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Kinetics of docosahexaenoic acid ethyl ester accumulation in dog plasma and brain ☆

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ABSTRACT

This study explores dog plasma and brain fatty acid composition achieved after long-term supplementation at high DHA doses. A 90% concentrate of DHA Ethyl Ester (DHA-EE) administered by oral gavage to Beagle dogs at doses of 100, 500, 1000, and 2000 mg/kg bw/day for 8 weeks resulted in DHA increases in both plasma and brain. In a subsequent 9-month study, DHA-EE was administered at 150, 1000 and 2000 mg/kg bw/day. Plasma DHA increased between 150 and 1000 mg/kg bw/day but not between 1000 and 2000 mg/kg bw/day and there were increases from Day 1to 92 but not between days 92 and 273. Doses > 500 mg/kg bw/day in the 8-week and all doses in the 9-month study resulted in DHA increases in the brain. The dose of 150 mg/k gbw/day is sufficient to achieve maximal brain concentrations if DHA is administered chronically. For shorter than 6 months of supplementation, higher doses are required.

1. Introduction

Docosahexaenoic acid (DHA), C22:6n-3, is a long-chain omega-3 polyunsaturated fatty acid. Numerous reports from professional and expert committees have advocated increased intake of omega-3 fatty acids and suggested that DHA is an essential nutrient for many life stages, but especially during growth and development [1]. DHA is an integral component of neural membrane phospholipids that impacts brain structure and function [2], and DHA supplementation has positive effects on cognitive performance in elderly individuals [3,4]. In addition, DHA is believed to have beneficial effects for adults due to its anti-inflammatory [5] and triglyceride lowering properties [6]. Highly purified prescription ethyl esters of long-chain omega-3 fatty acids are used for the treatment of severe hypertriglyceridemia [7].

Omega-3 fatty acids have been investigated for its benefits not only in humans but in companion animals as well. Studies of the effects of DHA supplementation during perinatal growth on neurodevelopment in puppies indicated improved visual acuity related to greater retinal sensitivity, and increased learning in concurrent discrimination tasks [8,9]. Supplementation with DHA may benefit dogs of all ages as demonstrated by studies describing some of the neurologic, renal, cardiovascular, immune, and musculoskeletal effects of elevated blood levels of n-3 fatty acids, especially DHA [10,11].

Most commercial pet foods contain a source of omega-3 fatty acids, mainly DHA and eicosapentaenoic acid (EPA). Despite the benefits of DHA and EPA supplementation in dogs, potential risks associated with usage of these omega-3 fatty acids are not well investigated. Although some attempts in this direction have been made [10,12], comprehensive studies involving supplementation with high doses of DHA and/or EPA for extended periods are lacking. This study explores the plasma and brain fatty acid composition of dog tissues achieved after long-term supplementation at high DHA doses.

The two studies described herein began with a dose-ranging study in dogs in which DHA-EE was administered via oral gavage to Beagle dogs for 8 weeks at doses of 0, 100, 500, 1000, and 2000 mg/kg bw/day. DHA-EE is a highly concentrated ethyl ester of DHA containing 90% of DHA by weight (900 mg/g). The DHA is derived from the heterotrophic microalga *Crypthecodinium cohnii* in a controlled fermentation process, and the resulting triglyceride oil (DHA single-cell oil [DHASCO®]) is extracted and refined according to current Good Manufacturing Practices. DHASCO® oil naturally contains 480–600 mg/g DHA (48% to 60% by weight). This study provided both tolerability and fatty acid compositional information for a subsequent 9-month study. The objective of the 9-month study was to evaluate plasma and brain fatty acid compositional alterations as a function of time and DHA-EE dose when administered orally for 9 months (273 days) at doses of 0, 150, 1000, and 2000 mg/kg bw/day and to determine the persistence of such alterations after a 2-month recovery period. Toxicological findings of the study are described in detail in a separate

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publication [13].

In the 9-month dog study, the lowest dose of 150 mg/kg bw/day was selected based on the maximum expected clinical human dose of DHA-EE of 4 g/day. The 4 g/day dose corresponds to 120 mg/kg bw/day in dogs according to the allometric scaling conversion based on body surface area correction factors [14]. The high dose of 2000 mg/kg bw/day was selected based on the compositional results of the 8-week study. The dose of 1000 mg/kg bw/day was selected as an intermediate dose to exhibit a potential gradient of effects.

Plasma samples were collected from all animals on Days 28 and 56 in the 8-week study and on Days 1, 92 and 273 in the 9-month study for the analysis of fatty acids. Estimates of the maximum concentration of DHA in plasma (Cmax), time of occurrence to a maximum DHA concentration (Tmax), and area under the curve (AUC) for oral administration of DHA-EE were determined in accordance with accepted practices [15]. In addition, DHA levels in plasma and brain were analyzed at the end of the treatment period in both studies as well as at the end of the recovery period in the 9-month study.

2. Materials and methods

Both studies were conducted at Charles River Laboratories Preclinical Services in Ohio, USA in compliance with the Good Laboratory Practice (GLP) regulations. Plasma samples collected during the treatment period were analyzed for DHA concentration at MPI Research (Pennsylvania, USA) by a validated gas chromatography/flame ionization detection method and in accordance with GLP. Brain and plasma samples collected at the study termination (both the main phase and recovery) were analyzed for fatty acid content at DSM Nutritional Products (Columbia, Maryland, USA) according to a validated analytical method using GLC with a flame ionization detector (below).

2.1. Animals

Male and female Beagle dogs were approximately 7 months of age at the time of randomization in both studies with body weights ranging from 6.1 to 7.7 kg for the males and 5.5–6.9 kg for the females. The animals were allowed to acclimate to the laboratory environment for 11 days prior to the first day of dosing (Day 1). Before randomization, the animals were weighed and examined in detail. Animals determined to be suitable as test subjects were randomly assigned to groups by a stratified randomization scheme designed to achieve similar group mean body weights. Prior to the main phase termination in the 9-month study, recovery animals were randomly selected with no restrictions on randomization. The animals were housed individually in suspended stainless steel cages during acclimation and while on study. Housing and care were as specified in the USDA Animal Welfare Act (9 CFR, Parts 1, 2, and 3) and as described in the Guide for the Care and Use of Laboratory Animals [16]. Teklad Dog Diet 2025 designed to support gestation, lactation, and growth from Harlan Laboratories was provided as a daily ration throughout the studies. The diet contained 26.0% of protein and 10.5% of fat; the energy density of the diet was 3.5 kcal/g. The principal fatty acids (FAs) were palmitic (1.9% of the diet or 18.0% of total FAs), stearic (0.5% of the diet or 4.8% of total FAs), oleic (3.4% of the diet or 32.4% of total FAs), linoleic (2.8% of the diet or 26.7% of total FAs) and α-linolenic acid (0.2% of the diet or 1.9% of total FAs). The diet did not contain any DHA or EPA. In addition, the diet contained 600 mg/kg of cholesterol, as well as the minerals and vitamins as prescribed by the NRC [17]. An approximate 300-gram ration of feed was provided daily to each dog beginning on the day after receipt.

2.2. Experimental design

In the 8-week study, DHA-EE and vehicle were administered once daily by oral gavage on Days 1–56 according to the experimental design illustrated in Table 1. Study animals (3 per sex per group) were terminated on Day 57.

In the 9-month study, DHA-EE and vehicle were administered once daily by oral gavage on Days 1–273 (Table 2). Main study animals (3 per sex per group) were terminated on Day 274, and the recovery animals (2 per sex per group) on Day 330.

2.3. Test compounds

The vehicle/control article used in both studies was corn oil. The test article contained at least 900 mg/g of DHA-EE (equivalent to 817 mg/mL), 3–4% of oleic acid (% of total fatty acids) and 1–2% of docosapentaenoic acid (22:5 n-3). It did not contain any EPA. DHA-EE was administered neat to the High dose animals and was diluted with the corn oil for the lower dosage groups (refer to Tables 1 and 2). The dose volume for each animal was 2.2 mL/kg bw/day and was based on the most recent body weight measurement. Diet samples collected at several time points during the study were analyzed for homogeneity and concentration. Concentration results were considered acceptable if the difference between the mean value found and the targeted concentration was $\leq 15\%$. Homogeneity results were considered acceptable if the RSD of the mean value at each sampling location was $\leq 5\%$. All samples met these acceptance criteria. In both studies, test articles were handled in the same way. DHA-EE and corn oil were stored frozen at $-20\ ^{\circ}C$ and were transferred to storage at $5\ ^{\circ}C \pm 3\ ^{\circ}C$ no more than 7 days before first use. Test materials were placed at ambient temperature and stirred for a minimum of 1 h before dose administration.

2.4. Kinetics

The blood samples (approximately 4 mL/sample) were collected via venipuncture of the jugular vein into tubes containing

Table 1
Experimental design of the 8-week study.

Group	No. of animals		Test material	DHA-EE Dose (mg/kg bw/day)	Dosing regimen
	Male	Female			
Control	3	3	Corn Oil	0	Once daily oral gavage on Days 1–56
Low	3	3	DHA-EE	100	
Mid	3	3		500	
Mid-High	3	3		1000	
High	3	3		2000	

Table 2
Experimental design of the 9-month study.

Group	No. of main (Recovery) animals		Test material	DHA-EE Dose (mg/kg bw/day)	Dosing regimen
	Male	Female			
Control	3 (2)*	3 (2)*	Corn oil	0	Once daily oral gavage on Days 1–273
Low	3 (2)*	3 (2)*	DHA-EE	150	
Mid	3 (2)*	3 (2)*		1000	
High	3 (2)*	3 (2)*		2000	

* The number in parenthesis indicates the number of animals in the recovery phase of the experiment.