[CANCER RESEARCH 59, 5671–5673, November 15, 1999]

Advances in Brief

A New Ligand for the Peroxisome Proliferator-Activated Receptor-γ (PPAR-γ), GW7845, Inhibits Rat Mammary Carcinogenesis[1]

Nanjoo Suh, Yongping Wang, Charlotte R. Williams, Renee Risingsong, Tona Gilmer, Timothy M. Willson, and Michael B. Sporn[2]

Department of Pharmacology and Norris Cotton Cancer Center, Dartmouth Medical School, Hanover, New Hampshire 03755 [N. S., Y. W., C. R. W., R. R., M. B. S.], Glaxo Wellcome Research and Development, Research Triangle Park, North Carolina 27709 [T. G., T. M. W.]

Abstract

We have tested a new ligand for peroxisome proliferator-activated receptor-γ, GW7845, as an inhibitor of experimental mammary carcinogenesis, using the classic rat model with nitrosomethylurea as carcinogen. Rats were first treated with a single dose of nitrosomethylurea (50 mg/kg body weight, i.p.). Starting 1 week later, they were fed GW7845, at either 60 or 30 mg/kg of diet, for 2 months. This agent significantly reduced tumor incidence, tumor number, and tumor weight at both doses. This is the first report of the use of a ligand for peroxisome proliferator-activated receptor-γ to prevent experimental breast cancer.

Introduction

The continuing magnitude of the breast cancer problem with respect to incidence, morbidity, and mortality requires further drug discovery to prevent this disease (1). The use of tamoxifen, raloxifene, and fenretinide as clinically proven, effective agents to suppress breast carcinogenesis (2–4) indicates that chemoprevention is a viable strategy for the prevention of breast cancer in women. Current research in this area is driven by the need to discover new agents that will be more effective and have fewer side effects. In this brief communication, we report the first use of a new and highly potent ligand for the nuclear receptor, PPAR-γ,[3] GW7845 to inhibit experimental mammary carcinogenesis *in vivo.*

PPAR-γ is a transcription factor belonging to the nuclear receptor superfamily (5–7) and forms functional heterodimers with the retinoid X receptor (8). PPAR-γ is of great current interest because it mediates the antidiabetic effects of several TZDs that are now in widespread clinical use for treatment of type 2 diabetes (9, 10). The PPARs bind a variety of naturally occurring fatty acids and eicosanoids with low micromolar affinity (6). Interestingly, PPAR-γ has a preference for polyunsaturated fatty acids (11), dietary components that have been shown to lower the incidence of cancer in experimental animals (12, 13), although the clinical relevance of these observations remains unclear (12, 14).

Synthetic PPAR-γ ligands have been shown to inhibit growth of several human tumor cell lines in culture (15–17) and, most notably, to induce growth arrest and differentiation in primary cultures of human liposarcoma cells, both *in vitro* and *in vivo* (18, 19). In contrast, there have been conflicting reports on the effects of the TZD class of PPAR-γ ligands in experimental colon carcinogenesis (20–22). The mechanism of inhibition of growth of tumor cells by ligands for PPAR-γ is not well understood (23). For the present study, reported here, the availability of a potent member of a new class of ligands for PPAR-γ, GW7845 (24), has enabled us to test this agent for inhibition of mammary carcinogenesis in the classic rat model that uses NMU as carcinogen.

Materials and Methods

Cell Culture and Differentiation Assays. GW7845 was dissolved in DMSO (0.01 M), and aliquots were frozen at −20°C. Serial dilutions were made in DMSO before addition to cell culture media. The 3T3-L1 preadipocyte cells were obtained from American Type Culture Collection, grown to confluency in DMEM/5% calf serum, and then treated once with compounds in DMEM/10% fetal bovine serum. Every 2 days thereafter, medium was changed to DMEM/10% fetal bovine serum without added compounds. Cells were harvested on day 6, and as a marker of differentiation, glycerol 3-phosphate dehydrogenase was measured in lysates, using a standard assay for consumption of NADH at 340 nm (25).

Mammary Carcinogenesis Studies. A total of 159 female Sprague Dawley rats (Taconic Farms, Germantown, NY) received i.p. injections of NMU (50 mg/kg body weight) when 21 days old, as described by Thompson *et al.* (26). One week later, the rats were randomly assigned to one of six experimental groups (Table 1). GW7845 and tamoxifen were blended into the diets as described previously (27) and were fed to the rats continuously, either alone or in combination, for the duration of the experiment. Rats were killed after 2 months (CO_2 inhalation), and breast cancers were enumerated and weighed at autopsy.

Other. The Fisher exact test and the Mann-Whitney rank test were used to evaluate the statistical differences between the treatment groups; all *P* values shown are two-sided. Institutional guidelines for proper and humane use of rats were observed.

Results and Discussion

GW7845 is a tyrosine analogue (Fig. 1), rather than a TZD such as troglitazone, rosiglitazone, and pioglitazone (the ligands for PPAR-γ in current clinical use). Unlike the TZDs, GW7845 has been optimized for potency on PPAR-γ (24) and is significantly more potent than either rosiglitazone or troglitazone when assayed for induction of adipogenic differentiation in the fibroblastic cell line, 3T3-L1 (25), as shown in Fig. 2.

We have performed two separate but identical long-term experiments to demonstrate the chemopreventive efficacy of GW7845. Given the widespread use of tamoxifen as an agent to prevent breast cancer, we have also looked at potential synergism between GW7845 and tamoxifen. The results in both experiments were essentially identical; therefore, we have pooled the data in Table 1.

GW7845 was well tolerated at the doses fed (Table 1), and rats treated with this agent weighed the same as controls. Table 1 shows that GW7845 had significant inhibitory effects on mammary carcinogenesis regardless of whether tumor incidence, numbers of tumors per rat, or ATB (the average weight of a rat's tumor at autopsy) was

Received 8/23/99; accepted 10/5/99.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] This work was supported by the National Foundation for Cancer Research, NIH Grant R01 CA78814, and DOD/AMRD Award 17-99-1-9168. M. B. S. is Oscar M. Cohn Professor, and Y. W. is a Howard Hughes Medical Institute predoctoral fellow.

[2] To whom requests for reprints should be addressed, at Department of Pharmacology, Dartmouth Medical School, 7650 Remsen, Hanover, NH 03755. Phone: (603) 650-6557; Fax: (603) 650-1129; E-mail: Michael.Sporn@dartmouth.edu.

[3] The abbreviations used are: PPAR-γ, peroxisome proliferator-activated receptor-γ; TZD, thiazolidinedione; NMU, nitrosomethylurea; ATB, average tumor burden.

Table 1 *Prevention of breast cancer by GW7845 and tamoxifen*

Treatment[a]	No. of tumor-free rats/total no. of rats (P_1; P_2)[b]	Average no. of tumors (P_1; P_2)[b]	ATB (P_1; P_2)[b]	Rats with 3 or more tumors (P_1; P_2)[b]	Rats with tumor burden >5 g (P_1; P_2)[b]
Control (vehicle)	5/42	2.4	5.6	22/42	18/42
GW7845 Hi	8/21 (0.02)	1.1 (0.002)	1.7 (0.002)	2/21 (0.0009)	1/21 (0.002)
GW7845 Lo	7/21 (0.08)	0.8 (<0.0001)	1.5 (0.0004)	0/21 (<0.0001)	2/21 (0.009)
Tamoxifen	5/33	1.6 (0.02)	2.4 (0.02)	7/33 (0.008)	6/33 (0.03)
Tamoxifen + GW7845 Hi	9/21 (0.009; 0.03)	0.9 (0.0002; 0.03)	0.9 (0.0002; 0.05)	0/21 (<0.0001; 0.03)	0/21 (0.0002)
Tamoxifen + GW7845 Lo	12/21 (0.0003; 0.002)	0.6 (<0.0001; 0.001)	1.3 (0.0001; 0.01)	1/21 (0.0002)	3/21 (0.03)

[a] Doses used were as follows: 60 mg GW7845/kg diet (GW7845 Hi); 30 mg GW7845/kg diet (GW7845 Lo); and 0.5 mg tamoxifen/kg diet. All animals (21 days old) received an i.p. injection of 50 mg NMU/kg body weight 1 week before starting the feeding of chemopreventive agents.

[b] P_1 is the value for the comparison of rats treated with chemopreventive agents with control rats treated with vehicle alone; P_2 is the value for the comparison of rats treated with tamoxifen + GW7845 with rats treated with tamoxifen alone.

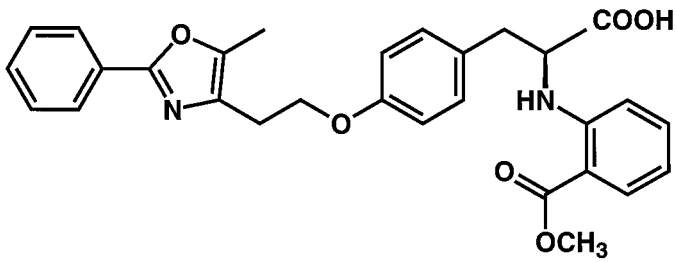

Fig. 1. Structure of GW7845.

measured. The effects on ATB are particularly interesting; GW7845 effected a 70% reduction in this index. Striking effects of GW7845 on tumor multiplicity and weight were seen (Table 1) when the number of rats with three or more tumors or the number of rats with a tumor burden >5 g were scored. Both doses of GW7845 appeared equally effective in all parameters measured. To evaluate possible synergy with tamoxifen, we deliberately chose a very low dose of this agent, which is only marginally effective (27, 28). As seen in Table 1, although some statistically significant additive effects were seen with the combination of GW7845 and tamoxifen, there was little evidence in these experiments for a strong synergy between the two.

These initial experiments *in vivo* establish GW7845 as an agent worthy of further consideration for chemoprevention of cancer. Further studies in other organ systems in which PPAR-γ plays an important role, as well as potential synergy with other agents for which there is a mechanistic basis (*e.g.*, selective ligands for the retinoid X receptor), should now be pursued, as well as further evaluation of the mechanism of suppression of carcinogenesis by PPAR-γ.

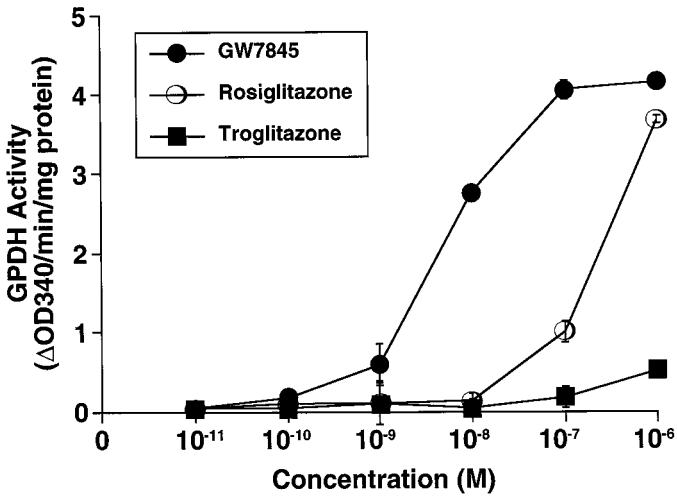

Fig. 2. GW7845 is more potent than either rosiglitazone or troglitazone in induction of adipogenic differentiation in 3T3-L1 fibroblasts. Adipogenesis was measured after 6 days of treatment, as described (25), using a glycerol 3-phosphate dehydrogenase assay as a marker. *OD340*, absorbance at 340 nm; *bars*, SE.

Acknowledgments

We thank Tammy Frazer for expert assistance in preparation of the manuscript. Marilyn Brown and her staff, especially Jennifer Marcroft and Catherine LaBarre, have provided excellent animal care.

References

1. Landis, S. H., Murray, T., Bolden, S., and Wingo, P. A. Cancer statistics, 1999. CA Cancer J. Clin., *49:* 8–64, 1999.
2. Fisher, B., Costantino, J. P., Wickerham, D. L., Redmond, C. K., Kavanah, M., Cronin, W. M., Vogel, V., Robidoux, A., Dimitrov, N., Atkins, J., Daly, M., Wieand, S., Tan-Chiu, E., Ford, L., and Wolmark, N. Tamoxifen for prevention of breast cancer: report of the national surgical adjuvant breast and bowel project P-1 study. J. Natl. Cancer Inst., *90:* 1371–1388, 1998.
3. Cummings, S. R., Eckert, S., Krueger, K. A., Grady, D., Powles, T. J., Cauley, J. A., Norton, L., Nickelsen, T., Bjarnason, N. H., Morrow, M., Lippman, M. E., Black, D., Glusman, J. E., Costa, A., and Jordan, V. C. The effect of raloxifene on risk of breast cancer in postmenopausal women: results from the MORE randomized trial. JAMA, *281:* 2189–2197, 1999.
4. Veronesi, U., De Palo, G., Marubini, E., Costa, A., Formelli, F., Mariani, L., Decensi, A., Camerini, T., Del Turco, M. R., Di Mauro, M. G., Muraca, M. G., Del Vecchio, M., Pinto, C., D'Aiuto, G., Boni, C., Campa, T., Magni, A., Miceli, R., Perloff, M., Malone, W. F., and Sporn, M. B. Randomized trial of fenretinide to prevent second breast malignancy in women with early breast cancer. J. Natl. Cancer Inst., *91:* 1847–1856, 1999.
5. Mangelsdorf, D. J., Thummel, C., Beato, M., Herrlich, P., Schutz, G., Umesono, K., Blumberg, B., Kastner, P., Mark, M., Chambon, P., and Evans, R. M. The nuclear receptor superfamily: the second decade. Cell, *83:* 835–839, 1995.
6. Kliewer, S. A., and Willson, T. M. The nuclear receptor PPARγ–bigger than fat. Curr. Opin. Genet. Dev., *8:* 576–581, 1998.
7. Kliewer, S. A., Lehmann, J. M., and Willson, T. M. Orphan nuclear receptors: shifting endocrinology into reverse. Science (Washington DC), *284:* 757–760, 1999.
8. Kliewer, S. A., Umesono, K., Noonan, D. J., Heyman, R. A., and Evans, R. M. Convergence of 9-*cis* retinoic acid and peroxisome proliferator signaling pathways through heterodimer formation of their receptors. Nature (Lond.), *358:* 771–774, 1992.
9. Lehmann, J. M., Moore, L. B., Smith-Oliver, T. A., Wilkison, W. O., Willson, T. M., and Kliewer, S. A. An antidiabetic thiazolidinedione is a high affinity ligand for peroxisome proliferator-activated receptor γ (PPARγ). J. Biol. Chem., *270:* 12953–12956, 1995.
10. Spiegelman, B. M. PPAR-γ: adipogenic regulator and thiazolidinedione receptor. Diabetes, *47:* 507–514, 1998.
11. Xu, H. E., Lambert, M. H., Montana, V. G., Parks, D. J., Blanchard, S. G., Brown, P. J., Sternbach. D. D., Lehmann, J. M., Wisely, G. B., Willson, T. M., Kliewer, S. A., and Milburn, M. V. Molecular recognition of fatty acids by peroxisome proliferator-activated receptors. Mol. Cell, *3:* 397–403, 1999.
12. Rose, D. P. Dietary fatty acids and cancer. Am. J. Clin. Nutr., *66:* 998S–1003S, 1997.
13. Ip, C. Review of the effects of trans fatty acids, oleic acid, n-3 polyunsaturated fatty acids, and conjugated linoleic acid on mammary carcinogenesis in animals. Am. J. Clin. Nutr., *66:* 1523S–1529S, 1997.
14. Willett, W. C. Specific fatty acids and risks of breast and prostate cancer: dietary intake. Am. J. Clin. Nutr., *66:* 1557S–1563S, 1997.
15. Mueller, E., Sarraf, P., Tontonoz, P., Evans, R. M., Martin, K. J., Zhang, M., Fletcher, C., Singer, S., and Spiegelman, B. M. Terminal differentiation of human breast cancer through PPARγ. Mol. Cell, *1:* 465–470, 1998.
16. Elstner, E., Müller, C., Koshizuka, K., Williamson, E. A., Park, D., Asou, H., Shintaku, P., Said, J. W., Heber, D., and Koeffler, H. P. Ligands for peroxisome proliferator-activated receptorγ and retinoic acid receptor inhibit growth and induce apoptosis of human breast cancer cells *in vitro* and in BNX mice. Proc. Natl. Acad. Sci. U S A, *95:* 8806–8811, 1998.
17. Brockman, J. A., Gupta, R. A., and DuBois, R. N. Activation of PPARγ leads to inhibition of anchorage independent growth of human colorectal cancer cells. Gastroenterology, *115:* 1049–1055, 1998.
18. Tontonoz, P., Singer, S., Forman, B. M., Sarraf, P., Fletcher, J. A., Fletcher, C. D., Brun, R. P., Mueller, E., Altiok, S., Oppenheim, H., Evans, R. M., and Spiegelman,

B. M. Terminal differentiation of human liposarcoma cells induced by ligands for peroxisome proliferator-activated receptor γ and the retinoid X receptor. Proc. Natl. Acad. Sci. U S A, *94:* 237–241, 1997.
19. Demetri, G. D., Fletcher, C. D., Mueller, E., Sarraf, P., Naujoks, R., Campbell, N., Spiegelman, B. M., and Singer, S. Induction of solid tumor differentiation by the PPARγ ligand troglitazone in patients with liposarcoma. Proc. Natl. Acad. Sci. U S A, *95:* 3951–3956, 1999.
20. Sarraf, P., Mueller, E., Jones, D., King, F. J., DeAngelo, D. J., Partridge, J. B., Holden, S. A., Chen, L. B., Singer, S., Fletcher, C., and Spiegelman, B. M. Differentiation and reversal of malignant changes in colon cancer through PPARγ. Nat. Med., *4:* 1046–1052, 1998.
21. Lefebvre, A. M., Chen, I., Desreumaux, P., Najib, J., Fruchart, J. C., Geboes, K., Briggs, M., Heyman, R., and Auwerx, J. Activation of the peroxisome proliferator-activated receptorγ promotes the development of colon tumors in C57BL/6J-APC-Min/+ mice. Nat. Med., *4:* 1053–1057, 1998.
22. Saez, E., Tontonoz, P., Nelson, M. C., Alvarez, J. G., Ming, U. T., Baird, S. M., Thomazy, V. A., and Evans, R. M. Activators of the nuclear receptor PPARγ enhance colon polyp formation. Nat. Med., *4:* 1058–1061, 1998.
23. Gelman, L., Fruchart, J-C., and Auwerx, J. An update on the mechanisms of action of the peroxisome proliferator-activated receptors (PPARs) and their roles in inflammation and cancer. Cell. Mol. Life Sci., *55:* 932–943, 1999.
24. Cobb, J. E., Blanchard, S. G., Boswell, E. G., Brown, K. K., Charifson, P. S., Cooper, J. P., Collins, J. L., Dezube, M., Henke, B. R., Hull-Ryde, E. A., Lake, D. H., Lenhard, J. M., Oliver, W., Jr., Oplinger, J., Pentti, M., Parks, D. J., Plunket, K. D., and Tong, W. Q. *N*-(2-Benzoylphenyl)-L-tyrosine PPARγ agonists. 3. Structure-activity relationship and optimization of the N-aryl substituent. J. Med. Chem., *41:* 5055–5069, 1998.
25. Suh, N., Wang, Y., Honda, T., Gribble, G. W., Dmitrovsky, E., Hickey, W. F., Maue, R. A., Place, A. E., Porter, D. M., Spinella, M. J., Williams, C. R., Wu, G., Dannenberg, A. J., Flanders, K. C., Letterio, J. J., Mangelsdorf, D. J., Nguyen, L., Porter, W. W., Ren, R. F., Roberts, A. B., Roche, N. S., Subbaramaiah, K., and Sporn, M. B. A novel synthetic oleanane triterpenoid, 2-cyano-3,12-dioxoolean-1,9-dien-28-oic acid, with potent differentiating, antiproliferative, and anti-inflammatory activity. Cancer Res., *59:* 336–341, 1999.
26. Thompson, H. J., McGinley, J. N., Rothhammer, K., and Singh, M. Rapid induction of mammary intraductal proliferations, ductal carcinoma in situ and carcinomas by the injection of sexually immature female rats with 1-methyl-1-nitrosourea. Carcinogenesis (Lond.), *16:* 2407–2411, 1995.
27. Anzano, M. A., Smith, J. M., Uskokovic, M. R., Peer, C. W., Mullen, L. T., Letterio, J. J., Welsh, M. C., Shrader, M. W., Logsdon, D. L., Driver, C. L., Brown, C. C., Roberts, A. B., and Sporn, M. B. 1α,25-Dihydroxy-16-ene-23-yne-26,27-hexafluorocholecalciferol (Ro24–5531), a new deltanoid (vitamin D analogue) for prevention of breast cancer in the rat. Cancer Res., *54:* 1653–1656, 1994.
28. Gottardis, M. M., and Jordan, V. C. Antitumor actions of keoxifene and tamoxifen in the *N*-nitrosomethylurea-induced rat mammary carcinoma model. Cancer Res., *47:* 4020–4024, 1987.