

INVESTIGATION OF ORAL FENRETINIDE FOR TREATMENT OF GEOGRAPHIC ATROPHY IN AGE-RELATED MACULAR DEGENERATION

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Background: Excessive accumulation of retinol-based toxins has been implicated in the pathogenesis of geographic atrophy (GA). Fenretinide, an orally available drug that reduces retinol delivery to the eye through antagonism of serum retinol-binding protein (RBP), was used in a 2-year trial to determine whether retinol reduction would be effective in the management of geographic atrophy.

Methods: The efficacy of fenretinide (100 and 300 mg daily, orally) to slow lesion growth in geographic atrophy patients was examined in a 2-year, placebo-controlled double-masked trial that enrolled 246 patients at 30 clinical sites in the United States.

Results: Fenretinide treatment produced dose-dependent reversible reductions in serum RBP-retinol that were associated with trends in reduced lesion growth rates. Patients in the 300 mg group who achieved serum retinol levels of $\leq 1 \mu\text{M}$ ($\leq 2 \text{ mg/dL}$ RBP) showed a mean reduction of 0.33 mm^2 in the yearly lesion growth rate compared with subjects in the placebo group ($1.70 \text{ mm}^2/\text{year}$ vs. $2.03 \text{ mm}^2/\text{year}$, respectively, $P = 0.1848$). Retinol-binding protein reductions $< 2 \text{ mg/dL}$ correlated with further reductions in lesion growth rates ($r^2 = 0.478$). Fenretinide treatment also reduced the incidence of choroidal neovascularization (approximately 45% reduction in incidence rate in the combined fenretinide groups vs. placebo, $P = 0.0606$). This therapeutic effect was not dose dependent and is consistent with anti-angiogenic properties of fenretinide, which have been observed in other disease states.

Conclusion: The findings of this study and the established safety profile of fenretinide in chronic dosing regimens warrant further study of fenretinide in the treatment of geographic atrophy.

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Geographic atrophy (GA) is a late stage manifestation of atrophic age-related macular degeneration (AMD), a blinding disease for which there is no available treatment. Although the exact pathophysiologic

mechanisms leading to the development of GA have not been elucidated, there is substantial evidence that implicates excessive accumulation of lipofuscin within the retinal pigment epithelium (RPE) as a pathological component of the disease process.^{1–3} Lipofuscin comprises incompletely digested cellular debris from spent photoreceptor outer segment disks and contains chemically modified residues of proteins and lipids.⁴ The aberrant accumulation of lipofuscin is believed to interfere with normal cellular function of the RPE, leading to RPE cell death and dysfunction/loss of overlying photoreceptors because of the absence of trophic influences from the RPE.^{5,6}

A prominent molecular component of lipofuscin is the toxic vitamin A (retinol)-based compound,

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N-retinylidene-*N*-retinylethanolamine (A2E). The compound accumulates within RPE phagolysosomes in an age-dependent and light-dependent manner and is known to have numerous deleterious effects on RPE cells, including generation of reactive oxygen species,^{7,8} impairment of lysosomal degradative functions,^{9,10} induction of proapoptotic proteins,¹¹ complement activation,¹² and, at high concentrations, acting as a cationic detergent to perturb biologic membranes.¹³ Based on these findings, A2E is thought to be a principal cytotoxic compound within ocular lipofuscin.

N-retinylidene-*N*-retinylethanolamine and related compounds produce a distinct autofluorescent emission that is readily detectable by fundus autofluorescence (FAF) photography. Advances in FAF imaging have permitted monitoring of autofluorescence patterns in vivo and have facilitated diagnosis of GA.^{14–16} Areas of increased FAF are observed at the boundaries of GA lesions and are associated with reductions in both scotopic and photopic retinal sensitivity.^{17–19} Prospective analysis of FAF patterning in GA patients has demonstrated that new atrophic lesions develop in areas with increased FAF, indicating that increased FAF precedes both development and enlargement of GA lesions.^{16,17,20–22} These findings are consistent with the theory that cytotoxic fluorophores, such as A2E, are present within areas of increased FAF. The compounds that contribute to FAF are derived from circulating retinol, which is delivered to the RPE by serum retinol-binding protein (RBP).^{23,24} Therefore, reduction in delivery of RBP-retinol to the RPE is theorized to reduce accumulation of A2E and related fluorophores and slow lesion growth in GA patients.

Fenretinide (*N*-(4-hydroxyphenyl)retinamide, 4-HPR) is a synthetic derivative of vitamin A that has had extensive human exposure in various disease states over the past 25 years (e.g., rheumatoid arthritis, psoriasis, and various cancers).^{25–30} Although these studies have found limited efficacy for fenretinide in treating systemic diseases, the acute and chronic exposure in human patients (approximately 9,000 patient-years) has been useful to establish long-term safety and tolerability of fenretinide.^{26–28} A consistent side effect observed in all human clinical trials of fenretinide is delayed dark adaptation (DDA).^{27,28,30–32} This effect has been attributed to reduced levels of visual chromophore within the visual cycle.^{31–33}

Fenretinide possesses a unique chemical structure that allows it to compete with retinol for binding to RBP and cause rapid elimination of the RBP–fenretinide complex in urine.^{34,35} This effect is dose dependent and reversible upon drug cessation.^{25–28} Retinol-binding protein is the

primary vehicle for retinol delivery to extrahepatic target tissues and possesses a single high-affinity binding site for retinol, resulting in a 1:1 M RBP-retinol stoichiometry in serum.^{24,36} Therefore, fenretinide-mediated reductions of circulating RBP are matched by proportional reductions of serum retinol. Because RPE is uniquely dependent upon retinol delivery by circulating RBP,^{36,37} fenretinide treatment preferentially reduces retinol concentrations in the eye.

In a preclinical proof-of-concept study, fenretinide was shown to reduce circulating RBP-retinol in a dose-dependent manner and dramatically slow the production of A2E and related compounds in the Stargardt (*abca4*–/–) animal model.³⁸ Based upon these data, clinical findings of increased FAF in GA patients, and the documented safety profile of fenretinide, a 2-year phase II trial was initiated in GA patients. This proof-of-concept study was conducted to gather preliminary evidence to determine the safety and efficacy of daily oral fenretinide to slow lesion growth in patients with GA secondary to dry AMD.

Materials and Methods

Study Design

The GA fenretinide trial was a 2-year, phase II, double-masked, randomized, placebo-controlled, multicenter proof of concept trial to determine the safety and efficacy of oral fenretinide at 2 doses (100 mg and 300 mg daily) in subjects with GA secondary to dry AMD (ClinicalTrials.gov Identifier: NCT00429936). Subjects signed written informed consent before determination of their full eligibility. The inclusion criteria were as follows: male or female, 50 years to 89 years with GA within 500 μ m of fovea, total atrophic area of 1 to 8 disk areas (2.54–20.32 mm²) not characterized as either focal or patchy by FAF photography and best-corrected visual acuity of 20/20 to 20/100 (Snellen equivalent using the Early Treatment Diabetic Retinopathy Study chart). Baseline FAF images were evaluated by masked readers at the GRADE Reading Center (University of Bonn, Bonn, Germany). Subjects with active choroidal neovascularization (CNV) in the study eye were excluded from participation; a history of CNV in either eye was allowed.

Safety and Efficacy Assessments

Assessments of drug safety and efficacy were performed at baseline and at regular intervals including blood chemistries, hematology, and incidence/severity of adverse events (AEs). Blood samples were

collected at regular intervals during the study for quantitation of the parent drug, the primary metabolite (*N*-(4-methoxyphenyl)retinamide), RBP, and serum retinol (Apuit, Inc, Edinburgh, United Kingdom). Retinal imaging included color fundus (CF) photography, FAF, and fluorescein angiography. Retinal images were evaluated by masked readers at the Digital Angiographic Reading Center (DARC, New York, NY). Retinal thickness and macular volume measurements were obtained using Stratus optical coherence tomography (OCT). The masked OCT data were evaluated at the OCT Reading Center at Duke University (Durham, NC). Best corrected visual acuity and contrast sensitivity were assessed by clinical site investigators. Onset of CNV was diagnosed by clinical site investigators and confirmed by masked readers at DARC. A validated Night Vision Questionnaire was used to determine the degree of DDA. A grade value was assigned based on responses to five questions related to vision in dim light conditions. The percent of patients reporting DDA and mean grades for DDA severity were determined from the Night Vision Questionnaire data. Lesion growth rates (annualized aggregate lesion size growth, square millimeter per year) were calculated using at least 3 measurements (data from baseline and 2 later time points past 6 months). The primary evaluation of efficacy was based on comparison of annualized lesion growth rates among the treatment groups as measured by either CF or FAF.

Study Treatment

The active drug substance, fenretinide, has the chemical name of *N*-(4-hydroxyphenyl)retinamide (4-HPR) and was formulated as a 100 mg softgel capsule with polysorbate 80 and corn oil (16 oblong) for oral administration. A total of 246 patients were randomly assigned (1:1:1) into the placebo ($n = 82$), 100 mg ($n = 80$) or 300 mg ($n = 84$) treatment groups. The doses were self-administered daily after the evening meal. Patients also received daily PreserVision Lutein Soft Gels Eye Vitamins without beta carotene.

Statistical Analysis

Safety analyses were performed on all randomized subjects who received at least one administration of the allocated dose. All analyses on the safety population were based on data as observed through the whole extent of exposure. The efficacy analysis was performed on an intent-to-treat basis among all subjects. This population was defined as all randomized subjects who received at least one administration of the allocated dose and who had at least two follow-up visits. The primary efficacy measure (annualized

change in aggregate lesion size growth) was evaluated only for patients completing at least 18 months of treatment. The last observation carried forward method for missing data was not used. For each subject, the variable was the first-order slope of lesion size as a function of time on treatment at each assessment fitted by linear regression without an intercept. A natural log transform on lesion size was applied to linearize the slope. The efficacy analysis compared the mean slopes between the active and placebo treatment groups. An analysis of covariance model was used to evaluate the homogeneity of the individual slope estimates of the subjects. Baseline lesion size was included as a covariate. Supportive analyses included analysis of the slopes as derived above but using a nonparametric analysis of variance. A repeated-measures model of the log of the GA lesion growth across all time points was used to estimate the slope of the change for each treatment group and differences in the slopes between the groups. For the repeated-measures analysis, the comparison was the average slope across all patients as opposed to the average of all slopes obtained for each patient. Treatment group, site, and visit month were considered as fixed effects. A covariance structure appropriate to the spacing of the time points was used, and each subject was treated as a random effect. No imputation for missing data was carried out.

Results

Study Population

The study population was demographically and clinically comparable across the three treatment groups. The patients were predominantly whites with a median age of approximately 80 years. There were no significant differences in baseline best corrected visual acuity or lesion sizes. Demographic data and baseline ocular characteristics for the treatment groups are provided in Table 1 and Table 2, respectively.

Study Withdrawal and Adverse Events

Over the 2-year study period, a total of 68 patients were lost to early withdrawal. Reasons for study withdrawal are provided in Table 3. A listing of AEs, which accounted for withdrawal rates of 6.1%, 17.5%, and 20.2% in the placebo, 100 mg, and 300 mg groups, respectively, are shown in Table 4. Among the reported AEs, only disorders of skin/subcutaneous and eye were determined to be drug related. Skin and subcutaneous disorders (rash and pruritus) were expected and observed at the expected frequencies. A

Table 1. Demographic Data for Patients Enrolled in the Fenretinide GA Trial

	Placebo (n = 82)	Fenretinide 100 mg (n = 80)	Fenretinide 300 mg (n = 84)
Age, years			
Median	80.0	79.5	79.0
Minimum	55	58	53
Maximum	89	89	90
Gender, n (%)			
Male	30 (36.6)	28 (35.0)	39 (46.4)
Female	52 (63.4)	52 (65.0)	45 (53.6)
Ethnicity, n (%)			
Hispanic/Latin	1 (1.2)	0	3 (3.6)
Not Hispanic/Latin	81 (98.8)	80 (100.0)	81 (96.4)
Race, n (%)			
White	81 (98.8)	80 (100.0)	83 (98.8)
Black/African American	1 (1.2)	0	0
Other race	0	0	1 (1.2)

description of specific eye disorders among patients is provided in Table 5. Delayed dark adaptation, referred to in the Medical Dictionary for Regulatory Activities (MedDRA) coding tables as “Night Blindness,” was an expected primary side effect of fenretinide therapy. In the combined fenretinide groups, 2.5% of patients (4 of 163) withdrew because of DDA complications. Visual disturbance was cited as a reason for study withdrawal by 4 patients in the 300 mg group. Patients experiencing this phenomenon described transient patterns of color or darkened areas within their visual field upon waking or during an immediate exposure to bright light. The molecular/biochemical basis for these disturbances is unclear. All visual disturbances were resolved shortly after drug cessation. One patient in the 100 mg group and 3 patients in the 300 mg group withdrew because of reduced visual acuity. The percentage of patients with lesions that involved, or encroached upon, the fovea at study baseline was highest in the 300 mg group (81%), followed by the 100 mg group (72%) and placebo (70%). Thus, it is possible that the incidence of reduced visual acuity in the 300 mg group may be attributable to a disproportionately higher percentage of foveal involvement in this group at baseline.

Other Adverse Events Not Leading to Study Withdrawal

Ocular AEs that were reported during the trial but did not lead to study withdrawal are listed in Table 6 (percent of patient reports). There was a marked reduction in the incidence of CNV onset among patients in fenretinide treatment groups (discussed in “Choroidal Neovascularization Incidence”). Dose-related AEs, such as conjunctivitis, lacrimation, and blurred vision were anticipated based upon previous fenretinide trials.^{27,28,32} Incidence of DDA (night blindness) was also assessed by questionnaire (see “Materials and Methods”). Analysis of patient reports showed that 29.3% of patients in the placebo group experienced DDA. The incidence of DDA reports increased by 7% to 8% in patients treated with fenretinide. Severity of DDA, as assessed by grade values obtained from the Night Vision Questionnaire, was determined to be dose dependent (discussed in “Delayed Dark Adaptation”). The most prominent dose-related AE reported during the trial was visual disturbance. As discussed above, this phenomenon was transient and reversible and did not contribute significantly to withdrawal rates. Nonocular AEs (blood chemistries, etc.) were not significantly different among the treatment groups.

Serum Retinol-Binding Protein

Temporal analysis of serum RBP concentrations showed a dose-dependent reduction of serum RBP in fenretinide-treated patients. Nadir RBP levels were observed after 3 months of daily drug administration. At nadir, mean RBP concentrations in the 100 mg and 300 mg treatment groups were approximately 3 mg/dL and 2 mg/dL, respectively. Relative to baseline RBP concentrations, the nadir RBP values corresponded to reductions of approximately 50% in the 100 mg group and approximately 70% in the 300 mg group (Figure 1). Changes in serum retinol concentrations were consistent with changes in serum RBP throughout the study period (not shown). Cessation of fenretinide intake at study end (Month 24) resulted in return of RBP levels toward baseline levels over the subsequent month.

Table 2. Baseline Ocular Characteristics for Patients Enrolled in the Fenretinide GA Trial

	Placebo (n = 82)	Fenretinide 100 mg (n = 80)	Fenretinide 300 mg (n = 84)
Mean BCVA	66.57	68.59	68.12
Lesion size by CF, median ± SD, mm ²	8.17 ± 4.50	8.10 ± 4.78	9.06 ± 5.03
Lesion size by FAF, median ± SD, mm ²	8.55 ± 4.84	8.33 ± 5.10	9.02 ± 5.26

BCVA, best-corrected visual acuity; CF, color fundus photography; FAF, fundus autofluorescent photography.

Table 3. Study Withdrawal Rates in the Fenretinide GA Trial

	Placebo (n = 82), n (%)	Fenretinide 100 mg (n = 80), n (%)	Fenretinide 300 mg (n = 83), n (%)
Early withdrawal from study	14 (17.1)	28 (35.0)	26 (31.0)
Reason for study exit			
Subject withdrew consent	8 (9.8)	12 (15.0)	8 (9.5)
Protocol violation	1 (1.2)	0	1 (1.2)
Lost to follow-up	0	2 (2.5)	0
Adverse event	5 (6.1)	14 (17.5)	17 (20.2)

Visual Acuity

Analysis of visual acuity in the fenretinide trial showed a 2-year loss of 10 to 11 letters in all treatment groups (Figure 2). There was a slightly greater, albeit statistically insignificant, visual acuity loss in the 300 mg dosage group at 12 months to 18 months. This effect could be attributed to a greater rate of foveal involvement (discussed above) and/or higher incidence of events at the ocular surface (e.g., dryness or lacrimation and mild conjunctivitis) in patients within the 300 mg group. The stasis of visual acuity loss in the 300 mg group from 18 to 24 months and at the end of the 1-month follow up period indicates that the observed loss was a transient effect unrelated to drug exposure.

Delayed Dark Adaptation

Analysis of dark adaptation deficits was based upon responses to the Night Vision Questionnaire. Each group demonstrated progressive DDA deficits (lower grade) during the 2-year study period. Although there

Table 4. Incidence of Adverse Events Leading to Study Withdrawal

System Organ Class, Preferred Term	Placebo (n = 82), n (%)	Fenretinide 100 mg (n = 80), n (%)	Fenretinide 300 mg (n = 83), n (%)
Cardiac disorders	1 (1.2)	2 (2.5)	0
Eye disorders	0	3 (3.8)	8 (9.6)
Gastrointestinal	2 (2.4)	3 (3.8)	1 (1.2)
Investigations	0	2 (2.5)	1 (1.2)
Neoplasms	2 (2.4)	0	2 (2.4)
Nervous system	0	3 (3.8)	2 (2.4)
Respiratory	1 (1.2)	0	1 (1.2)
Skin and subcutaneous	0	3 (3.8)	2 (2.4)
Vascular	1 (1.2)	1 (1.3)	0

Table 5. Report of Eye Disorders Leading to Study Withdrawal

MedDRA Term	Fenretinide 100 mg (n = 80)	Fenretinide 300 mg (n = 83)
Night blindness	1	3
Visual disturbance	0	4
Visual acuity reduced	1	3
Dry eye	1	0
Macular degeneration	1	1

Values represent number of patient reports.
MedDRA, Medical Dictionary for Regulatory Activities.

was a dose-dependent trend for increased DDA, there were no statistically significant differences in the magnitude of DDA among the 3 treatment groups (Figure 3A). Analysis of DDA grade as a mean percentage change from baseline showed the most profound effect in the 300 mg group. At the end of the study period, patients in the 300 mg group had a mean reduction of approximately 38% in their baseline DDA grade compared with an approximate 28% reduction for patients in the 100 mg group and an approximate 16% reduction for patients in the placebo group (Figure 3B).

Lesion Growth Rates

Analysis of annualized lesion growth rates as a function of serum RBP concentration in each treatment group is shown in Figure 4. In this analysis,

Table 6. Adverse Events Not Leading to Study Withdrawal

System Organ Class, MedDRA* Term	Placebo (n = 82)	Fenretinide 100 mg (n = 80)	Fenretinide 300 mg (n = 83)
Cataract	12.2	11.3	13.3
CNV	18.3	8.8	9.6
Conjunctivitis	0	1.3	4.8
Dry eye	3.7	6.3	3.6
Lacrimation increased	1.2	3.8	7.2
Night blindness	29.3	36.3	37.3
Retinal hemorrhage	7.3	12.5	7.2
Vision blurred	2.4	6.3	8.4
Visual acuity reduced	69.5	66.3	71.1
Visual disturbance	7.3	18.8	26.5

Values represent percent of patients reporting in each treatment group.
MedDRA, Medical Dictionary for Regulatory Activities.

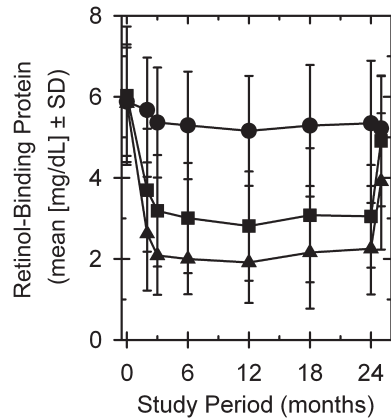


Fig. 1. Analysis of serum retinol-binding protein concentration in GA patients. Retinol-binding protein levels were measured from serum samples obtained from whole blood. Samples were taken at baseline and 1, 3, 6, 12, 18, 24, and 25 months. The 25-month time point constitutes a 1-month follow-up RBP value. Retinol-binding protein levels were quantified by enzyme-linked immunoabsorbent assay. The data show mean RBP levels ($\pm$ SD, mg/dL). Sample group numbers varied according to patient participation throughout the study period. Circles, placebo; squares, 100 mg fenretinide; triangles, 300 mg fenretinide.

growth rate slopes for each patient were calculated using the lesion size data from baseline and 2 later time points after 6 months (data from color fundus photography). These data were plotted as a function of the corresponding mean RBP concentration for each patient during the same time period. The median lesion growth rates for each treatment group and the corresponding patient numbers are provided in the Figure 4 legend. A least squares regression line was calculated

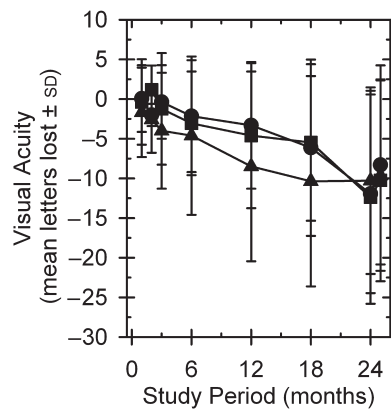


Fig. 2. Analysis of visual acuity loss in GA patients. Measures of best-corrected visual acuity in GA patients were determined with EDTRS charts. Visual acuity assessments were performed at baseline and 1, 3, 6, 12, 18, 24, and 25 months. The 25-month time point constitutes a 1-month follow-up visual acuity assessment. The data show EDTRS letters lost (mean $\pm$ SD) relative to group baseline values (Table 2). Sample group numbers varied according to patient participation throughout the study period. Circles, placebo; squares, 100 mg fenretinide; triangles, 300 mg fenretinide.

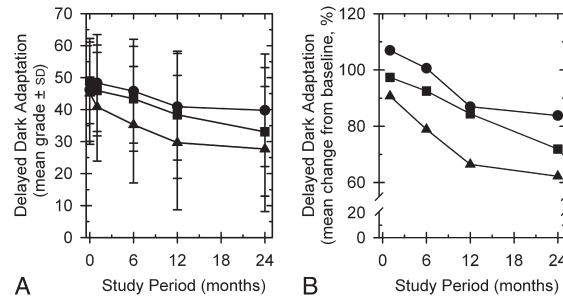


Fig. 3. Assessment of DDA in GA patients. A validated Night Vision Questionnaire was used to assess the extent of DDA in GA patients. Assessments were performed at baseline and 1, 6, 12, and 24 months. **A**, DDA grade (mean $\pm$ SD) for each treatment group during the study period. **B**, the change in extent of DDA as a percent of the baseline value for each treatment group. Sample group numbers varied according to patient participation throughout the study period. Circles, placebo; squares, 100 mg fenretinide; triangles, 300 mg fenretinide.

for the data points in each plot to examine the correlation between lesion growth rate and RBP concentration (solid lines). A trend for reduced lesion growth rates with reduced serum RBP levels was observed for patients in the 300 mg group. This trend appeared more pronounced at RBP levels <2 mg/dL. Therefore, an analysis of lesion growth rates for patients who achieved RBP levels of ≤ 2 mg/dL was performed. A similar analysis was performed for fenretinide-treated patients with serum RBP levels >2 mg/dL. The resulting data revealed a positive correlation for reduced lesion growth rates and RBP reductions <2 mg/dL for patients in the 300 mg fenretinide group ($r^2 = 0.478$, dashed line). Twenty-one patients in

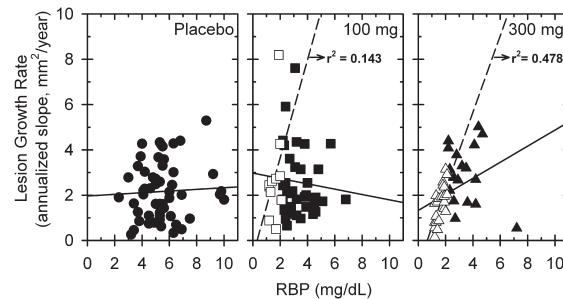


Fig. 4. Correlation between annualized lesion growth rate and RBP levels in GA patients. Lesion growth rate slopes (mm^2/year) were calculated for patients in each treatment group and plotted against their corresponding RBP concentration (mg/dL) as described in the text. The median lesion growth rates for the treatment groups are as follows: Placebo (circles, $n = 56$): 2.03 ± 1.24 ; 100 mg fenretinide (squares, $n = 45$): 2.14 ± 1.66 ; 300 mg fenretinide (triangles, $n = 41$): 1.95 ± 1.22 . A least squares regression analysis using all data points in each plot is shown (solid lines). In the fenretinide treatment groups, a least squares regression analysis was calculated for patients meeting the RBP criteria of ≤ 2 mg/dL (dashed line). The median lesion growth rates for these groups are as follows: 100 mg fenretinide (open squares, $n = 10$): 2.53 ± 2.13 ; 300 mg fenretinide (open triangles, $n = 21$): 1.70 ± 0.77 . The correlation coefficients (r^2) are provided in the figure insets.

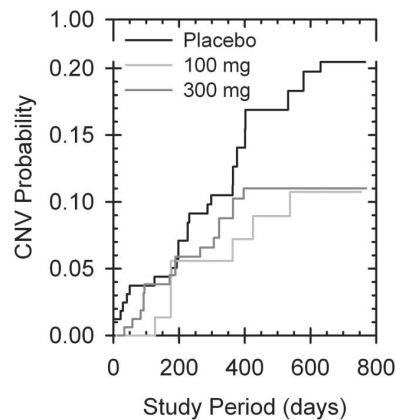


Fig. 5. Kaplan-Meier survival analysis for onset of CNV in GA patients. Patients with active CNV in the study eye were excluded from participation in the trial; a history of CNV in either study or fellow eye was allowed. Choroidal neovascularization events and diagnoses were ascertained as described in "Materials and Methods". The analysis shows time to first CNV event in either the study or fellow eye of GA patients. Treatment group identifications are provided in the figure inset.

the 300 mg group (51%) met the RBP criteria of ≤ 2 mg/dL. The median lesion growth rate in this group of patients represents a slowing of 0.33 square millimeter per year compared with placebo (1.70 ± 0.77 mm²/year vs. 2.03 ± 1.24 mm²/year, $P = 0.1848$). A similar trend was observed for patients in the 100 mg fenretinide group (dashed line); however, the limited number of patients achieving the reduced RBP criteria ($n = 10$) and the large variability in lesion growth rates (2.53 ± 2.13 mm²/year) precludes a definitive conclusion. There was no relationship between lesion growth rates and RBP at RBP levels >2 mg/dL (data not shown). It should be noted that the lesion growth rate slopes calculated from the FAF image data were in excellent agreement with data from color fundus photography ($96 \pm 3\%$).

Choroidal Neovascularization Incidence

The incidence of CNV onset was monitored as part of the safety assessment protocol. Analysis of time to first CNV event, in either the study eye or fellow eye, showed a markedly reduced incidence of CNV events in the fenretinide treatment groups during the second year of the study (Figure 5). This effect showed no dose dependency at the two studied doses. Calculated odds ratios showed a 2.2-fold increased risk for a CNV event in patients within the placebo group compared with patients in the combined fenretinide arms during the 2-year study (95% CI, $P = 0.0606$). A patient-based analysis of CNV incidence (at least 1 CNV event in either the study eye or fellow eye) showed a rate of 18.3% in the placebo group, 8.8% in the

Table 7. Incidence of CNV Onset in Patients Enrolled in the Fenretinide GA Trial

	Placebo (n = 82), N (%)	Fenretinide 100 mg (n = 80), N (%)	Fenretinide 300 mg (n = 83), N (%)
No CNV event	67 (81.7)	73 (91.3)	75 (90.4)
≥ 1 CNV event	15 (18.3)	7 (8.8)	8 (9.6)

100 mg fenretinide group, and 9.6% in the 300 mg fenretinide group (Table 7). In each of the 3 groups, the majority of CNV events ($>90\%$) occurred in the study eye. To determine whether the incidence of CNV events in the study eye could be attributed to previous CNV in the fellow eye, an analysis of study eye CNV onset in patients with previous CNV in the fellow eye was performed. Among 6 patients in the placebo group with a documented history of fellow eye CNV, 2 patients (33%) experienced a CNV event in the study eye. In the combined fenretinide arms, there was a total 26 patients with a history of fellow eye CNV at baseline. Only 1 of these 26 patients (4%) developed CNV in the study eye (Table 8).

Discussion

The current study was a phase II proof-of-concept trial that was designed to determine whether reductions in serum RBP-retinol would lead to reduced lesion growth in GA patients. Data from this trial show a trend for reduced lesion growth rates in patients in the 300 mg fenretinide group who achieved serum RBP levels of ≤ 2 mg/dL, which equates to a retinol concentration of ≤ 1 μ M. Fifty-one percent of patients in

Table 8. Incidence of CNV Onset in Patients with Fellow Eye CNV History

	Placebo (n = 82), n (%)	Fenretinide 100 mg (n = 80), n (%)	Fenretinide 300 mg (n = 83), n (%)
Patients with fellow eye CNV history	6 (7.3)	13 (16.3)	13 (15.7)
New CNV* (with fellow eye CNV)	2 (33)	0 (0)	1 (8)

*New CNV diagnosed in study eye.

the 300 mg treatment group who completed the 2-year study achieved this level of RBP reduction. In these patients, there was a mean reduction of 0.33 mm² in the yearly lesion growth rate, compared with placebo. Progressive reductions of RBP <2 mg/dL correlated with further reductions of lesion growth rates ($r^2 = 0.478$). Because of the limited number of patients achieving the RBP reduction criteria, the growth rate difference versus placebo did not achieve statistical significance ($P = 0.1848$). However, the data provide important information regarding the threshold level of serum RBP-retinol reduction required to effect a slowing of lesion growth in GA patients.

Fenretinide has been extensively studied in human cancers^{25–30} and is known to reduce serum RBP-retinol in a dose-dependent/reversible manner and also reduce retinoid content in ocular tissues.^{31–33,38} Clinical findings that have implicated retinol-based compounds, such as A2E, in GA lesion expansion and disease progression^{16,17,20–22} form the basis for repurposing of fenretinide to treat GA. The identification of A2E in human retinal lipofuscin³⁹ and the known cytotoxicity of A2E^{7–13} provide support for this therapeutic approach. However, an etiologic role for A2E in GA pathogenesis has not been definitively established and the time course required to effect slowing of A2E biosynthesis, via retinol reduction, in a clinically meaningful manner is not known.

The compound A2E is generated as a by-product of the visual cycle. Therefore, the effect of fenretinide on slowing A2E biosynthesis is predicated upon reduction of retinoids in the visual cycle. Proof of concept for this mechanism has been established in the Stargardt (*abca4*–/–) animal model, which accumulates massive amounts of A2E because of the absence of the ABCR transporter protein.³⁸ In *abca4*–/– mice, fenretinide-mediated reductions of serum RBP-retinol led to proportional reductions of visual cycle retinoids and A2E over 1-month period. Importantly, whereas the steady-state reduction of serum RBP-retinol occurred within approximately 3 days, reduction of visual cycle retinoids and A2E required at least 28 days of dosing.

In the present study, nadir RBP-retinol levels in fenretinide-treated patients were achieved after 3 months of daily dosing. However, trends for reduced lesion growth rates in the 300 mg group did not occur until after 12 months. There were no differences in lesion growth rates among patients before 12 months regardless of serum RBP-retinol levels. Additionally, reports of DDA (a subjective measure of change in visual function) were most prevalent among patients in the 300 mg group and severity was greatest during the 12- to 24-month time period.

Collectively, findings in the Stargardt mouse model and GA patients indicate a latency period between time to achieve steady-state serum RBP-retinol levels and attainment of steady-state effects in the eye (i.e., reduction of visual cycle retinoids and A2E). The gradual depletion of ocular retinoids during fenretinide treatment is believed to be the result of compensatory mechanisms that act to conserve retinoid levels in the eye during periods of vitamin A deficiency.⁴⁰ It is tempting to speculate that a more prolonged exposure to fenretinide may have produced a greater effect against lesion growth.

It is noteworthy that the observed “therapeutic threshold” of retinol reduction ($\leq 1 \mu\text{M}$) observed to elicit a slowing of lesion growth rates in the present study has been associated with specific effects on visual cycle function in previous clinical investigations of fenretinide. In these studies, the most pronounced effects on visual function (e.g., elevated dark-adapted thresholds and delay in timing of rod–cone break times as determined by dark adaptometry and electroretinography) were observed in fenretinide-treated patients with serum retinol levels $< 1 \mu\text{M}$.^{31–33} Consistent with these data are findings from epidemiologic studies of vitamin A deficiency, collectively involving hundreds of human subjects, which show that DDA is elicited when serum retinol concentrations drop below $1 \mu\text{M}$.^{41,42} Therefore, although the objective measures of visual function were not performed in the present study, it is likely that GA patients achieving retinol levels of $\leq 1 \mu\text{M}$ would have had measurable changes consistent with reduced visual chromophore in the retina.

Analysis of visual acuity in the fenretinide trial showed a 2-year loss of 10 to 11 letters in all treatment groups. This rate of visual acuity loss is consistent with the natural history of GA.^{43,44} Visual acuity loss is not highly correlated with lesion progression in GA patients because the lesions are initially outside the fovea.^{44,45} Therefore, slowing of lesion growth over a 2-year period in the fenretinide trial was not expected to significantly impact visual acuity.

Notably, fenretinide treatment was associated with a marked reduction of CNV onset in GA patients. This effect was not dose dependent and is consistent with antiangiogenic properties of fenretinide that have been observed in oncology studies.^{46,47} In GA patients treated with either dose of fenretinide, the rate of CNV incidence was reduced by approximately 45%, relative to placebo. Previous investigations of CNV incidence in GA subjects have shown that subjects with CNV in one eye have an increased risk of developing CNV in the contralateral eye.⁴⁸ In the current study, differences in CNV incidence between placebo and the fenretinide groups could not be attributed to a disproportionate risk

in the placebo group. In fact, the risk of CNV onset (based on previous CNV history at baseline) was increased by more than twofold in the fenretinide groups, relative to the placebo. Despite increased risk in the fenretinide groups, only 1 of 26 patients with a history of fellow eye CNV developed CNV in the study eye, compared with 2 of 6 patients in the placebo group.

In summary, fenretinide treatment was found to be safe and well tolerated and produced a trend for reducing the growth of GA lesions. This therapeutic effect was associated with a requisite reduction of serum RBP-retinol and a latency period that led to steady-state changes in the eye. Fenretinide treatment also reduced the onset of CNV in GA subjects, reducing the risk for conversion from dry to wet AMD. The absence of a dose-dependent effect for prevention of CNV onset provides further support for antiangiogenic properties of fenretinide that are not dependent on RBP reduction. Collectively, the data from this trial provide compelling support for further investigations of the therapeutic potential of fenretinide in the treatment of AMD.

Key words: age-related macular degeneration, fenretinide, geographic atrophy, lipofuscin, *N*-retinylidene-*N*-retinylethanolamine, retinal pigment epithelium, retinol-binding protein, vitamin A.

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