Biopharmaceutic Factors in Parenteral Administration of Vitamin E

HAROLD L. NEWMARK, WILLIAM POOL, JACOB C. BAUERNFEIND, and ELMER De RITTER ˣ

Abstract □ When properly formulated, micellar-type aqueous dispersions of tocopheryl acetate are administered intravenously or intramuscularly to dogs, the rate-limiting step in the bioavailability of the physiologically active free tocopherol is the rate of hydrolysis of the acetate ester. A similar dispersion of free tocopherol yields blood levels of tocopherol many fold higher than those obtained with the acetate ester after intravenous injection and also yields much greater increases in blood levels of free tocopherol after intramuscular injection than the acetate ester formulation, particularly in the early period after the dose.

Keyphrases □ Vitamin E—biopharmaceutic factors, parenteral administration, rate-limiting step □ Tocopheryl acetate—biopharmaceutic factors, parenteral administration, rate-limiting step □ Bioavailability—tocopheryl acetate from parenteral formulations

α-Tocopherol (vitamin E) exists naturally in foods primarily in the free, unesterified form. For food fortification, pharmaceutical dosage forms, and animal supplementation, the esters, principally the acetate and succinate, are used because of their greater stability to oxidation. When administered orally to animals and humans, both esters and free α-tocopherol are fully active biologically as nutritional sources of vitamin E, indicating ready hydrolysis of the esters in the GI tract (1, 2).

Parenteral preparations containing α-tocopheryl acetate have been available commercially and for investigational use for some time, but little or no information is available concerning the kinetics of the hydrolysis needed to provide the biologically active free tocopherol from these dosage forms. A recent request from a medical research group concerned with the problems of open heart surgery prompted an investigation of this problem. During such surgery, the blood is oxygenated under high oxygen tension in an extracorporeal apparatus. This stresses the lipid antioxidant (*e.g.*, free α-tocopherol) reserves of the erythrocyte membranes, resulting in excessive, undesirable oxidative hemolysis of red cells.

The tocopherol levels in the blood simultaneously drop to very low levels, well below the acceptable minimum of 0.5 mg/100 ml. Apparently the large pools of tocopherol stored elsewhere in the body are not mobilized rapidly enough into the blood to correct this stress. Parenteral administration of *dl*-α-tocopheryl acetate in micellar aqueous dispersions surprisingly did not correct the rapid decline of the free tocopherol levels in the blood in this stress situation[1].

Rindi and Perri (3) administered a 5% aqueous emulsion of *dl*-α-tocopheryl acetate intramuscularly to humans and demonstrated a rapid increase in plasma tocopheryl acetate content, reaching a maximum at 8 hr after injection. The free tocopherol level, however, increased only slowly, reaching a maximum at about 30 hr after injection.

Table I—Formulas of Parenteral Vitamin E Products

Component	*dl*-α-Tocopheryl Acetate, 50 mg/ml	*dl*-α-Tocopherol, 50 mg/ml
dl-α-Tocopheryl acetate[a]	50 mg	—
dl-α-Tocopherol[a]	—	50 mg
Polyoxyethylated fatty acid derivative[b]	4% (w/v)	10% (v/v)
Glycerin	5% (w/v)	—
Ethyl alcohol	—	10% (v/v)
Propylene glycol	—	10% (v/v)
Benzyl alcohol	—	1% (v/v)
Thimerosal	0.1 mg	—
Disodium edetate	0.1 mg	0.1 mg
Sodium chloride	—	9 mg
Sodium acetate	0.4 mg	0.3 mg
Acetic acid	0.1 mg	2.5 mg
Water for injection, *q.s.* to	1.0 ml	1.0 ml
pH	6.0–6.6	About 4
Packaging	10-ml multiple-dose vials	2-ml single-dose ampuls

[a] Hoffmann-La Roche Inc., Nutley, N.J. [b] Emulphor EL 620, GAF Corp., New York, N.Y.

Table II—Increases in Blood Plasma Levels of Tocopherol and Tocopheryl Acetate in Dogs after Intramuscular Injection of an Aqeuous Emulsion of *dl*-α-Tocopheryl Acetate (5 mg/kg)

Blood Sample, Time after Injection	Dog Number 1106	1412	1556	Mean
Predose Level of Tocopherol, mg %				
	0.99	1.03	0.93	0.98
Increase in Level of Tocopherol, mg %				
7.5 min	0.00	0.00	0.00	0.00
15 min	0.00	0.00	0.01	0.003
30 min	0.09	0.01	0.01	0.04
60 min	0.17	0.09	0.05	0.10
2 hr	0.17	0.18	0.06	0.14
4 hr	0.31	0.26	0.15	0.21
8 hr	0.31	0.61	0.61	0.51
24 hr	0.31	0.57	0.61	0.50
Increase in Level of Tocopheryl Acetate, mg %				
7.5 min	0.3	0.5	0.1	0.3
15 min	0.3	2.0	0.9	1.1
30 min	0.6	2.9	1.2	1.6
60 min	0.7	5.0	1.6	2.4
2 hr	1.0	4.2	1.7	2.3
4 hr	1.1	4.1	1.9	2.4
8 hr	0.9	1.8	0.9	1.2
24 hr	0.2	0.1	0.4	0.2

[1] M. K. Horwitt, St. Louis University College of Medicine, personal communication.

Table III—Increases in Blood Levels of Tocopherol and Tocopheryl Acetate in a Dog after Intravenous Injection of an Aqueous Emulsion of *dl*-α-Tocopheryl Acetate (5 mg/kg)

Blood Sample, Time after Injection	Free Tocopherol, mg %	Tocopheryl Acetate, mg %
Predose	0.81	0.00
	Increase after Injection	
7.5 min	0.18	4.1
15 min	0.18	2.8
30 min	0.19	2.4
60 min	0.17	2.0
2 hr	0.21	1.7
4 hr	0.45	1.0
8 hr	0.45	1.0
24 hr	0.16	0.9

The present study was designed to obtain biopharmaceutical information for a more effective formulation of parenteral vitamin E.

EXPERIMENTAL

Materials—Aqueous dispersions of 5% *dl*-α-tocopherol NF and *dl*-α-tocopheryl acetate NF were prepared with the compositions shown in Table I. These dispersions represent modifications of formulas of injectable aqueous emulsions of fat-soluble vitamins described by Aiello and Bauernfeind (4).

These preparations are almost optically clear, have no droplets visible in a light microscope, and can be considered colloidal or micellar dispersions. The free tocopherol, being more difficult to emulsify, required more of the surfactant, a polyoxyethylated fatty acid derivative. The preparations were sterilized by bacteriological filtration through Selas candles and were aseptically subdivided into sterile ampuls and vials.

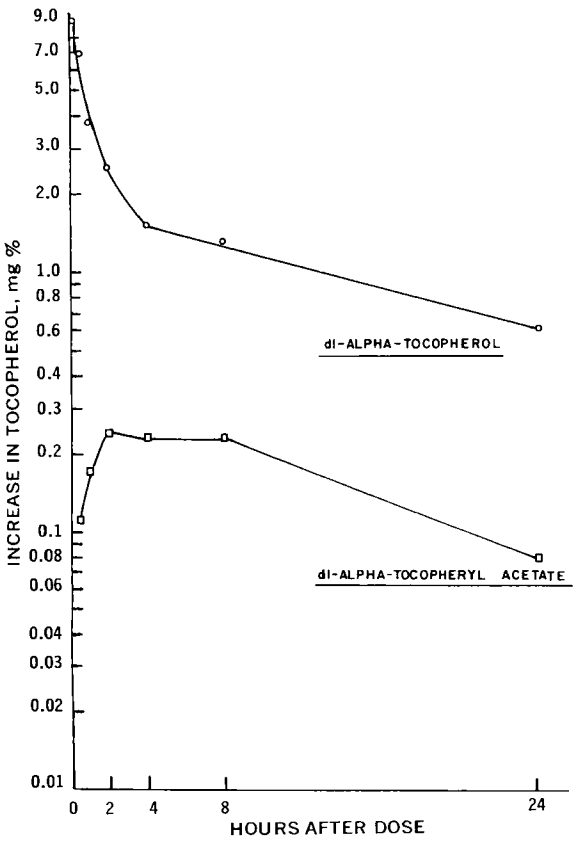

Figure 1—*Increase in blood plasma levels of tocopherol in the dog after intravenous injection of* dl-α-*tocopherol and* dl-α-*tocopheryl acetate at 5 mg/kg.*

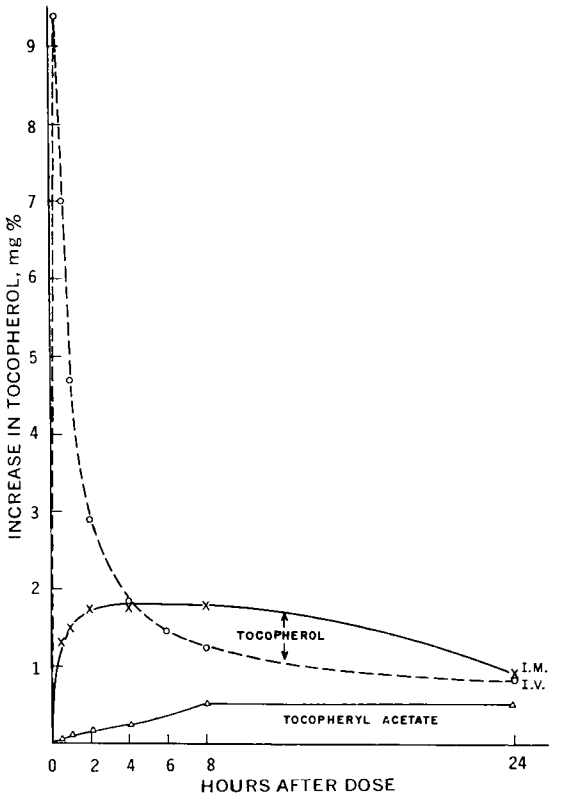

Figure 2—*Increase in tocopherol levels in dog blood plasma after injection with 5 mg/kg of* dl-α-*tocopherol (intravenous and intramuscular) or* dl-α-*tocopheryl acetate (intramuscular). Predose control levels were subtracted in each case to obtain the value shown; each curve represents the average for three dogs.*

Methods—Dogs, ~ 10 kg, were injected with 5 mg/kg of either *dl*-α-tocopherol or *dl*-α-tocopheryl acetate in the aqueous parenteral formulations. Each dog received a single injection, either intramuscularly in the gluteal muscle area or intravenously in the cephalic vein. Blood samples were drawn from each dog before drug administration and at various times after injection.

The heparinized blood samples were centrifuged and the plasma was analyzed for free tocopherol by the Emmerie–Engel color reaction utilized in the method of Quaife *et al.* (5). Plasma samples obtained from dogs that received *dl*-α-tocopheryl acetate were analyzed for free tocopherol content both before and after alkaline hydrolysis in the presence of an antioxidant, 4′-hydroxyacetanilide. The difference between these analyses represents tocopherol equivalent to the tocopheryl acetate present.

In a separate series of tests, samples of freshly drawn dog and human blood were incubated at 37° with *dl*-α-tocopheryl acetate, using the described parenteral formulation diluted 1 to 50 in 0.9% saline with 0.2 ml added to 4 ml of blood to provide a level of 5 mg/100 ml of blood. Aliquots were withdrawn at intervals and assayed for free α-tocopherol to determine the effect of whole blood in effecting hydrolysis of the acetate ester.

RESULTS AND DISCUSSION

Intramuscular injection of 5 mg/kg of *dl*-α-tocopheryl acetate in three dogs gave increases in plasma levels of free and esterified tocopherol (Table II). These data are similar to those reported for single intramuscular injections of 300 mg of an aqueous dispersion of *dl*-α-tocopheryl acetate into humans (about 5 mg/kg in 20 humans) (3). The results again demonstrate that the ester moves fairly rapidly into the bloodstream from the intramuscular site for this aqueous dispersion but that the hydrolysis to the physiologically active free tocopherol is quite slow. Apparently, little hydrolysis occurs at the injection site in the muscle or elsewhere. The similarity of these dog tests with the data of the previously mentioned human tests confirmed the suitability of the dog as an experimen-

Table IV—*In Vitro* Hydrolysis Tests of *dl*-α-Tocopheryl Acetate[a] Added to Dog or Human Blood

Sample	Hours at 37°	Dog No. 322 (Heparinized)	Dog No. 372 (Untreated)	Human Blood Sample	
				Subject 1	Subject 2
		Free Tocopherol, mg %			
Control (no tocopheryl acetate)	0	1.30	1.20	1.48	1.68
	2.0	—	—	1.43	1.64
Test (added tocopheryl acetate)	0.5	1.33	1.16	1.47	1.72
	1.0	1.36	1.07	1.43	1.66
	2.0	1.23	1.22	1.43	1.66
	3.0	1.26	1.25	—	—

[a] Aqueous emulsion (Table I) diluted 1 to 50 in 0.9% saline and 0.2 ml added to 4 ml of whole blood to provide a level of 5 mg of *dl*-α-tocopheryl acetate/100 ml.

tal animal for design of an improved parenteral formulation of vitamin E.

Intravenous administration of *dl*-α-tocopheryl acetate in a single dog at 5 mg/kg showed a similar slow hydrolysis of the ester (Table III). Although the acetate persists in the blood for many hours, only a small increase in free tocopherol levels is observed.

The increases in levels of free tocopherol in blood plasma obtained after a single intravenous administration of 5 mg/kg of *dl*-α-tocopheryl acetate (average of two dogs) or 5 mg/kg of *dl*-α-tocopherol (average of two dogs) are shown in Fig. 1. The high peak tocopherol level in the plasma of dogs receiving free tocopherol intravenously is consistent with the value expected based on the dog's blood volume. There was a many fold greater elevation of plasma levels of free tocopherol over pretreatment levels after intravenous administration of free tocopherol than with esterified tocopherol. This fact indicates again that the hydrolysis of tocopheryl acetate is the major rate-limiting step in bioavailability.

Samples of human and dog whole blood showed no significant increase in free tocopherol when inoculated with *dl*-α-tocopheryl acetate and incubated at 37° (Table IV). These results suggest that the blood itself does not have enzymes capable of rapid hydrolysis of the acetate ester.

Table V—Increase in Blood Plasma Levels of Tocopherol after Intramuscular Injection of an Aqueous Emulsion of *dl*-α-Tocopherol (5 mg/kg)

Time after Injection	Dog Number			Mean
	1103	1112	1118	
7.5 min	0.8	1.0	0.0	0.6
15 min	0.8	1.7	0.3	0.9
30 min	0.9	1.9	1.0	1.3
60 min	1.5	1.7	1.4	1.5
2 hr	1.7	2.1	1.4	1.7
4 hr	1.7	2.1	1.4	1.7
8 hr	1.7	1.9	1.9	1.8
24 hr	0.4	1.4	0.8	0.9

The data in Table V demonstrate that the emulsion of free tocopherol produces a fairly rapid and substantial rise in blood levels of tocopherol in the dog after intramuscular injection. Although the levels in the first 4 hr were lower than those obtained after intravenous administration, the area under the 24-hr curves (Fig. 2) was practically the same for both routes, indicating complete absorption of the free tocopherol from the injection site in the muscle.

Figure 2 also illustrates the much more rapid increases in blood levels of the physiologically active free tocopherol after intramuscular injection of the aqueous dispersion of free tocopherol as compared to that of tocopheryl acetate. Hydrolysis of the ester *in vivo* proceeds rather slowly, with the peak level of free tocopherol occurring 8 hr after intramuscular administration of the acetate ester and being maintained almost constant up to at least 24 hr.

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[x] To whom inquiries should be directed.