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Bioavailability of intramuscular vitamin E acetate in rabbits

J. L. PEDRAZ*, B. CALVO*, A. BORTOLOTTI, A. CELARDO, M. BONATI, *Laboratory of Clinical Pharmacology, Istituto di Ricerche Farmacologiche "Mario Negri", Via Eritrea 62, 20157 Milano, Italy*

Abstract—The bioavailability of α-tocopherol acetate and α-tocopherol (vitamin E) was assessed in male rabbits given 50 mg kg^{-1} doses according to a randomized design. After intramuscular injection of α-tocopherol acetate in colloidal aqueous solution, a mean absolute bioavailability of 65% was calculated for the acetate and 35% for the physiologically active compound, α-tocopherol. Comparison of the kinetic profiles after intravenous and intramuscular administration of the acetate and intravenous injection of α-tocopherol, revealed absorption of α-tocopherol acetate from the site of injection and hydrolysis of the acetate to be potential limiting steps in the bioavailability of α-tocopherol. Intramuscularly injected α-tocopherol acetate in olive oil (the only formulation available in a few European countries) proved completely bio-unavailable. It thus appears necessary to re-assess the utility of current vitamin E supplementation, since the only formulations available offer poor bioavailability.

α-Tocopherol (vitamin E), one of the biological antioxidants, is currently receiving attention concerning its efficacy in preventing or reducing the incidence of severe clinical conditions associated with adverse oxidation events in premature newborns (retrolental fibroplasia, intraventricular haemorrhage, bronchopulmonary dysplasia, haemolytic anaemia) and in adults (coronary, rheumatic and hypertensive heart diseases) (Bieri et al 1983; Roberts & Knight 1987).

α-Tocopherol is practically insoluble in water and is oxidized by atmospheric oxygen and light (Sebrell & Harris 1954). Because of their greater stability, the esters of α-tocopherol (acetate, succinate, nicotinate) are commonly used in different formulations, particularly intramuscular preparations of the acetate. Oral administrations are bioavailable but have a slow absorption rate (particularly in the preterm newborn, who have immature gastrointestinal function and in whom pathological states may affect oral absorption (Morselli et al 1980). Thus is it of limited use in acutely raising blood levels (Bell et al 1979). Then, too, intravenous administration resulted in a tragedy (Martone et al 1986), with the deaths of 38 infants who had received the drug. However, the esters, also, are poorly soluble in aqueous solutions. So, different formulae (micellar aqueous dispersion, colloidal aqueous solutions, olive and sesame oil solutions) have been prepared to improve the bioavailabilty of α-tocopherol acetate and also its hydrolysis to α-tocopherol, the active moiety.

* Permanent address: Departamento de Farmacia y Tecnologia Farmaceutica, Universidad de Salamanca, Spain.
Correspondence to: M. Bonati, Laboratory of Clinical Pharmacology, Istituto di Ricerche Farmacologiche "Mario Negri", Via Eritrea 62, 20157 Milano, Italy.

There have been a number of studies about the bioavailability of vitamin E in animals (Newmark et al 1975; Phelps 1981), human neonates (Bougle et al 1986) and adults (Bateman & Uccellini 1985; Baker et al 1986). These studies, using different doses, timing, routes of administration and formulations, tend to give piecemeal information rather than a systematic picture. Thus, to date, no information is available concerning the intramuscular bioavailability of the one parenteral formulation in olive oil available in Italy and other European countries.

The present study was designed to compare the disposition profile of this formulation and a standard colloidal aqueous solution, currently available only in a few countries.

Materials and methods

According to a randomized design six male New Zealand rabbits (Charles River, Italy), 3–3·5 kg, were given 50 mg kg^{-1} of: i) (±)-α-tocopherol acetate in colloidal solution (Ephynal, Hoffman-LaRoche, Switzerland) intravenously (i.v.) (in one of the marginal ear veins) and intramuscularly (i.m.) (deep into the thigh muscle); ii) (±)-α-tocopherol i.v., as a galenical aqueous dispersion (Phelps 1981); iii) (±)-α-tocopherol acetate in olive oil (Evion Forte, Bracco, Italy).

Blood samples (0·5–1 mL) were drawn from a vein in the non-injected ear in heparinized syringes at various times up to 72 h. The samples were stored at −20°C until assay. Blood concentrations of α-tocopherol acetate and α-tocopherol were determined by the HPLC method of Celardo et al (1988).

A two-week wash-out period was left between one treatment and the next, to allow a return to similar blood trough levels of vitamin E in all the rabbits after each treatment (4·3 ± 0·8). Hematocrit, body weight, food and water consumption were checked daily during the study.

Blood concentration-time data after different doses, minus the basal value, were analysed according to a non-compartmental analysis and the parameters were obtained from the usual relationships (Gibaldi & Perrier 1982). The highest sample concentration observed was taken as the peak concentration. The "lag time (observed)" for absorption or formation was estimated as the midpoint between the times of collection of the first sample above assay sensitivity (for α-tocopherol acetate) or trough level (for α-tocopherol) and the sample immediately before it. Total body clearance (CL) after i.v. administration was calculated as $CL = dose^{iv}/AUC^{iv}$, where AUC is the area under the curve from zero to infinity, estimated using the trapezoidal

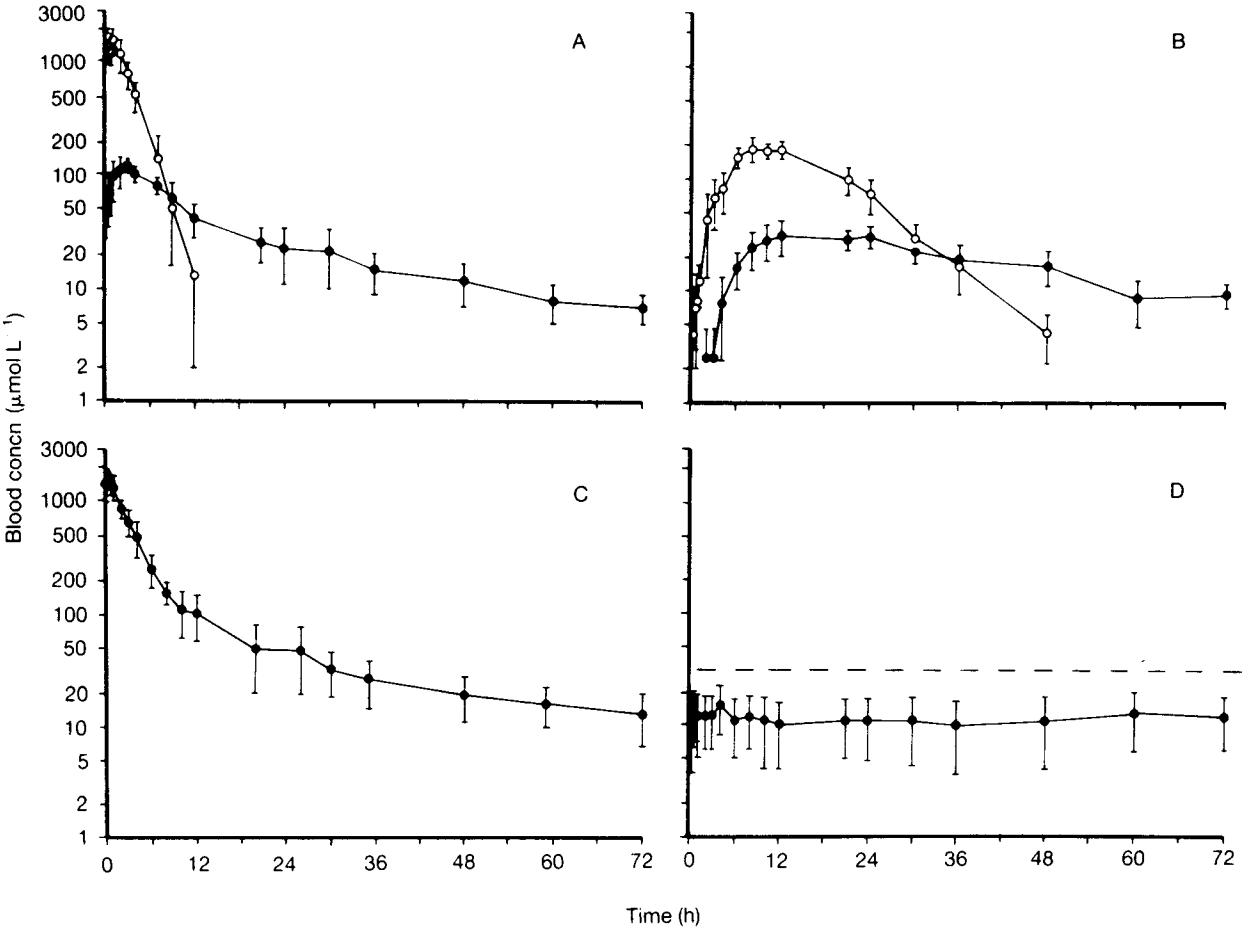

FIG. 1. Semilogarithmic plot of mean (±s.d.) blood α-tocopherol acetate (○) and α-tocopherol (●) concentrations vs time in six male rabbits receiving 50 mg kg^{-1} i.v. (A) and i.m. (B) α-tocopherol acetate in colloidal aqueous solution, i.v. α-tocopherol in aqueous dispersion (C), and i.m. α-tocopherol acetate in olive oil formulation (D). Dashed line (D) depicts the upper 95% confidence level of trough concentrations of vitamin E. Basal levels of α-tocopherol have been subtracted in A, B and C.

rule and extrapolated from the last assayed sample to infinity by the final elimination rate constant (λ_z) calculated by log-linear least-squares fit of the last five points of the blood samples. The systemic availability (F) of α-tocopherol acetate was estimated from $F = AUC^{im} \cdot dose^{iv} / AUC^{iv} \cdot dose^{im}$. The systemic availability of α-tocopherol after administration of its acetate was estimated from $F = AUC^{acetate} \cdot dose^{iv} / AUC^{iv} \cdot dose^{acetate}$, where $AUC^{acetate}$ is the AUC of α-tocopherol after different doses of its acetate, expressing the doses as molar. The effect of the different doses on pharmacokinetic values was assayed by Student's *t*-test and one-way analysis of variance (ANOVA) and probabilities (*P*) less than 0·05 were considered statistically significant.

Results and discussion

All rabbits completed each phase of the study. Mean blood concentration-time profiles of the two tocopherols after different doses are shown in Fig. 1, and estimated pharmacokinetic parameters in Table 1. When α-tocopherol acetate was given i.v. as a colloidal aqueous solution (Fig. 1 A) a rapid log-linear decline was observed and a mean elimination rate constant of 0·47 h^{-1} and corresponding mean half-life of 1·6 h were calculated. After 24 h the acetate was no longer detected in the bloodstream. α-Tocopherol blood concentrations rapidly reached maximum levels of 129±26 μmol L^{-1} at a t_{max} of 2·6±0·5 h. Then, peak concentrations declined slower than for the acetate, and a mean elimination half-life of 34 h was calculated.

After i.m. α-tocopherol acetate as a colloidal aqueous solution (Fig. 1 B), the compound was detectable in the first blood sample (5 min), then levels rose up to 200±39 μmol L^{-1} at 10·3±1·8 h. The slope of the blood concentration decline was about six times lower than after i.v. administration (λ_z 0·11 h^{-1}; $t_{\frac{1}{2}}$ 6·1 h; $P < 0{\cdot}001$), giving evidence that absorption of α-tocopherol acetate from the i.m. injection site is a rate-limiting step in its systemic and absolute availability, and suggesting that the process is more complex than a simple first- or zero-order one, as shown for other water-insoluble compounds (Hirano et al 1981). Thus most of the dose of α-tocopherol acetate is quickly absorbed, but a small fraction is absorbed very slowly and absorption persists long after the time of peak drug concentration in blood. In fact, the compound was measurable in the blood up to 48 h after i.m. injection. The infinite areas beyond the sampling series never exceeded 10% after either route of injection. Calculated absolute i.m. bioavailability of α-tocopherol acetate administered in colloidal formulation ranged from 51 to 75 %, average 65%. Statistically significant differences ($P < 0{\cdot}001$) of λ_z and AUC in opposite directions justify the calculated close values for total body clearance after both routes (Table 1).

The fact that absorption of α-tocopherol acetate from the injection site represents a limiting step of its kinetic profile is also supported by the α-tocopherol blood-concentration curves. Intravenous α-tocopherol acetate gave a rapid increase of α-tocopherol blood levels (Fig. 1 A) with a C_{max} of 129±26 μmol L^{-1} at a t_{max} of 2·6±0·5 h, significantly

Table 1. Pharmacokinetic parameters of α-tocopherol acetate and α-tocopherol in male rabbits given 50 mg kg^{-1} i.v. and i.m.

	F	λ_z (h^{-1})	$t_{\frac{1}{2}} \lambda_z$ (h)	AUC (μmol h L^{-1})	CL (L h^{-1} kg^{-1})
α-Tocopherol acetate					
i.v.†	1	0·47±0·11	1·6±0·7	5568±811	0·019±0·003
i.m.†	0·65±0·08*	0·11±0·01*	6·1±0·7	3616±503*	0·020±0·003
α-Tocopherol					
i.v.‡	1	0·025±0·006	28·5±7·1	7895±1918	0·015±0·003
i.v. acetate†	0·35±0·07*	0·022±0·007	33·9±12·6	2307±479*	0·017±0·005
i.m. acetate†	0·26±0·04**	0·022±0·003	35·5±7·4	1878±471**	0·016±0·006

* $P < 0·001$ compared with i.v.
** $P < 0·05$ compared with i.v. acetate
† after Ephynal injection
‡ after galenical dispersion injection

different from i.m. α-tocopherol acetate (Fig. 1 B), with its C_{max} of 34±7·1 μmol L^{-1} at a t_{max} of 21·8±5·1 h. A mean lag-time of 1·5±0·8 h was observed in the appearance of α-tocopherol into bloodstream.

These findings suggest that hydrolysis to the physiologically active compound must also take into account as a potential limiting step in the bioavailability of the two tocopherols. Estimated AUC values after i.v. administration of 50 mg $kg:^{-1}$ α-tocopherol (Fig. 1 C) were 3·5 times (7895 vs 2307 μmol h L^{-1}, Table 1) those after i.v. injection of the acetate, again supporting the role of hydrolysis in availability.

On the basis the absolute bioavailability value calculated for the acetate after i.m. administration (65%), a significant difference ($P < 0·05$) was found between the calculated bioavailability of α-tocopherol after the i.v. and i.m. acetate, ranging from 28 to 43% (average 35%) and from 21 to 31% (average 26%), respectively. Values for λ_z, $t_{\frac{1}{2}} \lambda_z$ and CL were close for α-tocopherol after different routes (Table 1).

After i.m. administration of α-tocopherol acetate in olive oil it was not detected in the bloodstream of all six animals over the whole study. α-Tocopherol blood levels remained constantly in the non-supplemented range (Fig. 1 D), showing the complete lack of i.m. bioavailability of this formula.

These findings agree with previous indications that α-tocopherol acetate in sesame oil is poorly absorbed (if at all) in neonates (Pantoja et al 1984), and confirm the ineffectiveness of α-tocopherol acetate in oil formulations. The use of high viscosity injection vehicles, such as glycerol, cottonseed oil, sesame oil, or polyethylene glycols—and here olive oil—is accompanied by poor intramuscular absorption (Niazi 1979). It was also confirmed that the hydrolysis of the acetate to α-tocopherol is a rate-limiting step in the bioavailability of the compound, as previously reported in dogs (Newmark et al 1975), as is the rate of absorption from the site of injection to the bloodstream.

Taking into account that the therapeutic efficacy of α-tocopherol supplementation was related to blood (or plasma) levels and how soon these are achieved (Lemons & Maisels 1985), even though the colloidal aqueous solution was more bioavailable than the oil, it still is not the best possible formulation. Further biopharmaceutical work is needed to produce more appropriate products.

In accordance with Phelps' statement (Phelps 1984), the results reported here suggest the need for meticulous evaluation of the formulation to be used for tocopherol supplementation. Although particular caution must be used in extrapolating experimental findings to clinical indications, the need for extreme care in the use and clinical evaluation of routine vitamin E formulations is confirmed. The choice (or the lack of choice) of formulations with poor bioavailability may have profound therapeutic implications in current clinical practice.

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