Plasma Retinol Level Reduction by the Synthetic Retinoid Fenretinide: A One Year Follow-up Study of Breast Cancer Patients

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ABSTRACT

Fenretinide (HPR) is a synthetic retinoid which has been shown to cause a reduction in the incidence of carcinogen-induced epithelial tumors in experimental animals, and it has been chosen to be tested as a chemopreventive agent in humans. A study on plasma concentrations of HPR, of its metabolite N-(4-methoxyphenyl)retinamide (MPR), and on its effects on endogenous retinol was performed in groups of 14 to 18 breast cancer patients who received p.o. daily doses of placebo or 100, 200, and 300 mg of HPR for 6 mo and subsequently 200 mg for an additional 6 mo. After the first 5 mo of treatment, there was a linear relationship between doses of HPR administered and HPR, MPR, and retinol levels. HPR and MPR levels increased with the increase in dose, whereas retinol levels decreased, and the reduction was statistically significant compared with the placebo group after all the doses tested. Plasma retinol binding proteins (RBP) decreased proportionally to retinol (r = 0.96). The effect of HPR on retinol and RBP occurred early, since retinol and RBP levels had already been decreased, compared with the initial levels, by 38% and 26%, respectively, 24 h after a 200-mg HPR dose. After 12 mo of treatment, in patients treated with 200 mg daily, the dose chosen for a chemopreventive trial, HPR and retinol levels were similar to those found at 5 mo, suggesting no drug accumulation and no further retinol reduction, whereas MPR levels were higher. Following interruption of treatment, as HPR decreased, retinol increased with a linear relationship between log levels (r = 0.78); after about 50 days, HPR was present in trace amounts, and retinol levels were in the range of those of the placebo group. These data show that HPR treatment lowers retinol and RBP plasma concentrations. This effect is related to HPR levels and is reversible on cessation of HPR administration.

INTRODUCTION

Retinoids, the synthetic and natural analogues of vitamin A, block the phenotypic expression of cancer in vitro, and some of them have been shown to be effective inhibitors of carcinogen-induced epithelial tumors in experimental animals (1, 2). Among the synthetic retinoids tested against mammary and urinary carcinogenesis, fenretinide or HPR seems to be promising in terms of its effectiveness relative to toxicity. In fact, it has been shown to reduce urinary bladder carcinoma in mice (3) and the development of breast cancer induced in rats by 4-[(butyl-N-(4-hydroxybutyl)]nitrosamine (3) has been shown to reduce urinary bladder carcinogenesis in mice by 4-[N-buty1-N-(4-hydroxybutyl)]nitrosamine (3) as well as the number of alveolar nodules which spontaneously develop in C3H/He mice (4) and the development of breast cancer induced in rats by N-methyl-N-nitrosourea, with relatively low toxicity (5).

For these reasons HPR has been chosen to be tested clinically as a chemopreventive agent. Its effectiveness in preventing contralateral breast cancer is being tested in breast cancer patients with no axillary lymph node metastases who have already undergone radical surgery by administering it daily at the dose of 200 mg, a dose chosen in a randomized phase I trial (6). Since several studies have been reported on the effect in humans of some retinoids, both as preventive and as therapeutic anticancer agents (7), but nothing is known about their influence on endogenous vitamin A, our aim was to investigate the effects of this therapy on plasma retinol levels. Besides, since long-term administration of HPR is required in the ongoing chemoprevention trial in breast cancer patients, we investigated about the levels of HPR, of its metabolite MPR (8, 9), and about its effects on retinol levels during 1-yr treatment.

MATERIALS AND METHODS

Drug, Patients, and Protocol. HPR, administered in capsules of 100 mg, and placebo, consisting of capsules of a similar size and containing the same excipients, were supplied by McNeil Pharmaceutical (Spring House, PA).

Groups of 14 to 18 patients, who were participating in a Phase I trial of HPR (6), were included in this pharmacological study. The characteristics of the patients and of the protocol have been previously described (6). Briefly, all the patients had a histologically confirmed diagnosis of breast cancer with no axillary lymph node metastases and had undergone modified mastectomy or quadrantectomy, axillary dissection and radiotherapy 1 to 3 yr prior to study entry. Each patient signed an informed consent prior to starting HPR, and women able to bear children were asked to use measures to avoid pregnancy during treatment because HPR is known to be teratogenic (10). Their median age was 50 yr (range 35 to 65 yr). They were randomized into 4 groups taking p.o. daily doses of 100 (Group 1), 200 (Group 2), 300 (Group 3) mg of HPR, or placebo (Group 4) for the first 6 mo and subsequently all the groups received 200 mg for the following 6 mo. The daily dose was taken after supper.

Sample Collection and Analytical Procedures. Blood samples were collected, if not otherwise specified, about 12 h after the last daily dose, a time chosen as the most feasible in order to have all the samples at the same interval from drug administration. Blood samples were collected in heparinized tubes wrapped in aluminum foil, and all the procedures were performed in the dark to prevent exposure to light. Samples were centrifuged at 1500 × g for 15 min at 4°C, and the separated plasma was kept frozen at -20°C until analysis, never for more than 3 wk. Plasma was then analyzed for HPR, MPR, and retinol content as previously reported (11). Acetonitrile was added to the samples, which were then vortex mixed in the dark, allowed to sit for 10 min, and centrifuged at 10,000 × g for 5 min to pellet the precipitated proteins. One hundred µl of the supernatants were analyzed on a Perkin-Elmer Series 2/1 liquid chromatograph fitted with a C18 (5 µm) reverse-phase column (125 × 4.6 mm) and a C18 precolumn (Perkin-Elmer, Milan, Italy). The mobile phase consisted of CH3CN:H2O:CH3COOH (75:23:2, v:v:v) delivered at a flow rate of 2 ml/min. Detection was performed with a Perkin-Elmer LC95 absorbance detector at 365 nm which is the absorption maximum for HPR and which also allows a good sensitivity for both MPR and retinol. Peak areas were integrated with a Gilson data analysis system (Gilson, Middleton, WI). Quantitative evaluation of HPR, MPR, and retinol was performed by comparing the peak areas in the samples with those from reference standard curves set up with different known amounts of the three reference standards in plasma. The limits of detectability were 5, 15, and 50 ng/ml for HPR, MPR, and retinol, respectively, and the standard curves were linear up to 2500 ng/ml. The recovery was 98 ± 3% for HPR, 93

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2. To whom requests for reprints should be addressed.

3. The abbreviations used are: HPR, fenretinide or N-(4-hydroxyphenyl)-all-trans-retinamide; MPR, N-(4-methoxyphenyl)retinamide; RBP, retinol binding protein.
± 5% for MPR, and 94 ± 6% for retinol. The coefficient of variation of HPR, MPR, and retinol in the plasma of 3 patients, each one in 5 replicates, was within 5%. HPR and MPR were kindly provided by McNeil Pharmaceutical. All-trans-retinol (Sigma, St. Louis, MO) was added from stock solutions prepared in CH₃CN containing the antioxidant butylated hydroxytoluene according to the method of Nierenberg and Lester (12). Eretinate-free acid (Ro 10-1670), kindly provided by Roche-Milan, Italy, was used as an internal standard by adding it in concentrations of 500 ng/ml to the CH₃CN used to precipitate the proteins. The presence of retinoic acid was also assayed by cochromatography using as reference standard all-trans-retinoic acid (Sigma).

RBP and Prealbumin Detection. RBP and prealbumin plasma levels were detected by radial immunodiffusion with LC-Partigen-RBP and M-Partigen-prealbumin plates (Behring, Marburg, West Germany), respectively.

Statistical Analysis. Regression analysis was carried out to study the regression of HPR, MPR, and retinol plasma levels in relation to HPR doses. Correlation analysis was performed to study the relationships between retinol and RBP plasma levels and between retinol and HPR plasma levels. A two-way analysis of variance was performed on the levels of HPR, MPR, and retinol only of those patients thoroughly examined (i.e., at 5, 9, and 12 mo), and the differences between the means at 12 and 9 mo versus 5 mo were evaluated by Dunnet's t test.

RESULTS

High-Performance Liquid Chromatography Separation. The representative chromatograms of plasma taken from placebo- and HPR-treated patients are reported in Fig. 1. The retention times were the following: internal standard RO 10-1670, 3.30 min; HPR, 4.60 min; retinol, 6.20 min; and MPR, 8.70 min. In HPR-treated patients (Fig. 1A), besides HPR, MPR, and retinol, several other peaks were found with retention times shorter than that of HPR. Among these peaks, the one which coeluted with the front of the solvent and which presumably was constituted of more than one metabolite had a total area much higher than that of HPR and MPR.

No peak could be detected with the retention time of retinoic acid (5.50 min) in plasma of both placebo- and HPR-treated patients.

Plasma Levels of Retinol and RBP and Their Relationship following HPR Treatment. To further investigate the effect of HPR on retinol, the plasma of 12 patients taking daily the 200-mg dose was assayed for retinol and RBP levels at baseline and 24 h after the first HPR administration; in 4 of these patients plasma levels were also assayed 12 h after the seventh administration. A good correlation (r = 0.96) was found between the plasma retinol and RBP concentrations in these patients as shown in Fig. 3. As can be seen from the results reported in Table 1, retinol and RBP levels were already reduced 24 h after the first HPR dose in all treated patients with a mean reduction of 38% and 26%, respectively. Prealbumin concentrations, also assayed in these patients, were not affected by HPR treatment at any time they were tested (data not shown).

HPR, MPR, and Retinol Plasma Levels during 12 Months of Administration of Different Dosage Regimens. As reported in "Materials and Methods," after 6 mo of daily treatments with 100 (Group 1), 200 (Group 2), 300 (Group 3) mg of HPR, and placebo (Group 4), all the patients were changed to the 200-mg...
dose for another 6-mo period. HPR, MPR, and retinol levels of some patients in each dosage regimen were assayed at 5, 9, and 12 mo to evaluate whether, during repeated treatments lasting 1 yr, HPR and/or MPR levels accumulate or retinol levels are further decreased. The plasma of 12, 10, 6, and 11 patients in groups 1, 2, 3, and 4, respectively, was assayed each time, and the means with the standard deviations of the results obtained are reported in Table 2.

Patients of Group 1 had at 9 and 12 mo, while taking the 200-mg dose, higher HPR and MPR levels and lower retinol levels than those found at 5 mo while taking the 100-mg dose. Patients of Group 2, who were on the 200-mg dose for the whole year, showed no differences in HPR and retinol levels evaluated at 5, 9, and 12 mo, whereas MPR levels increased at 12 mo. Patients who started with 300 mg (Group 3) had, at 5 mo, higher levels of HPR and MPR than those of Groups 1 and 2, and these levels remained still higher at 9 and 12 mo after having received 200 mg, thus suggesting drug accumulation with a 300-mg/day schedule. In spite of higher HPR and MPR levels at 12 mo, retinol levels were similar to those found in the other groups. In patients of Group 4, HPR treatment during the last 6 mo caused, as expected, a significant reduction in retinol levels.

Table 1 Plasma concentrations of retinol and RBP at baseline and 24 h after administration of 200 mg of HPR.

<table>
<thead>
<tr>
<th>Retinol (ng/ml)</th>
<th>RBP (mg/100 ml)</th>
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<tr>
<td>Patient</td>
<td>Baseline</td>
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<tr>
<td>1</td>
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DISCUSSION

The results reported herein show that p.o. HPR treatment causes, in humans, an early reduction of both retinol and RBP plasma concentrations and that this effect is reversible on cessation of HPR treatment. Moreover, we have shown that, during 1 yr of daily treatments with HPR at the dose of 200 mg, the dose chosen (6) to be administered in a chemopreventive trial in breast cancer patients, no increase in HPR levels and no further retinol reduction were found compared with those found at 5 mo. The reduction of retinol found after all the doses of HPR tested was linearly related to the dose and well evidenced by the relationship found between HPR and retinol levels at different times from drug interruption.

The effect of HPR on plasma retinol levels has been previously shown to be present also in Sprague-Dawley rats (10, 11), the same strain of rats in which the effectiveness of HPR as chemopreventive agent of carcinogen-induced breast cancer has been proven (5). A similar early and marked reduction of retinol plasma levels has been reported also after all-trans-retinoic acid administration in rats (13). Although it has been suggested by some authors that HPR activity in tissues may be mediated by its conversion to retinoic acid (14), it seems unlikely that the effect of HPR on plasma retinol levels is due to its biotransformation to this compound. In fact, detectable levels of retinoic acid were not found in the plasma of these HPR-treated patients, and other authors never observed an increase in tissue levels of retinoic acid after administration of HPR to rats (8, 15).

Plasma retinol levels are homeostatically controlled over the physiological range of liver vitamin A concentrations (16). In fact retinol is stored in the liver as retinyl esters, which are hydrolyzed into retinol and mobilized from the liver and transported in the blood as a 1:1 complex between retinol and the physiological range of liver vitamin A concentrations (16).
endogenous retinol levels on the chemopreventive activity of their rapid reversal when therapy is discontinued, reported in combine with the protein opsin, function as visual pigment of complex release might account for this effect.

HPR. Evidence reported during these last years on the role of taking 300 mg for 6 mo (6).

patients with basal cell carcinoma treated with high doses (800 mg) of HPR (19), and, consequently, maintain night vision. retinal, one of the isomers of the aldehyde of retinol, is able to... of the data and critical and useful advice about the manuscript, Loredana Cleris for excellent technical help, and Laura Zanesi for secretarial assistance.

retinoids.

diets, not only with increased levels of vitamin A but also deficient or marginally low in vitamin A, on the prevention of carcinogen-induced tumors (21, 22) points out the complex nature of the in vivo chemopreventive effect of vitamin A and retinoids.

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