}$) were known *a priori* from the 9 sheep which were dosed with labelled DL- α -tocopherol. This information was used to influence the baseline estimate by adding a penalty to the objective function (OF) which is to be minimized (equation 8).

$$OF = \sum_{i=1}^n \frac{(y_o - y)^2}{V_1} + \text{Ln } V_1 + \frac{(C_o - C)^2}{V_2} + \text{Ln } V_2 \quad [8]$$

Where y_o is the observed value of the dependent variable, y is the predicted value of the dependent variable, V_1 is the predicted variance of the predicted observation, C is the current baseline estimate, and C_o and V_2 are the *a priori* mean and variance of the population parameters. It should be noted that the penalty is not taken into account in the computation of the final log likelihood.

Deconvolution analysis

The instantaneous absorption rate of tocopherol after IP administration of [^{14}C] or [^3H] labelled DL- α -tocopherol was tentatively estimated using a method of discrete deconvolution as previously described (Toutain *et al.*, 1988). Briefly, a deconvolution analysis is based on modeling the disposition kinetics of tocopherol as a linear and time invariant (stationary system). Labelled-tocopherol absorption was modeled by the following convolution integral (equation 9):

$$C(t) = \int_0^t F(t - \tau) S(\tau) d(\tau) \quad [9]$$

Where $C(t)$ is the observed plasma specific activity of tocopherol at any time t , $S(\tau)$ is an unknown function (*ie* the input rate which is to be calculated), $F(t)$ is the response function to a unit impulse and τ is the integration variable. For $F(t)$, mean parameters obtained in another experiment (Karpinski *et al.* Hidiroglou, 1990) were used. In the present experiment [^3H] or [^{14}C]D- α -tocopherol specific activity was modeled according to a 3-open compartment model with elimination from the central compartment; specific activity was described by a triexponential equation of the form (equation 10):

$$C(t) = Y_1 e^{-\lambda_1 t} + Y_2 e^{-\lambda_2 t} + Y_3 e^{-\lambda_3 t} \quad [10]$$

Where $C(t)$ is the specific activity at time t , Y_1 , Y_2 and Y_3 are the coefficients of exponential terms and λ_1 , λ_2 and λ_3 are exponents. The mean values of these different parameters were obtained from 25 sheep: $Y_1 = 105\,347 \text{ dpm}/\mu\text{g}$, $Y_2 = 27\,902.2 \text{ dpm}/\mu\text{g}$, $Y_3 = 3\,973.8 \text{ dpm}/\mu\text{g}$, $\lambda_1 = 1.562 \text{ h}^{-1}$, $\lambda_2 = 0.06170 \text{ h}^{-1}$ and $\lambda_3 = 0.00598 \text{ h}^{-1}$. These values were estimated from Karpinski and Hidiroglou (1990).

Deconvolution is an ill-defined problem due to measurement error in $C(t)$. To improve the analysis, raw data (plasma specific activity after IP administration) were smoothed with an unweighted least-square spline function (subroutine E0 2BAF from the NAG library, document 135470).

Statistics

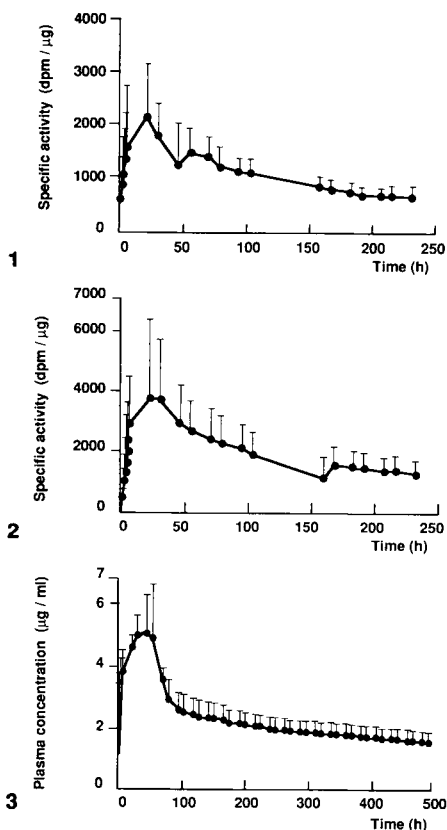
Arithmetic means and standard deviations were calculated. For half-lives, harmonic means were calculated, and variance was estimated using a jackknife technique (Lam *et al.*, 1985). Statistical comparisons were made using either Student's *t*-test or 1-way analysis of variance (ANOVA). Comparison among groups was carried out with the Newman-Keuls test.

RESULTS

Non compartmental analysis (labelled tocopherol)

The mean plasma specific activity (dpm/ μg) or concentrations ($\mu\text{g/ml}$) after IP administration of [^{14}C]DL- α -tocopherol (1 $\mu\text{Ci/kg}$ bw; dose 1), [^3H]DL- α -tocopherol (4 $\mu\text{Ci/kg}$ bw; dose 4) and unlabelled DL- α -tocopherol acetate (100 mg/kg bw) are shown in figures 1, 2 and 3 respectively.

After administration of labelled tocopherol, specific activity increased progressively to reach an observed peak value of $2\,127 \pm 1\,002 \text{ dpm}/\mu\text{g}$ (dose 1) and $4\,112 \pm 2\,534 \text{ dpm}/\mu\text{g}$ (dose 4) after a delay of $26.0 \pm 4.0 \text{ h}$ and $33.6 \pm 8.8 \text{ h}$ post-injection



Figs 1–3. 1. Mean \pm SD profile of observed plasma specific activity after intraperitoneal administration of [^{14}C]D- α -tocopherol (1 $\mu\text{Ci}/\text{kg}$ bw) to 4 sheep. 2. Mean \pm SD profile of observed plasma specific activity after intraperitoneal administration of [^3H]DL- α -tocopherol (4 $\mu\text{Ci}/\text{kg}$ bw) to 5 sheep. 3. Mean \pm SD profile of observed plasma D- α -tocopherol concentrations after intraperitoneal administration of DL- α -tocopherol acetate (100 mg/kg bw) to 5 sheep.

respectively. Thereafter, concentrations decreased slowly up until the last sampling time (232 h post injection). Just before administration of unlabelled DL- α -tocopherol acetate the mean control plasma D- α -

tocopherol concentration for the 5 sheep was 1.30 ± 0.157 mg/ml. After unlabelled tocopherol administration, plasma tocopherol increased progressively to reach an observed peak value of 5.53 ± 1.14 $\mu\text{g}/\text{ml}$ after a delay of 38.4 ± 13.2 h. Thereafter, plasma concentrations decreased progressively to return to baseline values after a delay which was not possible to determine graphically.

The *MRTs* of specific activity were 195 ± 59.6 h (dose 1) and 212 ± 45.9 h (dose 4); the difference was not significant (Student's *t*-test; $P > 0.05$). Due to the presence of baseline values, the *MRT* of D- α -tocopherol after administration of DL- α -tocopherol acetate was not calculable by statistical moments.

Compartmental modeling

Based upon Akaike's information criterion, none of the tested models was shown to be systematically superior to the others. Nevertheless, for simplicity and to favor comparison with other or further studies, we selected the most conventional model, *ie* the monocompartmental model with a first-order rate constant of absorption. Accordingly, the apparent half-time of elimination was 99.6 ± 28.3 h (dose 1) and 121.3 ± 38.5 h (dose 4). After administration of the unlabelled DL- α -tocopherol acetate, the terminal half-life of D- α -tocopherol was 75.0 ± 49.7 h; differences between labelled and unlabelled tocopherol were not significant ($P > 0.05$, ANOVA). The apparent half-life of absorption was estimated to 4.35 ± 5.50 (dose 1), 4.10 ± 1.35 (dose 4) and 5.7 ± 3.2 h (unlabelled 100 mg/kg); differences were not significant ($P > 0.05$, ANOVA). T_{max} was 26.7 ± 11.57 h (dose 1), 21.5 ± 3.34 h (dose 4) and 25.3 ± 5.61 (100 mg/kg); differences were not signifi-

cant ($P > 0.05$, ANOVA). The volume of distribution (with respect to bioavailability) was 1.3 ± 0.57 l/kg (dose 1) 1.9 ± 0.48 l/kg (dose 4) and 13.68 ± 3.39 l/kg (100 mg/kg). This last value was significantly higher than the 2 others ($P < 0.01$, ANOVA and Newman-Keuls test).

Individual values of the different parameters are given in tables I, II and III for dose 1, dose 4 and the 100 mg/kg dose, respectively. Figures 4, 5 and 6 show the goodness of fit for a representative sheep for each of the 3 treatments.

Deconvolution analysis

Data obtained with labelled tocopherol were subjected to a deconvolution analysis. Figure 7 shows the time-development of the input process for dose 1 in a repre-

sentative sheep. For dose 1 ($2.2 \cdot 10^6$ dpm/kg) the initial input rate in the central compartment was $39\,241 \pm 39\,741$ dpm/kg/h, and increased rapidly to reach a maximum value of $57\,995 \pm 35\,883$ dpm/kg/h after a delay ranging from 3 to 12 h; then the input rate decreased rapidly. Half of the activity was absorbed after 38.13 ± 16.76 h and at that time, the input rate was $19\,546 \pm 10\,528$ dpm/kg/h; 90% of the activity was absorbed after 151.2 ± 27.3 h and, at that time the input rate was $3\,471.7 \pm 1\,238.2$ dpm/kg/h. The calculated amount resorbed was $2\,361\,000 \pm 712\,800$ dpm/kg, a value very close to the administered dose ($2.2 \cdot 10^6$ dpm/kg). Individual values are given in table IV. After dose 4 ($8.8 \cdot 10^6$ dpm/kg), the initial input rate was $76\,653 \pm 35\,312$ dpm/kg/h, the maximum input rate ($108\,273 \pm 62\,261$ dpm/kg/h) was observed after a delay ranging from less than 3 to 9 h. Half of the activity was absorbed after 44.3 ± 6.5 h

Table I. Pharmacokinetic parameters describing the disposition kinetics of DL- α -tocopherol specific activity in plasma after intraperitoneal administration of [^{14}C]DL- α -tocopherol ($1 \mu\text{Ci/kg}$ ie $2.2 \cdot 10^6$ dpm/kg/BW) to 4 sheep (monocompartmental model with a first-order rate constant of absorption).

Parameters (units)	Sheep (No)				Mean \pm SD
	1	2	3	4	
B_0 (dpm/ μg)	1 179.3	3 033.2	2 713.1	1 916.0	2 210.4 \pm 832.6
V_c/F (ml/kg)	2 148.6	930.3	1 278.6	939.6	1 324.3 \pm 572.9
K_a (h^{-1})	0.0981	0.358	0.118	0.0630	0.159 \pm 0.134
$t_{1/2} K_a$ (h)	7.06	1.94	5.9	11.0	4.35 \pm 5.50 ^a
K_{10} (h^{-1})	0.00415	0.00831	0.00669	0.00867	0.00696 \pm 0.00206
$t_{1/2} K_{10}$ (h)	167.0	83.3	103.5	79.9	99.6 \pm 28.3 ^a
MRT (0-inf) (h)	271.9	148.8	212.0	146.8	194.9 \pm 59.61
C_{max} (dpm/ μg)	1 025.62	2 773.6	2 283.6	1 394.7	1 869.38 \pm 801.34
T_{max} (h)	33.66	10.76	25.74	36.62	26.70 \pm 11.57

B_0 $K_a/K_a - K_{10}$: intercept; V_c/F : apparent volume of distribution; K_a : first-order rate constant of absorption; K_{10} : first-order rate constant of elimination; $t_{1/2} K_{10}$: half-life; C_{max} : maximum plasma concentration; T_{max} : time to reach C_{max} ; MRT (0-inf): mean residence time calculated by trapezoidal rule from time 0 to infinity. ^a Harmonic mean.

Table II. Pharmacokinetic parameters describing the disposition kinetics of DL- α -tocopherol specific activity in plasma after intraperitoneal administration of [^3H]DL- α -tocopherol ($4 \mu\text{Ci/kg}$, *ie* $8.8 \cdot 10^6$ dpm/kg bw) to 5 sheep (monocompartmental model with a first-order rate constant of absorption).

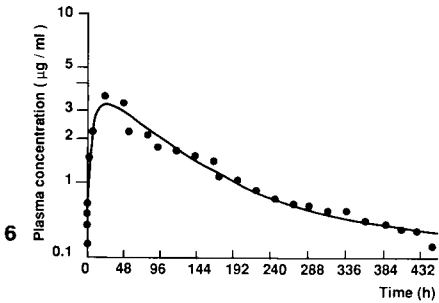
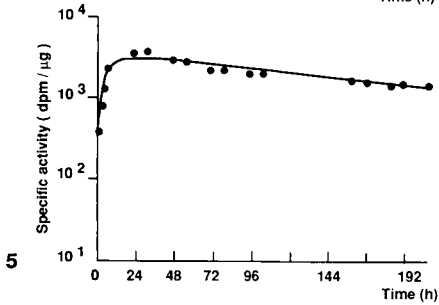
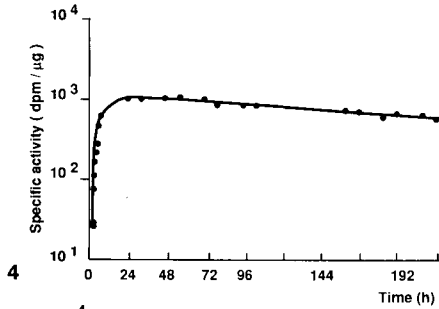
Parameters (units)	Sheep (No)					Mean \pm SD
	5	6	7	8	9	
B_0 (dpm/ μg)	3 544.6	1 916.5	6 948.3	2 318.9	4 623.9	3 870.4 \pm 2 023.1
V_c/F (l/kg)	1 900.6	2 496.0	1 248.7	2 276.1	1 704.0	1 925.1 \pm 488.9
K_a (h^{-1})	0.1285	0.2527	0.1567	0.1570	0.1446	0.1679 \pm 0.0488
$t_{1/2} K_a$ (h)	5.4	2.7	4.4	4.4	4.8	4.10 \pm 1.35 ^a
K_{10} (h^{-1})	0.00519	0.00363	0.00748	0.00651	0.00577	0.005 72 \pm 0.00145
$t_{1/2} K_{10}$ (h)	133.6	190.9	92.6	106.4	120.2	121.3 \pm 38.5a
MRT (0-inf) (h)	176.6	290.5	189.8	189.2	213.4	211.9 \pm 45.90
C_{max} (dpm/ μg)	3 097.0	1 801.6	5 965.5	2 021.0	4 044.8	3 386.0 \pm 1 698.9
T_{max} (h)	26.01	17.03	20.38	21.11	23.2	21.5 \pm 3.3

B_0 $K_a/K_a - K_{10}$: intercept; V_c/F : apparent volume of distribution; K_a : first-order rate constant of absorption; K_{10} : first-order rate constant of elimination; $t_{1/2} K_{10}$: half-life; C_{max} : maximum plasma concentration; T_{max} : time to reach C_{max} ; MRT (0-inf): mean residence time calculated by trapezoidal rule from time 0 to infinity. ^a Harmonic mean.

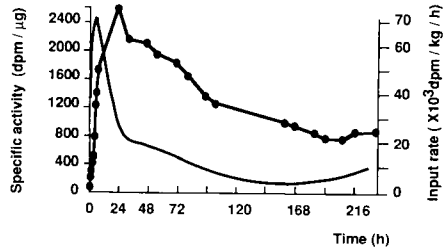
Table III. Pharmacokinetic parameters describing the disposition kinetic of D- α -tocopherol in plasma after intraperitoneal administration of DL- α -tocopherol acetate (100 mg/kg bw) to 5 sheep (monocompartmental model with a first-order constant of absorption).

Parameters (units)	Sheep (No)					Mean \pm SD
	10	11	12	13	14	
B_0 ($\mu\text{g/ml}$)	3.85	2.40	3.01	4.31	3.80	3.47 \pm 0.76
V_c/F (l/kg)	11.80	18.95	15.12	10.57	11.98	13.68 \pm 3.39a
Base ($\mu\text{g/ml}$)	1.25	1.50	1.23	1.33	1.19	1.30 \pm 0.13
K_a (h^{-1})	0.101	0.0780	0.111	0.224	0.0923	0.121 \pm 0.0587
$t_{1/2} K_a$ (h)	6.7	8.9	6.2	3.1	7.5	5.7 \pm 3.2b
K_{10} (h^{-1})	0.0118	0.0173	0.00371	0.00549	0.00782	0.00922 \pm 0.00544
$t_{1/2} K_{10}$ (h)	58.6	39.9	186.6	126.2	88.6	75.0 \pm 49.7b
C_{max} ($\mu\text{g/ml}$)	4.15	3.06	3.90	5.26	4.21	4.11 \pm 0.78
T_{max} (h)	23.9	24.8	31.6	17.0	29.2	25.3 \pm 5.61

B_0 $K_a/K_a - K_{10}$: intercept; K_a : first-order rate constant of absorption; K_{10} : first-order rate constant of elimination; $t_{1/2} K_{10}$: half-life; C_{max} : maximal concentration; T_{max} : time to reach C_{max} . ^a Calculations take into account the fact that the racemate was injected while only the α -form was measured; ^b Harmonic mean.



Figs 4–6. 4. Semilogarithmic plot of observed (•) and adjusted (-) plasma specific activity (dpm/μg) versus time (h) after an intraperitoneal administration of [^{14}C]DL- α -tocopherol (1 $\mu\text{Ci/kg}$ bw) to a representative sheep. 5. Semilogarithmic plot of observed (•) and adjusted (-) plasma specific activity (dpm/μg) versus time (h) after an intraperitoneal administration of [^3H]DL- α -tocopherol (4 $\mu\text{Ci/kg}$ bw) to a representative sheep. 6. Semilogarithmic plot of observed (•) and adjusted (-) plasma D- α -tocopherol ($\mu\text{g/ml}$) versus time (h) after an intraperitoneal administration of DL- α -tocopherol acetate (100 mg/kg bw) to a representative sheep.



7 Fig 7. Instantaneous input rate (dpm/kg/h) (-) and observed plasma specific activity (dpm/μg) (•••) after an intraperitoneal administration of [^{14}C]DL- α -tocopherol (1 $\mu\text{Ci/kg}$ bw) to a representative sheep.

and, at that time, the input rate was $36\,611 \pm 23\,590$ dpm/kg/h. 90% of the activity was absorbed after 162.3 ± 11.24 h and, at that time, the input rate was $6\,642 \pm 2\,634$ dpm/kg/h. The amount resorbed as calculated by deconvolution was $4\,596\,000 \pm 1\,943\,000$ dpm/kg, a value lower than the actual administered dose ($8.8 \cdot 10^6$ dpm/kg) suggesting an incomplete availability ($52.2 \pm 22.07\%$). Individual values are given in table V.

DISCUSSION

The selection of an appropriate route of administration is a prerequisite for safe and successful vitamin E therapy. When comparing different routes of administration, 2 main factors must be taken into account: bioavailability, *ie* the fraction which actually reaches the systemic circulation, and local tolerance. In sheep, it was shown that DL- α -tocopherol acetate given orally was not as effective in raising α -tocopherol in the blood as when given intramuscularly. Despite the fact that the IM route provides a relatively higher availability than the oral route, its use suffers from major draw-

Table IV. Bioavailability (rate and extent) of α -tocopherol after [^{14}C]DL- α -tocopherol intraperitoneal administration ($1 \mu\text{Ci}/\text{kg} = 2.2 \cdot 10^6 \text{ dpm}/\text{kg}/\text{bw}$) (deconvolution analysis).

Parameters (units)	Sheep (No)				Mean \pm SD	
	1	2	3	4		
Amount resorbed ($10^3 \text{ dpm}/\text{kg}$)	1 740	2 964	2 993	1 748	2 361	\pm 712.8
F(%)	79.10	134.0	136.1	79.4	107.2	\pm 32.3
Input rate at time zero (dpm/kg/h)	14 930	97 399	32 113	12 524	39 241.5	\pm 39 740.8
Maximum input rate (dpm/kg/h)	22 689	102 599	70 428	36 264	57 995	\pm 35 883
Time of maximum input rate (h)	3	3	6	12	NC	
50% absorption time (h)	60	19.5	39	34	38.13	\pm 16.76
input rate at 50% absorption time (dpm/kg/h)	8 815	33 801	19 774	15 973	19 545.7	\pm 10 527
90% absorption time (h)	183	129	165	128	151.2	\pm 27.28
input rate at 90% absorption time (dpm/kg/h)	2 184	5 161	3 329	3 213	3 471.7	\pm 1 238.2

F(%) Bioavailability expressed as percent resorbed; NC: not calculated.

Table V. Bioavailability (rate and extent) of α -tocopherol after [^3H]DL- α -tocopherol intraperitoneal administration ($4 \mu\text{Ci}/\text{kg} = 8.8 \cdot 10^6 \text{ dpm}/\text{kg}$) (deconvolution analysis).

Parameters (units)	Sheep (No)					Mean \pm SD	
	5	6	7	8	9		
Amount resorbed ($10^3 \text{ dpm}/\text{kg}$)	4 579	2 905	7 299	2 609	5 588	4 596	\pm 1 943
F(%)	52.04	33.02	82.94	29.65	63.5	52.2	\pm 22.07
Input rate at time zero (dpm/kg/h)	51 782	57 465	132 903	51 029	90 088	76 653	\pm 35 312
Maximum input rate (dpm/kg/h)	90 853	57 464	202 427	51 029	139 594	108 273	\pm 63 261
Time of maximum input rate (h)	6	< 3	9	< 3	< 3	NC	
50% absorption time (h)	42	52.5	49	36	42	44.3	\pm 6.50
input rate at 50% absorption time (dpm/kg/h)	26 528	15 747	75 003	23 208	42 568	36 611	\pm 23 590
90% absorption time (h)	169.5	177	150	153	162	162.3	\pm 11.24
Input rate at 90% absorption time (dpm/kg/h)	6 395	5 291	8 496	3 168	9 860	6 642	\pm 2 634

F(%) Bioavailability expressed as percent resorbed; NC: not calculated.

backs. In sheep, it was shown for different commercial formulations that vitamin E compounds were strongly irritating to the tissue at the injection site. In addition, since tocopherol is taken up by local drainage lymph glands and stored locally for a considerable period (more than 16 weeks), this route of administration is considered unacceptable on both economic and hygienic (residue) grounds (Dickson *et al*, 1986). In searching for an appropriate administration route for vitamin E, another major point deserves attention; for pharmaceutical dosage forms, esters, principally acetate and succinate, are used because of their greater stability to oxidation (Newmark *et al*, 1975). These esters are only prodrugs and the active moiety (tocopherol) must be released by hydrolysis. After IM injection of the acetate ester in dogs, rapid absorption was reported but hydrolysis of the ester to the physiologically active free tocopherol was quite slow. This was explained by the fact that blood itself has no enzymes capable of rapid hydrolysis of the acetate ester (Newmark *et al*, 1975). The major site of tocopherol ester hydrolysis is the liver. This explains the theoretical advantage of a route of administration for which a prodrug is mainly drained by the portal circulation to arrive directly in the liver to undergo a possible first-pass hydrolysis. In this respect, the IP route deserves attention as a drug delivered into the peritoneal cavity may exit by 3 different routes: directly into the systemic circulation, by large terminal lymphatics called lacunae and located in the diaphragm (Tsilibary and Wissig, 1983), and mainly by the portal circulation which implies a first passage through the liver prior to entry into the systemic circulation (Luka *et al*, 1971). This suggests that IP treatment may present a significant pharmacokinetic advantage for esters which must be hydrolysed by the liver. In sheep, bioavailability of α -tocopherol after DL- α -tocopherol acetate administra-

tion (100 mg/kg) was 20% higher for the IP than for the intraruminal route (Hidioglou and Charmley, 1990). The disposition kinetics of DL- α -tocopherol in sheep plasma and tissues following a single IP administration of DL- α -tocopherol was reported (Hidioglou and Charmley, 1990) but the sampling schedule did not allow modeling of peritoneal tocopherol absorption.

The present study provides some insight into vitamin E disposition after IP administration. The use of labelled DL- α -tocopherol allows estimation of the relevant kinetic parameters of vitamin E disposition without confounding effects of physiological vitamin E. After IP administration the *MRT* was about 1 week. To our knowledge, the *MRT* has never been estimated for vitamin E. From parameters of equation 10 obtained in the literature (see *Materials and Methods*) the *MRT* of specific activity after an IV administration of [³H]D- α -tocopherol can be estimated according to equation 11 (Yamaoka *et al*, 1978b):

$$MRT = \frac{(Y_1/\lambda_1^2 + Y_2/\lambda_2^2 + Y_3/\lambda_3^2)}{(Y_1/\lambda_1 + Y_2/\lambda_2 + Y_3/\lambda_3)} \quad [11]$$

ie about 100 h; the mean absorption time (MAT) after IP administration can be tentatively calculated according to equation 12 (Riegelman and Collier, 1980).

$$MAT = MRT_{IP} - MRT_{IV} \quad [12]$$

leading to a *MAT* for the IP route of about 100 h. It must be recalled that after a delay corresponding to the *MAT*, 63% of the material has been theoretically absorbed if the drug obeys a monocompartmental model with a first-order rate constant of absorption (Riegelman and Collier, 1980). From our deconvolution study, it can be calculated that $77.06 \pm 7.33\%$ of the tocopherol has been absorbed after a delay of 100 h, which is consistent with this rough non-compartmental estimation.

A compartmental approach was used to describe and model the time-course of specific activity. Several monocompartmental models were tested (first vs zero-order absorption, continuous vs discontinuous absorption). As none of these models was systematically better than others, we selected the most frequently used model, *ie* the monocompartmental model with continuous first-order absorption rate. In sheep, after labelled tocopherol intravenous administration, a 3-compartment model was selected to model the time-course of specific activity (Hidiroglou and Karpinski, 1987; Karpinski and Hidiroglou, 1990). After IP administration the slow process of absorption does not allow separation of the distribution and elimination processes: the specific activity was modeled with a lumped-parameters approach with a single parameter (K_{10}) describing the overall process of distribution and elimination. The half-life of the terminal phase ($t_{1/2} K_{10}$) after the administration of labelled DL- α -tocopherol was estimated to be 99.3 ± 28.3 and 121.3 ± 38.5 h. These values are similar to that obtained after an IV administration of [^3H]D- α -tocopherol (118 ± 18 h) (Karpinski and Hidiroglou, 1990). This suggests that the terminal phase observed after IP administration is likely to represent an elimination process (not a flip-flop situation) and that the disposition of [^3H]D- α -tocopherol is likely to be similar to that of [^{14}C]DL- α -tocopherol.

A lumped-parameters approach is a current practice for modelling peritoneal absorption. As noted by others (Flessner *et al*, 1984) this type of approach does not allow one to explain (or more simply to accurately describe) the complex process that actually governs the transport between the peritoneal cavity and the plasma. The present study exemplifies this situation as no simple input function was able to satisfactorily describe the entry

rate of α -tocopherol in plasma. Using a single first-order rate constant of absorption (K_a), the absorption half-life was estimated to be 4–6 h, a figure suggesting a relatively rapid absorption of tocopherol from the peritoneum. This is not consistent with our non-compartmental estimate of the *MAT* of about 100 h. In order to correctly qualify the entry rate of labelled α -tocopherol (specific activity) in circulation, a deconvolution approach was used to identify the instantaneous entry rate. Deconvolution requires 2 basic assumptions, *ie* linearity and stationarity: linearity of tocopherol disposition has been demonstrated (Sternberg and Pascoe-Dawson, 1959) and there is no evidence of non-stationarity in tocopherol disposition. The output function of a linear function (plasma specific activity after IP administration) is given by the convolution of the input function (which is the process under investigation) and by the disposition function of the system (as obtained after IV administration). Deconvolution yield the input function when both output and disposition functions are known. In the present study, only the output function was experimentally determined, the disposition function being obtained from the literature (Karpinski and Hidiroglou, 1990). Despite this limitation, a realistic input function was identified with some major characteristics. First, the entry rate was not immediately maximum (as with a single first-order absorption rate) but reached a maximum value only after a delay ranging from less than 3 h to 12 h. This could be due to the fact that the solute can leave the peritoneal cavity by bulk flow through stomata located in the diaphragm (Tsilibary and Wissig, 1983). The egress of a fraction of tocopherol by lymphatics after IP administration is supported by the very high concentration of tocopherol found in the lungs after this mode of administration (Hidiroglou *et al*, 1990). Secondly, it was calculated that half

the available dose was absorbed within 38–44 h and 90% within 150–160 h; the origin of this relatively slow process of absorption remains unclear. Similarly, we have no explanation for the difference in the apparent systemic availability between the 2 labelled doses tested, *ie* an apparent 100% availability for dose 1, and only about 50% for dose 4.

Many kinetic studies concerning vitamin E have been carried out using labelled tocopherol. In the present experiment, we observed a major difference in the kinetic parameters derived from specific activity following labelled DL- α -tocopherol administration and D- α -tocopherol plasma level following DL- α -tocopherol acetate administration at a pharmacological dose (100 mg/kg): the apparent volumes of distribution (V_D/F) were 13.68 ± 3.39 l/kg vs 1–2 l/kg for unlabelled and labelled tocopherol respectively. The most likely explanation is a poor availability of D- α -tocopherol after DL- α -tocopherol acetate administration. Another possible explanation could be a difference in the distribution of tocopherol enantiomers. Indeed, in the present experiments the injected material (labelled and unlabelled) was the DL- α -tocopherol and the measured plasma parameters were the specific activity of labelled DL- α -tocopherol and plasma concentrations of D- α -tocopherol for the unlabelled test article. A higher volume of distribution for the D-form than for the L-form could be a factor explaining some of the differences between unlabelled and labelled test articles.

In conclusion, the intraperitoneal route seems to be convenient for administration of tocopherol formulations, but more information is required to qualify the exact process of absorption (rate and extent), especially for the different possible formulations and esters.

ACKNOWLEDGMENTS

The authors thank Dr R Garcia-Villar for criticism and help in the preparation of the manuscript. The present work is contribution No 1679 of the Animal Research Center, Canada.

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