Pilot Study of Rosiglitazone Therapy in Women with Breast Cancer: Effects of Short-term Therapy on Tumor Tissue and Serum Markers

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Abstract

Purpose: Peroxisome proliferator-activated receptor γ (PPARγ) is a steroid nuclear receptor that is activated by natural compounds such as specific fatty acids and synthetic drugs such as thiazolidinedione antidiabetic agents. Expressed in normal and malignant mammary epithelial cells, activation of PPARγ is associated with antiproliferative effects on human breast cancer cells in preclinical studies. The purpose of this study was to test the hypothesis that PPARγ ligand therapy might inhibit tumor growth and progression in human breast cancer.

Experimental Design: We conducted a pilot trial of short-term (2-6 weeks) treatment with the thiazolidinedione rosiglitazone in 38 women with early-stage (T_{is}-T_2, N_{0-1}, M_0) breast cancer, administered between the time of diagnostic biopsy and definitive surgery.

Results: Short-term treatment with rosiglitazone (8 mg/d) did not elicit significant effects on breast tumor cell proliferation using Ki67 expression as a measure of cell proliferation and surrogate marker of tumor growth and progression. In pretreatment tumors notable for nuclear expression of PPARγ by immunohistochemistry, down-regulation of nuclear PPARγ expression occurred following rosiglitazone administration ($P = 0.005$). No *PPARG* mutations were identified, and the incidence of P12A and H446H polymorphisms did not differ relative to U.S. controls ($P = 0.5$). Treatment with rosiglitazone resulted in increased serum adiponectin ($P < 0.001$), decreased insulin levels ($P = 0.005$), and increased insulin sensitivity ($P = 0.004$). Rosiglitazone was well tolerated without serious adverse events.

Conclusion: Our data indicate that short-term rosiglitazone therapy in early-stage breast cancer patients leads to local and systemic effects on PPARγ signaling that may be relevant to breast cancer.

Peroxisome proliferator-activated receptor γ (PPARγ) is a ligand-activated transcription factor homologous to other members of the steroid receptor superfamily, including the retinoid, vitamin D, thyroid, estrogen, and progesterone receptors. PPARγ binds to specific DNA sequences, known as peroxisome proliferator response elements, forming a heterodimer with retinoid X receptor to initiate transcription of downstream genes (1–3). Ligands for PPARγ include specific fatty acids, eicosanoids, and synthetic compound (4–6).

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Synthetic ligands for PPARγ include the thiazolidinediones, a class of oral hypoglycemic drugs that reduce hyperglycemia and hyperinsulinemia in insulin-resistant states. Rosiglitazone (Avandia, Glaxo SmithKline Beecham, Philadelphia, PA) is a thiazolidinedione in clinical use for treatment of type 2 diabetes mellitus. Activation of PPARγ regulates the expression of insulin-responsive genes involved in the regulation of glucose and fatty acid metabolism. Rosiglitazone reduces blood glucose levels and hyperinsulinemia in rodent models of type 2 diabetes mellitus, which seems to be mediated by the action of insulin in liver, muscle, and fat tissue.

Highly expressed in adipose tissue, PPARγ is also expressed by benign and malignant breast epithelium (7–9). Thiazolidinedione activators of PPARγ seem to promote tumor cell differentiation and inhibit tumor cell proliferation (7). *In vivo* studies using xenograft or carcinogen-induced models of mammary tumorigenesis indicate inhibitory effects of PPARγ ligand treatment (10, 11). Furthermore, PPARγ haploinsufficiency ($PPAR\gamma^{+/-}$) also confers increased susceptibility to dimethylbenz(*a*)anthracene-induced mammary carcinogenesis (12). These studies suggest that PPARγ ligands could serve as negative regulators of breast cancer development and progression.

We conducted a pilot trial of rosiglitazone to test the hypothesis that ligand activation of PPARγ in human breast cancer leads to the inhibition of tumor growth and progression.

To this end, short-term treatment with rosiglitazone in women with breast cancer during the period between the diagnostic breast biopsy and definitive surgical procedure of lumpectomy or mastectomy was initiated to prospectively evaluate the effect of this PPARγ ligand in modulating tumor cell proliferation. Differences in Ki67 nuclear staining in tumor specimens from the initial biopsy versus subsequent reexcision served as the primary study end point, using Ki67 expression as a surrogate measure of tumor growth. Secondary objectives included determination of (*a*) tolerability and toxicity of rosiglitazone during the short-term treatment of women newly diagnosed with breast cancer; (*b*) differences in tumor cell differentiation in pretreatment and posttreatment tumor samples; (*c*) the association of *PPARG* gene mutations in breast cancer specimens; and (*d*) alterations of exploratory serum markers of insulin resistance and rosiglitazone administration.

Patients and Methods

Patients. Women newly diagnosed with breast cancer following core needle or nonexcisional biopsy, to undergo definitive surgery for breast cancer for T_{is}, T_1, T_2 tumors, were eligible to participate. Given the inhibitory effects of PPARγ ligands on normal and malignant mammary epithelial proliferation *in vitro* and mammary carcinogenesis in animal models, we viewed both *in situ* and invasive breast cancers as potentially responsive to rosiglitazone. Subjects were screened for sufficient tumor tissue in biopsy blocks as well as residual tumor in the breast by clinical examination and/or imaging studies. Ineligibility criteria included abnormal fasting blood glucose or liver function tests and history of diabetes mellitus, heart failure, peripheral edema, and/or liver disease.

For purposes of comparing *PPARG* germ-line variant frequencies in cases (those with breast cancer) and controls, we used genomic DNA extracted from 110 unrelated white (of Northern and Western European origin) populational controls, who were healthy volunteers resident in the central Ohio region.

Trial design. An open-label clinical trial was conducted at The Ohio State University with approval of the Institutional Review Board of The Ohio State University and Human Subjects Review Board of the Department of Defense. After obtaining informed consent, rosiglitazone was administered orally at 4 mg twice a day for 2 to 6 weeks until the morning of lumpectomy or mastectomy. Patients used a daily diary to record all adverse events that occurred during the treatment period. Study personnel also contacted patients at least once during the treatment period to assess compliance and any potential problems. Formalin-fixed, paraffin-embedded tumor samples from the biopsy and lumpectomy or mastectomy procedures were used for the correlative tissue analyses. Serum alanine transaminase levels were checked before study entry, every 2 weeks while on the study medication, and at 4 weeks following the last dose of drug on the day of definitive breast cancer surgery. Additional blood samples were obtained at baseline, day of surgery, and 1 month after surgery from patients participating in serum biomarker analyses.

Statistical considerations

Sample size. Our projected sample size of 34 patients was based on an estimated effect size from published studies of proliferation indices of 0.5 with a two-sided α of 0.05 and a power of 0.8 using a paired *t* test or Wilcoxon matched-pairs signed-ranks test.

For the genetic analyses, we based sample size calculations on tests of the primary hypotheses that polymorphisms in *PPARG* will be associated with disease status (13, 14). Each of the described polymorphisms occurs at a single nucleotide, with only 2 bases observed in the population, such that the sample size of 34 subjects would be more than sufficient to detect association when a minimum of two (P12A and H446H) and a possible four simultaneous comparisons are done.

Analysis. Analysis of proliferation was done using paired *t* tests and Wilcoxon matched-pairs signed-ranks tests of data from specimens taken at initial biopsy and at surgery following rosiglitazone treatment. Analysis of adiponectin and insulin sensitivity over three time points was done using ANOVA; if the overall *P* value was significant, paired *t* tests were used to compare data at time points. Analysis of categorical data was done using Fisher's exact test. Discordance in change in categories of nuclear and cytoplasmic staining intensity was analyzed using the McNemar test. Two-tailed $P \leq 0.05$ was considered statistically significant. Analyses were done using SPSS 14.0 (SPSS, Inc., Chicago, IL).

For *PPARG* mutation analysis, 110 controls as described above were matched to cases with a control/case ratio of 2.97. The statistical power calculations were done assuming a control/case ratio of 2, to provide conservative power calculations. The data from Zhou et al. (13) were used to estimate the allele frequencies and association effect size. The association test seeks evidence that the polymorphic allele is associated with disease. The resulting contingency tables included both alleles for each individual and implicitly assumed that the two alleles are independent (conditioned on disease status). We used a type I error probability of 0.05, and all proposed statistical comparisons were two sided. Based on standard normal approximations implemented in S-Plus 3.4 (Mathsoft, Inc., Needham, MA) and for the sample size of 34, the power to detect the association at the two sites exceeds 0.75. After a Bonferroni correction for multiple comparisons, the power at the two sites is ~ 0.7.

Serum analyses

Fasting blood draws were obtained from all subjects at the time of enrollment (time 0), every 2 weeks while on study drug and/or day of surgery (time 1), and ~ 30 days following the last dose on the day of surgery (time 2). These samples were processed by the Ohio State University Clinical Laboratories for alanine transaminase, with glucose and insulin levels obtained at time 0 and time 2. For 17 subjects, additional sera from blood samples collected at the time of enrollment, day of surgery, and 1 month after surgery were stored at $-80^{\circ}C$ until analysis. Samples were analyzed in duplicate for C-peptide of insulin by RIA (Diagnostic Systems Laboratories, Inc., Webster, TX); insulin by chemiluminescence methods (Diagnostic Products Corporation, Los Angeles, CA); glucose by immobilized enzyme technology using the YSI 2300 Stat Plus Glucose and Lactate Analyzer (YSI International, Yellow Springs, OH); insulin-like growth factor (IGF)-I and IGF binding protein 3 using the Advantage chemiluminescence autoanalyzer (Nichols Diagnostics, San Clemente, CA); leptin and adiponectin by RIA (Linco Research, St. Charles, MO); and interleukin-6 and matrix metalloproteinase-9 (total) by ELISA (R&D Systems, Minneapolis, MN). All assays were done according to the manufacturers' instructions at the General Clinical Research Center of The Ohio State University.

Histologic analysis

Routine staining with H&E was done on all tumor tissue specimens. Immunohistochemistry was done with 4-μm-thick tissue sections using standard techniques. Following deparaffinization and rehydration through xylenes and graded ethanol solutions, slides were quenched for 5 min in a 3% hydrogen peroxide solution to block endogenous peroxidase activity. Antigen retrieval was done by incubating slides in citrate buffer pH 6.1 (DAKO, Carpinteria, CA) in a vegetable steamer. Slides were then placed on a DAKO Autostainer (DAKO) and incubated with either primary antibody to PPARγ (Santa Cruz Biotechnology, Santa Cruz, CA) at 1:12 dilution for 60 min or Ki67 (DAKO, M7240) at 1:150 dilution for 30 min at room temperature. Following blocking for endogenous biotin with an avidin/biotin blocking system (DAKO), the Labeled Streptavidin-Biotin Complex Plus (DAKO) was used. Slides were then counterstained in Richard Allen hematoxylin, dehydrated

through graded ethanol solutions, and coverslipped. Slides were analyzed blindly, with assessment of Ki67 expression as percent of positive tumor cells and PPARγ expression as 0 to 3+ staining intensity of tumor cell nucleus and/or cytoplasm.

DNA extraction and *PPARG* analysis

Tissues from 10-μm-thick sections of paraffin-embedded tumor and case-matched normal breast tissue were scraped from slides or obtained via laser capture microdissection as needed. The samples were deparaffinized, followed by DNA isolation using a modified QIAamp (Qiagen, Valencia CA). As previously described, DNA amplification was done and confirmed in agarose gels, followed by loading samples onto 15% to 65% gradient gels for differential gradient gel electrophoresis (13). Migration patterns were compared with historic sample controls.

Results

Patients and treatment. Between February 2002 and August 2004, 39 patients were enrolled in the trial at the Ohio State University. Thirty-eight patients completed the study. One subject withdrew to undergo surgery at an earlier date; with only 2 days of study medication, she was not eligible for evaluation. Based on self-reports and pill counts, compliance to the treatment was excellent at nearly 100%. All subjects completed the study with a 1 month postoperative visit and blood draw. Four subjects did not have sufficient tumor tissue for the correlative analyses despite pre-enrollment screening efforts to avoid this outcome. Additional blood samples were obtained from 17 subjects for serum biomarker analyses. Patient and tumor characteristics are depicted in Table 1.

Rosiglitazone does not alter cell proliferation. Ki67 is a nuclear protein expressed by proliferating cells, thus indicative of tumor cell proliferation. Analysis of Ki67 immunoreactivity as either the mean percentage of immunostained cell nuclei or as the observed range of heterogeneous staining did not disclose statistically significant differences between Ki67 expression in pretreatment and posttreatment tumor samples (Table 2). Discontinuation of hormone replacement therapy or oral contraceptive use at the time of breast cancer diagnosis did not significantly affect the overall results ($n = 6$), with decreased Ki67 expression levels in three subjects and stable values in the other three patients.

Similarly, comparison of H&E-stained sections from pretreatment and posttreatment tumor specimens failed to identify differences in tubule formation, mitotic activity, and nuclear morphology as measures of tumor cell differentiation (data not shown).

Rosiglitazone effects on PPARγ expression in breast tumor specimens. PPARγ immunostaining intensity was scored from 0 (none) to 3+ (highest) in breast cancer tissue specimens pre- and post-rosiglitazone treatment ($n = 34$), with instances of heterogeneous staining indicated as a range of intensity scores. PPARγ expression did not differ significantly when assessed as increased, decreased, or unchanged from baseline levels, with overlap between the range of pretreatment or posttreatment values designated as unchanged ($P = 0.24$; Table 3). However, using absolute values of mean intensity scores, PPARγ immunostaining decreased with short-term rosiglitazone therapy ($P < 0.001$). Baseline nuclear and cytoplasmic staining intensities of 1.01 ± 0.88 (SD) and 1.29 ± 0.71 decreased to 0.37 ± 0.57 ($P = 0.004$) and 0.9 ± 0.49 ($P = 0.21$), respectively. When considering only cases with baseline PPARγ immunostaining levels of 1+ to 2+ ($n = 17$; i.e. those with potential for decreasing or increasing to 0 or 3+, respectively), nuclear PPARγ expression decreased significantly in 84% (14 of 17) of patients following administration of rosiglitazone ($P = 0.005$; Table 4).

***Somatic mutations in* PPARG.** In 37 cases of matched tumor and normal breast tissue specimens, we did not identify any subjects with somatic mutations in *PPARG*. One case did not have sufficient tissue for analysis. There were two patients with the P12A polymorphism, one with the H446H polymorphism, and five with both polymorphisms. No significant differences in the distribution of alleles at each site relative to U.S. controls ($P = 0.5$) were detected.

Rosiglitazone treatment increases adiponectin levels and insulin sensitivity. In 17 patients, fasting serum samples obtained pretreatment and posttreatment (day of surgery and then 1 month after surgery) were analyzed for insulin, glucose, C-peptide, IGF-I, IGF binding protein 3, adiponectin, leptin, interleukin-6, and matrix metalloproteinase-9 levels (Table 5). Glucose levels obtained before initiation of rosiglitazone treatment were significantly lower than both posttherapy time points ($P = 0.019$), but all of the levels were within normal limits, ranging from ~55 to 70 mg/dL and ~64 to 85 mg/dL, respectively. Insulin levels were lower on the last day of

Table 1. Patient and tumor characteristics

Age range (median), y	27-85 (52)
Race, *n* (%)	
White	36 (95)
African American	2 (5)
Menopausal status, *n* (%)	
Pre- or peri-menopausal	16 (42)
Postmenopausal	19 (50)
Unknown	3 (8)
Hormone use, *n* (%)	
None	22
Past history	8
Current at diagnosis	8
Tumor size, *n* (%)	
T_{is}	2 (5)
T_{1mic}	2 (5)
T_{1a}	2 (5)
T_{1b}	4 (11)
T_{1c}	16 (42)
T_2	12 (32)
Axillary nodal status, *n* (%)	
Negative	29 (76)
Positive	9 (24)
Stage, *n* (%)	
0	2 (5)
I	20 (53)
IIA	8 (21)
IIB	8 (21)
Estrogen receptor	
Negative	10
Positive	26
Progesterone receptor	
Negative	15
Positive	21
HER-2/neu overexpression	
Negative	28
Positive	8

Table 2. Ki67 expression in biopsy and surgical specimens

Pre-rosiglitazone (mean)	Post-rosiglitazone (mean)	Δpre-post (Δmean)*
<5	<5	↔
<5	<5	↔
5	5	↔
70-90 (80)	70-90 (80)	↔ (↔)
5-10 (7.5)	5-10 (7.5)	↔ (↔)
70-80 (75)	70-80 (75)	↔ (↔)
20-40 (30)	20-40 (30)	↔ (↔)
10-30 (20)	10-30 (20)	↔ (↔)
60-90 (75)	60-90 (75)	↔ (↔)
30-50 (40)	30-50 (40)	↔ (↔)
5-10 (7.5)	5-10 (7.5)	↔ (↔)
10	5-10 (7.5)	↔ (↓)
5-10 (7.5)	5	↔ (↓)
20-40 (30)	20-30 (25)	↔ (↓)
40-60 (50)	30-50 (40)	↔ (↓)
10-39 (20)	5-10 (7.5)	↔ (↓)
10-30 (20)	10-20 (15)	↔ (↓)
0-10 (5)	<5	↔ (↓)
10	10-20 (15)	↔ (↑)
5-10 (7.5)	10-20 (15)	↔ (↑)
30-40 (35)	30-50 (40)	↔ (↑)
10-30 (20)	20-30 (25)	↔ (↑)
10-20 (15)	20-40 (30)	↔ (↑)
5-10 (7.5)	10-20 (15)	↔ (↑)
10	10-20 (15)	↔ (↑)
10-20 (15)	5	↓ (↓)
20-30 (25)	5	↓ (↓)
20-40 (30)	5-10 (7.5)	↓ (↓)
10	5	↓
20-30 (25)	<1	↓ (↓)
5-10 (7.5)	<5	↓ (↓)
<1	10	↑

NOTE: Ki67 expression was assessed as percentage of cell nuclei with immunostaining in 32 evaluable cases, with the range of staining indicated for instances of heterogeneity. Change (Δ) between pretreatment and posttreatment values is indicated as increased (↑), decreased (↓), or unchanged (↔). Unchanged values include those with overlap between ranges of pretreatment and posttreatment staining.
*P nonsignificant at >0.05.

treatment (day of surgery) compared with both the pretreatment and 1 month posttreatment levels (P = 0.005). As calculated by the quantitative insulin sensitivity check index [QUICKI = 1 / [log(I) + log(G)]; ref. 15], insulin sensitivity increased with rosiglitazone relative to pretreatment and posttreatment values (P = 0.004). Adiponectin levels significantly increased with rosiglitazone administration, as samples obtained on the day of surgery had significantly higher levels than those obtained before therapy or 1 month after discontinuation of the study drug (P < 0.001 and P < 0.001, respectively). The adiponectin levels at these first and last time points did not differ (P = 0.99).

Levels of C-peptide, as a measure of insulin secretion and disposal, did not differ significantly between the pretherapy and day of surgery (P = 0.90) and 1 month posttreatment (P = 0.07), but the 1 month posttreatment levels were higher than those on the day of surgery (P = 0.024) with an overall significant decrease between treated and untreated conditions (P = 0.014). IGF-I (P = 0.91), IGF binding protein 3 (P = 0.98), leptin (P = 0.57), interleukin-6 (P = 0.86), and matrix metalloproteinase-9 (P = 0.61) did not differ significantly between any of the time points.

Tolerability and toxicity of rosiglitazone. Rosiglitazone was tolerated without any serious or unexpected adverse events. Nineteen of 38 (50%) subjects reported no adverse events, whereas 19 patients experienced single or multiple adverse events. Most of the reported events seemed to be unrelated to the study intervention and were grade 1 or 2 in severity. Table 6 summarizes the rosiglitazone-related adverse events. Transient grade 1 elevations of alanine transaminase were observed in two subjects and probably related to rosiglitazone therapy. One of the subjects had initiated chemotherapy with doxorubicin and cyclophosphamide before the 30-day postoperative visit, and the second instance occurred with the alanine transaminase level obtained the day after surgery rather than before the procedure. Upper respiratory infection symptoms, a known side effect of rosiglitazone, were reported by one subject and also likely related to the study intervention. Five of six reported headaches (four grade 1 and one grade 2) seemed possibly related to rosiglitazone.

Discussion

Despite preclinical evidence of the antiproliferative effects of thiazolidinediones in breast cancer, we did not detect significant differences in Ki67 expression between pretreatment and posttreatment tumor tissues in this prospective study of rosiglitazone therapy in early-stage breast cancer patients. Rosiglitazone at 8 mg yields a maximum serum concentration ($C_{max} \pm$ SD) of 598 ± 117 mg/mL (Avandia package insert) or 1.67 μmol/L, which is within the 1 to 10 μmol/L range used to induce antiproliferative effects on mammary epithelial cells *in vitro* (9, 16). The lack of effect on Ki67 expression may relate to the short treatment period as well as the use of a dose capable of insulin sensitization, but not tumor suppression, on an acute basis. Indeed, serum indices of increased insulin sensitivity showed systemic effects of the drug in the subset of subjects tested. Possibly, combination therapy with an retinoid X receptor ligand or other agent might have yielded significant short-term effects on tumor cell proliferation, as suggested by preclinical studies (10, 17).

Additionally, heterogeneity of tumor features, such as expression of specific cofactors and/or corepressors, stage of disease, and certain patient characteristics may confound the results (18–20). For example, a recent study suggests that the coactivator amplified-in-breast cancer 3 is needed for the

Table 3. Cross-tabulation of changes in nuclear and cytoplasmic PPARγ expression relative to baseline

Change in cytoplasmic PPARγ	Change in nuclear PPARγ			
	Decreased	Increased	Unchanged	Total
Decreased	13	0	2	15
Increased	0	1	3	4
Unchanged	2	2	11	15
Total	15	3	16	34

Table 4. Cross-tabulation of PPARγ expression pretreatment and posttreatment

	Staining intensity score	Nuclear PPARγ post-rosiglitazone							Total
		0.00	0.25	0.50	0.75	1.00	1.50	2.00	
Nuclear PPARγ pre-treatment	0.00	3	0	1	0	0	0	2	6
	0.25	2	1	0	1	1	0	0	5
	0.50	1	0	1	0	0	0	0	2
	0.75	0	1	0	0	0	0	0	1
	1.00	5	0	1	0	2	0	0	8
	1.25	0	1	1	0	0	0	0	2
	1.50	3	0	0	0	0	1	0	4
	2.00	2	0	1	0	0	0	0	3
	3.00	3	0	0	0	0	0	0	3
Total		19	3	5	1	3	1	2	34

	Staining intensity score	Cytoplasmic PPARγ post-rosiglitazone							Total
		0.00	0.25	0.50	0.75	1.00	1.50	2.00	
Cytoplasmic PPARγ pre-rosiglitazone	0.00	0	0	0	0	1	0	0	1
	0.50	0	0	1	1	0	0	0	2
	0.75	1	0	1	1	0	0	0	3
	1.00	0	2	3	1	6	4	1	17
	1.50	0	0	0	0	1	1	1	3
	2.00	1	0	2	0	2	0	0	5
	3.00	0	0	0	0	3	0	0	3
Total		2	2	7	3	13	5	2	34

inhibition of cell proliferation by PPARγ (21). Studies from our own group also indicate that responsiveness to rosiglitazone requires at least one functional *PTEN* allele such that tumors with homozygous *PTEN* deletions or promoter mutations would prove refractory to treatment (22). Furthermore, signaling cross-talk between PPARγ and estrogen receptor could lead to differential effects of PPARγ ligand activation in breast cancer (23, 24). These reports raise the possibility that specific molecular profiles of the breast cancer may predict response to rosiglitazone and perhaps other thiazolidenediones. Despite a decrease in Ki67 immunostaining after rosiglitazone administration in seven subjects, our small sample size did not support subset analyses to assess correlation with specific tumor and/or patient characteristics (e.g., estrogen receptor expression, tumor size, and menopausal status).

Intriguingly, rosiglitazone treatment is associated with down-regulation of nuclear PPARγ expression in breast cancers with a minimum threshold level of PPARγ expression. Our data indicate that tumors with nuclear PPARγ immunostaining intensity at 1+ to 2+ are likely to respond to rosiglitazone with decreased nuclear expression, whereas PPARγ expression does not change significantly if baseline levels are low to negligible. Decreased expression of nuclear PPARγ raises the possibility of receptor activation and subsequent degradation, as observed *in vitro* (25), and may indicate rosiglitazone-mediated effects on breast cancer cell signaling in specific tumors. Localization of this nuclear receptor to the cytoplasm is of uncertain significance. Although tumor cell proliferation seems to be unaffected by short-term rosiglitazone therapy, further studies are needed to evaluate other end points of PPARγ signaling that might affect mammary carcinogenesis.

Of interest is the increase in serum adiponectin levels as a result of rosiglitazone administration. Expressed in adipose tissue and strongly induced during *in vitro* differentiation of preadipocytes, adiponectin is a 30-kDa protein secreted by adipose tissue with insulin-sensitizing and anti-inflammatory effects (26, 27). PPARγ activation stimulates adiponectin production *in vitro* and *in vivo*, and circulating adiponectin levels seem to be markedly suppressed in insulin-resistant subjects with dominant negative PPARγ mutations (P467L, V290M; ref. 28). Thus, adiponectin seems to be useful as an early and specific biomarker for PPARγ activation.

Serum adiponectin levels may also inversely correlate with breast cancer risk based on case-control studies in women newly diagnosed with breast cancer (29). Additionally, adiponectin is a potential endogenous inhibitor of angiogenesis, suppressing endothelial cell proliferation and migration *in vitro* and neovascularization *in vivo* (30). Adiponectin levels also correlate inversely with insulin resistance, a factor associated with increased risk for breast cancer (31). Our data indicate that adiponectin levels in early-stage breast cancer patients can be increased by short-term drug intervention. The potential therapeutic implications of modulating adiponectin levels require further study.

We initially found somatic *PPARG* mutations in exons 3 and 5 with loss of function in 4 of 55 sporadic colon cancers originating mainly from Finland (32), but a subsequent study screening exons 3 and 5 in *PPARG* in nearly 400 clinical samples (including 37 breast cancers) and cell lines suggests that mutations in these specific exons occur rarely in human malignancies (33). Additionally, screening of 170 primary colorectal tumors and 12 liver metastases failed to identify a loss-of-function point mutation in exon 6 (K422Q) detected in colon cancer cell lines (34). With analyses limited to specific *PPARG* exons, however, these latter two reports are somewhat problematic for evaluation of a putative tumor suppressor gene. Nonetheless, our analysis of 37 women with breast cancer for

Table 5. Serum markers of insulin resistance and PPARγ activation ± rosiglitazone therapy

	Time	Subjects (*n*)	Mean (SD)	*P*
Adiponectin	0	17	16.39 (7.54)	<0.001
	1	17	29.69 (10.12)	
	2	17	16.87 (7.13)	
C-peptide	0	17	1.51 (1.11)	0.014
	1	17	1.28 (0.93)	
	2	17	2.65 (1.96)	
Glucose	0	17	63.09 (17.24)	0.019
	1	16	74.09 (18.28)	
	2	17	77.90 (12.74)	
IGF-I	0	17	161.48 (45.37)	0.905
	1	17	161.28 (49.89)	
	2	17	167.35 (39.42)	
IGFBP3	0	17	2,154.49 (570.04)	0.976
	1	17	2,142.23 (425.14)	
	2	17	2,178.38 (448.94)	
IL-6	0	13	4.66 (5.57)	0.864
	1	14	5.76 (7.74)	
	2	13	4.70 (3.81)	
Insulin	0	16	7.15 (4.61)	0.005
	1	17	4.09 (2.50)	
	2	17	8.83 (4.80)	
Leptin	0	17	14.41 (6.95)	0.569
	1	16	13.06 (5.73)	
	2	16	15.43 (6.08)	
MMP-9	0	17	842.41 737.41)	0.609
	1	17	652.30 (530.25)	
	2	17	914.09 (1,018.62)	
QUICKI	0	16	3.32 (0.70)	0.004
	1	17	3.93 (0.85)	
	2	17	3.14 (0.43)	

NOTE: Serum samples were obtained at baseline (time 0), last day of drug (time 1), and 30 d after the last dose (time 2).
Abbreviations: IGFBP3, IGF binding protein 3; IL-6, interleukin-6; MMP-9, matrix metalloproteinase 9.

mutation of exons II, 1, 2, 3, 4, 5, and 6 did not detect any known somatic or germ-line *PPARG* mutations. Furthermore, the frequency of PPARγ gene polymorphisms in these breast cancer patients followed the distribution of the control population. Although the Pro12Ala polymorphism has been associated with body mass index and insulin sensitivity (35, 36), the small number of study subjects with a specific PPARγ polymorphism precluded correlation with tumor and/or patient characteristics. *PPARG* mutations do not seem to account for tumor response to PPARγ activation by specific ligands in this subset of breast cancer patients.

Previous clinical trials have evaluated the efficacy of thiazolidinedione therapy in subjects with advanced metastatic cancer, conducted with troglitazone before its withdrawal from clinical use. A pilot study of patients with unresectable advanced liposarcoma indicated potential for differentiating effects with troglitazone (37). Although troglitazone seemed to induce stable or decreased prostate-specific antigen levels in 41 patients with metastatic prostate cancer in a phase II study (38), a randomized placebo-controlled trial of rosiglitazone in men with increasing prostate-specific antigen levels following treatment for prostate cancer showed no effects on disease progression and prostate-specific antigen doubling time (39). Additionally, phase II studies of troglitazone in metastatic colorectal and refractory metastatic breast cancer failed to show beneficial effects on disease progression (40, 41). In the latter trial of 22 stage IV breast cancer patients with an 8-week median treatment duration, troglitazone did not prevent disease progression. Together with our findings that short-term thiazolidinedione exposure does not significantly influence the proliferation of established tumors at a surgically respectable stage, clinical trial results in advanced and early-stage breast cancer do not support a role for such drugs as single-agent therapy at the current dosing regimen. These negative findings lead us to speculate that antiproliferative effects may require longer-term and/or combination therapy with other nuclear receptor agonists; alternatively, another hypothesis to test is whether intervention with PPARγ ligand therapy at earlier stages of carcinogenesis can prevent progression to preinvasive and invasive disease. Future clinical studies of rosiglitazone could also target a specific molecular subtype of breast cancer to optimize response based on the expression of other nuclear receptors, cofactors, and downstream signaling factors that influence the activity of PPARγ (20, 21).

Not surprisingly, short-term treatment of nondiabetic breast cancer patients with rosiglitazone is well tolerated. Fifty percent of the subjects did not experience any adverse events, and reported adverse events were mild to moderate in severity and generally unrelated to the study intervention. Although the eligibility criteria led to selection of subjects at low risk for anticipated complications (e.g., absence of history of liver disease, diabetes mellitus, cardiac disease, edema), this study shows that rosiglitazone treatment is a tolerable, feasible intervention in women with breast cancer.

In summary, our study shows that short-term treatment with rosiglitazone does not significantly alter tumor cell proliferation, as assessed by Ki67 immunostaining of pretreatment and posttreatment tumor tissues, although the changes

Table 6. Adverse events caused by rosiglitazone

Adverse event	Grade 1	Grade 2	Relationship to therapy
↑Alanine transaminase	2		2 probably related
Headache	5		4 possibly related, 1 unrelated
		1	1 possibly related
Chest cold/upper respiratory infection		2	1 probably related, 1 unrelated

NOTE: Nineteen of 38 subjects reported adverse events. Unrelated adverse events: fatigue (4), nausea (4), reduced appetite (1), increased appetite (1), dry mouth (2), back pain (1), wound infection (1), dyspepsia (1), vomiting (1), lightheadedness (1), hot flashes (1), sore throat (1), sinus infection (1), migraine headaches (2).

in PPARγ expression from baseline suggest the potential for receptor-mediated signaling following ligand activation. The specific gene targets for PPARγ-mediated signaling in this setting require further elucidation and may relate to insulin sensitization and other metabolic effects. Indeed, the increase in serum adiponectin and the increase in insulin sensitivity point to potential risk reduction benefits with longer-term treatment. Such effects may also prove ineffectual in treating established breast cancers on a short-term basis or as single-agent therapy. Potentially specific tumor and/or patient characteristics may influence responsiveness to rosiglitazone and other PPARγ ligands and could be addressed in future studies.

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