Chemoprevention of Mammary Carcinoma by LGD1069 (Targretin):
An RXR-selective Ligand

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Abstract

Recently, 9-cis retinoic acid, a high affinity ligand for retinoid acid receptors and retinoid X-receptors (RXRs), was shown to have efficacy superior to all-trans retinoic acid as a chemopreventive agent in the N-nitroso-N-methylurea-induced rat mammary carcinoma model. To further explore the specific contribution RXR activation may play in suppression of carcinogenesis, the efficacy of LGD1069 (Targretin), an RXR-selective ligand, in the N-nitroso-N-methylurea-induced rat mammary tumor model was studied. LGD1069-treated animals showed a 90% reduction in tumor burden and tumor incidence compared with vehicle-treated rats with an efficacy similar to that achieved with tamoxifen. LGD1069 was very well tolerated during 13 weeks of chronic therapy with no classic signs of “retinoid-associated” toxicities. These data demonstrate that LGD1069, an RXR-selective ligand, can act as a highly effective and benign chemopreventive agent for mammary carcinoma.

Introduction

As the search for optimal chemopreventive agents continues, the major attributes of this class of agents must be safety as well as efficacy. Emphasis on a favorable drug profile is especially crucial because these chemopreventive agents will be given for long periods of time to a population of individuals with a high risk of cancer. The induction of breast cancer by the carcinogen NMU has served as an in vivo model to identify agents that suppress mammary carcinogenesis. Several classes of compounds have been identified that are highly efficacious in inhibiting mammary carcinogenesis. Of note, two of these agents, anti-estrogens and retinoids, inhibit both the incidence and number of NMU-induced tumors (1, 2).

The anti-estrogen TAM is the endocrine treatment of choice for the therapy of hormone-dependent breast cancer. TAM is well tolerated by most patients and has been administered safely to cohorts of breast cancer patients continuously for 5–10 years. In recent clinical trial overviews, TAM has been shown to significantly increase disease-free survival and decrease mortality in early- and late-stage breast cancer (3, 4). However, several significant side effects have been described after chronic administration of TAM in breast cancer patients. Notably, long-term TAM administration experimentally and clinically, stimulates the growth and increases the incidence of endometrial carcinomas (3, 4).

Retinoids, such as ATRA and 13-cis RA, have also been shown to have efficacy as chemoprevention agents in both preclinical models and human clinical trials (5–7). However, in human clinical trials, chronic treatment has often been associated with significant toxicities, which include mucocutaneous toxicity, thereby limiting their use for chemoprevention (6, 8). Recently, Anzano et al. (1) have elegantly demonstrated that 9-cis RA has superior efficacy when compared with ATRA for preventing NMU-induced tumors in an experimental chemoprevention model. Because 9-cis RA is a ligand for both RARs and RXRs, whereas ATRA binds only to the RAR subtypes, it is possible that activation of the RXRs contributed to the superior activity of 9-cis RA in this model.

In an attempt to dissect distinct biological pathways mediated by RAR and RXR signal transduction, numerous laboratories have synthesized retinoids with receptor subtype selectivity (9, 10). LGD1069 (Targretin) was identified as a selective RXR ligand that is devoid of significant RAR binding and transactivation of RAR-responsive genes. This is in contrast to compounds such as TNPB and AM580, which selectively bind and activate the RARs (10). These ligands, as well as others, have provided the unique opportunity to pharmacologically dissect the role that each receptor pathway contributes to the control of cell proliferation, differentiation, and apoptosis. For example, using a series of RXR- and RAR-selective ligands, we have recently demonstrated that in leukemic cells, activation of RAR pathways regulates cell proliferation and differentiation, whereas activation of RXR pathways leads to the induction of apoptosis (11). These observations suggest that there are distinct biological processes controlled by RAR and RXR pathways.

To specifically examine whether RXR activation contributes to the suppression of carcinogenesis, we have examined the ability of the RXR-selective ligand LGD1069 to alter tumor formation in the NMU-induced mammary tumor model. In addition, we compared the efficacy of LGD1069 with that of TAM, an agent previously shown to be highly efficacious in this model. We demonstrate that LGD1069 significantly decreases tumor incidence and tumor multiplicity compared with vehicle-treated animals. The maximum inhibitions in tumor incidence and multiplicity are similar to those achieved with TAM. In addition, none of the classic side effects associated with many of the current retinoids (ATRA, 13-cis RA) has been observed in these experiments with LGD1069. The favorable therapeutic index of LGD1069 suggests that it may be efficacious, either as a single agent or in combination with other endocrine agents, as an agent for the chemoprevention of breast cancer.

Materials and Methods

Formulation of Test Compounds. LGD1069 (Targretin, Ligand Pharmaceuticals, Inc., San Diego, CA) was suspended in an aqueous solution composed of 10% w/v polyethylene glycol (Glassman Pharmaceutical, San Diego, CA) and 90% of 1% w/v carboxymethylcellulose (Sigma, St. Louis, MO). LGD1069 (30 or 100 mg/kg) was administered orally to animals at 5 ml/kg body weight by 16-gauge gavage needle (Popper and Son’s, New Hyde Park, NY). TAM (Sigma) was formulated in purified sesame oil (Croda, Parsippany, NJ) by first dissolving in a small volume of ethanol and then evaporating the ethanol under a stream of gas.
purified nitrogen to solubilize the TAM into the sesame oil. Rats were given s.c. a volume of 0.1 ml containing either 50 or 150 μg/kg of TAM.

Treatment of Animals. NMU (Sigma) was formulated as an aqueous solution of 10 mg/ml by wetting NMU powder with 3% acetic acid and dissolving it in sterile saline (1). Fresh solutions of NMU were injected within 30 min of preparation. Virgin female Harlan Sprague Dawley rats (Harlan Sprague Dawley, Indianapolis, IN), 50 days of age, were injected i.v. in the tail vein with 5 mg of NMU/100 g body weight as described previously (1). Rats were housed in a United States Department of Agriculture-registered facility in accordance with NIH guidelines for the care and use of laboratory animals. All animals received food (Harlan Teklad LM485-7012, Indianapolis, IN) and acidified water ad libitum. Animals were examined for tumors and weighed weekly. One week after carcinogen administration, rats were randomized into experimental groups and treated 5 days a week with LGD1069 or TAM.

Uterine Wet Weight Assay. Immature 21-day-old female Harlan Sprague Dawley rats were housed and administered test compounds under identical conditions described above. E2 (Sigma) was administered s.c. in a similar manner to TAM. After 3 days of treatment, all animals were sacrificed the next day, and uterine wet weights were obtained after blotting on filter paper.

Retinoid Binding and Cotransfection Assays. Retinoid binding studies using baculovirus-derived receptors and cotransfection studies were completed using methods described previously (12, 13).

Hormone Assays and Determination of LGD1069 Plasma Levels. E2 was measured using a 125I-labeled-radioimmunoassay standard kit modified for use with rat serum (Diagnostic Systems, Webster, TX). Progesterone was measured using H1-[1,2,6,7,16,17]progesterone and rabbit anti-progesterone antibody (Fitzgerald Labs, Concord, MA). Radioactivity counts were quantified using donkey anti-rabbit conjugated scintillation proximity assay bead technology (Amer sham, Chicago, IL). Rat prolactin was measured using a standard radioimmunoassay kit (Amersham).

LGD1069 concentrations in 0.5-ml plasma samples were quantified using external standardization and isocratic high-pressure liquid chromatography with UV detection at 262 nm. Standards in control EDTA rat plasma ranged from 0.5 to 30 μM. Extracts and protein precipitation were accomplished by vortexing (~30 sec) with 5 volumes of methanol. After chilling at ~20°C (~1 h) and centrifuging, supernatants were evaporated in a vacuum and reconstituted in high-pressure liquid chromatography mobile phase (CH3CN/10 mM NH4OAc/HOAc; 80:20:1, v/v/v). After additional centrifugation, supernatants were injected into a Microsorb-MV, 4.6 × 250-mm, 5-μm, C-18 column (Rainin Instrument Co., Inc., Woburn, MA). Separations were at 40°C with a flow rate of 1.5 ml/min.

Statistical Methods. Differences in the significance of tumor incidence between groups was determined by ANOVA followed by Fisher’s Protected LSD test. Differences in average numbers of tumors were determined by ANOVA followed by unpaired Student’s t test. Analysis of hormone levels, uterine wet weights, and LGD1069 levels were determined by ANOVA followed by unpaired Student’s t test. All statistical tests were computed using SuperANOVA software version 1.11 (Berkeley, CA).

Results

RRX Selectivity of LGD1069. The retinoid receptor specificity of LGD1069 was examined using a competitive ligand binding assay with recombinantly expressed retinoid receptors generated from baculovirus extracts (Fig. 1A). LGD1069 has a binding dissociation constant (Kd) ≤30 nM for three RXR subtypes. In comparison, LGD1069 has a very weak binding affinity for the RARs, with KdS in the range of 4200–7600 nm. Thus, in the ligand-binding assay, this compound is ~100-fold selective for the RXRs compared with the RARs. To examine the transcriptional activity of this compound, transient co-transfection assays of the individual retinoid receptors, along with an appropriate reporter molecule, were used. LGD1069 produces a concentration-dependent increase in transactivation for all three RRX subtypes with EC50s that range from 30 to 46 μM (Fig. 1B). Its potency and efficacy for the RXRs is similar to 9-cis RA (12, 13). In contrast, LGD1069 is completely inactive in cotransfection assays for RARα and only at high concentrations in the micromolar range does it weakly activate RARβ and RARγ. At 10 μM, the activity of LGD1069 on RARβ and RARγ is ~20% (relative normalized response) of the maximal response elicited by the naturally occurring RAR activator ATRA. Although Dawson et al. (14) have reported a lower RXR selectivity for LGD1069 due to higher EC50 for RARβ and RARγ, these differences in selectivity may be caused by differences in reporter constructs used in the bioassays by our two groups. However, our combination of cotransactivation and competitive ligand-binding data presented here and elsewhere (9) strengthens the fact that LGD1069 is a weak activator of RARs. Thus, within a defined concentration window, LGD1069 functions as an RRX-selective ligand.

Efficacy of LGD1069 in NMU Rat Mammary Carcinoma Model. To examine the ability of LGD1069 to alter the induction of NMU-induced breast tumors, Harlan Sprague Dawley rats were randomized into groups 1 week after carcinogen and treated (5 days a week) with vehicle, LGD1069 (p.o.), or TAM (s.c.). The doses of LGD1069 chosen in this study are in a range that we have demonstrated to be efficacious in a human squamous cell carcinoma mouse xenograft model and very well tolerated in chronic dose-ranging studies in the rat (data not shown). The doses of TAM chosen have been shown to be effective in preventing the appearance of mammary tumors in this model (2). In the control group, the rats achieved a 100% tumor incidence after 10–12 weeks (Fig. 2A). LGD1069 was highly efficacious at inhibiting the incidence of NMU-induced tumors at both 30 and 100 mg/kg. LGD1069 at both doses and TAM at 150 μg/kg significantly inhibited (10–12 weeks after NMU) the incidence of tumor formation with equivalent efficacy compared with vehicle-treated animals (P < 0.001). Final tumor incidence for the 30 and 100 mg/kg LGD1069-treated animals was 22% (4 of 18) and 12% (2 of
TAM at 150 \( \mu \text{g/kg} \) (•), LGD1069 at 30 \( \text{mg/kg} \) (○), or LGD1069 at 100 \( \text{mg/kg} \) (★). Animals were treated from weeks 1—12, after NMU injection, with vehicle (○), TAM at 50 \( \mu \text{g/kg} \) (△), and LGD1069 at 150 \( \mu \text{g/kg} \) (●). LGD1069 at both doses evaluated was at least as efficacious as the respective controls at weeks 10, 11, and 12 (\( P < 0.001 \)).

Animals were dosed daily (5 days/week). Curves indicated by the * are significantly different from controls at weeks 10, 11, and 12 (\( P < 0.001 \)).

The mean tumor burden (e.g., multiplicity) in vehicle-treated animals was 3.0 tumors per animal (Fig. 2B). The tumor multiplicity in TAM-treated animals at the 150-\( \mu \text{g/kg} \) dose was 0.25 tumors per rat. No significant difference (\( P > 0.05 \)) in tumor burden (1.81 tumor per rat) was observed in animals treated with TAM at 50 \( \mu \text{g/kg} \). No differences were noted in the overall volumes of tumors that appeared in LGD1069- or TAM-treated animals compared with vehicle-treated animals. Tumor morphologies in vehicle-treated animals were classified as invasive highly cellular adenocarcinomas. Similarly, all tumors from treatment groups were identical in histomorphology to vehicle-treated tumors with no apparent changes in grade or character (data not shown).

LGD1069 Side Effect Profile: Hormonal Perturbations and Dose-limiting Toxicities. The growth and development of mammary tumors in the NMU model is dependent on both sex steroid (progesterone and estrogen) and peptide hormones (prolactin and growth hormone; Ref. 15). We sought to determine whether LGD1069 altered the hormonal milieu of NMU-treated animals and disrupted the progression of tumor growth through an alteration in these endocrine signaling pathways. Serum hormone levels in animals sacrificed after 12 weeks of LGD1069 or TAM treatment were analyzed and compared with those from vehicle-treated animals. Results from serum hormone measurements demonstrated no significant alterations in serum estrogen and progesterone levels in the vehicle- and LGD1069-treated animals (Table 1). Additionally, no significant alterations in prolactin levels were observed in animals treated with LGD1069 or TAM when compared with vehicle-treated animals. Measurement of prolactin was conducted from serum samples obtained in the late afternoon (under minimal stress) to exclude changes from diurnal and stress variation. These data suggest that the inhibitory properties of LGD1069 on suppression of carcinogenesis were not due to a perturbation in the levels of sex hormones.

Animal weight has been an easily observable and accurate means to examine the side effect profile and tolerability of pharmacological agents. LGD1069-treated animals exhibited no significant decreases in animal weight at doses tested throughout this experiment (data not shown). In contrast, body weight for both TAM-treated groups was ~90% of vehicle-treated control animals. This observation is consistent with previous reports for TAM (1). No apparent toxicities were noted for LGD1069-treated animals in this study except for a slight alopecia under the mouth of one animal (1 of 16) treated at the 100-\( \mu \text{g/kg} \) dose. Therefore, one can conclude that LGD1069 was very well tolerated at doses in which it was highly efficacious. Finally, to correlate the dose administered with the observed biological activity of LGD1069, we examined the plasma concentrations of LGD1069 in this study. The plasma levels of LGD1069 at 30- and 100-\( \mu \text{g/kg} \) doses were in the low micromolar range, 3 h after administration of the final dose (Table 1), and there is a concentration-dependent increase in the plasma level with increasing doses of compound. These plasma concentrations of LGD1069 have also been shown to be readily achievable in clinical studies.

**Effect of LGD1069 on Uterine Wet Weight.** Another indicator of change in endocrine function can be monitored by measuring differences in uterine wet weight after different pharmacological treatments. LGD1069 significantly reduced (\( P < 0.05 \)) mean uterine weight at both 30 and 100 \( \mu \text{g/kg} \) compared with vehicle-treated controls was 3.0 tumors per animal (Fig. 2B). The tumor multiplicity was greatly reduced to less than one tumor per animal in all LGD1069-treated animals. LGD1069-treated animals achieved a significant (\( P < 0.001 \)) 10-fold reduction in tumor burden to 0.33 tumors per rat. No significant difference (\( P > 0.05 \)) in tumor burden that appeared in LGD1069- or TAM-treated animals compared with vehicle-treated animals.

**Table 1. Endocrine levels, uterine wet weight, and LGD1069 plasma concentrations in NMU-induced rats after treatment with LGD1069 or TAM**

<table>
<thead>
<tr>
<th>Group</th>
<th>[Estradiol] (pg/ml)</th>
<th>[Progesterone] (ng/ml)</th>
<th>[Prolactin] (ng/ml)</th>
<th>Uterine wet weight (g)</th>
<th>[LGD1069] (μg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>23.6 ± 4.2</td>
<td>32.0 ± 5.8</td>
<td>49.7 ± 9.2</td>
<td>0.64 ± 0.04</td>
<td>NA</td>
</tr>
<tr>
<td>30 mg/kg LGD1069</td>
<td>22.0 ± 4.5</td>
<td>39.9 ± 7.7</td>
<td>59.9 ± 27.2</td>
<td>0.48 ± 0.06 †</td>
<td>3.32 ± 0.40</td>
</tr>
<tr>
<td>100 mg/kg LGD1069</td>
<td>16.3 ± 2.7</td>
<td>35.3 ± 5.8</td>
<td>30.3 ± 9.0</td>
<td>0.40 ± 0.04 †</td>
<td>5.82 ± 0.88</td>
</tr>
<tr>
<td>50 ( \mu \text{g/kg} ) TAM</td>
<td>18.1 ± 2.9</td>
<td>27.8 ± 3.7</td>
<td>76.8 ± 8.0</td>
<td>0.46 ± 0.03 †</td>
<td>NA</td>
</tr>
<tr>
<td>150 ( \mu \text{g/kg} ) TAM</td>
<td>9.6 ± 1.0 ‡</td>
<td>25.6 ± 3.4</td>
<td>74.1 ± 9.2</td>
<td>0.33 ± 0.02 ‡</td>
<td>NA</td>
</tr>
</tbody>
</table>

† Values significantly different from control (\( P < 0.05 \)).

‡ NA, not applicable.
animals (Table 1). Mean uterine wet weight was reduced by >25% in both LGD1069-treated groups. Animals chronically treated with both doses of TAM also had mean uterine wet weights that were comparable to LGD1069-treated animals. Uterine histology of TAM-treated animals showed decreases in epithelial proliferation and increases in stromal/myometrial proliferation (compared with vehicle-treated animals) that were consistent with the partial agonist activity of TAM in the uterus (data not shown). In contrast to TAM, no stimulatory activity of stromal/myometrial cellular compartments of the uterus was observed in animals treated with LGD1069; a slight antagonism of the estrogenized epithelial morphology was noted in LGD1069 animals (100 mg/kg) compared with vehicle-treated animals (data not shown).

One of the major concerns of TAM therapy is that its "estrogenic" agonist activity in the uterus may lead to an increase in endometrial carcinomas. In the nonestrogenized environment of the immature rat, the estrogenic activities of TAM or E2 can be readily measured as an increase in uterine wet weight. Thus, the immature rat can be used as a sensitive bioassay to evaluate the ability of LGD1069 to antagonize TAM- or E2-stimulated uterine wet weight gain. As expected, E2 (2.0 µg/animal) or TAM (1 mg/kg) alone stimulated the mean uterine wet weight in animals treated for 3 days by almost 3- and 2-fold, respectively, over vehicle-treated animals (Fig. 3). LGD1069 (100 mg/kg) administered alone had no effect on uterine wet weights compared with vehicle-treated animals. Unexpectedly, LGD1069 administered in conjunction with E2 or TAM significantly (P < 0.05 and P < 0.001) antagonized the increases in uterine wet weight observed with either of these two agents in a dose-dependent manner. Maximum inhibitions of E2- and TAM-stimulated uterine wet weight achieved with LGD1069 were 35 and 25%, respectively. These results demonstrate that LGD1069 has no estrogenic activity as a single agent and may in fact at least partially obviate the unwanted side effects of TAM agonism in the uterus during combination therapy.

Discussion

An ideal chemoprevention agent must be tolerable and cause no significant decline in quality of life for high-risk but otherwise normal healthy patients. The anti-estrogen TAM, the most widely used hormonal therapy for the treatment of breast cancer, is the first agent to be tested in long-term multicentered chemoprevention breast cancer trials (3). TAM, in addition to its proven antitumor efficacy, has been reported to have significant cardiovascular protective and beneficial lipid lowering effects. However, the short-term use of TAM is associated with hot flashes in women, and the long-term use of TAM is associated with an increase of endometrial carcinoma in postmenopausal women (3, 4). Despite these concerns, the overall drug safety profile of TAM and its relatively safe clinical record make it a highly attractive agent for long-term use. Thus, it is imperative that any future compound chosen for use in breast cancer prevention trials have a safety profile that is equal to or superior to TAM.

LGD1069 had no deleterious effects on rats chronically treated at dose levels used in these experiments. The ability of chronic administration of LGD1069 to completely suppress the formation of mammary tumors underscores the importance of the minimal side-effect profile of LGD1069. No traditional retinoid toxicities, such as mucocutaneous toxicity, were noted in rats treated with either dose level of LGD1069 used in these NMU studies. Furthermore, bone fractures and osteopathy recorded historically in other animal studies with comparable doses of ATRA are completely absent at maximally tolerated doses of LGD1069 in the rat. Finally, no decreases in body weights were observed in LGD1069-treated animals at either dose used in the present NMU studies.

LGD1069 (Targretin) is presently undergoing human Phase I/IIa clinical testing (16, 17) and is the first RXR-selective ligand to be tested in humans. Similar to the present preclinical studies, LGD1069 is well tolerated in these trials over a wide dose range. In general, traditional retinoid-induced toxicities (e.g., mucocutaneous toxicities, headaches, and hypertriglyceridemia) have been observed at a lower frequency and severity (compared with other retinoids) with LGD1069 at doses of ≤500 mg/m2/day (17). At doses of 400 mg/m2/day, the peak plasma levels of LGD1069 are ~8.3 µM (17). Therefore, the plasma concentrations of LGD1069 achieved in the present preclinical study are readily attainable at the dose levels that are well tolerated in human clinical trials. In these early clinical trials, antitumor responses have been seen in patients with cutaneous T-cell lymphoma, and some patients with metastatic non-small cell lung carcinoma have experienced stable disease for periods of more than 3–13 months (16, 17). These observations suggest several clinical areas for future clinical studies with LGD1069.

Over the past 10–15 years, other retinoids, such as ATRA, 4-N-(hydroxyphenyl)retinamide, and 13-cis RA, which have very little or no RXR activity, have been used in the clinic for chemoprevention with some encouraging results, but their use has been limited due to certain toxicities (6–8). In the NMU model, it has been recently reported that 9-cis RA, which has RXR activity, is the most efficacious of all retinoids tested in this model (1). Additionally, Anzano et al. (1) have reported a significant decrease average tumor weight at necropsy from 9-cis RA-treated animals compared with vehicle-treated animals. Although we observed no decrease in tumor volumes for LGD1069-treated animals compared with vehicle-treated animals, the direct comparison of NMU tumor weights and volumes may not be comparable parameters for tumor burden in this model. In these experiments, we have shown that the RXR-selective ligand LGD1069 is highly efficacious and can achieve maximum antitumor effects without dose-limiting toxicities. Additionally, work done in this laboratory indicates that LGD1069 can reduce, in a dose-dependent manner, the volume of skin papillomas in the 7,12-dimethylbenz(a)anthracene-induced SENCAR mouse two-stage skin papilloma model. These data suggest that LGD1069 should be examined in human clinical trials in a chemoprevention/chemointervention mode.

13. J. Strasser, unpublished data.
CHEMOPREVENTION OF MAMMARY CANCER BY RXR-SELECTIVE LIGAND

The observations that LGD1069 is highly efficacious with a very favorable therapeutic index raises the question of whether direct activation of RXR will lead to suppression of carcinogenesis. A series of molecular and cellular studies has provided evidence indicating that RXRs play a critical role in several endocrine signaling pathways. RXR may function as a transcriptional activator and transduce a signal: (a) as a RXR homodimer; (b) as a heterodimeric partner for the RARs; (c) as a heterodimeric partner with other intracellular receptors, which include thyroid hormone receptors, vitamin D receptors, and peroxisomal proliferator-activated receptor; and (d) as a heterodimeric partner for a number of orphan receptors such as LXR and NGFI-B (18, 19). Thus, in the context of the NMU model, LGD1069 may suppress the formation of breast tumors by several mechanisms. LGD1069 could act by: (a) directly activating RXR-specific pathways; (b) enhancing activity of endogenous retinoids that activate the RARs; and (c) weakly activating RAR signaling pathways (LGD1069 can weakly activate RARβ and RARγ at high concentrations). Although the specific mechanisms of action for inhibiting NMU-induced tumors by RXR ligands remain to be explored, these experiments do definitively demonstrate the efficacy of LGD1069 in inhibiting the incidence and multiplicity of NMU-induced rat mammary tumors. Future studies will help uncover additional RXR-mediated mechanisms that are distinct from the biology of traditional RAR signaling pathways.

Finally, apart from the antitumor effects of LGD1069, we have presented uterine wet weight data that suggests that LGD1069 may also have some anti-estrogenic effects on the uterus. TAM has been well documented to act as a partial estrogenic antagonist, decreasing uterine wet weight in the estrogensensitive environment of the mature intact rat and increasing uterine wet weight in the low to nonestrogenic environment of the immature rat. However, we did not anticipate that LGD1069 would decrease mean uterine wet weights in the intact mature rat and blunt both E₂- or TAM-stimulated uterine wet weight increases in the immature rat.

Recently, evidence for anti-estrogenic behavior of retinoids in the stroma and myometrial compartments of the rat uterus have been reported (21). We have reported that 9-cis RA (a RAR and RXR pan-agonist) has antiproliferative effects in TAM-responsive and resistant human breast cancer cells (22). Emerging data also suggest that the possible molecular mechanism for the anti-estrogenic activity of LGD1069 may be through an inhibition of estrogen receptor transactivation. We have reported previously that 9-cis RA can inhibit estrogen receptor activation of an estrogen response element-reporter construct in a transient cotransfection assay (22). In addition, RXR/peroxisomal proliferator-activated receptor heterodimers have been shown to inhibit transactivation by the estrogen receptor through competitive binding for estrogen response elements (23). In total, these data suggest that agents such as LGD1069 may have anti-estrogenic properties that may complement the use of TAM in long-term treatment of breast cancer patients.

Because the toxicities between TAM and LGD1069 appear to be nonoverlapping, additional studies are planned to investigate whether synergistic or additive effects of TAM and LGD1069 can be observed as has been reported for other retinoids (1, 5, 24). These data definitively demonstrate that the shifting activity from RAR to RXR is highly efficacious in the NMU model with a more benign side-effect profile. The present observations suggest that this new class of ligands has a favorable therapeutic index and should offer new opportunities for the development of next-generation therapeutic agents for chemoprevention.

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References

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