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Original Paper

Long-term Effects of Fenretinide on Retinal Function

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The long-term effects of the synthetic retinoid fenretinide (4-HPR) on retinal function were studied by electroretinogram (ERG) in 24 women treated for a median of 30.5 months and in 18 untreated controls belonging to a phase III intervention trial. The six outcome measures were: a wave implicit time, peak-to-peak amplitude and implicit time of *b* wave following both cone stimulation and maximal cone–rod stimulation in the dark-adapted eye. Multivariate analysis of covariance was applied to evaluate the joint effect on the whole set of ERG measures taking into account their inter-relationship. Predictive factors with a significant effect on ERG measures were: (1) a qualitative interaction between age and treatment duration and (2) the squared (parabolic) function of plasma retinol. Individually, the *b* wave implicit time following cone stimulation was the only ERG measure significantly influenced by the predictors, indicating a primary effect of 4-HPR on retinal photoreceptor sensitivity without significant alterations of the inner nuclear layer. Thus, in contrast to previous reports at higher dose, administration of 4-HPR at 200 mg/day seems to exert subtle alterations of retinal function as measured by ERG. © **1997 Elsevier Science Ltd. All rights reserved.**

Key words: retinoids, retinal effects, cancer prevention

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INTRODUCTION

4-HPR (fenretinide or *N*-4-(hydroxyphenyl)retinamide), a synthetic amide of all-*trans*-retinoic acid, is currently under investigation in controlled clinical trials for the prevention of several solid tumours in at risk patients [1]. Interim analyses of some of these studies have demonstrated the high tolerability of this compound despite prolonged consumption [2, 3], a particularly remarkable finding when compared to other retinoids [4].

However, one important issue is the precise definition of the entity and degree of night blindness that has been associated with 4-HPR administration. Early case reports employing high doses of 4-HPR (600–800 mg daily) showed significant alterations of ERG (electroretinogram) and impaired dark adaptation [5–7], a finding which prompted the halt of further investigation with this compound at such doses. Pharmacological studies subsequently showed that administration of 4-HPR induces a dose-dependent decrease of plasma retinol and its binding protein [8] which play an essential function in night vision [9]. Conversely, at the daily dose of 200 mg currently used in chemoprevention trials, a systematic assessment of the long-term effects on objective ERG measures has not been carried out.

We recently studied the effects of 200 mg 4-HPR administration in a cohort of women by means of the Goldmann–Weekers dark-adaptometer, a psychophysical test which measures the subjective perception of light by the dark-adapted eye [10]. Elevations of scotopic thresholds were found in approximately 50% of the treated women compared to 6% in untreated controls and were linearly dependent on plasma retinol decline. However, half of the patients with elevated rod thresholds were asymptomatic, a finding in keeping with previous studies in subjects with intestinal or liver diseases [11, 12], thus leaving the real-life implications of the findings unsettled.

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These findings prompted us to study ERG measures in a controlled setting to assess the long-term effects of the drug (and drug-induced retinol decline) on retinal function.

MATERIALS AND METHODS

A cohort of 42 consecutive patients with previously operated stage I breast cancer belonging to a large-scale trial of chemoprevention of contralateral breast cancer [13] entered the study. 24 women receiving 4-HPR (R.W. Johnson Pharmaceutical Research Institute, Spring House, Pennsylvania, U.S.A.), 200 mg daily (2 capsules at dinner) with a 3-day drug suspension at the end of each month and 18 randomised, untreated controls were included in the study. Median treatment time was 30.5 months (range 6–44). Patient characteristics were balanced for tumour characteristics (not shown) and body mass index (weight/height^2, mean (±SE) 24.3 (±0.5) in the cases versus 25 (±0.7) in the controls), while treated women were slightly older: mean (±SE) age 57.6 (±1.2) versus 53.5 (±0.9) years. All women were tumour free and in good general condition and none were receiving medication other than 4-HPR. Informed consent was obtained from each patient after the study had received Institutional Review Board approval. All subjects underwent a complete ophthalmological examination to exclude ocular diseases or significant refractive errors.

The ERGs of both eyes were recorded simultaneously using an Amplaid SD15 amplifier according to the guidelines for standard clinical ERG [14], and modified as follows: photopic testing was first begun 10 min after adaptation to a Ganzfeld background luminance of 30 cdm^{-2}. A standard flash of 3 cdm^{-2}.s was delivered to elicit the cone response. Partial scotopic testing was then performed after the patient adapted to the dark for 15 min. The maximal combined (cone–rod) response was produced by the white standard flash in the dark-adapted eye every 5 s. Because the aim of the study was to reflect the real-life conditions of adaptation, the pure rod response was not recorded. Since there were no significant differences between the responses of the two eyes for any of the patients, the responses were averaged for comparison between the experimental and control group. The human ERG shows two prominent peaks in potential, the *a* and *b* waves (Figure 1). Six measures were considered as end points: the implicit time (ms) from prestimulus baseline to the *a* wave peak, and the peak-to-peak implicit time and amplitude (μvolt) of the *b* wave, in the photopic and scotopic phase of the test, respectively. The *a* wave amplitudes were not considered in the analysis since errors in the definition of the trigger baseline could not be excluded.

Immediately before ERG recording, a blood sample was taken for measurements of plasma concentrations of 4-HPR, its principal metabolite *N*-4-(methoxyphenyl)retinamide and retinol, that were performed by HPLC as previously described [8]. In the treated patients, ERGs and blood sampling were performed at different intervals from the monthly drug holiday, but kept at a relatively constant interval from the last 200 mg drug intake (mean ± SE, 13 h, 42 ± 40 min).

The effect of 4-HPR on ERG measures adjusted for the possible confounding effect of a number of covariates (age, BMI, plasma retinol) was assessed by multivariate analysis of covariance, which has the advantage of estimating the expected differences on all responses, simultaneously taking into account their intercorrelations [15, 16].

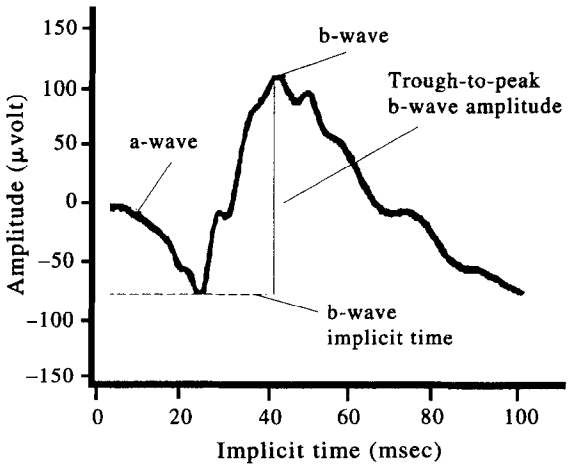

Figure 1. Example of ERG tracing following cone–rod stimulation in the dark-adapted eye.

Treatment was expressed as a categorical predictor with the control group as reference and the treated group expressed either as a whole (i.e. drug versus no treatment) or divided into two subsets by the median duration of treatment, or median plasma concentration of drug (384 ng/ml) or metabolite (284.5 ng/ml). The effect of each treatment category relative to the reference category (no treatment) was assessed using dummy (0, 1) variables.

The final multivariate model was subsequently tested in univariate multiple regressions (one for each ERG measure) using Bonferroni's procedure to adjust the nominal significance level for multiple tests, i.e. by dividing the nominal level by the number of response measures [15]. All analyses, including model diagnostics, were carried out using SPSS/PC+ (SPSS Inc., Chicago, Illinois, U.S.A.) and SAS (SAS Institute Inc., Cary, North Carolina, U.S.A.) packages. All *P* values were two-tailed.

RESULTS

Compared to baseline plasma retinol levels decreased by a mean of approximately 75% during 4-HPR administration (from basal 587.5 ± 36.3 to 143.9 ± 14.8 ng/ml, $P < 0.000$), while no significant variations occurred in control women (from basal 553.8 ± 39.2 to 585.7 ± 25.2 ng/ml).

Table 1. Results of multivariate analysis of covariance on the whole set of ERG measures

Predictor	Wilks' lambda	*F*-test	DF	*P* value
Treatment duration	0.501	1.93	12/56	0.05
Age	0.658	2.42	6/28	0.05
Plasma retinol	0.582	3.35	6/28	0.01
Squared plasma retinol	0.502	4.63	6/28	0.00
Age by plasma retinol	0.696	2.04	6/28	0.09
Age by treatment duration	0.399	2.72	12/56	<0.01
Overall	0.288	3.46	16/64	<0.01

F-test, multivariate test; DF, degrees of freedom; Overall, regression model including all predictors.

Table 2. Results of univariate analysis of covariance on individual ERG measures

Response	*F*-ratio	DF	*P* value
Photopic *a*IT	2.27	8/33	0.25
Scotopic *a*IT	1.15	8/33	0.93
Photopic *b*A	1.27	8/33	0.87
Scotopic *b*A	1.59	8/33	0.67
Photopic *b*IT	3.22	8/33	0.04
Scotopic *b*IT	2.12	8/33	0.31

IT, implicit time; A, amplitude; *F*-test, univariate test. DF, degrees of freedom; *P* value, significance level adjusted by Bonferroni's procedure

The set of predictive factors which explained the joint variability of ERG measures is shown in Table 1. The data indicate that the behaviour of ERG measures as a whole was affected by a significant interaction between age and treatment duration and by the parabolic correlation with plasma retinol concentrations. Results were similar when the treated group was subdivided by the median plasma concentration of drug or metabolite (data not shown). Subsequent analysis of covariance on individual ERG measures demonstrated that the light-adapted *b* wave implicit time was the only ERG measure significantly influenced by the predictors (Table 2). Figure 2 exemplifies the behaviour of the *b* wave implicit time as a function of the interaction between age and treatment duration. While no change of *b* wave implicit time is observed as a function of age in both untreated and women treated for less than 30.5 months, this measure is delayed in older women and earlier in younger women who were treated long-term with 4-HPR. The parabolic dependence of photopic *b* wave implicit time on plasma retinol levels is illustrated in Figure 3.

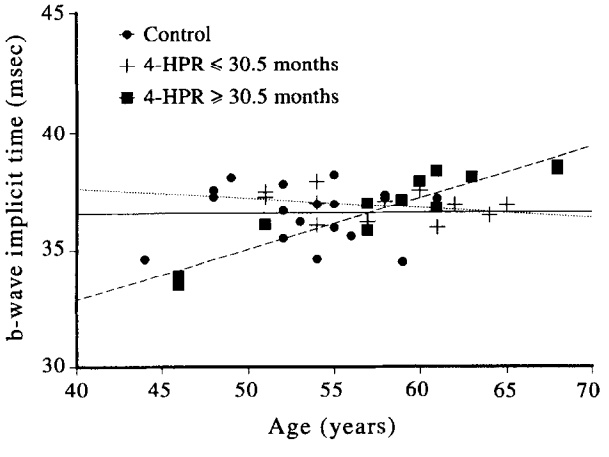

Figure 2. Behaviour of wave implicit time following cone stimulation as a function of age and duration of 4-HPR administration (•, untreated; +, short-term treated; ■, long-term treated). Points represent observed values; lines represent interpolations with the values predicted by the regression model (——, untreated; ····, short-term treated; - - -, long-term treated).

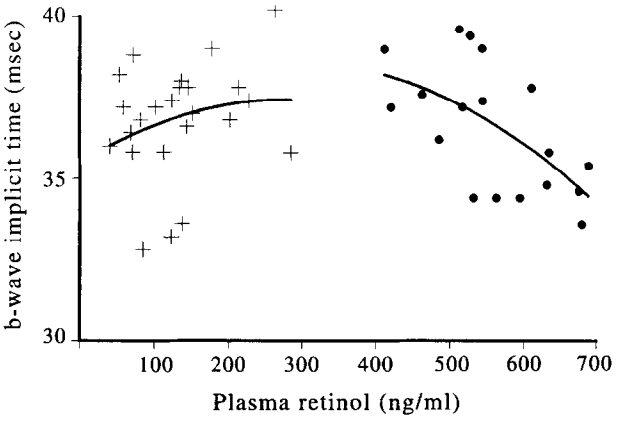

Figure 3. Behaviour of *b* wave implicit time following cone stimulation as a function of plasma retinol concentrations. Data are presented both as observed values (•, untreated; +, treated) and as curves predicted by the regression function. No significant effect of treatment duration was observed (data not shown).

While in the control population, the implicit time tends to be shorter as plasma retinol concentrations increase, a reverse effect is observed in 4-HPR treated women, where an earlier response is observed at minimal retinol concentrations. A similar parabolic dependence on plasma retinol concentrations was observed for the *b* wave amplitude in the dark adapted eye (data not shown).

DISCUSSION

Previous case reports employing intermediate–high doses (400–800 mg) of 4-HPR have shown significant alterations of ERG entailing severe blunting of the amplitude of the rod *b* wave at 800 mg/day [5] or reduction of the same parameter at doses of 400–600 mg/day [6, 7]. At the daily dose of 200 mg, which is currently employed in cancer chemoprevention trials, only anecdotal observations have been reported [3, 17]. However, because of the obvious interest in revealing even subtle alterations of retinal function induced by pharmacological agents in a preventive context and, similarly, of the inherent difficulty in establishing normal thresholds for ERG measures, a larger controlled study was warranted.

Our results show that 200 mg 4-HPR administration induces an overall pattern of ERG effects that depend on (1) a qualitative interaction between age and treatment duration and (2) the squared function of plasma retinol concentrations. Individually, the only ERG measure that was significantly affected by the set of predictors was the light-adapted *b* wave implicit time, which reflects the photoreceptor activity [18]. Conversely, the activity of the inner nuclear layer of the retina, which is commonly reflected by the trough-to-peak *b* wave amplitude [18], is not substantially affected at this dose of the retinoid, in contrast to previous reports at higher doses [5–7]. Taken together, the data seem to suggest a dose-dependent degree of inhibition of retinal function induced by the retinoid. In addition, the biphasic dependence of *b* wave measures on plasma retinol illustrates a fine regulatory mechanism through which the

retinal photoreceptor and the inner neural component govern the process of visual adaptation.

A trend towards a mild inhibition of retinal receptor function following prolonged duration of intervention was observed in the older women. Specifically, changes of *b* wave implicit time were contrasting depending on patient age, with delayed latencies being observed in older women. Similarly, we previously observed a heightened risk of altered psychophysical thresholds as measured by dark-adaptometry with increasing treatment time [10], ageing and obesity being risk factors for diminished night vision due to a strong association with lower plasma retinol concentrations [19]. However, the alterations of dark-adaptometry normalised upon treatment cessation [10].

A primary effect of 4-HPR on the *b* wave differs from the results observed with the use of 13-*cis*-retinoic acid [20, 21] or with the synthetic retinoid etretinate [22]. Indeed, the mechanisms of action affecting retinal function appear to vary among retinoids. For instance, previous reports indicate that night blindness induced by the intake of all-*trans*-retinoic acid may be due to its selective interference with the 11-*cis*-retinol dehydrogenase in the eye [23]. As regards 4-HPR, while early experiments in the rat have suggested the lack of direct inhibition of visual pigment formation [24], a recent study by Lewis and Phang [25] seems to provide evidence for a retinoid interference with the compensatory mechanisms that would normally be acting to conserve a decreased ocular supply of vitamin A. Moreover, the visual effects induced by 4-HPR may be related to a reduced availability of circulating retinol [26] due to an inhibition of the secretion of the retinol/retinol binding protein complex into plasma from both liver and extrahepatic tissues [27, 28]. A selective interference with normal vitamin A absorption has also been suggested [29].

Interestingly, natural retinoids, such as all-*trans*-retinoic acid and 13-*cis*-retinoic acid, may also induce similar but less pronounced effects on retinol levels, presumably because of the lower bioequivalent doses adopted in the clinic and the shorter half-life, while aromatic retinoids, such as etretinate, have a lower affinity than retinol for its binding protein [30].

In clinical terms, we have recently observed that low-dose vitamin A supplementation can normalise scotopic thresholds during conventional or high-dose 4-HPR administration [10, 31], but it is not known whether this supplementation may affect the preventive potential of the retinoid.

In conclusion, administration of 4-HPR at the daily dose of 200 mg exerts subtle changes on the electrophysiology of visual adaptation that pertain essentially to the *b* wave implicit time, indicating a primary effect on retinal photoreceptor sensitivity.

1. Costa A, Formelli F, Chiesa F, Decensi A, De Palo G, Veronesi U. Prospects of chemoprevention of human cancers with the synthetic retinoid fenretinide. *Cancer Res* 1994, **54** (suppl), 2032–2037.
2. Chiesa F, Tradati N, Marazza M, *et al.* Prevention of local relapses and new localizations of oral leukoplakia with the synthetic retinoid Fenretinide (4-HPR). Preliminary results. Oral Oncology. *Eur J Cancer* 1992, **28B**, 97–102.
3. Rotmensz N, De Palo G, Formelli F, *et al.* Long-term tolerability of Fenretinide (4-HPR) in breast cancer patients. *Eur J Cancer* 1991, **27**, 1127–1131.
4. Smith MA, Parkinson DR, Cheson DC, Friedman MA. Retinoids in cancer therapy. *J Clin Oncol* 1992, **10**, 839–864.
5. Kaiser-Kupfer MI, Peck GL, Caruso RC, Jaffe MJ, Di Giovanna JJ, Gross EG. Abnormal retinal function associated with fenretinide, a synthetic retinoid. *Arch Ophthalmol* 1986, **104**, 69–70.
6. Kingston TP, Lowe NJ, Winston J, Heckenlively J. Visual and cutaneous toxicity which occurs during *N*-(4-hydroxyphenyl)-retinamide therapy for psoriasis. *Clin Exp Dermatol* 1986, **11**, 624–627.
7. Modiano MR, Dalton WS, Lippman SM, Joffe L, Booth AR, Meyskens FL Jr. Ocular toxic effects of fenretinide. *J Natl Cancer Inst* 1990, **82**, 1063.
8. Formelli F, Carsana R, Costa A, *et al.* Plasma retinol level reduction by the synthetic retinoid Fenretinide: a one year follow-up study of breast cancer patients. *Cancer Res* 1989, **49**, 6149–6152.
9. Fulton AB, Hansen RM, Underwood BA, Shwachman H, Barg DC. Scotopic threshold and plasma retinol in cystic fibrosis. *Invest Ophthalmol Vis Sci* 1982, **23**, 364–370.
10. Decensi A, Torrisi R, Polizzi A, *et al.* Effect of the synthetic retinoid fenretinide on dark adaptation and the ocular surface. *J Natl Cancer Inst* 1994, **86**, 105–110.
11. Russell RM, Smith VC, Multack R, Krill AE, Rosenberg IH. Dark-adaptation testing for diagnosis of subclinical vitamin-A deficiency and evaluation of therapy. *Lancet* 1973, **11**, 1161–1164.
12. Patek AJ, Haig C. The occurrence of abnormal dark adaptation and its relation to vitamin A metabolism in patients with cirrhosis of the liver. *J Clin Invest* 1939, **18**, 609–616.
13. Veronesi U, De Palo G, Costa A, Formelli F, Marubini E, Del Vecchio M. Prevention of breast cancer with retinoids. *Monogr Natl Cancer Inst* 1992, **12**, 93–97.
14. International Standardization Committee, International Society for the Clinical Electrophysiology of Vision. Standard for clinical electroretinography. *Arch Ophthalmol* 1989, **107**, 816–819.
15. Hand DJ, Taylor CC. *Multivariate Analysis of Variance and Repeated Measures.* London, Chapman and Hall, 1993, 62–63.
16. Krzanowski WJ. *Principles of Multivariate Analysis.* Oxford, Oxford University Press, 1993, 449–473.
17. Costa A, Rotmensz N, Campa T, Magni A, Assimakopulous G. Safety and tolerability of retinoids. In De Palo G, Sporn M, Veronesi U, eds. *Progress and Perspectives in Chemoprevention.* New York, Raven Press, 1992, 69–76.
18. Hood DC, Birch DG. A computational model of the amplitude and implicit time of the b-wave of the human ERG. *Visual Neurosci* 1992, **8**, 107–126.
19. Torrisi R, Parodi S, Fontana V, *et al.* Factors affecting plasma retinol decline during long-term administration of the synthetic retinoid fenretinide in breast cancer patients. *Cancer Epidemiol Biomarkers Prev* 1994, **3**, 507–510.
20. Brown RD, Grattan CEH. Visual toxicity of synthetic retinoids. *Br J Ophthalmol* 1989, **73**, 286–288.
21. Weleber RG, Denman ST, Hanifin JM, Cunningham WJ. Abnormal retinal function associated with isotretinoin therapy for acne. *Arch Ophthalmol* 1986, **104**, 831–837.
22. Weber U, Melnik B, Goerz G, Michaelis L. Abnormal retinal function associated with long-term etretinate? *Lancet* 1988, **i**, 235–236.
23. Law WC, Rando RR. The molecular basis of retinoic acid induced night blindness. *Biochem Biophys Res Commun* 1989, **161**, 825–829.
24. Crouch RK, Goletz P. Fenretinide does not block visual pigment formation in the rat. *J Ocular Pharmacol* 1988, **4**, 253–257.
25. Lewis KC, Phang JM. Effects of chronic administration of *N*-(4-hydroxyphenyl)retinamide (4-HPR) on vitamin A metabolism in the eye. *Proc Am Assoc Cancer Res* 1995, abs. 1571, p. 263l.
26. Lewis KC, Zech LA, Phang JM. Effects of *N*-(4-hydroxyphenyl) retinamide supplementation on vitamin A metabolism. *Cancer Res* 1994, **54**, 4112–4117.

27. Berni R, Formelli F. *In vitro* interaction of fenretinide with plasma retinol-binding protein and its functional consequences. *Fed Eur Biochem Soc Lett* 1992, **308**, 43–45.
28. Smith JE, Lawless DC, Green MH, Moon RC. Secretion of vitamin A and retinol-binding protein into plasma is depressed in rats by *N*-(4-hydroxyphenyl)retinamide (fenretinide). *J Nutr* 1992, **122**, 1999–2009.
29. Dew SE, Wardlaw SA, Ong DE. Effects of pharmacological retinoids on several vitamin A-metabolizing enzymes. *Cancer Res* 1993, **53**, 2965–2969.
30. Berni R, Clerici M, Malpeli G, *et al.* Retinoids: *in vitro* interaction with retinol-binding protein: an influence on plasma retinol. *FASEB J* 1993, 7, 1179–1184.
31. Decensi A, Bruno S, Torrisi R, Parodi S, Polizzi A. Pilot study of high dose fenretinide and vitamin A supplementation in bladder cancer. *Eur J Cancer* 1994, **30A**, 1909–1910.

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