The Synthetic Retinoid Fenretinide Lowers Plasma Insulin-like Growth Factor I Levels in Breast Cancer Patients

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Abstract

We studied the effect of fenretinide [N-(4-hydroxyphenyl)retinamide (4-HPR)], a synthetic analogue of retinoic acid, on plasma insulin-like growth factor I (IGF-I) levels in a consecutive cohort of stage I breast cancer patients belonging to a randomized phase III trial of breast cancer chemoprevention. Thirty-two women receiving 4-HPR 200 mg/daily and 28 untreated controls entered the study. IGF-I levels were determined, after acid-ethanol extraction, on plasma obtained at randomization and after a mean time of 10.8 ± 0.3 months.

At baseline, there was no difference in IGF-I levels between the two groups (152.9 ± 9.4 versus 159.2 ± 7.0 ng/ml in treated and control group, respectively). After follow-up time, while plasma IGF-I levels were unchanged in control patients (163.3 ± 7.4 ng/ml; P = 0.5), they were significantly reduced to 134.6 ± 8.1 ng/ml in the patients treated with 4-HPR (P = 0.003 and P = 0.011 versus baseline and control values, respectively). Multiple regression analysis showed that treatment was the only determinant of IGF-I decline. Moreover, the interaction between treatment and age was significant, in that the decrease of IGF-I levels induced by 4-HPR administration was much more pronounced in younger patients, while an age-related decline was observed in controls.

We conclude that the synthetic retinoid 4-HPR lowers circulating IGF-I levels in early breast cancer patients. Although the importance of this observation for the clinical prevention of breast cancer remains to be established, it further substantiates the rationale of the combination of 4-HPR with tamoxifen, which is known to decrease IGF-I as well and to act synergistically with the retinoid in preclinical models.

Introduction

The IGF3 family, including IGF-I and IGF-II, their receptors and binding proteins, appears to play a crucial role in the growth of several human malignancies (1). IGF-I is a potent mitogen for transformed breast epithelium, in which it seems to act through a paracrine mechanism, being synthesized by the stroma surrounding the tumor (2).

Retinoids, the natural and synthetic analogues of vitamin A, have been proven effective in inhibiting carcigenesis in experimental models in vitro and in vivo (3). The mechanisms through which retinoids exert their antiproliferative and differentiating effects are still unclear, however. Recently, the binding of natural retinoids (all-trans-retinoic acid, 9-cis-retinoic acid) with specific nuclear receptors (RAR and RXR) and subsequent induction of gene transcription has been well documented (4). A number of hypotheses on the resulting steps of retinoid action have been made, among which interactions with several growth factor pathways seem plausible. Retinoids have been shown to induce the synthesis of TGF-Î² (5), a growth factor which negatively modulates cancer growth (6). Moreover, retinoic acid is able to inhibit IGF-I induced growth in breast cancer cell lines (MCF-7) (7). This inhibition is associated with the induction of the expression of the insulin-like growth factor binding protein 3 mRNA in the same system (7).

4-HPR, a synthetic amide of retinoic acid, has proven active in preventing the growth of MCF-7 cells (8) and N-nitroso-N-methylurea-induced breast cancer in rats (9). A phase III clinical trial was undertaken at the National Tumor Institute of Milano, Italy, in 1987 to test the effectiveness of 4-HPR as a chemopreventive agent for contralateral breast cancer (10). To shed light on the potential of the above-mentioned findings in a clinical setting, we investigated the effects of 4-HPR administration on plasma levels of IGF-I in a subset of these patients. Our results are the first arising from a clinical trial that show the ability of a retinoid to lower circulating levels of IGF-I.

Subjects and Methods

Subjects. We studied a cohort of 60 consecutive breast cancer patients participating in a large multicentric randomized phase III trial of breast cancer chemoprevention, who were attending the National Cancer Institute of Genova for periodic follow-up. All patients had stage I cancer (T1 N0), had undergone surgery in the previous 10 years, and had received no adjuvant systemic therapy. A detailed description of inclusion criteria has been published elsewhere (10). Thirty-two women assigned to receive 4-HPR (RW Johnson Pharmaceutical Research Institute, Springhouse, PA), 200 mg p.o. daily, and 28 randomized controls entered the study. Menstrual status, time from surgery and BMI, expressed as weight (kg) divided by squared height (m²), were recorded for each patient. No endocrine or metabolic alterations were present at randomization. Charts of all patients were reviewed in order to exclude the occurrence of drug-induced alterations of hepatic or metabolic function or variations in body weight >5%. IGF-I levels were determined at randomization and during follow-up at a mean interval of 10.8 ± 0.4 months. Blood samples were obtained between 9 and 12 a.m. during periodic clinical examination. Plasma were separated by centrifugation and stored at −20°C until assayed.

Methods. IGF-I was measured by radioimmunoassay after acid ethanol extraction, using commercially available kits purchased from the Nichols Institute (San Juan Capistrano, CA). The IGF-I content was expressed as ng/ml. Sensitivity of the assay was 0.06 ng/ml. Intrassay and interassay coefficients of variation were 2 and 7.5%, respectively. The investigator who carried out the assay was blinded to patient allocated arm.

Statistical Analyses. Data were computed using SPSS statistical package. Since IGF-I levels were normally distributed, as assessed by Kolgomorov-Smirnov nonparametric test, absolute values were analyzed using Student’s t test for dependent and independent samples. Simple linear regressions were performed to determine dependence of IGF-I on age, BMI, and time from surgery. In order to evaluate the dependence of IGF-I levels on 4-HPR administration controlling for the influence of other variables, multiple regression analysis using the difference (Δ) of IGF-I levels between follow-up and base-
line values as dependent variable was performed. Statistical significance was defined as two-tailed \( P < 0.05 \). All results were given as the mean ± SE.

Results

Patient characteristics are detailed in Table 1. There was no significant difference between treated and control patients as regards BMI, menstrual status, time from surgery, and the interval between the two determinations of IGF-I and age, although patients receiving 4-HPR were slightly older than controls (\( P = 0.51 \)).

At baseline, no difference was evident in IGF-I levels between the two groups (152.9 ± 9.4 versus 159.2 ± 7.0 ng/ml in 4-HPR and control group, respectively; \( P = 0.59 \)) after a mean time of 10.8 ± 0.3 months, IGF-I levels declined significantly in treated patients compared with baseline (134.6 ± 8.1 ng/ml; \( P = 0.003 \)), with a mean reduction of 15.3 ± 5%, while no change was shown in controls (163.3 ± 7.4 ng/ml; \( P = 0.5 \)). Posttreatment values were also significantly lower when compared to follow-up values in controls (\( P = 0.011 \)) (Fig. 1).

When all 60 patients were considered together, a significant inverse relationship was observed between IGF-I and age, both in baseline and follow-up values (\( r = -0.42, P < 0.001 \), and \( r = -0.37, P < 0.01 \), respectively). When linear regression analyses were performed in the two groups separately, a significant inverse relationship was observed in controls (\( r = -0.53, P < 0.01 \) for baseline, and \( r = -0.62, P < 0.001 \) for follow-up values, respectively), while no such correlation was seen after 4-HPR administration (\( r = -0.12; P = \text{not significant} \)).

Multiple regression analysis, using delta IGF-I as the dependent variable and age, treatment, BMI, and menstrual status as covariates, showed that treatment was the only determinant of IGF-I decrease. In addition, the interaction between treatment and age was significant, indicating a different, age-dependent behavior of \( \Delta \) IGF-I in the two groups (Table 2). Specifically, the decrease induced by 4-HPR administration was much more pronounced in patients aged under 50 years (mean reduction, 26.7 ± 11.6%), while an age-related decline was seen in controls (Fig. 2).

No relationship was observed between IGF-I levels and BMI or time from surgery (data not shown). No alteration of hepatic function was recorded during 4-HPR administration.

Discussion

A large body of experimental evidence supports a biological role of IGFs in the proliferation of breast cancer cells (see references reviewed in Ref. 1). IGF-I is a more potent mitogen for breast cancer cell lines than estradiol and epidermal growth factor. Moreover, IGF-I receptor, which mediates the mitogenic action of both IGFs, has a higher expression in breast cancer cells than in normal epithelium, and the binding of the growth factor is enhanced in malignant compared to normal breast tissue (1). The blockade of the IGF-I receptor through the monoclonal antibody aIR-3 has been proposed as a clinical strategy for inhibiting tumor growth (11). Since IGF-I levels in the tumor microenvironment may be affected either by \textit{in loco} synthesis or by plasma levels (12), another clinical approach might be the interference with circulating levels of the growth factor, which derive mainly from hepatic synthesis (13).

Several endocrine strategies have proven effective in affecting plasma IGF-I levels (14–17). In particular, tamoxifen has been shown to induce a 15–25% decline in IGF-I levels both versus pretreatment (17) and versus placebo-treated patient values (15). In the present work, we show that treatment with the synthetic retinoid 4-HPR is also able to induce a significant lowering of circulating IGF-I.

The mechanisms through which the retinoid exerts this effect are unclear. A time-related decline can be excluded since control levels, determined after the same time from randomization, were unchanged. A nonspecific effect of the retinoid on hepatic synthesis is also very unlikely since no alteration of liver function has been reported even after long-term 4-HPR administration (18). Moreover, retinoic acid has been shown to scarcely affect IGF-I mRNA levels in cultured hepatocytes (19).

IGF-I hepatic production is mainly regulated by growth hormone (GH) (13). However, since retinoic acid has been shown to increase GH gene expression in pituitary cells \textit{in vitro} (20), an interference with this pathway seems unlikely as well.

One possible explanation of retinoid action involves TGF-β, which has been shown to negatively affect the synthesis of IGF-I (1). Moreover, in fibroblasts \textit{in vitro}, TGF-β increases the synthesis of the insulin-like growth factor binding protein 3 (21), the major protein bound to circulating IGF-I, the expression of which has been associated with growth inhibition of MCF-7 cells by retinoic acid (7). Thus, the increased expression of this growth factor may account for the observed decline of IGF-I.

Interestingly, the decrease in circulating IGF-I induced by 4-HPR administration was particularly relevant in women aged under 50 years, thus suggesting a potential interaction of the retinoid with sex hormones. Indeed, the hormonal regulation of IGF-I synthesis appears to be complex, with estrogens acting as dose-dependent stimulatory or inhibitory agents and tamoxifen showing an inhibitory effect (15). Steroid and retinoid receptors belong to the same superfamily (4); a mutual interaction at the molecular level, therefore, cannot be excluded. In addition, previous \textit{in vitro} and \textit{in vivo} studies have shown an additive or even synergistic effect of 4-HPR with tamoxifen in the growth inhibition of mammary tumor (22, 23).

Finally, our observation of a marked IGF-I decline in younger women is in keeping with preliminary data on the clinical chemopreventive activity of 4-HPR in breast cancer which seems to be evident in women younger than 45 years of age (4) and leads us to speculate that the decline in plasma IGF-I may represent at least one of the mecha-

<table>
<thead>
<tr>
<th>Table 1 Patient characteristics</th>
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<tbody>
<tr>
<td>Characteristic</td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td>Age (yr)</td>
</tr>
<tr>
<td>(range, 37–67)</td>
</tr>
<tr>
<td>BMI</td>
</tr>
<tr>
<td>Premenopause</td>
</tr>
<tr>
<td>Postmenopause</td>
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<tr>
<td>Time from surgery (months)</td>
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<tr>
<td>Follow-up* (months)</td>
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</tbody>
</table>

* Interval between the two determinations of IGF-I levels.

Fig. 1. Mean (± SE) plasma IGF-I levels (ng/ml) at randomization (●) and during follow-up (●) in 4-HPR and control group. *, \( P = 0.003 \) versus baseline values; **, \( P = 0.011 \) versus follow-up values of 4-HPR.
Table 2 Multiple regression analysis using the difference (Δ) of IGF-I levels (ng/ml) between follow-up and baseline as dependent variable

<table>
<thead>
<tr>
<th>Variable</th>
<th>β</th>
<th>SE(β)</th>
<th>95% Confidence interval</th>
<th>Test</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>42.78</td>
<td>49.30</td>
<td>-55.98 - 141.53</td>
<td>0.87</td>
<td>0.389</td>
</tr>
<tr>
<td>Treatment</td>
<td>-167.09</td>
<td>65.02</td>
<td>-297.34 - -36.85</td>
<td>-2.57</td>
<td>0.013</td>
</tr>
<tr>
<td>Age</td>
<td>-0.76</td>
<td>0.97</td>
<td>-2.70 - 1.17</td>
<td>-0.15</td>
<td>0.432</td>
</tr>
<tr>
<td>Age treatment</td>
<td>2.73</td>
<td>1.24</td>
<td>0.24 - 5.21</td>
<td>2.19</td>
<td>0.032</td>
</tr>
</tbody>
</table>

* Overall F test = 4.98; P = 0.004; $r^2 = 0.22$. β, regression coefficient; SE(β), SE of β.

* Student’s t test with 56 degrees of freedom.

In conclusion, treatment with 4-HPR is able to induce a significant decrease in plasma IGF-I levels. This observation, together with the known ability of tamoxifen to inhibit circulating and tissue levels of IGF-I (12), provides further rationale for testing the combination of these two agents in the chemoprevention of breast cancer.

References

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