

# Intracameral and Lenticular Penetration of Locally Applied Stable Isotope-labeled Vitamin E

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**Purpose:** To confirm the aqueous humor and lens dynamics of 1% deuterium-labeled  $\alpha$ -tocopherol acetate ( $D_3$ -VEA) solution.

**Methods:** The concentrations of  $D_3$ -VEA and  $D_3$ - $\alpha$ -tocopherol ( $D_3$ -VE), derived from  $D_3$ -VEA, in the aqueous humor and lens were measured after continuous instillation of 1%  $D_3$ -VEA into the cul-de-sac of rat eyes for 1 or 3 weeks.  $D_3$ -VEA and  $D_3$ -VE concentrations were determined by gas chromatography/mass selective detector.

**Results:** In the aqueous humor, the concentrations of  $D_3$ -VEA and  $D_3$ -VE were, respectively, 93.1 and 9.4 ng/mL after 1 week, and, respectively, 498.9 and 21.5 ng/mL after 3 weeks. In the lens, they were, respectively, 15.0 and 9.8 ng/g after 1 week, and 6.1 and 4.8 ng/g after 3 weeks.

**Conclusion:** The penetration levels of  $\alpha$ -tocopherol acetate by eyedrop application were confirmed in the aqueous humor and lens. **Jpn J Ophthalmol 2001;45:125–127** © 2001 Japanese Ophthalmological Society

**Key Words:** Aqueous humor, deuterium-labeled vitamin E acetate, gas chromatograph/mass selective detector, lens, rat.

## Introduction

In a previous study, the authors reported that  $\alpha$ -tocopherol acetate (VEA) eye drops delayed the progression of lens opacification induced by steroid and naphthalene treatment.<sup>1,2</sup> However, the dynamics in the anterior segment of the eye after instillation of VEA eye drops have not yet been studied.

This study was carried out to investigate the VEA and  $\alpha$ -tocopherol (VE) concentration in the anterior segment of the eye after continuous instillation of deuterium labeled-VEA ( $D_3$ -VEA) eyedrops.

## Materials and Methods

The  $D_3$ -VEA eyedrops (1%, pH 6.5, ratio of osmolarity:1.0) (Figure 1) were manufactured by emul-

sification with Tween 80 by the Santen Pharmaceutical Company, Ltd (Osaka).

### Protocol for Instillation of $D_3$ -VEA Eyedrops

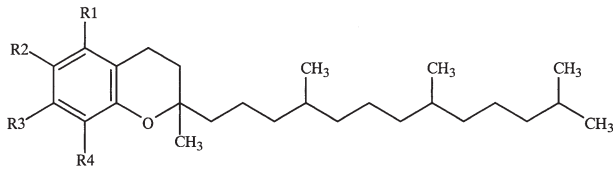
Forty-eight Brown-Norway rats (8-week-old, female; Sankyo Labo, Ishikawa), were fed normal chow and were divided into 4 groups (12 rats/group): Groups 1 and 3 were untreated and groups 2 and 4 were administered  $D_3$ -VEA eye drops 5 times daily (5  $\mu$ L/eye, 2-hour intervals) for 1 or 3 weeks, respectively.

### Preparation of Aqueous Humor and Lens

In groups 1 and 2, the rats were sacrificed by decapitation 30 minutes after the fourth instillation on day 7. Rats in groups 3 and 4 were sacrificed 30 minutes after the fourth instillation on day 21. The rats used in this experiment were maintained and used according to ARVO guidelines, and the animal care guidelines of Kanazawa Medical University. The aqueous humor was collected using a micro-syringe with a 27-G needle and the lens was removed from

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- R1, R3, R4=CH<sub>3</sub>, R2=OH →  $\alpha$ -tocopherol (VE)  
 R1, R3, R4=CH<sub>3</sub>, R2=CH<sub>3</sub>COO →  $\alpha$ -tocopherol acetate (VEA)  
 R1=CD<sub>3</sub>, R3, R4=CH<sub>3</sub>, R2=OH → 5-CD<sub>3</sub>- $\alpha$ -tocopherol (D<sub>3</sub>-VE)  
 R1=CD<sub>3</sub>, R3, R4=CH<sub>3</sub>, R2=CH<sub>3</sub>COO → 5-CD<sub>3</sub>- $\alpha$ -tocopherol acetate (D<sub>3</sub>-VEA)  
 R1, R3=H, R4=CH<sub>3</sub>, R2=OH →  $\delta$ -tocopherol ( $\delta$ -VE)  
 R1, R3=H, R4=CH<sub>3</sub>, R2=CH<sub>3</sub>COO →  $\delta$ -tocopherol acetate ( $\delta$ -VEA)

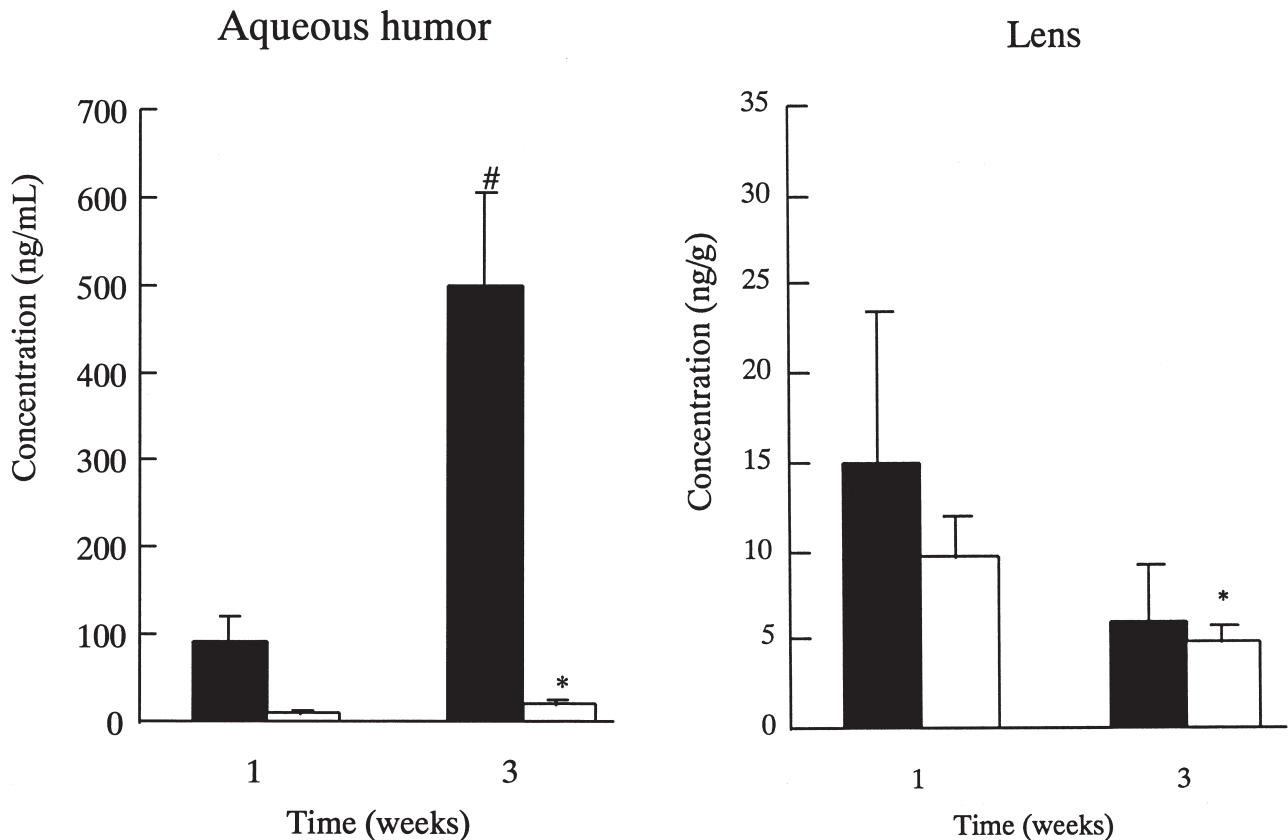
**Figure 1.** Structures of  $\alpha$ -tocopherol,  $\alpha$ -tocopherol acetate, 5-CD<sub>3</sub>- $\alpha$ -tocopherol, 5-CD<sub>3</sub>- $\alpha$ -tocopherol acetate,  $\delta$ -tocopherol and  $\delta$ -tocopherol acetate.

the eyeball. Aqueous humor or lens materials from 6 eyes of 3 rats were gathered as a single test sample, thus yielding a total of 4 samples for each group of 12 animals at each testing time.

### Determination of D<sub>3</sub>-VEA and D<sub>3</sub>-VE

Internal standard (IS), which contained both 0.2  $\mu$ g/mL of  $\delta$ -VE and  $\delta$ -VEA (Figure 1), was added (25  $\mu$ L) to each sample of aqueous humor. The sample was evaporated and derivatized with pentafluoropropionic anhydride at 55°C for volatility. Water (0.2 mL) was added to stop the derivatization. The sample was extracted with benzene and the upper layer (organic layer) was applied to a silica column (100 mg/mL). The organic layer was evaporated and 50  $\mu$ L of ethyl acetate was added to the residue (final sample). The final sample was applied to gas chromatography/mass selective detector analysis.

Each lens sample was homogenized with 25  $\mu$ L (0.2  $\mu$ g/mL) of IS and 1 mL of methanol, and then centrifuged. The supernatant was evaporated, benzene was added to the residual sample and it was applied to a silica column, and prepared for analysis in the same manner as the aqueous humor, above.<sup>3</sup>



**Figure 2.** Concentrations of D<sub>3</sub>-VEA and D<sub>3</sub>-VE in (left) aqueous humor and (right) lens. ■: D<sub>3</sub>-VEA, □: D<sub>3</sub>-VE. Aqueous humor or lens materials from 6 eyes of 3 rats were gathered as a single test sample, thus yielding a total of 4 samples for each group of 12 animals at each testing time. (left) Aqueous humor: mean  $\pm$  SE (N = 4). <sup>#</sup>*P* < .05 vs D<sub>3</sub>-VEA at 1 week (Aspin-Welch *t*-test). <sup>\*</sup>*P* < .05 vs D<sub>3</sub>-VE at 1 week (Student *t*-test). (right) Lens: mean  $\pm$  SE (N = 4, D<sub>3</sub>-VE at 1 week: N = 3). <sup>\*</sup>*P* < .05 vs D<sub>3</sub>-VE at 1 week (Student *t*-test).

## Results

### *Concentrations of D<sub>3</sub>-VEA and D<sub>3</sub>-VE in the Aqueous Humor*

In the aqueous humor, the concentration of D<sub>3</sub>-VEA was 93.1 ng/mL after 1 week of continuous instillation, with a more than five-fold increase (498.9 ng/mL) after 3 weeks of continuous instillation. D<sub>3</sub>-VE (derived from D<sub>3</sub>-VEA) concentrations after the first and third weeks were 9.4 ng/mL and 21.5 ng/mL, respectively (Figure 2, left).

### *Concentrations of D<sub>3</sub>-VEA and D<sub>3</sub>-VE in the lens*

The D<sub>3</sub>-VEA concentrations in the lens after the 1st and 3rd weeks were 15.0 ng/mL and 6.1 ng/mL; D<sub>3</sub>-VE concentrations were 9.8 ng/mL and 4.8 ng/mL, respectively. The concentrations of D<sub>3</sub>-VEA and D<sub>3</sub>-VE after the 3rd week showed a decrease in comparison with those after the 1st week (Figure 2, right).

D<sub>3</sub>-VEA and D<sub>3</sub>-VE could not be detected in the aqueous humor and lens samples of the untreated groups.

## Discussion

The authors reported that VEA eyedrops delayed the progression of lens opacification induced by steroid and naphthalene treatment in a previous study.<sup>1,2</sup> The purpose of the current study was to investigate the VEA penetration in the aqueous humor and lens after continuous VEA instillation.

In this penetration study, D<sub>3</sub>-VEA ophthalmic solution was used to determine the exogenous VEA and VE because endogenous VE exists in the aqueous humor and lens. We could confirm exogenous VEA and VE in the aqueous humor and lens.

In the aqueous humor, VEA and VE concentrations increased more in the 3-week treatment group than in the 1-week treatment group (Figure 2, left). The increase in concentration was in proportion to

the length of the treatment period. On the other hand, VEA and VE concentrations in the lens decreased more in the 3-week treatment group than in the 1-week treatment group (Figure 2, right). Although the cause of the decrease is not known, it is possible that the growth period of the rat correlated with the VE distributional change in the lens. The authors previously reported that (1) the lens VE concentration in a 4-month-old rat had decreased 50% compared with that in a 1-month-old rat and that (2) the VE concentrations in the anterior, posterior, and equatorial cortices of 4- and 12-month-old rats had decreased to one third of those in a 1-month-old rat. In contrast, the VE concentration in the nuclear part had decreased to two thirds of that in a 1-month-old rat.<sup>3</sup> On the other hand, it has been reported that the wet weight of the rat lenses showed a linear increase until 4 months, and then the weight increase ratio stabilized.<sup>4</sup> The lens wet weight increase<sup>4</sup> and VE concentration decrease<sup>3</sup> in the lens cortex occurred at the same time, indicating that these phenomena might be related. The regulation mechanism of VE concentration in the eye is not known. In the next study, the authors will investigate the intraocular mechanism of VE action.

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