Breast Tissue Accumulation of Retinamides in a Randomized Short-term Study of Fenretinide


Departments of Clinical Cancer Prevention and Biostatistics, The University of Texas M. D. Anderson Cancer Center, Houston, Texas 77030, and Cancer Care Center of Southern Arizona, Departments of Internal Medicine and Surgery, College of Medicine, University of Arizona, Tucson, Arizona 85724

ABSTRACT

Purpose: The synthetic retinoid N-(4-hydroxyphenyl)retinamide [4-HPR (or fenretinide)] has preclinical and clinical preventive activity in breast carcinogenesis. 4-HPR and its metabolites have been shown to accumulate in the mammary tissue of rodents. We assessed levels of 4-HPR and its major metabolite, N-(4-methoxyphenyl)retinamide (4-MPR), in plasma and in normal and neoplastic breast tissue obtained from women treated with 4-HPR.

Experimental Design: We randomly assigned 14 women with suspected or very recently diagnosed breast cancer to receive 100, 200, or 300 mg of 4-HPR daily for 3–12 days before scheduled biopsy, lumpectomy, or mastectomy. Using high-performance liquid chromatography, we measured post-4-HPR-treatment concentrations of 4-HPR and 4-MPR in plasma and breast tissue obtained during surgery.

Results: Breast tissue and plasma retinamide (4-HPR plus 4-MPR) concentrations increased significantly with short-term oral administration of 4-HPR. Retinamide levels increased in a linear and dose-related fashion in plasma, whereas they peaked and plateaued at 200 mg/day in breast tissue. The total retinamide concentration in breast tissue exceeded that in plasma at each 4-HPR dose. The highest mean tissue:plasma retinamide ratios were achieved at 200 mg/day: 639.5 ± 253.8 versus 190.6 ± 91.9 ng/ml (4.8:1) for 4-HPR and 594.4 ± 201.9 versus 130.5 ± 37.8 ng/ml (6.6:1) for 4-MPR. Plasma retinol levels decreased in association with increasing 4-HPR doses. Two patients experienced grade 1 toxicity at the 300 mg/day dose.

Conclusions: These findings indicate that retinamides preferentially accumulate in human breast tissue (versus plasma). 4-HPR tissue concentrations at 200 mg/d were equivalent to those that inhibit growth and induce apoptosis of breast cancer cells in vitro. Previous clinical and correlative laboratory results suggest that 4-HPR may reduce risk in premenopausal women, who are more prone (than are postmenopausal women) to estrogen receptor (ER)-negative breast cancer development. The present results and previous data (including in vitro 4-HPR activity against ER-negative breast cancer) support further study of 4-HPR in the setting of premenopausal/ER-negative breast cancer prevention.

INTRODUCTION

Retinoids, the natural and synthetic derivatives of vitamin A, have chemopreventive activity in certain in vivo settings (1, 2). Although over 1500 retinoids have been tested, only a few have advanced into clinical prevention trials. Compared with natural retinoids (e.g., 9-cis-, 13-cis-, and all-trans-retinoic acids), synthetic retinoid analogues can be less toxic, more inhibitory, more tissue-specific, and therefore more suitable for cancer chemoprevention (3, 4). 4-HPR (or fenretinide) is a synthetic retinoid with in vitro activity in breast cancer cells (5–7) and a higher therapeutic index than that of natural retinoids in breast and certain other animal carcinogenesis models (8, 9).

Over 20 years ago, Moon et al. (1, 8, 10) showed that 4-HPR was active in preventing carcinogen-induced mammary tumors in rodents. It inhibited the appearance of second mammary tumors after excision of the first palpable tumor in a rat mammary model and exhibited strong antiproliferative effects in carcinogen-induced ductal branching and mammary end-bud proliferation (1, 8, 10, 11). More recently, Green et al. (12) reported that 4-HPR selectively suppressed the development and progression of hyperplastic lesions and carcinoma in situ in the N-methyl-N-nitrosourea rat mammary carcinogenesis model. Results of an important Phase III clinical trial suggest that 4-HPR may reduce the risk of contralateral breast primary tumors in premenopausal women (13), possibly because of 4-HPR effects on IGF-I (14, 15).

Animal (16) and very limited clinical (17) data have suggested that 4-HPR and its main metabolite, 4-MPR, concentrate at high levels in human breast tissue. This tissue accumulation may contribute importantly to the selective preventive activity of 4-HPR in breast tissue in vivo. The experimental and clinical results outlined here led to the present prospective randomized clinical study to increase our understanding of the plasma and...
tissue kinetics of 4-HPR in the setting of breast cancer chemoprevention.

PATIENTS AND METHODS

Patients. Eligible study participants were women scheduled for biopsy to evaluate an abnormal breast examination or a suspicious lesion noted on mammography or for lumpectomy or mastectomy to remove a recently diagnosed malignancy. All study participants signed an informed consent approved by the Institutional Review Board of the University of Arizona. Participants randomly were assigned to receive 100, 200, or 300 mg/day of 4-HPR. The patients were instructed to begin taking the drug p.o. each day after their evening meal and to stop taking the drug on the day before surgery.

Collection and Processing of Blood and Breast Tissue. Blood was obtained at the time of study entry and again at the time of surgery (after 4-HPR treatment). Blood samples were drawn into heparinized tubes placed immediately on ice. Plasma was separated by centrifugation, aliquoted, and stored at −80°C. Breast tissue was obtained (both benign and, when available, malignant) for analysis from each patient during surgery. All specimens were kept shielded from light and stored at −80°C before analysis. 4-HPR, 4-MPR, and ROH levels.

4-HPR, 4-MPR, and ROH Analyses in Plasma and Breast Tissue. Breast tissue samples were weighed and homogenized in a tissue homogenizer with a 1:6 ratio of solution containing 0.05% EDTA and 0.05% ascorbic acid. 4-HPR, 4-MPR, and ROH were extracted from plasma and breast tissue homogenates as described previously (18). One hundred μl of N-(4-ethoxyphenyl)-all-trans-retinamide (10 μg/ml in ethanol) were added to a 500 μl aliquot of plasma or breast tissue homogenate as an internal standard. Specimens were vortexed and deproteinized with 50 μl of 5% perchloric acid. Extraction was performed by adding 500 μl of ethanol acetate to the samples, which were vortexed and spun at 13,000 × g for 1 min. Organic supernatants were injected directly into the high-performance liquid chromatography system. 4-HPR (with 4-MPR) and ROH were detected at 365 and 340 nm, respectively. 4-HPR and N-(4-ethoxyphenyl)-all-trans-retinamide high-performance liquid chromatography reference standards were supplied by McNeil Pharmaceuticals (Spring House, PA).

Statistical Methods. Descriptive statistics were provided whenever appropriate. Differences in plasma and tissue levels of 4-HPR, 4-MPR, and ROH among the three dose groups were analyzed by one-way ANOVA. The differences between two dose groups were compared by the two-sample t test. All Ps were based on two-sided tests.

RESULTS

Patient Characteristics. Fourteen women with suspected or recently diagnosed breast cancer enrolled in the trial. All subjects took the drug for at least 72 h. The mean duration of 4-HPR treatment was 4.7 days (range, 3–12 days). The participants’ mean age was 52 years (range, 34–71 years). Six women were premenopausal. Eleven patients had breast cancer; three had benign lesions (shown by pathology examinations of biopsy or surgical specimens obtained after 4-HPR treatment).

Plasma Levels. Plasma retinamide (4-HPR and 4-MPR) levels had a direct dose-response relationship with 4-HPR intake (P = 0.02 for 4-HPR; P = 0.08 for 4-MPR; P = 0.03 for 4-HPR plus 4-MPR). 4-HPR plasma levels (mean ± SE) at 100, 200, or 300 mg/day of 4-HPR were 34.9 ± 12.5, 190.6 ± 91.9, and 415.9 ± 27.7 ng/ml, respectively. Plasma 4-MPR levels at the three 4-HPR doses were 47.5 ± 7.7, 130.5 ± 37.8, and 196.5 ± 28.8 ng/ml, respectively (Fig. 1). Compared with baseline plasma ROH levels, posttreatment plasma ROH levels were 45% lower in the 100 mg/day 4-HPR group, 56% lower in the 200 mg/day 4-HPR group, and 68% lower in the 300 mg/day 4-HPR group (P = 0.02).

Tissue Levels. 4-HPR and 4-MPR levels were measured in histologically normal and abnormal breast tissue. Concentrations of the two retinamides did not differ significantly between histologically normal and abnormal breast tissue (data not shown), which is consistent with a previous study (19). Therefore, the values we report are the mean value of concentrations in both normal and abnormal tissue. The mean tissue concentration of 4-HPR or 4-MPR in the 100 mg/day group was significantly lower compared with the two higher-dose groups (4-HPR, P = 0.047; 4-MPR, P = 0.020). At 4-HPR doses of 100, 200, or 300 mg/day, breast tissue levels of 4-HPR (mean ± SE) were 44.7 ± 10.7, 639.5 ± 253.8, or 423.1 ± 111.9 ng/ml, respectively. 4-MPR levels at these doses were 42.4 ± 5.7,
Breast Tissue Accumulation of Retinamides

594.4 ± 201.9, or 387.0 ± 47.2 ng/ml, respectively. Even though there was a continued rise in plasma retinamide levels between the 100 and 300 mg/day doses of 4-HPR, the tissue retinamide levels peaked and plateaued at the intermediate 200 mg/day dose (Fig. 2).

**Tissue-to-Plasma Kinetics.** Total retinamide (4-HPR plus 4-MPR) concentration was higher in breast tissue than in plasma at all dose levels studied. Tissue levels were nearly 5-fold higher for 4-HPR and 7-fold higher for 4-MPR at the 200 mg/d dose. The tissue:plasma ratios of retinamide concentrations were not as high at the 100 or 300 mg/day doses of 4-HPR. With respect to the 100, 200, and 300 mg/day doses of 4-HPR, the mean concentration (range) ratios of tissue:plasma 4-HPR were 1.9 (0.5–3.3), 4.8 (1.2–13.2), and 1.0 (0.2–1.6), respectively, for each dose level. For 4-MPR, the mean concentration (range) ratios were 0.8 (0.6–1.1), 6.6 (0.8–20.4), and 2.1 (1.4–2.8), respectively, for each dose level. The highest tissue:plasma ratio for the combined 4-HPR plus 4-MPR levels was seen at 200 mg/day.

**4-HPR Toxicity.** Only 2 of the 14 study patients (14%) experienced side effects, which were relatively mild (grade 1) and occurred at the highest 4-HPR dose (300 mg/day). Treated for 7 days, one of these patients had dry mouth, fatigue, and myalgia; treated for 4 days, the other experienced decreased appetite and mild nausea and headache.

**DISCUSSION**

The present prospective study is the largest and only randomized clinical trial that has assessed the accumulation of 4-HPR (or any of its metabolites) in breast tissue. We determined the plasma and breast tissue kinetics of 4-HPR and its main metabolite, 4-MPR, in women who underwent a breast biopsy or other surgical procedure because of an abnormal breast examination and/or mammogram. This presurgical design has been used in other studies (20). We found that both breast tissue and plasma concentrations of retinamides (4-HPR and 4-MPR) increased significantly with short-term oral administration of 4-HPR at doses of 100, 200, or 300 mg/day and that the concentrations in breast tissue were greater than those in plasma at every dose level. At nearly 5:1 for 4-HPR and nearly 7:1 for 4-MPR, the highest ratios of retinamides in breast tissue to those in plasma occurred at the 4-HPR dose of 200 mg/day. This dose level also marked the plateau level of retinamide concentrations in breast tissue, i.e., there was no further increase at the next higher dose level (300 mg/day). The retinamide concentrations in plasma were linear and dose related and accompanied by reciprocal reductions in retinol levels, which is consistent with prior studies (18, 21).

Our study’s mean concentration of 4-HPR in breast tissue at the dose of 200 mg/day was 639.5 ng/ml, or 1.6 μM. *In vitro* data indicate that this concentration could be biologically active *in vivo*. In cell culture, 1 μM 4-HPR can produce significant growth inhibition and apoptosis in breast cancer cells (5–7, 22, 23).

The absolute tissue concentrations of 4-HPR and 4-MPR in our study were similar, despite relatively higher concentrations of 4-HPR in the plasma. 4-MPR is less polar and more lipophilic than 4-HPR and therefore tends to accumulate more in tissues. The metabolism of 4-HPR to 4-MPR, which has been shown in mammary organ culture systems (24), may also have contributed to the high tissue levels of 4-MPR in the present study.

Studies in rats and mice found that 4-HPR has cancer-preventive activity in breast tissue and accumulates there at 4-fold higher concentrations than in plasma (10, 16, 25). Formenti *et al.* (17) measured 4-HPR and 4-MPR levels in the breast biopsies of three women and nipple discharge of one woman treated with 4-HPR (all within the ranges of 100–300 mg/day and from 5 to 252 days). The tissue concentrations were 1–8 times higher for 4-HPR and 3–36 times higher for 4-MPR than those in plasma. The nipple discharge concentrations were approximately 13 times higher for 4-HPR and 30 times higher for 4-MPR than those in plasma.

A strong body of recent mechanistic data support further study of 4-HPR in certain breast cancer prevention settings. 4-HPR can act through either retinoid receptor-independent or -dependent pathways (26–29) and has potent receptor-independent apoptosis-inducing activity (29–33). Several potential mechanisms of 4-HPR action (34–43), such as down-regulation of human telomerase reverse transcriptase catalytic subunit mRNA levels (38), ceramide generation (37), reactive oxygen species generation (35, 36, 41, 43), and IGF-I suppression (14, 15, 34), may bear on 4-HPR activity in the breast.
The relative contribution of 4-MPR to 4-HPR chemopreventive activity in breast tissue is unclear. 4-MPR has no receptor-dependent activity and induces apoptosis in bladder, head and neck, and lung cancer cell lines (44, 45). 4-MPR is active in a mouse skin carcinogenesis model (46), and ancillary clinical results from a Phase III breast cancer prevention trial of 4-HPR (13) suggest that 4-MPR is important in suppressing IGF-I (a marker of increased breast cancer risk (47). There are conflicting in vitro data, however, as to whether 4-MPR inhibits growth of breast cancer cells (5–7).

A provocative Phase III subset analysis suggested that 4-HPR prevented contralateral breast cancer in premenopausal women (13). Because ER-negative cancer is more prevalent in premenopausal women than in postmenopausal women and is sensitive to 4-HPR in vitro (5, 6, 22), the Phase III subset finding may have been due in part to 4-HPR effects in preventing ER-negative tumors. The search for agents (e.g., retinamides, retinoic X receptor-selective and other retinoids, and cyclooxygenase-2 inhibitors) capable of preventing ER-negative tumors (48–50) was intensified by the landmark Breast Cancer Prevention Trial results demonstrating that tamoxifen is active only against ER-positive tumors (51), which are less aggressive than are ER-negative tumors. 4-HPR combined with tamoxifen is a promising regimen for preventing ER-negative and ER-positive breast cancer. This combination is synergistic in cell culture (52) and animals (53) and did not produce any adverse drug interactions (e.g., increased toxicity or altered pharmacokinetics) in a Phase I pilot study (54). 4-HPR may also be synergistic with other agents, such as nonsteroidal anti-inflammatory drugs and other agents (37, 50, 55).

In conclusion, the present randomized clinical study found that 4-HPR and 4-MPR preferentially accumulate in breast tissue (versus plasma) after short-term administration of 4-HPR. Total retinamide concentration in breast tissue exceeded plasma concentration at each dose level (100, 200, and 300 mg/day), and 4-HPR at 200 mg/day produced the highest tissue:plasma 4-HPR and 4-MPR ratios. This provocative retinamide finding could be extended by studies in nipple aspirate or ductal lavage samples (56). With favorable tissue kinetics in this study and promising activity in other studies [including in vitro growth inhibition and apoptosis induction in ER-negative breast cancer (5, 6, 22)], 4-HPR deserves further clinical and translational study, including correlative assessments of apoptosis in pre- and post-4-HPR-treatment tissue samples, in the setting of premenopausal/ER-negative breast cancer prevention.

REFERENCES

3. Costa, A., Malone, W., Perloff, J., Ran}

2404 Breast Tissue Accumulation of Retinamides


Breast Tissue Accumulation of Retinamides in a Randomized Short-term Study of Fenretinide


Updated version
Access the most recent version of this article at:
http://clincancerres.aacrjournals.org/content/9/7/2400

Cited articles
This article cites 52 articles, 31 of which you can access for free at:
http://clincancerres.aacrjournals.org/content/9/7/2400.full.html#ref-list-1

Citing articles
This article has been cited by 7 HighWire-hosted articles. Access the articles at:
/content/9/7/2400.full.html#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.