Effects of Fenretinide (4-HPR) on Dark Adaptation

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Objectives: To assess the alterations in dark adaptation induced by low (200 mg/d) doses of fenretinide (4-HPR), to assess whether these effects were cumulative and whether they were reversible, and to attempt to elucidate the mechanism underlying the changes in night vision.

Design: Case series.

Setting: Outpatient eye clinic.

Patients: Twenty-two women enrolled in a breast cancer chemoprevention trial, and 18 normal control subjects.

Intervention: Measurements of absolute luminance thresholds during dark adaptation.

Main Outcome Measures: Parameters of an exponential model of the dark-adaptation function before, during, and after administration of fenretinide.

Results: The most conspicuous effect of fenretinide on dark adaptation was a significant delay in the timing of the rod-cone break ($P<.001$). A minimal elevation of the final cone threshold was also observed. These effects were reversible after fenretinide therapy was discontinued and did not seem to be cumulative. An inverse relationship between delay of the rod-cone break and plasma retinol concentration was found.

Conclusion: The dose of fenretinide used in this study produced clearly measurable, but not severe, changes in night vision, which were rarely symptomatic.


FENRETINIDE (N-[4-hydroxyphenyl]-retinamide) (4-HPR) is a synthetic retinoid used in clinical trials for cancer therapy and the prevention of malignant neoplasms. Reversible dark-adaptation changes and electroretinogram abnormalities associated with fenretinide chemotherapy (using a high dose of 800 mg/d) for basal cell carcinoma have previously been documented in our clinic. An abnormality in dark adaptation was also reported in a more recent study involving the use of a low dose of fenretinide (200 mg/d for a median treatment time of 32 months) for breast cancer chemotherapy. This effect was associated with a decline in plasma retinol concentration. In this study, we measured the dark adaptation of women who were enrolled in a breast cancer prevention trial using a low dose of fenretinide (200 mg/d) for 4 months.

The main objective of this study was to ascertain whether this low dose of fenretinide induced measurable and clinically significant abnormalities in dark adaptation. In 17 patients, their dark-adaptation function while they were receiving fenretinide was compared with their own baseline measurement before exposure to the drug. In 22 patients (the 17 women mentioned earlier plus 5 additional patients), the results were compared with those obtained in a group of normal control subjects. Secondary objectives of this study were addressed with subsets of this group of 22 patients. To investigate if the effects of fenretinide on dark adaptation were cumulative, 13 patients were evaluated twice while receiving fenretinide. To establish if the effects of fenretinide were reversible, we compared the dark-adaptation function before exposure to the drug and after discontinuing its use in 11 patients. Finally, in 17 patients, we calculated the correlation between plasma concentration of retinoids (retinol, fenretinide, and fenretinide’s principal metabolite, N-[4-methoxyphenyl]-retinamide [4-MPR]) and dark-adaptation findings (adaptometric measures), in an attempt to elucidate the mechanism underlying the changes in night vision.
PATIENTS AND METHODS

PATIENTS

Twenty-five consecutive patients were recruited for this study. All patients gave informed consent after the study had been approved by the National Cancer Institute Institutional Review Board. Three patients were excluded from the analysis, the first because her dark-adaptation function was abnormal in the baseline examination, the second because of her extreme unreliability for adaptometry, and the third because she developed symptoms compatible with the diagnosis of optic neuritis during the study. The remaining 22 patients were included in this analysis. Their age ranged from 39 to 58 years (mean±SD age, 48.30 ± 4.96 years; median age, 47 years). The patients received four 4-week cycles of oral fenretinide, 200 mg/d, during days 1 through 25, with a drug holiday during days 26 through 28. Additionally, patients received oral tamoxifen, 20 mg/d, starting on day 29 and thereafter continuously through the last 3 cycles.

The control group was composed of 18 normal volunteers (14 women and 4 men). Normal limits for the parameters of the dark-adaptation function were computed using the data obtained in these normal subjects, by calculating the 2-sided tolerance limits that include 90% of the population with 90% confidence.1

CLINICAL EVALUATION

All patients were asked to report any visual symptoms experienced while receiving fenretinide, and specifically whether any changes in night vision were noted. They underwent a complete ophthalmological examination on each occasion their dark-adaptation function was measured. This included manifest refraction, best-corrected visual acuity (ETDRS distance visual acuity charts, Lighthouse Low Vision Products, Long Island City, NY), slit lamp examination of anterior segments, ophthalmoscopy, and biomicroscopy of the retina.

ADAPTOMETRY

Dark adaptation was measured with a Goldmann-Weekar adapterometer, modified to adopt the von Bekesy threshold tracking method, following the same technique used in our prior study1 to allow a valid comparison. Patients were light adapted for 5 minutes with a 2700-candela/m² (cd/m²) Ganzfeld background. Then the absolute luminance threshold was measured continuously during dark adaptation until no further threshold changes could be detected in a 10-minute period. A central 11°diameter circular target with a 0.6-Hz flicker rate and a maximum luminance of 3.77 cd/m² was used. The patients viewed the target monocularly. Their pupils were not dilated, to minimize photophobia during the bleach period. Only the measurements obtained when the patients were observing the stimulus with their right eye were used for this analysis.

The cone limb and the rod limb of the dark-adaptation function were digitized and fit using a slight modification of a mathematical model that describes the course of adaptation adequately.5 The values for the 3 parameters of the following model (a, b, and c) were obtained using an iterative curve-fitting algorithm:

(1) Cone-mediated limb:
\[ \text{Threshold} = a_{cone} + b_{cone} \times e^{-\text{time}/c_{cone}} \]

(2) Rod-mediated limb:
\[ \text{Threshold} = a_{rod} + b_{rod} \times e^{-\text{time}/c_{rod}} \]

where, in each limb, \( a_{cone} \) or \( a_{rod} \) is the final dark-adapted threshold (in log candelas per square meter); \( b_{cone} \) or \( b_{rod} \) is the magnitude of dark adaptation (in log candelas per square meter); and \( c_{cone} \) or \( c_{rod} \) is the time constant of dark adaptation (in minutes). The time of the rod-cone break (\( t_b \)) was then found by equating

(3) \[ a_{rod} + b_{rod} \times e^{-\text{time}/c_{rod}} = a_{cone} + b_{cone} \times e^{-\text{time}/c_{cone}} \]

and solving for time using a numerical method.

Finally, since the observed onset of the rod-mediated limb is the rod-cone break, the parameters of the rod-mediated limb were recalculated using the original version of the model:

(4) \[ \text{Threshold} = a_{rod} + b_{rod} \times e^{-(\text{time} - t_b)/c_{rod}} \]

which yields an accurate value of \( b_{rod} \).

PLASMA RETINOID CONCENTRATION ASSAY

Plasma was separated from blood samples (7-10 mL) by centrifugation. All samples were protected from light and stored at -70°C until analysis. Retinoid concentration was determined by high-performance liquid chromatography using a modification of the Bugge et al method.6 A Beckman system (Beckman Instruments Inc, San Ramon, Calif) with an analytical C-18 column with no end-capping (Zorbax ODS Special, 5-µm spherical particles, 25 cm × 4.6-mm inner diameter, MacMod Analytical Inc, Chadds Ford, Pa) was used. Retinoid peaks were detected with a variable wavelength detector (Beckman) set at 360 nm. Because the peak absorption for retinol is 325 nm, determination of retinol concentration required modification of the assay to this wavelength. The assay was linear between 0 and 1 µmol of fenretinide and 4-MPR and between 0 and 1000 ng/mL of retinol concentration.

STATISTICAL ANALYSIS

The parameters of the dark-adaptation model of patients were compared with those of control subjects using the Student t test. To account for the possibility that the assumptions required by this test were not met by the sample, the data were also analyzed using the Mann-Whitney test.1 To compare the adaptation parameters in the same patient before and after administration of fenretinide, and in 2 different study cycles, a paired t test was used.7 These data were also analyzed using the Wilcoxon signed rank test8 for the considerations described previously. In all these tests, 7 variables were compared. To maintain the overall probability of making a type I error at the .05 level, the Bonferroni method was adopted. Therefore, for each pairwise comparison a significance level of .05 ÷ 7 = .007 was chosen. Regression and correlation (Pearson product-moment correlation coefficient and the Spearman rank correlation coefficient)9 were used to assess the association of one parameter of the dark-adaptation function (the rod-cone break) and plasma concentration of retinoids. Because 4 correlation coefficients were calculated, a significance level of .05 ÷ 4 = .0125 was adopted.
RESULTS

ABNORMALITIES IN DARK ADAPTATION INDUCED BY FENRETINIDE

When the parameters of the dark adaptation of patients who were receiving fenretinide were compared with their own baseline function, the rod-cone break was significantly delayed (mean ± SD, from 5.12 ± 1.19 to 8.43 ± 2.65 minutes) (P<.001 in both the paired t test and the Wilcoxon test). Additionally, a minimal (but statistically significant) elevation of the final cone threshold (a_cone) (from −2.48 ± 0.24 to −2.31 ± 0.19 log cd/m²) could be seen (P = .005, paired t test, and P = .009, the Wilcoxon test). This determined an equivalent slight increase in the magnitude of rod adaptation (b Российка) (P = .01 and P = .01, respectively) (see “Comment” section). All other parameters of the dark-adaptation function did not vary significantly.

A representative example of the fenretinide effect is shown in Figure 1, in which the dark-adaptation functions of patient 15 at baseline (open circles) and on day 24 of cycle 2 (solid circles) are superimposed. The rod-cone break was delayed from 4.24 to 7.46 minutes. The shape of the rod limb, which is determined by the time course of adaptation (c Российка), had not changed but was displaced to the right in the time axis. Therefore, the final rod threshold (a Российка) was unchanged, although it was reached later than at baseline. A slight elevation of the final cone threshold (from −2.35 to −2.09 log cd/m²) was also seen in this patient.

A comparison of the dark-adaptation function of all 22 patients who were receiving fenretinide with that of normal control subjects showed, again, a significant delay in the rod-cone break, which occurred, on average, 3.36 minutes later (P<.001 in both the Student t test and the Mann-Whitney test). In 16 of the 22 women, the rod-cone break occurred beyond the upper normal limit of 6.92 minutes. Also, a minimal but statistically significant elevation of the final cone threshold could be seen (P = .001, Student t test, and P = .002, Mann-Whitney test). In only 2 women, the final cone threshold was marginally higher (<0.03 log cd/m²) than the upper normal limit (−2.06 log cd/m²). This was associated with an equivalent increase in the magnitude of rod adaptation (b Российка). Mean and SD values for all parameters of the dark-adaptation function are displayed in the Table, which shows that the differences seen in the remaining parameters were not statistically significant. In all patients, the final rod threshold was normal (<4.27 log cd/m²).

Only 2 patients (patients 11 and 9, whose rod-cone break occurred at 8.21 and 13.04 minutes, respectively) reported subjective changes in dark adaptation, describing that they needed more time to adjust to a dark environment, beginning in the first or the second fenretinide cycle (patient 9 and patient 11, respectively). Two additional patients (patients 22 and 13, whose rod-cone break occurred at 14.76 and 7.46 minutes, respectively) reported visual symptoms that were unquestionably related to an alteration in their adaptation rate. Patient 22 reported not noticing a wall immediately after turning off the room lights during the first fenretinide cycle, and patient 15 described requiring more time “to see a window covered with a curtain in a dark room” during the fourth fenretinide cycle.

In all 4 patients, the final cone threshold was in the normal range. Best-corrected visual acuity and all other opthalmological findings were normal in all patients.

ARE THE EFFECTS OF FENRETINIDE CUMULATIVE?

To assess the potential cumulative nature of the effects described in the preceding section, we evaluated a subset of 13 patients in 2 different study cycles. The patients were evaluated on approximately the same day of each cycle (days 22-25, with the exception of 1 patient, who was assessed on day 16 in one cycle). Although in 8 of these patients the delay in the rod-cone break became more pronounced after subsequent chemoprevention cycles, we were unable to document significant changes in the study parameters when data from all 13 patients were pooled. The modest further rod-cone break delay (which increased from 8.53 ± 2.33 minutes to 9.15 ± 2.61 minutes) did not reach statistical significance (P = .14, paired t test, and P = .13, Wilcoxon test).

ARE THE EFFECTS OF FENRETINIDE REVERSIBLE?

In 11 patients, the reversibility of the effects of fenretinide was evaluated by comparing their dark-adaptation function measured before the onset of the study and after discontinuing drug use. The time between the last dose of fenretinide and the final measurement of dark adaptation ranged from 3 to 643 days (median, 59 days). No statistically significant differences in the rod-cone break or in a_cone were found between the baseline and final measurements. The rod-cone break, after showing a drug-induced increase to 8.22 ± 2.38 minutes from the baseline value of 5.20 ± 1.13 minutes, decreased to 5.24 ± 1.22 minutes after fenretinide was discontinued. Similarly, a_cone, which had changed slightly from the −2.44 ± 0.23 log cd/m² baseline value to −2.31 ± 0.21 log cd/m², reverted to the original level of −2.43 ± 0.24 log cd/m². As expected, all other parameters of the dark-adaptation function remained unchanged.

Figure 1. Dark-adaptation functions measured in patient 15 at baseline (open circles) and on day 24 of cycle 2 (solid circles). The smooth curves passing through data points were fit using the exponential model described in the “Adaptometry” subsection of the “Patients and Methods” section. cd indicates candela.
Parameters of the Dark-Adaptation Function in Patients and Control Subjects

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Patients (n = 22)</th>
<th>Controls (n = 18)</th>
<th>Student t Test</th>
<th>Mann-Whitney Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rod-cone break†</td>
<td>8.58 ± 2.77</td>
<td>5.22 ± 0.79</td>
<td>.001‡</td>
<td>.002‡</td>
</tr>
<tr>
<td>Rod-cone delay‡</td>
<td>5.21 ± 0.39</td>
<td>4.74 ± 0.29</td>
<td>.01</td>
<td>.01</td>
</tr>
<tr>
<td>Acone§</td>
<td>6.31 ± 1.12</td>
<td>6.04 ± 1.12</td>
<td>.45</td>
<td>.48</td>
</tr>
<tr>
<td>Bcone§</td>
<td>1.43 ± 0.27</td>
<td>1.21 ± 0.38</td>
<td>.30</td>
<td>.17</td>
</tr>
<tr>
<td>Acone§</td>
<td>2.46 ± 0.28</td>
<td>2.23 ± 0.25</td>
<td>.95</td>
<td>.87</td>
</tr>
<tr>
<td>Bcone§</td>
<td>1.09 ± 0.38</td>
<td>1.21 ± 0.38</td>
<td>.50</td>
<td>.47</td>
</tr>
<tr>
<td>Acone§</td>
<td>−4.75 ± 0.33</td>
<td>−4.74 ± 0.22</td>
<td>.01</td>
<td>.01</td>
</tr>
<tr>
<td>Bcone§</td>
<td>−1.99 ± 0.38</td>
<td>−1.21 ± 0.38</td>
<td>.50</td>
<td>.47</td>
</tr>
</tbody>
</table>

*Values are expressed as mean ± SD.
†Values are expressed in log candelas per square meter.
‡Results were significant.
§Values are expressed in minutes.

Figure 2. Rod-cone break as a function of the ratio between plasma retinol concentration during administration of fenretinide and baseline plasma retinol concentration. The curved lines enclose the 95% confidence band about the regression line.

**RELATIONSHIP BETWEEN DARK ADAPTATION AND PLASMA CONCENTRATION OF RETINOIDS**

The association between the rod-cone break delay and the plasma concentration of retinol, fenretinide, and its metabolite, 4-MPR, were assessed in 15 patients. All patients in whom we measured dark adaptation and plasma retinoid concentration in the same treatment cycle were included in this subset. There was a statistically significant inverse relationship between rod-cone break delay and the ratio between retinol level during administration of fenretinide and baseline (before administration of fenretinide) retinol concentration (ie, retinol concentration during fenretinide administration divided by baseline retinol concentration) (Figure 2). The value of \( r_p \) (Pearson product-moment correlation coefficient) was \(-0.62\) (\( P = .008 \)) and that of \( r_s \) (Spearman rank correlation coefficient) was \(-0.70\) (\( P = .005 \)). The relationship between rod-cone break delay and retinol concentration during administration of fenretinide followed a similar trend (\( r_s = −0.58 [ P = .02 \] and \( r_s = −0.64 [ P = .01 ] \)). There was a suggestion of a direct relationship between fenretinide plasma concentration and rod-cone break delay (\( r_p = 0.55 [ P = .02 ] \) and \( r_s = 0.66 [ P = .01 ] \)). However, in the last 2 cases, the \( P \) values for \( r_p \) did not reach statistical significance at the level chosen. No significant associations were seen between delay in the rod-cone break and 4-MPR concentration.

**COMMENT**

Our data show that low doses of fenretinide (200 mg/d) produced a modification of the dark-adaptation function, characterized by a conspicuous rod-cone break delay. Additionally, a modest, statistically but probably not clinically significant, elevation of the final cone threshold and an equally modest increase in the magnitude of rod adaptation (\( b_{c0} \)) were seen. These last 2 effects are not independent. An examination of the model described in the “Patients and Methods” section shows that the magnitude of rod adaptation is measured from the level of the cone plateau. Therefore, the slight increase in \( b_{c0} \) is a direct consequence of the elevation of the final cone threshold, rather than an “improvement” in rod adaptation.

An accurate estimation of dark adaptation in patients receiving low doses of fenretinide should not consist exclusively of a measurement of the final rod threshold, since this parameter, which is frequently abnormal in patients subjected to higher doses of this drug,\(^1\) was invariably normal in all women in this study. Because the results obtained comparing the patient group with a normal control group were equivalent to those seen when each patient was compared with her own baseline, pretreatment measurements, while useful, are nonessential since population-based norms are sufficient.

Measurements of dark adaptation in patients with mild vitamin A deficiency have also shown abnormalities during adaptometry that resemble those we saw in our patients.\(^8\) In that study, Kemp et al\(^8\) present data obtained in an asymptomatic patient undergoing progressive degrees of vitamin A deficiency. In the early stages of deficiency (see their Figure 4, curve B), their patient showed changes in dark adaptation that are remarkably similar to those seen in patient 16 in our study (Figure 1). This similarity suggests the hypothesis that the effects of fenretinide are primarily due to an alteration in retinal vitamin A metabolism. This hypothesis is supported by a study of the effects of long-term fenretinide administration on vitamin A metabolism in the eyes of rats.\(^9\) This study documented a steady decline in vitamin A concentration in the eyes of rats treated with fen-
retinoid, which exceeded the decline in plasma retinol concentration. Unlike vitamin A–deficient rats, which had a reduced ocular vitamin A turnover, rats exposed to fenretinide showed an increased turnover. Lewis et al. proposed that fenretinide may interfere with the compensatory mechanisms that attempt to conserve ocular vitamin A stores, and that the normal uptake and/or metabolism of vitamin A in the eye is also affected by the drug.

The measurements of plasma concentration of retinoids in our patients also argue in favor of this explanation for the fenretinide effect, as the rod-cone break delay was significantly correlated with the decline in plasma retinol ratio (ie, retinol concentration during fenretinide administration divided by baseline retinol concentration) (Figure 2). As expected, the increasing concentration of plasma fenretinide was associated with more extended rod-cone break delays, which did not reach the stringent level of statistical significance chosen. This was possibly owing to the low statistical power determined by the few patients included in this aspect of our study.

Although we did not perform densitometric studies in our patients, the fact that their final rod threshold was normal in all cases suggests that their retinal rhodopsin concentration was normal. Because the timing of rhodopsin synthesis during dark adaptation is determined by the concentration of opsins and 11-cis-retinal, a decline in the available pool of 11-cis-retinal may explain the rod-cone break delay seen in our patients (Trevor D. Lamb, PhD, oral communication, May 15, 1997). Bleaching an increased proportion of rhodopsin also results in a rod-cone break delay and may be an alternative explanation for our findings.

It is highly probable that one or more of the mechanisms described earlier are wholly responsible for the changes in our patients’ dark adaptation. However, an additional effect of fenretinide on adaptation mediated by a modification of pupillary motility, while unlikely, cannot be completely ruled out.

Our results show only partial agreement with a previous study concerning the alterations in the dark-adaptation function induced by fenretinide. Decensi et al. found, as we did, that the rod-cone break was significantly delayed in patients treated with fenretinide. In contrast to our findings, they also reported a significant increase in the final rod threshold that was not confirmed by our data. The most likely explanation for this discrepancy is the fact that, in their study, adaptometry was performed only during the first 25 minutes of dark adaptation. Frequently, when the rod-cone break is delayed, the final rod threshold is not reached in 25 minutes, which leads to an underestimate of this final threshold. It is also possible, though less likely, that a difference in the stimulus size used may be responsible for the discrepancies.

Fortunately, the modifications in dark adaptation seen in this study were fully reversible after the drug was discontinued. This suggests that fenretinide does not have retinotoxic effects. Because the interval between the day in which the drug therapy was discontinued and the day in which adaptometry was performed was variable and frequently long, this study cannot address the rate at which the fenretinide effects disappear. It is unlikely that the alterations in night vision described in this article were due to tamoxifen, since they were reversed after discontinuing use of fenretinide, even though the patients continued receiving tamoxifen.

Although the fenretinide effect was seen in 16 patients, only 2 patients reported subjective dark-adaptation changes, and another 2 described visual symptoms less likely to be related to an alteration in their adaptation rate. This is probably due to the fact that in an urban environment, rod vision is rarely required, and an alteration in the timing of the rod limb is unlikely to be symptomatic in the absence of concomitant alterations of cone-mediated vision. The modifications seen in the cone limb in our patients were probably too minimal to elicit symptoms. There was no obvious association between presence of symptoms and the degree of rod-cone break delay.

An encouraging result of this study is that, while the effects of fenretinide were consistent, predictable, and clearly measurable, they did not entail dramatic, clinically significant changes in the dark-adaptation function. This indicates that, if this drug is proven to be effective as a chemotherapeutic or chemopreventive agent, visual side effects would not preclude its use at these low doses. The long-term effects of fenretinide treatment on visual function, however, require further study.

Accepted for publication February 27, 1998.

We are grateful for the assistance provided by Linda Goodman, Anne Randall, Antoinette LaReau, and Dessie Koutandreas, who performed measurements of dark adaptation, refraction, and visual acuity in the patients included in this study. Also, we thank Lessie McCain, Linda Goodman, and Nanette McAtee, who acted as clinical coordinators for the project. Finally, we thank Elliot D. Feldman, PhD, for useful discussions regarding the application of exponential models.

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