Phase I/II Trial of all-trans Retinoic Acid and Tamoxifen in Patients with Advanced Breast Cancer

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ABSTRACT
Because tamoxifen and all-trans-retinoic acid (ATRA) have additive antitumor effects in preclinical systems, we performed a Phase I/II clinical trial of this combination in patients with advanced breast cancer.

Patients with potentially hormone-responsive advanced breast cancer were enrolled. All received 20 mg of tamoxifen by mouth daily. Consecutive cohorts of 3–6 patients were treated on odd-numbered weeks with ATRA at doses of 70, 110, 150, 190, or 230 mg/m²/day.

Twenty-six patients were entered in this trial; 25 were evaluable. A dose of 230 mg/m² ATRA produced unacceptable headache and dermatological toxicity, but doses ≤190 mg/m² were tolerable. Two of 7 patients with measurable disease responded. Seven of 18 patients with evaluable, nonmeasurable disease achieved disease stability for more than 6 months. Plasma AUCs on day 1 of successive weeks of treatment were stable over time. A nonsignificant decrease in serum insulin-like growth factor I levels was noted during treatment, but this trend was similar to that observed in three “control” patients treated with tamoxifen alone.

When given with daily tamoxifen, the maximum tolerated dose of ATRA that could be given on alternate weeks was 190 mg/m²/day. This schedule of ATRA resulted in repeated periods of exposure to potentially therapeutic concentrations of ATRA. Declines in the serum insulin-like growth factor I concentrations observed in patients treated with tamoxifen and ATRA were similar to those observed in patients treated with tamoxifen alone. Objective responses were observed, some in patients who had previously progressed while receiving tamoxifen, suggesting that further studies would be of interest.

INTRODUCTION
For at least a century it has been realized that breast cancer is subject to hormonal influences and that manipulations of the hormonal milieu can be used to treat the disease (1). Modern studies have demonstrated that estrogenic effects are mediated through the ER, leading to a greater understanding of the mechanisms by which hormonal responses are mediated. Furthermore, it is being increasingly recognized that a variety of other growth factors and their receptors may also be involved in supporting or suppressing the growth of breast cancer cells. Among these factors are IGF-I, IGF-II, epidermal growth factor, heregulin, transforming growth factor α, transforming growth factor β, and the retinoids (2–12). These factors, their receptors, and their signaling pathways interact to modulate the growth of breast cancer cells. Preclinical investigations have indicated that additive or superadditive effects can result from combination treatments directed at more than one of these systems. In particular, antiestrogens and retinoids have been found to be additive or synergistic in their antitumor effects in a number of studies (13, 14).

Retinoids are a broad class of agents that interact with at least two families of receptors to produce manifold effects on cellular growth, death, and differentiation (15). Retinoids can inhibit the process of carcinogenesis in a variety of tissues (16), and dramatic responses to therapy with ATRA have been observed in patients with acute promyelocytic leukemia (17–19). Retinoic acid and several synthetic retinoids have been shown to inhibit the growth of human breast cancer cell lines and to attenuate estrogen-stimulated growth in breast cancer cell lines (4–12). Several studies have indicated that tamoxifen or hydroxytamoxifen and a variety of retinoids, including ATRA, have additive or superadditive antiproliferative effects on breast cancer cell lines (14) and that ATRA can inhibit estrogen-stimulated protein synthesis (9, 11). Furthermore, fenretinide, 9-cis-retinoic acid, and, to a lesser extent, ATRA add to the inhibition by tamoxifen of nascent tumor formation in the methylnitrosourea rat mammary carcinoma system (13, 20).

Based upon these preclinical studies, we have performed a Phase I/II study of tamoxifen and ATRA. Because ATRA can induce its own metabolism, resulting in an increase in elimination and a consequent decrease in serum concentration with chronic daily administration, we chose to dose ATRA daily on

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2The abbreviations used are: ER, estrogen receptor; IGF, insulin-like growth factor; ATRA, all-trans-retinoic acid; ECOG, Eastern Cooperative Oncology Group; PS, performance status; MTD, maximum tolerated dose; AUC, area under the curve; IRMA, immunoradiometric assay; BP, binding protein; RAR, retinoic acid receptor.
alternate weeks of therapy. This schedule has been shown to result in higher serum concentrations during the initial days of therapy during the weeks when ATRA is given (21), and we performed serial pharmacokinetics studies in selected patients entered on this trial to document this observation in our patient population. We also performed serial studies of IGF-I to examine the biological effects of treatment with the combination of tamoxifen and ATRA.

PATIENTS AND METHODS

Eligibility

This trial was open to patients with advanced breast cancer who would generally be treated with a hormonal manipulation. That is, patients were required to have either stage IV or recurrent breast cancer that was ER positive, progesterone receptor positive, or of unknown hormone receptor status. Prior hormonal therapy in the metastatic or adjuvant setting, including tamoxifen therapy, was allowed. However, patients receiving such therapy were required to have either shown an objective response to the most recent hormonal manipulation or to have remained stable for a period of at least 12 weeks. No required period of hormonal withdrawal prior to entry on this study was specified in the treatment protocol. On entry into the study, patients were classified as being either "tamoxifen naive" (no prior tamoxifen or adjuvant tamoxifen discontinued > 6 months prior to recurrence) or "tamoxifen resistant" (prior response or disease stability with tamoxifen followed by progression, or progression on adjuvant tamoxifen). Patients were required to be of ECOG PS 0–2 and to have adequate major organ function as defined by laboratory criteria (WBC count, ≥ 3000/μl; hemoglobin, ≥ 8.0 mg/dl; platelet count, ≥ 100,000/μl; serum creatinine level, ≤ 1.5 mg/dl; bilirubin level, ≤ 1.5 mg/dl; and liver enzymes, ≤ 5 × normal). Prior adjuvant chemotherapy was allowed, but no chemotherapy for advanced disease could have been given. Patients could have either bidimensionally measurable or evaluable, nonmeasurable disease. All patients were required to give their informed consent to participation, and the study protocol was reviewed and approved by an institutional human investigations review board. Patients were excluded from participation if they had rapidly progressive or life-threatening disease that was felt to be an indication for chemotherapy (e.g., malignant involvement of more than ⅓ of the liver or lymphangitic pulmonary disease). Patients with a life expectancy of less than 3 months were ineligible, as were pregnant or breast-feeding patients. Blood tests to determine eligibility and tumor evaluations based on physical examination or routine X-ray studies were required to have been performed within 15 days of registration to the study. Special scans (e.g., computed tomography scans) to assess disease status were required to have been performed within 30 days of registration. Conventional response criteria were used, with complete disappearance of disease being classified as a complete response, and 50% or greater reduction in the product of the largest perpendicular diameters of all measurable lesions prospectively selected for evaluation being required for a partial response. Stability of bone lesions in conjunction with a partial response of measurable disease was classified as a partial response provided that bone pain did not worsen. Patients with bone-only disease were considered to have evaluable, nonmeasurable disease.

Treatment

All patients at all dose levels received 20 mg of tamoxifen daily, administered as a single oral dose at bedtime. In addition, patients received treatment with ATRA, administered p.o. in two divided doses daily. ATRA was given with breakfast and dinner, and patients were advised to take the agent with a fat-containing meal. The daily doses of ATRA studied were 70, 110, 150, 190, and 230 mg/m²/day. ATRA was administered in 10-mg capsules, so doses were rounded to the nearest 10 mg; if the daily dose could not be equally divided, the larger dose was given in the morning. Because the clearance of ATRA is rapidly induced with chronic administration (21, 22), ATRA was administered on days 1–7 of alternate weeks, being given on the first, third, and subsequent odd-numbered weeks of treatment. Following a standard Phase I trial design, successive cohorts of 3–6 patients were treated with progressively higher doses of ATRA until the MTD of ATRA that could be given in combination with tamoxifen was determined. The MTD of ATRA was defined as being one dose level lower than the dose level that produced grade 3–4 toxicity in 3 or more of 6 patients, with toxicity graded according to the National Cancer Institute Common Toxicity Criteria. Entry onto the next higher dose level was allowed only after all patients at the previous dose levels had been treated for a minimum of 4 weeks; grade 3–4 toxicity occurring for the first time after this 4 week period was considered in the determination of the MTD, despite the fact that escalation to higher dose levels might have occurred. Once the MTD was determined, additional patients were treated at the MTD to more fully characterize the toxicity of that dose level. Before escalation to the next higher dose could occur, it was necessary to fail 3–6 patients at all dose levels for a minimum of 4 weeks. Chemotherapy or hormonal therapy for breast cancer concurrent with participation in this trial was not allowed, but palliative radiation to lesions not being followed for measurable or evaluable disease was allowed as long as the indication for such treatment did not represent disease progression. Other investigational agents and other retinoids, including over the counter vitamin A supplements, were not allowed. Therapy was continued without dose modification for grade 0–2 toxicity at the discretion of the investigator and the patient. In the event of grade 3 toxicity, therapy with ATRA was withheld until resolution to grade 0–1 was documented. Thereafter, therapy was resumed at the next lower dose level (or a 50% dose reduction at the lowest dose level). Patients were removed from the study if the same grade 3–4 toxicity reoccurred after dose reduction. Patients experiencing grade 4 toxicity were removed from the study, unless continued therapy was deemed appropriate by the principal investigator and the patient, in which case, the guidelines for grade 3 toxicity were followed. A complete blood count with differential, serum chemistry profile, serum cholesterol level, serum triglyceride level, and toxicity evaluation were determined on day 7 of weeks 1 and 3. These same evaluations were performed in conjunction with a brief history and physical examination on day 1 of weeks 5 and 11 and every 6 weeks thereafter. Disease assessment was performed every 6 weeks when disease was evaluable on physical examination or
chest X-ray; disease assessable by computed tomographic or other scans was evaluated every 10–12 weeks. Treatment was continued until disease progression, unacceptable toxicity, or patient withdrawal.

**Laboratory Methods**

**Pharmacokinetics Studies.** Pharmacokinetics studies were performed on the first day of weeks 1, 3, 5, and 7 in selected patients treated at the 150, 190, and 230 mg/m² dose levels. Two patients treated at the 110 mg/m² dose level had studies performed on the first day of therapy and then on the seventh days of weeks 3, 5, and 7. One patient, who has remained on therapy for more than 2 years, also had studies performed on the first day of weeks 56 and 98 to investigate the pharmacokinetics of ATRA after prolonged administration. On each day of pharmacokinetics monitoring, the morning dose of ATRA was administered with 8 ounces of whole milk. Plasma samples were drawn prior to drug administration, every 30 min for 4 h after the dose of ATRA and then every hour for an additional 4 h. Samples were protected from light, and separated plasma was frozen at −20°C prior to shipment and analysis at the National Cancer Institute.

**ATRA Assay and Pharmacokinetics Analysis.** The plasma concentration of ATRA was measured by a previously described high-performance liquid chromatography method (21). The AUC from 0 to 8 h was calculated by the trapezoidal method (23). Samples below the limit of detection (0.03 μM) obtained immediately prior to the first detectable time point or immediately following the last detectable time point were set to 0.03 μM. All other samples below the limit of detection were set equal to 0. No extrapolation was carried out due to the limited number of data points available to estimate the terminal decay.

**IGF-I Studies.** Studies of IGF-I were performed at the Cleveland Clinic Foundation. Plasma IGF-I assays were monitored serially in all patients, with the exception of two patients on dose level 4. Serum samples were obtained prior to therapy and on day 6 of weeks 5 and 11. Because changes in serum IGF-I concentration have been described in patients receiving tamoxifen alone, three “control” patients who were receiving tamoxifen alone were serially studied in the same manner as patients entered on this trial. IGF-I was measured by a monoclonal antibody-based IRMA. Serum samples were subjected to acid ethanol extraction to remove BPs prior to the assay. Reagents for this assay were obtained from Diagnostic Systems Laboratory (Webster, TX). The minimum detection limit for this assay was 0.8 ng/ml, and the interassay variations were between 7 and 9% (coefficient of variation). The recovery of cold IGF-I added to serum samples before extraction ranged between 90 and 100% in this IRMA (24).

IGF-I BP-3 and BP-1 were also measured by IRMAs, and reagents for these were also obtained from Diagnostic Systems Laboratory. The IGF BP-3 assay is based on two polyclonal antibodies, each used as a capture and localizing antibody. The interassay variations were less than 10%. For BP-1, a monoclonal antibody was used as the capture antibody and was coated on the test tube surface, and a 125I-labeled polyclonal antibody was used as a localizing antibody. This assay is designed to eliminate differential antibody recognition of the various IGF BP-1 phosphoforms and is unaffected by the state of IGF BP phosphorylation (25). This allows for an accurate measurement of total IGF BP-1 (26). The interassay variations were less than 10%. There were no cross-reactivities with other BPs as reported by the manufacturer.

**Statistical Methods**

The Kruskal-Wallis test was used to test for a general dose effect (27), and the Jonckheere-Terpstra test was used to test for a monotonic dose effect in IGF-I, IGF BP-1, and IGF BP-3 levels and the ATRA AUC (28). The Wilcoxon signed rank test was used to test for overall changes from the first to the second and from the first to the third time points in IGF-I, IGF BP-1, and IGF BP-3 levels (29). Spearman rank correlation coefficients were computed for the association between the AUC (day 1) and the change in IGF-I, IGF BP-1, and 3 levels (30). Both absolute changes and percent changes were evaluated. The Kaplan-Meier method was used to estimate survival and time on study (31).

**RESULTS**

**Patient Characteristics.** A total of 26 patients were enrolled in this study. One patient was considered inevaluable because she did not take tamoxifen and was found to be otherwise noncompliant. Two patients withdrew early because of headaches that were intolerable to them. Although these complaints would otherwise have been graded as grade 2, because they were unacceptable to the patients and led to study withdrawal, they were classified as grade 3. The median age was 62 years (range, 41–74). Fifteen patients were of ECOG PS 0, 10 were ECOG PS 1, and 1 was ECOG PS 2. Other patient characteristics are as listed in Table 1. Seven patients were considered to have bidimensionally measurable disease, whereas the remainder had evaluable, nonmeasurable disease. Five patients were tamoxifen naive, as defined above, whereas 20 evaluable patients were tamoxifen resistant, having progressed after initially responding to or achieving a period of

### Table 1 Patient characteristics

<table>
<thead>
<tr>
<th>Patient characteristic</th>
<th>No. of patients</th>
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<td>Entered</td>
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<tr>
<td>Evaluable</td>
<td>25</td>
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<td>Bidimensionally measurable disease</td>
<td>7</td>
</tr>
<tr>
<td>Evaluable, nonmeasurable disease</td>
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</tr>
<tr>
<td>Median age, yr (range)</td>
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<td>Soft tissue/regional nodes</td>
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<td>Liver</td>
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prolonged disease stability with prior tamoxifen. One such patient was entered in the trial immediately after her second course of tamoxifen therapy for advanced disease. In all other cases, intervening hormonal therapy had been administered between the time of disease progression on tamoxifen therapy and entry onto this trial.

Toxicity. Table 2 summarizes the toxicity noted at each dose level. At all doses, headaches, nausea, bone pain, and skin changes were noted. The headaches were most severe during the first week of therapy, peaking at the end of the first week of therapy. These headaches were sometimes associated with nausea and occasionally with vomiting, although no other evidence of elevated cerebrospinal fluid pressure was noted. The headaches and nausea tended to subside during the weeks that ATRA was not administered. These symptoms would recur during the weeks of ATRA reintroduction, although their severity tended to wane with subsequent cycles of treatment. Similarly, dermatological reactions, consisting of erythema and desquamation, were dose-related and were most severe with the initial weeks of therapy. These reactions also peaked toward the ends of the weeks of ATRA administration and were most severe during the initial cycles of treatment, tending to decrease in severity with continuing therapy. Bone pain was noted intermittently. Life-threatening hypercalcemia was noted in one patient, but was felt to be due to disease progression.

As can be seen in Table 2, ATRA at a dose of 230 mg/m²/day was unacceptably toxic. At that dose level, three of five patients developed grade 3 toxicity. These grade 3 toxic effects consisted of a headache in one patient and dermatological toxicity in two patients (moist desquamation). Thus, the next lower dose level, 190 mg/m²/day, was the MTD. All patients entered at that dose level to more fully characterize the toxicity at the MTD. Two grade 3 headaches were recorded among a total of nine patients treated at that dose level. In one case, the headache would have been recorded as grade 2 had the patient not reported it to be unacceptably severe and withdrawn from the study. Thus, 190 mg/m²/day is the MTD of ATRA that can be given in conjunction with 20 mg of tamoxifen per day.

Antitumor Effects. The antitumor effects of treatment are summarized in Table 3. All seven of the patients with bidimensionally measurable disease had received tamoxifen previously, and five were tamoxifen resistant. Among the seven patients with measurable disease were observed two partial responses. One of these partial responses occurred in a lymph node metastasis, whereas the second was in a biopsy-proven liver metastasis. Both of these patients were treated at the 150 mg/m²/day dose level of ATRA. The patient with the nodal metastasis was tamoxifen resistant, having had a partial response to tamoxifen, followed by disease progression. After a transient response to megestrol acetate, she was entered on this trial. Her lymph node metastasis became nonpalpable, and her bone lesions remained stable. Although her measurable disease did not progress on study, she was removed from the trial after 27 months of therapy when she developed a contralateral node-positive breast cancer. The second patient had received prior tamoxifen as adjuvant therapy, but she was considered tamoxifen naive because she relapsed after having been off tamoxifen for more than 1 year. She has had near-complete resolution of her liver metastasis and improvement of bony metastases. She remains in partial remission after more than 36 months of therapy.

Among the 18 patients with evaluable, nonmeasurable disease, 7 had disease stabilization for more than 6 months. Five of these disease stabilizations have occurred among the 15 evaluable patients with tamoxifen-resistant disease. Two of the disease stabilizations were observed among the three patients who were tamoxifen naive. The median time on study for the 25 evaluable patients was 23.5 months.
Pharmacokinetics Studies. There was a significant degree of interpatient variability in plasma ATRA concentrations observed at all dose levels. The plasma AUCs on the first day of weeks 1, 3, 5, and 7 are shown in Fig. 1. Data are not available for all patients at the later time points because some patients were taken off the study prior to those times. At all dose levels studied, the plasma AUC on the first day of alternate weeks was similar to the AUC observed on the first day of treatment. Considerable intrapatient variability in AUC was also observed during the 7-week study period (Fig. 1). One of the two patients treated at the 110 mg/m² dose level demonstrated a significant decrease by day 7 of this alternate week schedule, with an average plasma AUC on day 7 of weeks 3, 5, and 7 of 89 μM·min compared with a day 1 AUC of 822 μM·min.

Peak ATRA concentrations observed the first day of treatment averaged 3.0, 1.0, 2.5, and 2.1 μM for the 110, 150, 190, and 230 mg/m² dose levels, respectively. No relationship between dose and AUC or peak concentration could be demonstrated.

One patient treated at the 150 mg/m² dose level, who has remained on study for more than 3 years, had additional pharmacokinetics studies performed on the first day of weeks 56 and 98 (Fig. 1, patient 1). The AUCs observed on these days, 68 and 117 μM·min, respectively, were similar to those achieved during the initial weeks of treatment.

IGF-I Studies. The changes over time in serum IGF-I levels are displayed in Fig. 2. For the patients receiving ATRA, the mean change in serum IGF-I concentration between the baseline and second (week 5) time point was a decrease of 28.47 ± 15.31 ng/ml (median, −8.0; range, −231 to 54). Between the baseline and third (week 11) time point, the mean change in IGF-I concentration was a decrease of 38.43 ± 21.46 ng/ml (median, −24; range −248 to 88 ng/ml). These differences were not statistically significant (P = 0.089 for week 5 versus baseline; P = 0.056 for baseline versus week 11). However, when the control patients, treated with tamoxifen alone, were included with the group receiving tamoxifen + ATRA, the overall change in serum IGF-I concentration became significant at both 5 and 11 weeks (mean, −34.02 ± 14.43; P = 0.023, and mean, −45.71 ± 19.08; P = 0.014, respectively). No significant relationships could be demonstrated between dose and change in IGF-I level at either 5 or 11 weeks (at 5 weeks, Kruskal-Wallis test for general dose effect: P = 0.58; Jonckheere-Terpstra test for monotonic dose effect: P = 0.47; at 11 weeks, Kruskal-Wallis test: P = 0.33; Jonckheere-Terpstra test: P = 0.22).

Serial studies of serum IGF BP-1 and IGF BP-3 concentrations did not show consistent significant changes over time (data not shown). Again, a significant relationship between ATRA AUC and changes in IGF-I could not be demonstrated.

DISCUSSION

Based upon the toxicity observed in this study, 20 mg of tamoxifen per day and ATRA at doses up to 190 mg/m²/day can be given together with acceptable toxicity. Unacceptable toxicity was noted when ATRA was escalated to a dose of 230 mg/m²/day. No grade 3 toxicities were noted in patients treated with ATRA at a dose of 150 mg/m². Thus, the MTD of ATRA when given with tamoxifen is similar to that of ATRA when given as a single agent (22). Although grade 2 headache and dermatological toxicity can be expected at the MTD, these toxicities tend to improve with chronic therapy, and this combination should be acceptable to patients with advanced disease or at a high risk for relapse.

The pharmacokinetics of ATRA showed a high degree of intra- and interpatient variability, and it must be recognized that only five patients underwent pharmacokinetics studies at all scheduled time points. The large number of 10-mg capsules required for the doses administered may have contributed to this variability, but this degree of variability has been observed in several studies that used a wide range of doses (21, 32–35). Intrapatient variability resulted in changes in AUC over time, but these changes were heterogeneous, and the consistent and profound declines in AUC observed with continuous drug administration were not observed. The intermittent schedule of ATRA administration resulted in repetitive periods of exposure to concentrations of ATRA normally only observed on the first day of treatment. This was not only true for the 7-week time period studied but also in one patient studied for longer than 1 year. This observation confirms a prior study (21) that suggests that an every-other-week schedule of ATRA administration offers a pharmacokinetic advantage over a chronic daily schedule. This schedule should be considered for further investigation in future clinical studies involving treatment with ATRA.

Both tamoxifen (36, 37) and fenretinide (38) therapies have been associated with suppression of serum IGF-I concentrations, and it has been suggested that this decline might be a mechanism by which antitumor effects are mediated (39, 40) or serve as an intermediate marker of drug effect (41). In our study, nonsignificant decreases in serum IGF-I concentrations were noted in patients treated with ATRA in combination with tamoxifen. Changes of a similar nature were noted in a small number of patients treated with tamoxifen alone, however, consistent with previous reports (36, 37). No relationship between ATRA dose or plasma AUC and change in IGF-I concentration could be demonstrated, although it must be recognized that the variability in both the ATRA pharmacokinetics and the serum IGF-I levels complicate such analyses. Similar declines were
noted in patients treated with tamoxifen alone, and it is only when this group of patients was included in the analysis that a significant decrease in serum IGF-I concentration over time could be demonstrated. Although the small patient numbers and biological variability of serum IGF-I levels prevent a definitive conclusion from being reached, these observations do not suggest that therapy with the combination of ATRA and tamoxifen is associated with effects on systemic IGF-I concentrations that are substantially greater than those of tamoxifen alone.

Objective responses in patients with measurable disease and periods of prolonged disease stability in patients with evaluable, nonmeasurable disease were observed. Of particular interest are (a) the relatively long duration of some of these responses, and (b) the observation of objective response or prolonged disease stabilization in patients who had previously shown evidence of acquired tamoxifen resistance. All of the responses in tamoxifen-resistant disease occurred after interven-

ing therapy with salvage hormonal therapy. Because second responses to tamoxifen have been observed after a tamoxifen-free period, it is not possible to definitively conclude that ATRA reversed tamoxifen resistance in any patient, nor would it be possible to differentiate such reversal of tamoxifen resistance from a response to single-agent ATRA. The data are consistent with the interpretation that ATRA reverses tamoxifen resistance in some patients, however, and clinical trials designed to test this hypothesis would be of interest.

The nature of the interactions between estrogen, retinoids, and their receptors has been the subject of considerable preclinical investigation. ERs and RARs are members of the steroid-thyroid receptor superfamily (15, 42, 43). ATRA is thought to be a RAR-α-specific ligand, although it may be metabolized to species that interact with retinoid X receptors (15, 42, 43). In studies in human breast cancer cell lines, the effects of ATRA are thought to be mediated through RAR-α (44, 45). In MCF-7 cells, ATRA can inhibit estradiol-stimulated growth and the
estradiol-induced increase in transforming growth factor α and pS2 gene expression (9). Exposure to ATRA results in increased ER expression in MCF-7 cell lines (8), and ER-positive cell lines have been found to have higher levels of RAR-α than ER-negative cell lines. Although ER-negative cell lines are generally resistant to the growth inhibitory effects of ATRA (45, 46), transfection of a functional ER gene into an ER-negative cell line can restore both RAR-α gene expression and sensitivity to the growth inhibitory effects of ATRA (47). The SKBr3 cell line, however, is ER negative but is responsive to the growth inhibitory effects of ATRA. This cell line expresses RAR-α mRNA at a 2-fold higher concentration than other ER-negative cell lines, which further suggests that the growth inhibition induced by ATRA is mediated through its interaction with RAR-α (45). Thus, the modulation of RAR-α mRNA expression seems to occur through both estrogen-dependent and estrogen-independent mechanisms. These observations suggest that some estrogen-unresponsive cells can remain responsive to retinoids.

A model adequately explaining these interactions between steroid hormones, retinoids, and their receptor families has not been advanced. Although it has not been demonstrated in the case of estrogen response elements, RAR-α can bind to the hormone response element for T3 and glucocorticoids (48). The ER-positive but tamoxifen-resistant RR MCF-7 subline may have an altered chromatin acceptor site for the ER-tamoxifen complex (8). This subline is resistant to ATRA, suggesting that the retinoid receptors may interact with these same antiestrogen acceptor sites. Alternatively, retinoids and estrogens may simply modulate a similar range of intermediaries of cellular proliferation, such as IGF-I or transforming growth factors α and β. ATRA does not stimulate reporter gene activity by the recently described ER-β (49), but other potential interactions between the retinoids and their receptors and ER-β have not, as yet, been described. The preclinical observations described above and the suggestive clinical results reported here imply that further investigation of the interactions of these two classes of agents should proceed at both the basic and clinical level. Clinical studies of antiestrogens and ATRA, as well as other, potentially less toxic, more selective, or more potent retinoids, are warranted.

REFERENCES


Phase I/II trial of all-trans retinoic acid and tamoxifen in patients with advanced breast cancer.

G T Budd, P C Adamson, M Gupta, et al.


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