Disposition kinetics and dosage regimen of vitamin E administered intramuscularly to sheep

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Three experiments were conducted to estimate the effects of single intramuscular (IM) administrations of vitamin E on blood plasma and tissue concentrations of α-tocopherol in sheep. In Expt 1, plasma kinetics of α-tocopherol in sheep (*n* 30) were investigated following IM administration of three doses (ten sheep/dose) of DL-α-tocopheryl acetate, (20, 40 and 60 mg/kg live weight). Plasma profiles of α-tocopherol consisted of a lag phase followed by an apparent first-order absorption and elimination phase. The rate of absorption and elimination, as well as the lag phase, were independent of the dose, but the extent of absorption was directly proportional to dose. In Expt 2 (eighteen experimental and five control sheep), the animals were injected as in Expt 1 and were killed at 0, 80 and 176 h. Increases in α-tocopherol levels in organs were much higher than in plasma. Some tissues such as liver, spleen, lung and adrenal appeared to exhibit rapid absorption and elimination phases. The amount absorbed was proportional to the dose administered. Other organs such as heart, kidney and pancreas had a slow elimination rate. In Expt 3, D-α-tocopherol was injected IM into ten sheep at either 604 mg or 1208 mg. The mean hepatic α-tocopherol concentrations in both groups rose rapidly and after 4 weeks of dosing its concentrations were higher than the predosing levels. The increase in hepatic tocopherol concentrations were higher following 1208 mg dosing than 604 mg D-α-tocopherol. No simple relationship existed between plasma and hepatic α-tocopherol concentrations. This suggests a difference in body mechanisms controlling vitamin E in blood and liver.

Vitamin E: Intramuscular vitamin E: Sheep

Information on the disposition kinetics of vitamin E compounds following their administration by various routes is important for the establishment of their bioavailability (Hidiroglou & Karpinski, 1987, 1988). In addition, studies on dosage regimens of vitamin E based on kinetic studies are essential for effective therapy of vitamin E deficiency (Colburn & Ehrenkranz, 1983). According to Graeber *et al.* (1977), intramuscular (IM) administration of 125 mg DL-α-tocopheryl acetate/kg body-weight to infants during the first week of life is sufficient to maintain adequate vitamin E status for several weeks. Recently, Hidiroglou & McDowell (1987) reported on pharmacokinetic plasma values in sheep following intramuscular administration of vitamin E in an oil carrier. However, some disturbances of the health of sheep (local swelling, oedema and lymphadenitis) were attributed by Behrens *et al.* (1975) to the use of IM injection of vitamins in oily carrier substances. The present study was designed to determine basic pharmacokinetic values of vitamin E in sheep after IM administration of vitamin E in emulsion.

MATERIALS AND METHODS
Animals

Yearling crossbred wethers, weighing 45–50 kg, were used. All animals originated from a flock born and raised in confinement. For 6 months before and during the experiment the animals were fed on a diet (*ad lib.*) consisting (g/kg) of grass silage 400, hay 400 and maize silage 200. The same sources of silage were used throughout.

Expt 1

Thirty sheep were dosed IM (gluteus) with DL-α-tocopheryl acetate. Because of practical constraints, the study was carried out in an unbalanced randomized block design. Sheep were grouped into six blocks of five sheep each, and doses of 20, 40 and 60 mg DL-α-tocopheryl acetate/kg body-weight were randomly assigned to one or two sheep within each block. Each block was studied in a distinct time-period and the design was structured so that each dose was administered to a total of ten sheep.

Samples of blood (approximately 5 ml) were drawn from the jugular vein of each wether using disposable needles and syringes containing heparin, at 0, 1, 3, 6, 10, 20 and 30 min, 1, 2, 3, 4, 5, 6 and 7 h, and thereafter twice daily for 20 d following the DL-α-tocopheryl acetate injection. The plasma was separated from the cellular blood components in a refrigerated centrifuge at 4°.

Expt 2

A total of twenty-three sheep were used. Five sheep were randomly assigned to a control group (no injection) and were killed on the first day. Six sheep were randomly assigned to each of the same dose preparations used in Expt 1, i.e. 20, 40 and 60 mg DL-α-tocopheryl acetate/kg body-weight. Three sheep from each dose group were killed at 80 h and the other three at 176 h after dosing. Various tissues (heart, hip muscle, neck muscle, lung, liver, spleen, pancreas, kidney, adrenal, omental fat and blood) were sampled and stored at −20° until they were analysed for their α-tocopherol content. During removal of all tissues, care was taken to dissect away superfluous adipose tissue.

Expt 3

Ten sheep were used. Five randomly selected sheep were given a single IM injection (gluteal muscle) of 604 mg D-α-tocopherol while the other five were injected IM with 1208 mg. Blood and liver (by biopsy) were sampled weekly. Biopsies were performed following endoscopic examinations by the method of Whitehair *et al.* (1988) which was adapted for sheep.

Materials

In Expts 1 and 2, the IM vitamin E preparation was a 300 g/l solution, of which the formula was (g): DL-α-tocopheryl acetate (96%) 300, benzyl alcohol 10, cremotor EL (glycerol–polyethylene glycol ricinoleate; emulsifying agent) 240, ethanol of Germany Pharmacopoeia (DAB 7) quality to 1 litre. This preparation was provided by BASF (Aktiengesellschaft, D-6700 Ludwigshafen, West Germany).

In Expt 3, the vitamin E preparation (Stuart product, Bedford, TX, USA) contained 201 mg D-α-tocopherol/ml compounded with ethyl alcohol (200 ml/l) and benzyl alcohol (10 ml/l) in emulsifiable base (polyoxyethylated fatty acid derivative).

Analytical procedures

Details of the analytical methods and instrumentation used for α-tocopherol analysis for blood and plasma and tissue have been reported previously (Hidiroglou, 1987).

Statistical methods

Plasma profiles following IM injection consisted of a lag phase followed by an apparent first-order absorption and elimination phase. Therefore, estimation of profiles in Expt 1 was based on non-linear least squares applied to the following model:

$$\log C_t = \log(A_0 + A_2) + e_t, \qquad \text{for } t \leqslant T$$
$$= \log\{A_0 + A_1 e^{-k_1(t-T)} - (A_1 - A_2) e^{-k_2(t-T)}\} + e_T, \quad \text{for } t > T$$

where C_t denotes tocopherol levels at time t, T represents the time at which first-order absorption begins, e_t is a random error term which is assumed to be normally and independently distributed with mean 0 and variance σ^2, and A and k are respectively intercept and rate parameters to be estimated. For each profile, the quoted model was fitted repeatedly using successive plasma sampling times as estimates of the lag phase. The optimal estimate of T was determined for each profile by using a minimum pooled residual mean-squared error criterion (Hudson, 1966). The corresponding estimated exponential equation was taken as the optimal representation of the profile. Parameter estimation was carried out using SAS non-linear regression (SAS Institute, 1982). As indicated in the model specification, analysis was carried out on the logarithmic scale. This analysis scale is appropriate for stabilizing the variances which appear to increase with increased concentrations.

The following measures were also used in characterizing the plasma profiles: AUC, the area under the plasma concentration v. time curve calculated by the trapezoidal rule; C_{max}, the maximum observed concentration; T_{max}, the time at which C_{max} occurred; C_0, the initial concentration; and C_{480}, the final measured concentration at 480 h.

Inter-dose comparisons of profile estimates and measures were based on analyses of variance applied to a model that included dose and block effects. Assessments of dose effects on areas and plasma tocopherol concentrations were carried out on '$AUC - 480 C_0$', $C_{480} : C_0$ and $C_{max} : C_0$ in order to adjust for baseline levels. These analysis were carried out on the log scale. First-order absorption and elimination measures, T and T_{max}, were analysed on the original scale. Averages on the log scale were transformed back to the original scale where they represent geometric means. Standard deviations on the log scale were multiplied by 100 to yield approximate coefficients of variation on the original scale.

For Expt 2, separate analyses were carried out for each organ, tissue and plasma. Tests were based on an analysis of variance of log concentrations applied to a model that included dose and slaughter time effects.

In Expt 3, separate analyses were carried out for the liver and plasma. A repeated-measures analysis of variance (Winer, 1971) was applied to the post-dosing log tocopherol concentrations adjusted for predosing levels (i.e. $\log(C_i/C_0)$, where $i = 1, 2, 3, 4$) to test for dose and time effects. Analyses were also carried out for $\log((C_i - C_0)/\text{dose})$, in order to determine whether concentration differences between the two groups were directly proportional to dose.

RESULTS

Expt 1

During the entire experiment, the animals were healthy. No local reactions in the area of the IM injections were observed and the body temperature taken at the same hour of the day did not deviate from the normal range. Excellent fits of the profiles were obtained for all animals. Summaries of the parameter estimates for the three groups are provided in Table 1 and examples of typical observed and estimated profiles for individual animals are presented in Fig. 1.

Table 1. *Expt 1*. Average exponential equation parameter estimates for plasma α-tocopherol in sheep* (C_t) *after intramuscular injections of 20, 40 and 60 mg* DL-*α-tocopheryl acetate/kg body-weight (BW)*

$$C_t = A_0 + A_2 \qquad\qquad t \leqslant T$$
$$= A_0 + A_1 e^{-k_1(t-T)} - (A_1 - A_2) e^{-k_2(t-T)} \qquad t > T$$

Dose (mg/kg BW)		Intercepts (μg/ml)				Rates (per h)		Lag phase (h)
		Pre-absorption		Final				
		A_1	$A_0 + A_2$	A_0		Absorption	Elimination	T
20	Geometric mean	6·63	1·10	1·31	Mean†	0·2064	0·0206	1·0
	CV (%)	45	22	31	SD	0·0865	0·0090	1·0
40	Geometric mean	10·49	1·14	1·62	Mean	0·1716	0·0228	0·4
	CV (%)	62	28	24	SD	0·0892	0·0123	0·3
60	Geometric mean	14·62	0·77	1·67	Mean	0·1450	0·0237	0·2
	CV (%)	82	50	32	SD	0·0790	0·0086	0·2

CV, coefficient of variation.
* For details, see p. 466.
† Arithmetic mean.
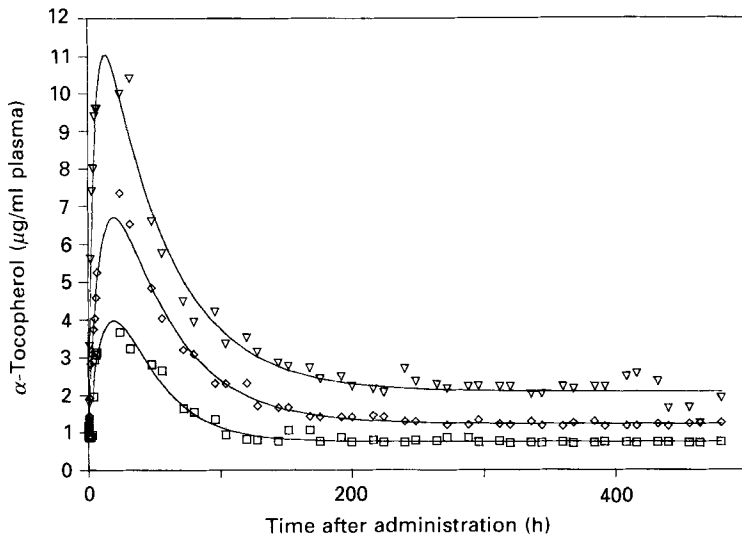

Fig. 1. Expt 1. Concentrations of plasma α-tocopherol in sheep after intramuscular injection of DL-α-tocopheryl acetate at 20 (□), 40 (◇), 60 (▽) mg/kg body-weight in sheep nos 6, 9 and 24 respectively. For details of procedures, see p. 466.

The lag phase ranged from 0·017 to 3 h. This was followed by a period of relatively rapid increases with peak concentrations occurring between 7 and 48 h. Elimination appeared to be well represented by a single exponential over the 20 d observation period; however, tocopherol levels at 480 h were generally significantly higher than initial levels as indicated by the $C_{480}:C_0$ ratios in Table 2. These increased levels at 480 h suggest that a substantial amount of tocopherol was absorbed in tissues and slowly redistributed into the plasma.

Table 2. *Expt 1*. Plasma α-tocopherol geometric means for profile summary statistics from exponential equations for sheep injected intramuscularly with 20, 40 and 60 mg DL-α-tocopheryl acetate/kg body-weight (BW)*

Dose (mg/kg BW)		C_0 (μg/ml)	AUC μg/ml per h	C_{max} (μg/ml)	C_{480} (μg/ml)		T_{max} (h)
20	Geometric mean	1·06	927·7	5·25	1·24	Mean†	25·5
	CV(%)	24	31	34	32	SD	7·6
40	Geometric mean	1·14	1210·6	7·69	1·58	Mean	26·4
	CV (%)	30	25	43	23	SD	7·6
60	Geometric mean	0·76	1278·0	8·92	1·53	Mean	28·0
	CV (%)	42	32	44	39	SD	4·2

C_0, initial concentration; AUC, area under the plasma concentration *v.* time curve; C_{max}, maximum observed concentration; C_{480}, final measured concentration at 480 h; T_{max}, time at which C_{max} occurred.
* For details, see p. 466.
† Arithmetic mean.

Presumably, an additional exponential elimination phase would become evident with a longer observation period.

There were no significant differences among the three dose groups with respect to absorption rate ($P > 0.05$) or elimination rate ($P > 0.05$; Table 1). The 20 mg/kg group had a longer lag phase ($P = 0.01$) than the 40 and 60 mg/kg dose groups. Differences in predose levels, $A_0 + A_2$, were not significant ($P > 0.05$) but were nevertheless used as covariates in inter-dose comparisons of intercept terms A_1 and A_0. Both A_1 and A_0 increased ($P < 0.01$) with increasing dose. In the case of A_1, the increases appeared to be directly proportional to dose (i.e. no significant differences ($P > 0.05$) in A_1/dose) indicating that a constant fraction of the dose was absorbed.

AUC, C_{max} and C_{480} measures are provided in Table 2. In each case, measures were significantly higher ($P < 0.01$) with increased dose. However, there were no significant differences ($P > 0.05$) in the ratios '(AUC$-480C_0$)/dose' for the three dose groups, indicating that amount absorbed after IM tocopherol injection was directly proportional to dose. In addition, there were no significant differences ($P > 0.05$) in the T_{max} values.

These observations suggest that tocopherol kinetics are not altered by dose. That is, the rates of absorption and elimination are independent of dose and the extent of absorption is directly proportional to dose.

Expt 2

Geometric means for plasma and tissue tocopherol levels in the second experiment are provided in Table 3.

Kidney and heart tissue had large increases in tocopherol content but differences between doses and differences between the 80 and 176 h observations were not significant ($P > 0.05$). A similar pattern was observed for tocopherol levels in the pancreas except that the 20 mg/kg dose group had an unusually high value at 176 h and there was a suggestion that tocopherol levels continued to increase over the 80–176 h time interval ($P = 0.08$). Tocopherol levels in omental fat also displayed a similar pattern except for the 20 mg/kg dose, which had a much lower value at 80 h.

The spleen and liver tocopherol levels at 80 h were proportional to dose ($P < 0.03$). By 176 h levels had dropped significantly ($P < 0.03$) but were still much higher than control levels ($P < 0.005$). The patterns for the adrenal and lung levels were similar to those of spleen and liver levels except that no decrease was noted between 80 and 176 h for the 20 mg/kg dose.

Table 3. *Expt 2. α-Tocopherol geometric means in tissues and plasma at slaughter for sheep injected intramuscularly with 20, 40 and 60 mg DL-α-tocopheryl acetate/kg body-weight (BW), 80 and 176 h post dosing*

	Concentration (µg/g)							
Dose (mg/kg BW)	0	20		40		60		CV (%)
Time (h)...	0	80	176	80	176	80	176	
Heart	1·0	14·6	21·0	22·6	14·3	20·0	19·3	35·0
Hip muscle	0·3	5·0	12·8	3·5	5·2	6·8	6·7	35·0
Neck muscle	0·3	3·7	6·5	4·7	5·0	5·9	6·3	26·7
Lung	1·6	16·5	16·3	39·0	14·1	61·9	17·2	36·0
Liver	2·8	25·5	17·4	46·2	16·3	52·0	22·0	41·0
Spleen	0·9	29·2	18·2	43·4	28·5	62·4	25·7	35·2
Pancreas	1·0	37·8	52·5	38·9	42·7	40·9	42·8	23·3
Kidney	1·5	10·2	9·5	10·2	11·7	13·4	9·6	26·1
Adrenal	3·9	28·1	29·3	39·7	24·1	60·3	31·2	47·4
Fat	1·9	4·6	13·4	12·5	10·3	9·7	11·6	49·9
Plasma*	1·7	3·4	2·3	3·6	2·6	4·5	2·3	12·0

CV, coefficient of variation.

* µg/ml.

Table 4. *Expt 3*. Alterations of α-tocopherol concentrations in the plasma (µg/ml) and liver (µg/g fresh tissue) of sheep injected intramuscularly with a single dose of D-α-tocopherol (604 or 1208 mg)*

		Concentrations† of α-tocopherol				
Time after dose (d)...		0	7	14	21	28
Dose (mg)						
Plasma	604	1·7	2·2	1·7	1·5	1·6
	1208	1·7	3·1	2·3	2·2	2·2
CV (%)			42·0	31·0	33·0	36·0
Statistical significance of difference: P‡			0·30	0·23	0·19	0·36
Liver	604	5·2	19·8	19·8	14·9	11·0
	1208	5·1	45·1	31·1	20·3	15·8
CV (%)			21·0	21·0	28·0	21·0
Statistical significance of difference: P‡			0·001	0·017	0·15	0·002

CV, coefficient of variation.

* For details, see p. 466.

† Geometric means.

‡ Based on an analysis of variance of log concentrations adjusted for baseline values.

For the hip and neck muscle there was no consistent dose–response relationship. These variations may be due to the percentage of fat or connective tissues in the muscle. Tocopherol levels in both tissues increased over the 176 h observation period except for hip muscle at 60 mg/kg, where no significant change was noted between 80 and 176 h.

As indicated in Expt 1, the plasma levels at 80 and 176 h were in the elimination phase, with the levels at 80 h being significantly higher ($P < 0.005$) than at 176 h. At 176 h, plasma levels had returned to baseline for the 20 mg/kg dose. Plasma levels for the 40 and 60 mg/kg doses were still significantly higher than control levels at 176 h.

Expt 3

In both the 604 and 1208 mg dose groups, hepatic α-tocopherol levels rose sharply during the first week following injection and subsequently decreased over the 28 d observation period (Table 4). At 28 d, the hepatic levels were still significantly higher ($P = 0.002$) than predosing levels. Hepatic α-tocopherol levels were also directly proportional to dose, and profiles of $\log ((C_i - C_0)/\text{dose})$ in the two dose groups were parallel. These observations indicate that hepatic α-tocopherol kinetics are dose-independent.

Plasma α-tocopherol levels also rose during the first week, but the increase was significant only for the 1204 mg group ($P = 0.04$). Plasma α-tocopherol levels declined after the first week and levels for days 14–28 were not significantly ($P > 0.05$) different from baseline values. No significant dose effects ($P > 0.05$) were noted for plasma α-tocopherol levels over the 7–28 d observation period.

DISCUSSION

The study of plasma α-tocopherol (Expt 1) showed a time lag between IM administration and the apparent onset of absorption. This lag may be the result of delayed hydrolysis of the acetylated form of the vitamin. When IM injection of DL-α-tocopheryl acetate is performed, the tissue is disrupted resulting in local trauma; then the absorption from the muscle may proceed directly into the blood or indirectly through the lymph. Muscle tissue has a rich supply of capillary vessels. However, there are few lymph vessels in muscle tissue. Following injection into a muscle mass, the DL-α-tocopheryl acetate in an emulsion vehicle would be distributed between the muscle fibres where mixing and continuity with one of the fluid compartments can occur.

The absorption of DL-α-tocopheryl acetate depends on the rate of transport of the DL-α-tocopheryl acetate molecule in the blood and its subsequent hydrolysis. It has been suggested by Gallo-Torres (1980) that only limited hydrolysis of DL-α-tocopheryl acetate to tocopherol occurs when the ester is given parenterally. The slow increase observed in plasma α-tocopherol levels might be due to slow deacetylation of the ester form, followed by a slow saturation of the tissue. It was noted that the final vitamin E values in the present experiment were higher than the initial plasma values. This indicates, as reported by Hidiroglou & McDowell (1987), a slow saturation of vitamin E in the various body tissues. In sheep following IM injection of D-α-tocopheryl acetate in aqueous suspension, Caravaggi *et al.* (1968) observed that the peak of vitamin E serum concentration was reached 8 h after its administration and this was followed by a slow decline.

In the present experiment, the rate of disappearance of vitamin E showed some variation within individuals. This may have been related to the difficulty of adequately controlling the geometry of the resulting depot in the site of penetration of vitamin E by the needle-injection technique. Moreover, the individual differences in the absorption of DL-α-tocopheryl acetate may have been related to the differences in muscle blood flow or to the extent of the absorption area, or both. When DL-α-tocopheryl acetate is administered by the IM route a depot is formed, then the acetylated form must leave the depot and reach the blood or lymph. Rindi & Perry (1958), following IM injection of DL-α-tocopheryl acetate in aqueous emulsion, reported the ester form to be the predominant form in the blood during the earlier part of the observation period until the 8th hour, and the free form to predominate at the end of the observation period (8th up to 48th hour). This gradual conversion may explain why some investigators have reported variable responses from parenterally-administered α-tocopherol ester forms (Newmark *et al.* 1975; Fujii, 1980).

The present results show that the rate of vitamin E absorption was proportional to the amount injected, and support the conclusion that the absorption of the ester form of

vitamin E from the injection site is preceded by a time lag which was probably related to the molecular mass of the DL-α-tocopheryl acetate dosed. In the first experiment, kinetic approaches have been applied to the evaluation of plasma tocopherol appearance and disappearance rates as well as metabolic pool size for plasma following IM injection of the ester form of vitamin E. The parameters used in the model provided great insight into the dynamics of vitamin E distribution in the body of the sheep. Plasma profiles were characterized by exponential equations with both absorption and elimination represented by single exponential terms. The extent of absorption, as measured by the area under the plasma concentration–time profile, was proportional to dose. Plasma tocopherol levels over the 480 h observation period appeared to plateau at a level which was significantly higher than predose tocopherol levels in the plasma.

In Expt 2, increases in tocopherol levels in tissues and organs were much higher than in plasma, with those in the spleen reaching 62·4 μg/g (at 80 h in the 60 mg dose), which was sixty-six times the control level.

Tocopherol profiles in the liver, spleen, adrenal and lung indicate rapid absorption and elimination of tocopherol, with the level of absorption being proportional to the dose administered. The heart, pancreas, kidney and fat also had rapid rates of absorption but the tocopherol levels did not change significantly with increased dose, indicating tissue saturation. There was also little change in tocopherol levels between 80 and 176 h post administration, suggesting that either elimination for these organs is very slow or that saturation levels are maintained through the redistribution of tocopherol from other tissues.

The hip and neck muscles appeared to act as tocopherol depots. Levels of tocopherol in hip and neck muscles were generally lower than in other tissues but continued to increase over the observation period. It further appears that vitamin E in muscles may have the slowest elimination rates. These tissues and the apparently-saturated organs such as the heart, kidney and pancreas may be responsible for maintaining the plasma tocopherol level at an elevated plateau for the observed 480 h period following tocopherol injection.

Hepatic storage of vitamin E was found to be good following parenteral administration of vitamin E in emulsion. According to Machlin & Gabriel (1982) rat liver and plasma levels of α-tocopherol appear to reflect relatively recent intake of vitamin E. According to Losowsky et al. (1972) neither liver nor plasma levels of vitamin E can be used as an index of absorption. Presumably this implies different rates of uptake, breakdown or turnover of α-tocopherol by different organs. The present findings show that no simple relationship exists between serum and hepatic stores of vitamin E. This suggests that the body mechanisms controlling serum levels of α-tocopherol are different from those controlling hepatic stores.

It is apparent from these findings that vitamin E can be supplied to sheep by means of IM injections of DL-α-tocopheryl acetate or D-α-tocopherol. However, there is still an unsolved problem, namely whether the administration of massive doses of well-absorbed vitamin E preparations constitutes an economical use of the vitamin in the livestock industry.

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