Fenretinide Breast Cancer Prevention Trial: Drug and Retinol Plasma Levels in Relation to Age and Disease Outcome

Franca Formelli, Tiziana Camerini, Elena Cavadini, Valentina Appierto, Maria Grazia Villani, Alberto Costa, Giuseppe De Palo, Maria Gaetana Di Mauro, and Umberto Veronesi

Abstract

Objectives: To assess, in women participating in a breast cancer prevention trial on fenretinide (4-HPR), the relationship of drug and retinol levels with the risk of second breast malignancy, taking into account age and menopausal status.

Methods: In a multicenter prevention trial, women with early breast cancer were randomly assigned to receive no treatment or 200 mg of 4-HPR/day for 5 years. Blood was collected at baseline and on a yearly basis during intervention from women recruited at the Istituto Tumori (Milan, Italy; 818 and 756 in the 4-HPR and control arm, respectively, who accounted for 53% of the participants in the trial). The plasma concentrations of 4-HPR, its main metabolite N-(4-methoxyphenyl) retinamide, and retinol were assayed by high-performance liquid chromatography. Three age ranges (≤45, 46–55, and ≥56 years), menopausal status at baseline, and disease outcome at a median follow-up of 97 months were taken into account in the analysis.

Results: Baseline retinol levels were significantly lower (P ≤ 0.05) in subjects ≤ 45 years than in older subjects, and among subjects in the age range 46–55 years, they were significantly higher (P ≤ 0.001) in those in postmenopause than in those in premenopause. Baseline retinol levels were not related to the risk of a second breast malignancy, 4-HPR and N-(4-methoxyphenyl)retinamide levels were not affected by menopausal status. They slightly, but significantly (P ≤ 0.05), increased with age (≥46 years versus ≤54 years) but only in disease-free subjects. Among subjects < 45 years, they were slightly, but significantly (P ≤ 0.05), higher in those subjects in which breast cancer recurred. 4-HPR treatment caused a retinol level reduction, which was strongly (r ≥ 0.71; P ≤ 0.001) related to pretreatment retinol levels.

Conclusions: Retinol plasma levels increased with age and after menopause and were not related to breast cancer recurrence. 4-HPR levels were lower in subjects < 45 years than in older subjects. The inverse relationship between drug plasma levels and 4-HPR preventative effects observed in young women suggests a role for 4-HPR plasma sequestration in 4-HPR biological activity.

Introduction

In March 1987, a multicenter randomized trial was started to assess the efficacy of the synthetic retinoid 4-HPR as a breast cancer preventive agent (1). The objective of the trial was to evaluate the efficacy of a 5-year intervention with 4-HPR versus no treatment in reducing the incidence of contralateral breast cancer or ipsilateral breast cancer in women operated on for early breast cancer. A different effect of 4-HPR was found depending on menopausal status, with a possible beneficial effect in premenopausal women and a reverse trend in postmenopausal women.

Similarly, studies on subgroups of women participating in the prevention trial had shown that 4-HPR affected circulating insulin-like growth factor-1 differently depending on age, with a greater reduction in women ≤ 50 years (mostly in premenopause) than in older women (2, 3). All together, these findings suggest an interaction between 4-HPR effects and menopausal status or age.

Information on 4-HPR pharmacokinetics and pharmacodynamics was obtained during a Phase I study, which was run to identify the dose to be administered in the breast cancer prevention study (4, 5). We demonstrated that 4-HPR, administered at 200 mg/day, resulted in average 4-HPR and 4-MPR plasma levels of ~1 μM, which remained constant over the 5-year treatment period (4) and caused a 70% decrease in plasma retinol levels (4, 5). In these studies, because of the few patients investigated, it was not possible to assess the potential influence of age or menopausal status on 4-HPR pharmacokinetics. Now with the availability of measurements of drug and retinol levels in plasma samples collected at baseline and during intervention from 1574 women (818 and 756 in the 4-HPR and control arm, respectively) participating in the Phase III prevention trial, it seemed appropriate to evaluate the association between drug plasma levels and disease outcome, taking into account the age and menopausal status of the patients. Moreover, because few and contradictory results (6–10) have been reported on the potential association between vitamin A (or retinol) and breast cancer, we assessed whether prerandomiza-

1 The abbreviations used are: 4-HPR, fenretinide; 4-MPR, N-(L-methoxyphenyl)retinamide; BMI, body mass index; RBP, retinol binding protein.
Blood was collected in heparinized tubes and wrapped in aluminum foil; all procedures were performed in the dark. After centrifugation, separated plasma was kept frozen at −20°C until analysis, never >3 weeks. Aliquots of 0.2 ml of plasma were added to 0.4 ml of CH₂CN containing butylated hydroxytoluene (125 μg/ml; Sigma, St. Louis, MO) as antioxidant, and the concentrations of 4-HPR, 4-MPR, and retinol were detected by high-performance liquid chromatography as described previously (4). The reference standards 4-HPR (molecular weight, M, 391,000) and 4-MPR (molecular weight, M, 405,000) were supplied by the R.W. Johnson Pharmaceutical Research Institute; retinol, used as reference standard, was obtained from Sigma.

Statistical Analysis. Menopausal status (before or after menopause), defined as the absence of menstruation for ≥1 year (1), and age at baseline were considered. Preliminary studies were performed on the relationship of retinol, 4-HPR, and 4-MPR with age by regression analysis in subjects who did not develop any evident disease and who developed contralateral and ipsilateral breast cancer (i.e., the disease conditions with the highest number of subjects). The regression of retinol on age was significant. In contrast, the regression of 4-HPR and 4-MPR was not significant even when subjects were divided according to menopausal status. As 4-HPR and 4-MPR were not related with age, subjects were divided into age ranges. Three age ranges were chosen: (a) ≤45; (b) 46–55; and (c) ≥56 years. The ranges ≤45 and ≥56 years were chosen because they included mostly pre and postmenopausal women, respectively. The range 46–55 years was chosen because it included both pre and postmenopausal women. As regards the regression analysis between retinol and age, when it was performed within each age range, it was significant only in the age range 46–55, which included pre and postmenopausal women. Therefore, in all of the analyses, plasma levels were subdivided according to the three age ranges, also taking into account menopausal status. The levels of 4-HPR, 4-MPR, and retinol were expressed as the mean ± SD. In all analyses, the values were log transformed to approximate a Gaussian distribution. In all statistical analyses, groups with one to three subjects were not considered for comparison, but their means were reported in tables for descriptive purposes.

Retinol levels at baseline were subdivided according to the three age groups, and menopausal status and the differences between the means were evaluated by Student’s t test (Table 2). The baseline retinol levels were then subdivided according to subsequent disease outcome, and the comparison of each mean versus that of subjects with no evident disease was carried out according to Dunnet’s t test (Fig. 1). The stability in time of retinol levels in control subjects was evaluated by analyzing the differences between the values at the beginning and end of the intervention period by Student’s paired t test in subjects who had blood drawn at both intervals (Table 3).

For 4-HPR-treated subjects, to analyze uniform time intervals during the 5-year intervention period, the following time ranges were considered: (a) 7–18 months (1 year); (b) 19–30 months (2 years); (c) 31–42 months (3 years); (d) 43–54 months (4 years); and (e) 55–66 months (5 years). The stability in time of 4-HPR, 4-MPR, and retinol levels was checked by: (a) regression analysis in all of the subjects with at least three measurements at different intervals from the beginning of treatment; and (b) Student’s paired t test in subjects who had blood drawn at 1 and 5 years (Table 4). Dunnet’s t test was carried out for comparison of the means of 4-HPR and 4-MPR levels in subjects with different disease outcome with those of subjects...
of the same age with no evident disease (Table 5). For comparison of the means of the different age groups within each type of disease condition, the Student’s t test on orthogonal contrasts was carried out according to the following criteria: (a) age group ≤ 45 years versus age group 46–55 years plus age group ≥56 years; and (b) age group 46–55 years versus age group ≥56 years (Table 5).

Correlation analysis was performed to study the relationship between each pair of variables considered for 4-HPR-treated patients in each age group. The variables considered were: (a) 4-HPR; (b) 4-MPR; (c) retinol during treatment; (d) retinol at baseline; (e) retinol difference (i.e., retinol at baseline minus retinol during treatment); (f) body surface area (m²); and (g) BMI (i.e., weight/height² expressed in kilograms and meters, respectively).

All of the analyses were performed using SAS software (13). Probability values reported are two sided. Values of \( P \leq 0.05 \) were considered significant.

Results

Subject Characteristics and Pretreatment Plasma Retinol Levels. The main characteristics of the subjects, whose blood was collected during the 4-HPR breast cancer prevention trial, are described in Table 1. The two treatment arms were well balanced and comparable with the whole study cohort (1).

Pretreatment retinol levels of subjects randomized in the 4-HPR and control arms are reported in Table 2, and they are compared with those of healthy female subjects. Only six subjects aged ≤45 years were in postmenopause in the 4-HPR \( (n = 3) \) and control \( (n = 3) \) arms, and only two subjects aged ≥56 years were in premenopause in the control arm. A comparison between pre and postmenopause was assessed only in the age group 46–55 years. In this age group, postmenopausal subjects had significantly higher retinol levels \( (P \leq 0.001) \) than premenopausal subjects. Among premenopausal subjects, retinol levels were lower \( (P \leq 0.05) \) in those aged ≤45 years than in those aged 46–55 years. There were no significant differences in retinol levels between the 4-HPR and control arms. The average retinol levels of subjects participating in the prevention trial were not statistically different from those of healthy female subjects.

To assess the value of retinol levels in predicting disease outcome and toxicity, the baseline retinol levels of subjects in the 4-HPR and control arm were subdivided according to subsequent disease conditions or toxicity and compared with the levels of subjects with no evident disease (Fig. 1). In the 4-HPR arm, subjects aged ≤45 years who experienced toxicity during 4-HPR treatment had initial retinol levels significantly lower \( (P \leq 0.05) \) than subjects of the same age with no evident disease. In the control arm, baseline retinol levels of the different groups did not differ from those of subjects with no evident disease, except for lower average retinol levels in women in the age group ≥56 years, who subsequently developed metastasis.

Retinol Plasma Levels of Subjects in the Control Arm during the 5-year Study. The stability of retinol levels in control subjects during the 5-year study was assessed by comparing retinol levels in subjects who had plasma samples drawn at baseline and at 5 years (Table 3). There were no changes from 0 to 5 years except for a significant increase in subjects aged 46–55 years who were in premenopause at baseline and had no evident disease.

4-HPR, 4-MPR, and Retinol Plasma Levels during the 5-year Study. For analysis of samples during 4-HPR intervention, the following criteria of selection were used to obtain consistent data: (a) last drug intake dose, 200 mg as one and not as two separate 100-mg daily doses; (b) blood sampling at 10–18 h from drug intake, i.e., between peak and trough concentrations at steady state (4); (c) samples with 4-HPR levels ≥ 150 ng/ml, corresponding on the basis of previous kinetics study (14), to the lower 95% tolerance limit for drug concentration at 18 h. After the selection criteria, 2,348 samples from 537 subjects in the 4-HPR arm were assessable for analysis of drug and retinol levels during the 5-year intervention period.

The eventual stability of 4-HPR, 4-MPR, and retinol levels during the intervention period was first assessed. To this aim, regression analysis was performed on 4-HPR, 4-MPR, and retinol levels for each subject with samples drawn at least at three different intervals during treatment. Among the 298 subjects analyzed, the regressions were significant only in very few cases (≤6% for 4-HPR and 4-MPR and 13% for retinol). The stability of drug and retinol levels was further analyzed by the paired t test in plasma levels of women who had plasma samples drawn at 1 and 5 years (Table 4). No changes in 4-HPR and 4-MPR plasma levels from 1 to 5 years were found. Retinol levels after the 5-year treatment were slightly lower than the levels at 1 year, and the differences were significant in the groups with no evident disease \( (P \leq 0.05 \) and 0.001). In the age group 46–55 years, no differences were found between the means of pre and postmenopausal women at 1 or 5 years. Therefore, all of the following analyses on subjects in the 4-HPR arm were performed, taking into account only the age groups and disease outcome.

Table 2. Retinol plasma levels (means ± SD in ng/ml) at baseline in subjects participating in the 4-HPR breast cancer prevention trial and in female healthy subjects

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Menopausal status</th>
<th>4-HPR prevention trial</th>
<th>Female healthy subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Group</td>
<td>(n)</td>
</tr>
<tr>
<td>≤45</td>
<td>Pre</td>
<td>(181)</td>
<td>541 ± 130.4</td>
</tr>
<tr>
<td>46–55</td>
<td>Post</td>
<td>(3)</td>
<td>555 ± 25.1</td>
</tr>
<tr>
<td></td>
<td>Pre</td>
<td>(211)</td>
<td>562 ± 123.0</td>
</tr>
<tr>
<td>≥56</td>
<td>Post</td>
<td>(169)</td>
<td>634 ± 132</td>
</tr>
</tbody>
</table>

<sup>a</sup> Menopausal status at baseline: pre, premenopausal; post, postmenopausal.
<sup>b</sup> CTR, controls.
<sup>c</sup> \( P \leq 0.05 \) versus subjects aged ≤45 years.
<sup>d</sup> \( P < 0.001 \) versus the same age subjects.

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Comparison of 4-HPR, 4-MPR, and Retinol Plasma Levels after 1 Year of Treatment. After the observation of drug and retinol stability during the 5-year treatment, comparison of mean levels for the different age groups according to disease outcomes and toxicity was performed at 1 year, i.e., when more samples were available. The mean levels of 4-HPR, 4-MPR, and retinol of subjects whose plasma was drawn after 1 year of treatment and the mean values of body surface area and BMI at baseline are reported in Table 5. The proportion of subjects, reported in Table 5, initially operated on for carcinoma in situ pT1 and pT2 who subsequently developed contralateral or ipsilateral breast cancer and metastasis was similar to that of subjects of the same age with no evident disease (data not shown).

Comparison of plasma levels was first assessed among age groups. In subjects with no evident disease, the 4-HPR and 4-MPR levels of the groups 46–55 and ≥56 years, which were not different from one another, were significantly higher ($P \leq 0.05$) than those of the group ≤45 years, despite concomitant larger body surface areas and BMI ($P \leq 0.005$). No difference was found among age groups in the other disease conditions.

The plasma levels of subjects with the different disease conditions were then compared with those of subjects with no evident disease. In subjects ≤45 years, with contralateral or ipsilateral breast cancer, the means of 4-HPR and 4-MPR levels were higher ($P \leq 0.05$), and the means of retinol levels were lower ($P \leq 0.05$) than those of subjects of the same age with no evident disease. In subjects of all age groups who developed metastasis, average 4-HPR and 4-MPR levels were higher than in subjects with no evident disease, although the differences were not statistically significant. In these subjects, the SD of 4-MPR levels was also higher. This was attributable to many subjects with abnormally high 4-MPR levels, i.e., ≥900 ng/ml. Among subjects who developed metastasis, those ≥56 years had a larger body surface area and higher BMI than subjects with no evident disease of the same age. The few subjects who experienced toxicity had smaller body surface areas (mean values 1.41 and 1.54 m$^2$) than subjects in the other groups.

![Fig. 1](Plasma retinol levels (means ± SD) at baseline according to subsequent disease outcome and toxicity (data referraling to fewer than three subjects are not reported). Empty columns, subjects in premenopause; gray columns, subjects in postmenopause. The number of subjects belonging to each group is reported inside the columns. CTR, controls; Cont. or ipsi. ca., contralateral or ipsilateral breast cancer; Met., metastasis; 2nd primary ca., second primary cancer; Tox., toxicity or abnormal laboratory tests. *, $P \leq 0.05$ versus same age subjects with no evident disease. Dunnet’s t test.)
Fenretinide Plasma Levels and Second Breast Malignancy

BMI, significant correlations were found only in subjects with the lowest mean value was 1.59 m² for subjects ≤ 45 years with no evident disease, suggesting a proportionally higher drug intake.

The correlations between each pair of variables reported in Table 5 were also assessed. Retinol at baseline, which is not reported in Table 5, and retinol decrease (i.e., retinol at baseline minus retinol during treatment) were also taken into account. Only subjects with no evident disease and contralateral or ipsilateral breast cancer, i.e., the disease conditions with the highest number of subjects, were considered in the analyses. When drug levels were correlated with body surface area and BMI, significant correlations were found only in subjects ≤ 45 years with no evident disease in which 4-HPR levels were inversely associated with body surface area (r = -0.38; P = 0.001) and in subjects ≤ 45 years who developed contralateral or ipsilateral breast cancer in which 4-MPR levels were directly associated with BMI (r = 0.61; P = 0.03; data not shown). A highly significant correlation was found between 4-MPR and 4-HPR levels (r ≥ 0.59; P ≤ 0.03) and between the retinol decrease and retinol levels at baseline (r ≥ 0.7; P ≤ 0.001) in subjects with no evident disease and in those with contralateral and ipsilateral breast cancer of all age ranges (data not shown). 4-HPR levels were inversely correlated with retinol levels during treatment (r ≥ 0.27; P ≤ 0.02) in subjects with no evident disease of all age ranges, whereas such a correlation was not found in subjects who developed contralateral or ipsilateral breast cancer (data not shown). As the number of subjects with no evident disease was larger than that of subjects with contralateral or ipsilateral breast cancer, for a more adequate comparison, the same correlation was also assessed on a number of subjects with no evident disease (randomly selected) equal, for each age range, to that of subjects with contralateral and ipsilateral breast cancer. The correlation between 4-HPR levels and retinol levels during treatment was still significant (r ≥ 0.48; P ≤ 0.05; data not shown).

Discussion
The primary objectives of the study were to assess, in subjects participating in a 4-HPR prevention trial, the influence of age and menopausal status on drug and retinol plasma levels and

### Table 3  Plasma retinol levels (means ± SD in ng/ml) in control patients at baseline and after 5 years

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Menopausal status</th>
<th>Year</th>
<th>No evident disease</th>
<th>Contralateral or ipsilateral breast cancer</th>
<th>Metastasis</th>
<th>Second primary cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤45</td>
<td>Pre</td>
<td>0 (63)</td>
<td>517 ± 106.5 (9)</td>
<td>493 ± 88.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>46–55</td>
<td>Pre</td>
<td>0 (76)</td>
<td>547 ± 126.6 (12)</td>
<td>522 ± 83.5 (7)</td>
<td>518 ± 180.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Post</td>
<td>0 (49)</td>
<td>597 ± 122.8 ±</td>
<td>515 ± 114.3</td>
<td>627 ± 215.7</td>
<td></td>
</tr>
<tr>
<td>≥56</td>
<td>Post</td>
<td>0 (82)</td>
<td>611 ± 154.8 (6)</td>
<td>546 ± 130.9 (9)</td>
<td>621 ± 170.0 (4)</td>
<td>684 ± 170.8</td>
</tr>
</tbody>
</table>

*Menopausal status at baseline: pre, premenopausal; post, postmenopausal.

### Table 4  4-HPR, 4-MPR, and retinol plasma levels (means ± SD in ng/ml) after 1- and 5-year 4-HPR treatments

<table>
<thead>
<tr>
<th>Age (year)</th>
<th>Treatment (year)</th>
<th>Subjects with</th>
<th>No evident disease</th>
<th>Contralateral or ipsilateral breast cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤45</td>
<td>4-HPR</td>
<td></td>
<td>(n) Premenopause</td>
<td>(n) Postmenopause</td>
</tr>
<tr>
<td>1</td>
<td>(42)</td>
<td></td>
<td>385 ± 118.6</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>354 ± 115.3</td>
<td></td>
<td>489 ± 154.5</td>
<td></td>
</tr>
<tr>
<td>4-MPR</td>
<td>372 ± 156.3</td>
<td></td>
<td>463 ± 110.1</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>348 ± 121.2</td>
<td></td>
<td>540 ± 161.8</td>
<td></td>
</tr>
<tr>
<td>Retinol</td>
<td>156 ± 85.3</td>
<td></td>
<td>124 ± 100.5</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>136 ± 67.3</td>
<td></td>
<td>73 ± 41.0</td>
<td></td>
</tr>
<tr>
<td>46–55</td>
<td>4-HPR</td>
<td></td>
<td>(35) 446 ± 137.2 (24)</td>
<td>459 ± 146.6 (5)</td>
</tr>
<tr>
<td>1</td>
<td>452 ± 149.4</td>
<td></td>
<td>414 ± 145.3</td>
<td>348 ± 102.9</td>
</tr>
<tr>
<td>5</td>
<td>437 ± 209.0</td>
<td></td>
<td>441 ± 178.2</td>
<td>425 ± 95.7</td>
</tr>
<tr>
<td>4-MPR</td>
<td>456 ± 212.6</td>
<td></td>
<td>433 ± 209.7</td>
<td>407 ± 163.2</td>
</tr>
<tr>
<td>5</td>
<td>164 ± 84.7</td>
<td></td>
<td>174 ± 88.9</td>
<td>177 ± 18.7</td>
</tr>
<tr>
<td>Retinol</td>
<td>144 ± 79.8</td>
<td></td>
<td>141 ± 65.5</td>
<td>165 ± 60.1</td>
</tr>
<tr>
<td>≥56</td>
<td>4-HPR</td>
<td></td>
<td>(26) 435 ± 137.6 (4)</td>
<td>473 ± 67.8</td>
</tr>
<tr>
<td>1</td>
<td>445 ± 154.3</td>
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<td>424 ± 42.7</td>
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<tr>
<td>5</td>
<td>460 ± 277.2</td>
<td></td>
<td>415 ± 86.5</td>
<td></td>
</tr>
<tr>
<td>4-MPR</td>
<td>480 ± 273.5</td>
<td></td>
<td>396 ± 86.4</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>171 ± 78.6</td>
<td></td>
<td>211 ± 132.0</td>
<td></td>
</tr>
<tr>
<td>Retinol</td>
<td>136 ± 88.0</td>
<td></td>
<td>120 ± 61.9</td>
<td></td>
</tr>
</tbody>
</table>

*P ≤ 0.05.  
b P ≤ 0.001 versus 1-year levels by Student’s t paired test.
induce a significant increase in retinol levels (19). Oral contraceptive therapy in young women has been found to mones on retinol plasma levels do not clarify the issue. In fact, the few studies that have explored the influence of sex hor-

circulating lipoproteins that occurs with aging. The results of levels found in older women might be linked to the increase in women pausal status. The reasons for the observed increase in women ––that aged 46–55 postmenopause had higher retinol levels than those in pre-

showed, during the 5-year trial, a significant increase in retinol levels, which were directly related, remained con-

In vitro 4-HPR correspond to the least effective concentrations in in vitro models. However, we showed previously that 4-HPR accumulates in the breast because its concentrations in nipple discharge were 10 times higher than those in plasma (4). 4-HPR and 4-MPR levels, which were directly related, remained con-

At randomization, the 4-HPR and control arms had com-

Evidence from our and previous studies (6, 8) suggests that retinol levels are not associated with a risk of breast cancer. Our results on the role of retinol levels as predictive of breast tumor recurrence indicate that, in women operated on for stage I breast cancer, retinol levels have no prognostic value for contralateral or ipsilateral breast cancer. Less conclusive are the results on the role of retinol levels in predicting breast cancer metastasis. Control subjects >56 years who subsequently de-

Evidence of our and previous studies (6, 8) suggests that retinol levels are not associated with a risk of breast cancer. Our results on the role of retinol levels as predictive of breast tumor recurrence indicate that, in women operated on for stage I breast cancer, retinol levels have no prognostic value for contralateral or ipsilateral breast cancer. Less conclusive are the results on the role of retinol levels in predicting breast cancer metastasis. Control subjects ≥56 years who subsequently de-

p.o. dose of 200 mg of 4-HPR resulted, at 10–18 h after administration, in 4-HPR and 4-MPR plasma levels of ~400 ng/ml, corresponding to ~1 μM. The 4-HPR concentrations found effective in suppressing the in vitro growth of tumor cells range from 1 to 10 μM (22). Therefore, plasma levels of 1 μM 4-HPR correspond to the least effective concentrations in in vitro models. However, we showed previously that 4-HPR accumulates in the breast because its concentrations in nipple discharge were 10 times higher than those in plasma (4). 4-HPR and 4-MPR levels, which were directly related, remained constant during the 5-year treatment. The results confirm previously reported data found in a small group of women participating in a Phase I trial (4).

With regard to the possible influence of age and meno-

pensive status on 4-HPR pharmacokinetics, the present data indicate that circulating 4-HPR and 4-MPR levels are not linked to menopausal status but that they are influenced by patient age. After identical daily doses during 1 year of treatment, subjects ≤ 45 years had slightly, but significantly, lower 4-HPR and 4-MPR plasma levels than the older subjects. This occurred despite the marked lower body surface area of women ≤ 45 years than those in the other age ranges. Lower drug absorption, higher metabolism to undetected metabolites, or higher biliary excretion might account for the lower 4-HPR and 4-MPR levels found in the youngest women. After their absorption from the gastrointestinal tract, retinoids, which are water insoluble compo-

Table 5 4-HPR, 4-MPR, and retinol levels (means ± SD in ng/ml) after 1-year 4-HPR treatment and body surface area and BMI (means ± SD) at baseline

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>No evident disease</th>
<th>Contralateral or ipsilateral breast cancer</th>
<th>Metastases</th>
<th>Second primary cancer</th>
<th>Toxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 45</td>
<td>4-HPR (70)</td>
<td>394 ± 123.5 (13)</td>
<td>473 ± 172.1 (5)</td>
<td>547 ± 177.7 (3)</td>
<td>366 ± 37.6</td>
</tr>
<tr>
<td></td>
<td>4-MPR</td>
<td>387 ± 186.0</td>
<td>503 ± 249.9 (9)</td>
<td>606 ± 418.4</td>
<td>406 ± 137.7</td>
</tr>
<tr>
<td></td>
<td>Retinol</td>
<td>152 ± 76.1</td>
<td>109 ± 58.0 (4)</td>
<td>189 ± 97.2</td>
<td>106 ± 128.8</td>
</tr>
<tr>
<td></td>
<td>m²</td>
<td>1.59 ± 0.12</td>
<td>1.60 ± 0.10</td>
<td>1.66 ± 0.26</td>
<td>1.41 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>BMI</td>
<td>22.45 ± 3.41</td>
<td>22.70 ± 2.59</td>
<td>24.86 ± 5.94</td>
<td>18.33 ± 0.39</td>
</tr>
<tr>
<td>46–55</td>
<td>4-HPR (107)</td>
<td>445 ± 156.9 (9)</td>
<td>411 ± 108.2 (9)</td>
<td>536 ± 317.1 (6)</td>
<td>377 ± 102.9</td>
</tr>
<tr>
<td></td>
<td>4-MPR</td>
<td>452 ± 234.9</td>
<td>409 ± 109.5</td>
<td>635 ± 490.9</td>
<td>322 ± 119.1</td>
</tr>
<tr>
<td></td>
<td>Retinol</td>
<td>177 ± 86.6</td>
<td>201 ± 121.7</td>
<td>137 ± 60.1</td>
<td>190 ± 39.5</td>
</tr>
<tr>
<td></td>
<td>m²</td>
<td>1.66 ± 0.13 (d)</td>
<td>1.62 ± 0.08</td>
<td>1.67 ± 0.12</td>
<td>1.84 ± 0.21</td>
</tr>
<tr>
<td></td>
<td>BMI</td>
<td>24.17 ± 3.77 (e)</td>
<td>23.61 ± 2.57</td>
<td>25.78 ± 4.29</td>
<td>27.09 ± 5.40</td>
</tr>
<tr>
<td>≥ 56</td>
<td>4-HPR (57)</td>
<td>427 ± 187.5 (5)</td>
<td>419 ± 106.9 (8)</td>
<td>512 ± 225.1 (6)</td>
<td>468 ± 234.4 (2)</td>
</tr>
<tr>
<td></td>
<td>4-MPR</td>
<td>421 ± 231.6</td>
<td>406 ± 167.7</td>
<td>525 ± 200.7</td>
<td>386 ± 85.8</td>
</tr>
<tr>
<td></td>
<td>Retinol</td>
<td>162 ± 85.0</td>
<td>168 ± 78.5</td>
<td>160 ± 64.2</td>
<td>146 ± 101.8</td>
</tr>
<tr>
<td></td>
<td>m²</td>
<td>1.68 ± 0.14 (d)</td>
<td>1.69 ± 0.15</td>
<td>1.76 ± 0.05 (d)</td>
<td>1.57 ± 0.15</td>
</tr>
<tr>
<td></td>
<td>BMI</td>
<td>24.98 ± 4.27 (e)</td>
<td>25.94 ± 4.21</td>
<td>28.30 ± 3.66 (d)</td>
<td>23.15 ± 1.42</td>
</tr>
</tbody>
</table>

*P ≤ 0.05 versus same age patients with no evident disease by Dunnett’s t test.

b. m², body surface area.

c. P ≤ 0.05.

d. P ≤ 0.005 versus patients ≤ 45 years, with no evident disease.

subjects treated with 4-HPR. Additional studies are necessary to clarify this issue.

Estimates of drug plasma levels demonstrated that a daily

prospectively evaluate their association with a second breast malignancy.

At randomization, the 4-HPR and control arms had com-

pensive status on 4-HPR pharmacokinetics, the present data indicate that circulating 4-HPR and 4-MPR levels are not linked to menopausal status but that they are influenced by patient age. After identical daily doses during 1 year of treatment, subjects ≤ 45 years had slightly, but significantly, lower 4-HPR and 4-MPR plasma levels than the older subjects. This occurred despite the marked lower body surface area of women ≤ 45 years than those in the other age ranges. Lower drug absorption, higher metabolism to undetected metabolites, or higher biliary excretion might account for the lower 4-HPR and 4-MPR levels found in the youngest women. After their absorption from the gastrointestinal tract, retinoids, which are water insoluble compo-

bil

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anced and the difference was small, we cannot draw conclusions on the role of drug plasma levels as predictive of 4-HPR efficacy. However, the results, in conjunction with the finding of higher drug plasma levels in older subjects who did not show any preventive benefit from 4-HPR treatment (1), suggest an inverse association between plasma drug levels and 4-HPR preventive effects. An explanation for this intriguing result might be that higher drug plasma levels reflect lower drug concentrations in the tissues where they are needed. It may be that the binding of 4-HPR to lipoproteins in older women and young nonresponding women might inhibit entry of the drug into cells. In vitro data on tumor cell lines support such a hypothesis. In in vitro assays, the addition of retinoids using serum-free conditions or low serum concentrations resulted in dose-dependent growth inhibitory effects, which decreased when serum or serum components were added (24–26). It has also been demonstrated that reduced growth inhibitory effects were associated with decreased retinoid uptake by the cells (25). The same applies to the in vitro growth inhibitory effects of 4-HPR.4

Although the difference was not statistically significant, subjects who subsequently developed metastases also had higher 4-HPR and 4-MPR levels than subjects with no evident disease. Moreover, a high percentage of these subjects had abnormally high (≥900 ng/ml) 4-MPR plasma levels. 4-HPR and 4-MPR are both lipopholic, and 4-MPR has a longer storage than 4-HPR in deep tissue compartments, presumably fat, from which it is slowly released (4). The abnormally high 4-MPR concentrations found in subjects who subsequently developed metastasis presumably reflect a high percentage of adipose tissue in these subjects.

The results of the 4-HPR breast tumor prevention trial have shown the good tolerability of the retinoid at 200 mg/day over a 5-year treatment (1, 12). The present results indicate that the few subjects who experienced 4-HPR toxicity and whose mean drug plasma levels are herein reported had a smaller body surface area than subjects who showed no toxicity. The data suggest that 4-HPR should be administered on the basis of body surface area. Moreover, 4-HPR toxicity was associated with low retinol levels before 4-HPR administration but only in subjects aged ≤45 years. This age group had the lowest pretreatment retinol levels, and the additional reduction caused by 4-HPR in these subjects may have resulted in retinol levels below threshold limits.

Regarding the effect of 4-HPR on retinol plasma levels, we had shown previously that 4-HPR reversibly decreases the plasma concentrations of retinol and of its transport protein, RBP (4, 5). We also showed that the high binding affinity of 4-HPR for RBP and the lack of binding of the complex 4-HPR–RBP to transthyretin (27) might account for the reduction of retinol secretion from retinol-secreting organs. Retinol levels after a 5-year treatment were always slightly, but significantly, lower than those found at 1 year. The difference, although it does not seem to be biologically relevant, might indicate 4-HPR accumulation in retinol-secreting organs like the liver. A new finding was that the reduction in retinol levels was strongly and directly correlated with pretreatment retinol levels. Therefore, pretreatment retinol levels may predict retinol level reduction by 4-HPR; in fact, the higher the initial values, the higher the absolute decrease, with the percentage decrease remaining the same. Another interesting observation was that the inverse correlation between 4-HPR and retinol levels during treatment was significant in subjects with no evident disease, whereas it was not significant in those in whom breast cancer recurred. This result seems to further support the evidence of differences in 4-HPR pharmacokinetics between subjects with no evident disease and those who did not benefit from 4-HPR treatment. Interestingly, differences in retinoid metabolism have been found between human tumors and the respective normal tissues (28).

In conclusion, in a prospective study in women participating in a breast tumor prevention trial with 4-HPR, we found that subjects ≤ 45 years had lower drug plasma levels than older subjects, and, among young subjects, those with breast cancer recurrence had higher drug levels. A tentative explanation for this unexpected finding could be related to drug interaction with serum proteins, drug sequestration in plasma, and consequent interference with the tumor preventive potential. The hypothesis of serum components as determinant of 4-HPR plasma levels and bioavailability should be investigated in future studies with the retinoid.

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References


Fenretinide Breast Cancer Prevention Trial: Drug and Retinol Plasma Levels in Relation to Age and Disease Outcome

Franca Formelli, Tiziana Camerini, Elena Cavadini, et al.


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