Factors Affecting Plasma Retinol Decline during Long-Term Administration of the Synthetic Retinoid Fenretinide in Breast Cancer Patients[1]

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Abstract

Administration of the synthetic retinoid Fenretinide lowers circulating retinol and may thus affect night vision. We have recently shown that plasma retinol levels below 100 ng/ml are associated with moderate alterations of the dark adaptometry test. To identify which patients are more likely to experience a decrease of plasma retinol under this threshold, we measured plasma levels of retinol, Fenretinide, and its metabolite 4-MPR in a cohort of 28 women receiving Fenretinide at the daily dose of 200 mg and studied their relationship with clinical characteristics such as age, menstrual status, body mass index, and time on treatment. Our results show that patients aged over 55 years with a higher percentage of adipose tissue had higher plasma concentrations of 4-MPR, which turned out to be the major determinant of the retinol decrease. This subgroup may thus deserve careful ophthalmological surveillance.

Introduction

Retinoids, the natural and synthetic analogues of vitamin A, have proven effective in inhibiting growth and inducing differentiation in experimental tumor models (1), and recently, the use of retinoids as chemopreventive agents has been proposed in the clinical setting (2, 3). 4-HPR,[3] a synthetic amide of all-*trans*-retinoic acid, is safe and well tolerated after long-term administration for prevention of contralateral breast cancer (4), a notable finding compared to other retinoids (5).

A peculiar effect of 4-HPR is to lower plasma levels of vitamin A (6) and thus potentially affect night vision. Nyctalopia and severe alterations of electroretinogram have been reported as the principal side effects in patients treated with high doses (400–800 mg) of 4-HPR as a therapeutic agent (7, 8).

We recently studied dark adaptation by means of Goldmann-Weekers adaptometry test in 34 women with breast cancer who were administered 4-HPR, 200 mg daily, and in 31 untreated controls (9). Our results showed a relevant effect of the drug in determining reduced dark adaptation with 23.5 and 26.5% of patients showing mild and moderate alterations of measured dark adaptability, respectively, compared to 6% of mild alterations in controls. However, the high rate of positive test patients complaining of no symptoms (about 50%) implied that the test was extremely sensitive in detecting even a subclinical deficiency of vitamin A, thus leaving the real-life implication of this finding undetermined. Moreover, we showed that the occurrence of dark adaptation alterations correlated closely with the decrease of plasma retinol and promptly reversed after drug discontinuation.

In order to better define the pattern of vitamin A decrease in 4-HPR-treated patients and thus identify which patients are at increased risk for diminished night vision, we measured circulating levels of retinol, 4-HPR, and its major metabolite 4-MPR, and studied their relationship with clinical characteristics of patients such as age, menstrual status, weight, BMI, and duration of intervention.

Our results indicate that women aged over 55 years with a higher percentage of adipose tissue had higher plasma levels of 4-MPR, which turned out to be the major determinant of retinol decline. This subgroup may thus deserve careful ophthalmological surveillance.

Patients and Methods

Twenty-eight patients with a stage I (T_{1-2}, N_0) breast cancer, participating in a randomized phase III trial of 4-HPR chemoprevention of contralateral cancer, entered the study. All patients were aged between 38 and 65 years, had undergone surgery within the preceding 10 years and had received no adjuvant systemic therapy. A detailed description of inclusion criteria is reported elsewhere (10). This group of patients was also part of the larger cohort who went through an extended ophthalmological evaluation the results of which have been published elsewhere (9).

Patients received 4-HPR (RW Johnson Pharmaceutical Research Institute, Springhouse, PA) 200 mg/os daily (two 100-mg capsules after the evening meal) with a 3-day drug holiday at the end of each month, in order to allow the recovery of plasma retinol levels.

Body weight and BMI, expressed as weight (kg) divided by squared height (m^2), which is generally considered a more reliable index of body fat than absolute weight, were recorded at the time of the ophthalmological examination.

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[3] The abbreviations used are: 4-HPR, Fenretinide; BMI, body mass index; RBP, retinol binding protein.

Table 1 Multiple regression of plasma retinol[a]

Variable	β	SE(β)	95% CI	Test	P
Constant	−1067.54	337.61	−1771.78–−363.31	−3.16	0.005
Menstrual status	−72.3	38.57	−152.76–8.14	−1.87	0.075
4-HPR	2.31	0.75	0.75–3.88	3.09	0.006
4-MPR	−0.29	0.90	−0.48–−0.10	−3.23	0.004
Age	21.97	6.42	8.58–35.36	3.42	0.003
BMI	1.31	4.72	−8.54–11.16	0.28	0.784
Time on treatment	3.40	1.14	1.02–57.9	2.97	0.007
4-HPR × age	−0.04	0.01	−0.07–−0.01	3.21	0.004

[a] Overall F test = 3.61; $P = 0.01$; $R^2 = 0.56$. β, regression coefficient; SE(β), SE of β; 95% CI, 95% confidence intervals for β; Test, Student's *t* test with 20 df; *P*, Student's *t* test significance level.

At the same time, a blood sample was drained at a mean interval of 13 h, 42 min ± 40 min after the last drug assumption. Blood samples were collected into heparinized tubes, wrapped in aluminum foil, and centrifuged at 1500 rpm for 20 min and plasma was separated and stored at −20°C until assayed. All assays were performed in the dark.

Plasma levels of retinol, 4-HPR, and 4-MPR were determined by high-performance liquid chromatography as previously described (11).

All statistical analyses were carried out using SPSS/PC+ software package (12). Multiple and linear regression analyses were performed to assess the dependence of each variable under study (plasma retinol, 4-HPR, 4-MPR) on a number of covariates, including age, menstrual status, BMI, and duration of intervention (13). Smoking was not taken into account as covariate since only 3 of 28 patients were smokers. Overall F test and R^2 index were computed to evaluate the goodness of model fitting, and the SE of each regression coefficient was used to perform significance test (Student's *t* test) and 95% confidence intervals of each coefficient. Univariate normality assumption was estimated by the nonparametric Kolgomorov-Smirnov test for each dependent variable. Normal probability plot and residual analysis was carried out to verify any violation of regression assumption and to detect the presence and the influence of outlying observations. All data are given as mean ± SE.

Results

The mean age of the 28 patients was 56.9 years (range, 42–68), mean BMI was 23.98 ± 0.49, and mean weight was 62.6 ± 2.6 kg. Seven women were premenopausal and 21 were postmenopausal. Basal retinol levels were 584.6 ± 31.76 ng/ml and decreased to 133.61 ± 12.7 ng/ml ($P < 0.001$) after a median treatment time of 32 months (range, 6–45). No relationship was found between retinol and age, BMI, or weight. After a mean time of 13 h, 42 min ± 40 min from the last drug assumption, plasma concentrations of 4-HPR were 387.61 ± 22.15 ng/ml and plasma levels of 4-MPR, the major metabolite of 4-HPR, were 295.43 ± 23.8 ng/ml. No relationship was observed between plasma levels of retinol, 4-HPR, and 4-MPR and the actual dose *pro kilo* administered to each patient (data not shown).

When multiple regression analysis using retinol levels as dependent variable was performed, a statistically significant inverse effect of plasma concentrations of 4-MPR on retinol was clearly indicated, while the dependence of plasma retinol on 4-HPR appeared more complex because of the interaction between 4-HPR concentrations and age (Table 1). Specifically, while plasma retinol decreased with age in women with higher levels of 4-HPR, an opposite trend was observed in patients with 4-HPR concentrations below the median (Fig. 1). A positive significant effect of the duration of intervention on plasma retinol was observed, while no effect of BMI was found.

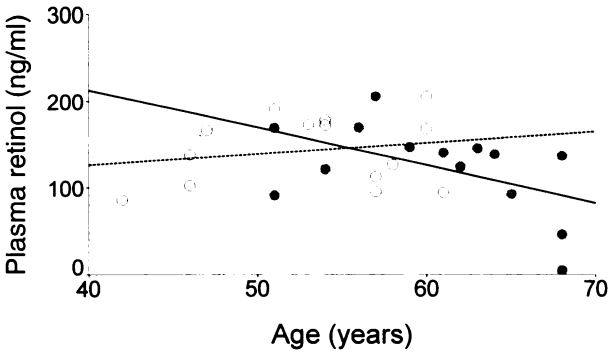

Fig. 1. Age-dependent behavior of plasma retinol levels in patients with plasma concentrations of 4-HPR above (full circles, solid line) or below (open circles, dashed line) the median value = 380.5 ng/ml (see text for a detailed explanation). Data are expressed as expected values.

Table 2 reports the results of multiple regression analysis performed using plasma concentrations of 4-MPR as dependent variable. A significant regression of 4-MPR on age, BMI, and 4-HPR levels is shown. The effect of all these variables on 4-MPR concentrations was modified by an interaction with age, as shown in Fig. 2 for the relationship between 4-MPR and BMI. Specifically, 4-MPR values increased with age in women with BMI above the median value, while no age-related effect was observed in patients with lower BMI. As a result, the difference of 4-MPR values between high and low BMI patients is negative in younger and positive in older women. On the other hand, when patients were categorized according to plasma levels of 4-HPR below or above the median, 4-MPR values increased with age in both groups, though with a different magnitude (data not shown).

When multiple regression was performed with plasma 4-HPR as dependent variable, results of univariate analysis were confirmed, showing 4-HPR levels to be dependent only on age (data not shown). Duration of intervention appeared not to affect either 4-HPR or 4-MPR concentrations (data not shown).

Discussion

Natural and synthetic retinoids can supply many biological properties of vitamin A, including its experimentally proven

Table 2 Multiple regression of plasma 4-MPR[a]

Variable	β	SEβ	95% CI	Test	*P*
Constant	6904.22	1754.41	3255.74–10552.71	3.94	<0.001
4-HPR	6.52	2.04	2.29–10.76	3.20	0.004
Age	−211.92	52.77	−321.66–−102.18	4.02	<0.001
BMI	−181.14	68.26	−323.09–−39.18	2.65	0.015
Age × age	1.59	0.52	0.52–2.67	3.09	0.006
BMI × age	3.31	1.18	0.86–5.77	2.80	0.010
4-HPR × age	−0.11	0.04	−0.19–−0.04	3.12	0.005

[a] Overall F test = 4.64; $P = 0.004$; $R^2 = 0.57$. β, regression coefficient; SE(β), SE of β; 95% CI, 95% confidence intervals of β; Test, Student's *t* test with 21 df; *P*, Student's *t* test significance level.

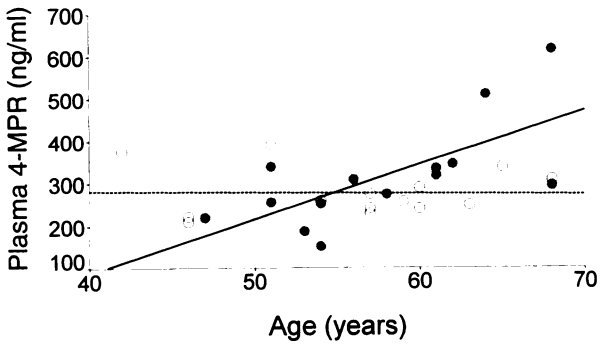

Fig. 2. Age-dependent behavior of plasma 4-MPR levels in patients with BMI above (full circles, solid line) and below (open circles, dashed line) the median value = 24.19 (see text for a detailed explanation). Data are expressed as expected values.

chemopreventive activity (14). Among various retinoids tested in clinical studies 4-HPR has been proven safe and well tolerated (15). However, like other retinoids, it is not able to supply the fundamental role of vitamin A in vision and in reproduction. The major clinical drawback in the administration of 4-HPR is, therefore, diminished night vision due to the drug-induced decrease of plasma retinol levels (6). The mechanisms through which the retinoid exerts this effect are not yet clear. 4-HPR has been shown to compete with retinol for the binding to RBP (16). The complex 4-HPR-RBP, however, does not bind transthyretin and is rapidly cleared from plasma due to its low molecular weight (16). In addition, it has recently been shown that 4-HPR induces specifically RBP secretion from the liver and its rapid accumulation in the kidney, thus suggesting that it may depress plasma retinol by reducing the amount of RBP available for the binding to retinol (17).

Our previous work has endeavored to determine a threshold for plasma retinol under which the occurrence of side effects, in particular dark adaptation impairment, may arise. We have shown that women with retinol levels <100 ng/ml are more prone to experience moderate alterations of dark adaptation (9). The aim of the present work was to predict which patients are more likely to experience such a dramatic drop of their circulating retinol during 4-HPR administration. Moreover, since 4-HPR is administered at the standard dose of 200 mg daily, it is conceivable that retinol decrease may be related to the actual dose *pro kilo* of the drug received by each patient. However, weight and the dose *pro kilo* do not seem to be critical in retinol decrease, while age and BMI appear to significantly affect, though indirectly, plasma retinol concentrations.

A major role in determining retinol decrease was played by 4-MPR. Interestingly, the complex regulation of plasma retinol and 4-MPR concentrations is attributable to a real biological (not simply confounding) effect of both BMI and age. Studies of 4-MPR metabolism in mice propose that 4-MPR, due to its high lipophilic structure, represents the storage form of the parent drug in adipose tissue (18). In humans, 4-MPR has a longer half-life than 4-HPR (54 *versus* 27 h) and has been found in higher concentrations in plasma after treatment interruption, suggesting its storage in tissues as occurs in mice (11, 19). Moreover, 4-MPR reaches higher concentrations in normal breast tissue (both epithelium and fat) and in nipple discharge than 4-HPR (11, 19). Our data seem to be consistent with this hypothesis: in women with higher BMI, 4-MPR may accumulate in adipose tissue, undergo slow release, and thus reach higher plasma levels. Contrary to the complex regulation of 4-MPR, 4-HPR levels appeared to be strictly dependent only on age. Previous studies have shown that pretreatment with drugs inducing the hepatic cytochrome P_{450} system significantly affect disposition and metabolism of 4-HPR, thus decreasing serum levels up to 50% (20). The reduction of cytochrome activity occurring with age might slow down drug metabolism and thereby account for the increased circulating levels observed in older patients.

Preliminary data obtained in breast cancer and melanoma cell lines suggest that the conversion to 4-MPR is necessary for the anticarcinogenic activity of 4-HPR (21). Our data highlight a critical role for the metabolite also in the toxicological profile related to the retinoid administration. Moreover, our data indirectly confirm that both the parent drug and the metabolite do not accumulate, their levels being stable even after long-term administration (11). This issue is crucial in a chemopreventive strategy which requires a prolonged, if not a life-spanning, administration.

Interestingly, plasma retinol levels tend to increase with time on treatment, so that a reduction in the incidence of alterations of dark adaptation with time might be expected. This could be related to the slight decrease of 4-MPR levels observed at 5 years of administration (11). The small number of observations does not allow to draw further conclusions.

In conclusion, our results suggest that patients older than 55 years of age, with higher relative percentage of adipose tissue (higher BMI), are more likely to have higher 4-MPR levels and consequently, to experience a dramatic decrease of their retinol levels. A careful follow-up of plasma retinol (*i.e.*, every 6 months) and an ophthalmological evaluation, through a questionnaire and, if needed, the Goldmann-Weekers adaptometry test, may be warranted in these patients.

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References

1. Sporn, M. B., and Roberts, A. B. Role of retinoids in differentiation and carcinogenesis. Cancer Res., *43:* 3034–3040, 1983.

2. Hill, D. L., and Grubbs, C. J. Retinoids and cancer prevention. Annu. Rev. Nutr., *12:* 161–181, 1992.

3. Omenn, G. S., Goodman, G., Thornquist, M., Grizzle, J., Rosenstock, L., Barnhart, S., Balmes, J., Cherniack, M. G., Cullen, M. R., Glass, A., Keogh, J., Meyskens, F., Jr., Valanis, B., and Williams, J., Jr. The β-carotene and retinol efficacy trial (CARET) for chemoprevention of lung cancer in high risk populations: smokers and asbestos-exposed workers. Cancer Res., *54* (Suppl.): 2038s–2043s, 1994.

4. Rotmensz, N., De Palo, G., Formelli, F., Costa, A., Marubini, E., Campa, T., Crippa, A., Danesini, G. M., Delle Grottaglie, M., Di Mauro, M. G., Filiberti, A., Gallazzi, M., Guzzon, A., Magni, A., Malone, W. F., Mariani, L., Palvarini, M., Perloff, M., Pizzichetta, M., and Veronesi, U. Long-term tolerability of fenretinide (4-HPR) in breast cancer patients. Eur. J. Cancer, *27:* 1127–1131, 1991.

5. Smith, M. A., Parkinson, D. R., Cheson, B. D., and Friedman, M. A. Retinoids in cancer therapy. J. Clin. Oncol., *10:* 839–864, 1992.

6. Formelli, F., Carsana, C., Costa, A., Buraneli, F., Campa, T., Dossena, G., Magni, A., and Pizzichetta, M. Plasma retinol reduction by the synthetic retinoid fenretinide: a one-year follow-up study of breast cancer patients. Cancer Res., *49:* 6149–6152, 1989.

7. Kaiser-Kupfer, M. I., Peck, G. L., Caruso, R. C., Jaffe, M. J., DiGiovanna, J. J., and Gross, E. G. Abnormal retinal function associated with fenretinide, a synthetic retinoid. Arch. Ophthalmol., *104:* 69–70, 1986.

8. Modiano, M. R., Dalton, W. S., Lippman, S. M., Joffe, L., Booth, A. R., and Meyskens, F. L. Ocular toxic effects of fenretinide. J. Natl. Cancer Inst., *82:* 1063, 1990.

9. Decensi, A., Torrisi, R., Polizzi, A., Gesi, R., Brezzo, V., Rolando, M., Rondanina, G., Orengo, M. A., Formelli, F., and Costa, A. Effect of the synthetic retinoid fenretinide on dark adaptation and the ocular surface. J. Natl. Cancer Inst., *86:* 105–110, 1994.

10. Veronesi, U., De Palo, G., Costa, A., Formelli, F., Marubini, E., and Del Vecchio, M. Chemoprevention of breast cancer with retinoids. Natl. Cancer Inst. Monogr., *12:* 93–97, 1992.

11. Formelli, F., Clerici, M., Campa, T., Di Mauro, M. G., Magni, A., Mascotti, G., Moglia, D., De Palo, G., Costa, A., and Veronesi, U. Five-year administration of Fenretinide: pharmacokinetics and effects on plasma retinol concentrations. J. Clin. Oncol., *11:* 2036–2042, 1993.

12. Norusis, M. J. SPSS/PC Base System User's Guide, Release 5.0, pp. 303–357. Chicago: SPSS Inc., 1992.

13. Kleinbaum, D. G., Kuppe, L. L., and Muller, K. F. Applied Regression Analyses and Other Multivariable Methods, pp. 102–103. Boston: PWS-KENT Publishing Company, 1988.

14. Goodman, D. S. Vitamin A and retinoids in health and disease. N. Engl. J. Med., *310:* 1023–1031, 1984.

15. Costa, A., Rotmensz, N., Campa, T., Magni, A., and Assimakopoulos, G. Safety and tolerability of retinoids. *In:* G. De Palo, M. Sporn, U. Veronesi (eds.), Progress and Perspectives in Chemoprevention of Cancer, pp. 69–76. New York: Raven Press, 1992.

16. Berni, R., and Formelli, F. *In vitro* interaction of fenretinide with plasma retinol-binding protein and its functional consequences. FEBS, *308:* 43–45, 1992.

17. Schaffer, E. M., Ritter, S. J., and Smith, J. E. *N*-(4-Hydroxyphenyl)retinamide (Fenretinide) induces retinol-binding protein secretion from liver and accumulation in the kidneys in rats. J. Nutr., *123:* 1497–1503, 1993.

18. Hultin, T. A., Filla, M. S., and McCormick, D. L. Distribution and metabolism of the retinoid, *N*-(4-methoxyphenyl)-all-trans-retinamide, the major metabolite of *N*-(4-Hydroxyphenyl)-all-*trans*-retinamide, in female mice. Drug Metab. Dispos., *18:* 175–179, 1990.

19. Mehta, R. G., Moon, R. C., Hawthorne, M., Formelli, F., and Costa, A. Distribution of Fenretinide in the mammary gland of breast cancer patients. Eur. J. Cancer, *27:* 138–141, 1991.

20. Hultin, T. A., McCormick, D. L., May, C. M., and Moon, R. C. Effects of pretreatment with the retinoid *N*-(4-Hydroxyphenyl)-all-*trans*-retinamide and phenobarbital on the disposition and metabolism of *N*-(4-Hydroxyphenyl)-all-*trans*-retinamide in mice. Drug Metab. Dispos., *16:* 783–788, 1988.

21. Mehta, R. R., Mehta, R. G., Hart, G. D., Hawthorne, M., Moon, R. C., and Das Gupta, T. K. Effect of 4-HPR on human breast carcinoma cells. Breast Cancer Res. Treat., *27:* A144, 1993.