Neuro
-endocrinology

Neuroendocrinology 2010;92(suppl 1):23–27
DOI: 10.1159/000314312

Published online: September 10, 2010

Role of the PPAR-γ System in Normal and Tumoral Pituitary Corticotropic Cells and Adrenal Cells

Massimo Mannelli[a] Giulia Cantini[a] Giada Poli[a] Monica Mangoni[a]
Gabriella Nesi[b] Letizia Canu[a] Elena Rapizzi[a] Elisa Borgogni[a] Tonino Ercolino[a]
Valentina Piccini[a] Michaela Luconi[a]

[a]Departments of Clinical Physiopathology and [b]Human Pathology and Oncology, DENOTHE Center of Excellence for Research, Transfer and High Education, University of Florence and Istituto Toscano Tumori, Florence, Italy

Key Words
Glitazones · Pituitary · Cushing's disease · Adrenal cancer

Abstract
PPAR-γ is a member of the nuclear hormone receptor superfamily of transcription factors, whose thiazolidinedione ligands (TZD) have been recently demonstrated to also possess anticancer properties in addition to their well-known insulin-sensitizer and glucose/lipid regulation activity. In this minireview, we summarize the current knowledge on PPAR-γ in normal and tumoral corticotropic pituitary and adrenal cells. The receptor expression has been shown in ACTH-secreting cells in both normal and adenomal pituitary as well as in normal and tumor adrenal cortex. Preclinical studies conducted both in vitro on tumor cells and in vivo on xenograft tumor models obtained by subcutaneous injection of cancer cells have evidenced the anticancer properties of TZD, in particular rosiglitazone (RGZ) and pioglitazone (PIO). In both pituitary and adrenocortical cancer, RGZ treatment results in inhibition of cell proliferation, through G0/G1 cell-cycle arrest and induction of cell apoptosis, leading to significant inhibition of tumor growth in the xenograft tumor models. In addition, since RGZ can reduce ACTH and corticosterone secretion in mouse corticotropic pituitary tumors, both RGZ and PIO have been used in the treatment of Cushing's disease with variable but generally unsatisfactory results. Discrepancies in the antitumor effects of TZD observed between successful preclinical and unsuccessful clinical studies may be particularly due to differences in treatment duration and doses used.

Introduction

PPAR-γ is a member of the nuclear hormone receptor superfamily of transcription factors, widely expressed in the organism, including adipose, vascular and immune cells.

Besides its well-known role in the regulation of metabolism, PPAR-γ has also recently emerged as a key regulator of inflammatory and immune responses [1] as well as of proliferation, differentiation and apoptosis in nor-

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

0028–3835/10/0925–0023$26.00/0

Accessible online at:
www.karger.com/nen

Massimo Mannelli
Department of Clinical Pathophysiology, University of Florence
Viale Pieraccini 6
IT–50139 Florence (Italy)
Tel. +39 055 427 1428, Fax +39 055 422 1931, E-Mail m.mannelli@dfc.unifi.it

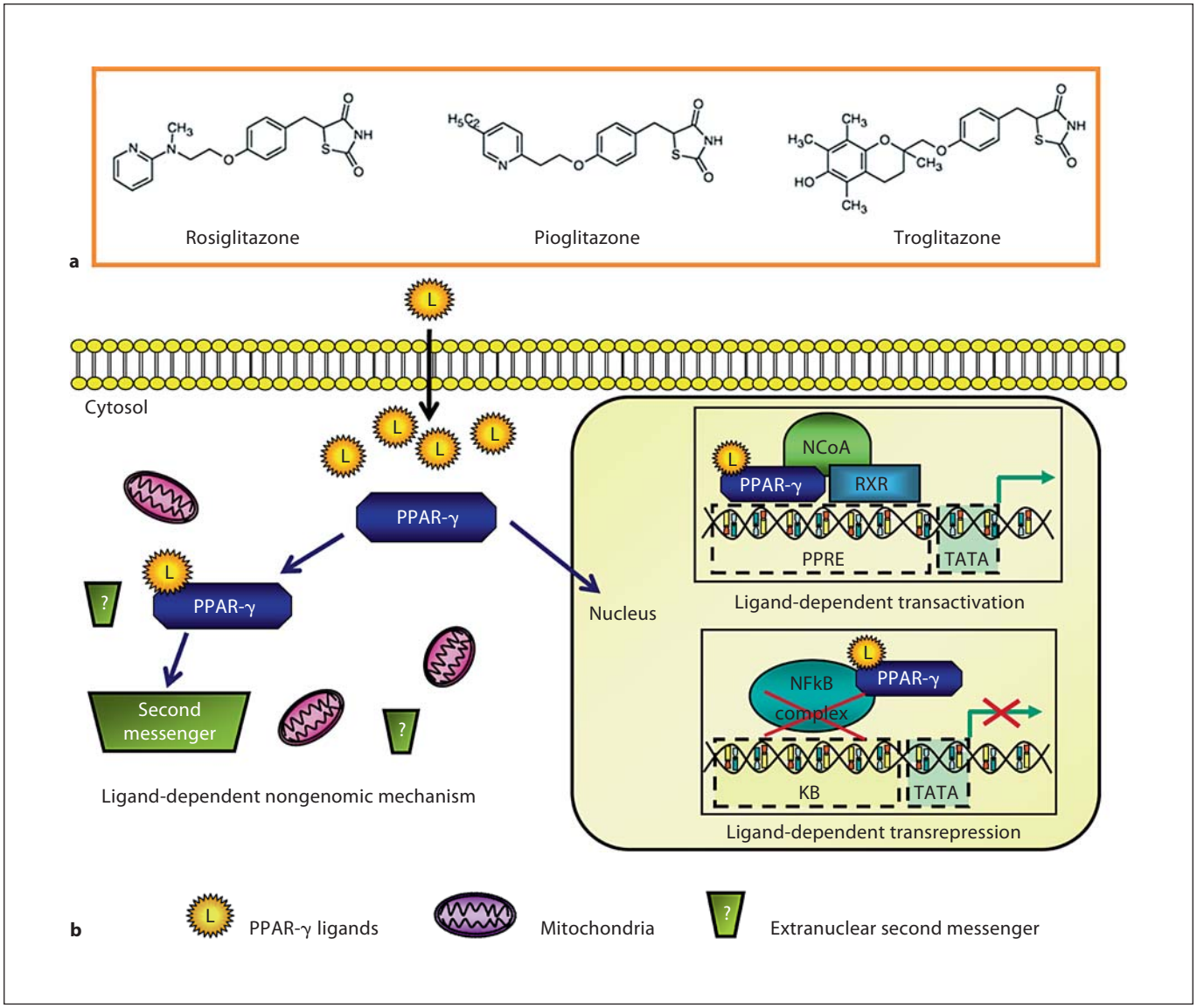

Fig. 1. a Thiazolidinediones and PPAR-γ mechanisms of action in the cell. Chemical structure of the most common PPAR-γ thiazolidinedione ligands. **b** Schematic illustration of genomic and nongenomic PPAR-γ mechanisms of action. NCoA = Specific nuclear PPAR-γ coactivator; RXR = retinoic acid receptor; PPRE = PPAR-γ responsive element; NFkB = nuclear factor kappa B; KB = NFkB responsive element; TATA = transcription start site (TATA box).

mal and tumor cells. In fact, its thiazolidinedione ligands (TZD), such as rosiglitazone (RGZ) and pioglitazone (PIO), have been shown to exert anticancer effects in several solid tumors [2] including tumors of the endocrine glands, namely pituitary and adrenal tumors.

The chemical structure of the most common PPAR-γ TZD ligands and the main receptor intracellular mechanisms of action are illustrated in figure 1.

PPAR-γ and Cushing's Disease

PPAR-γ is expressed in ACTH-secreting cells of normal human anterior pituitary as well as in ACTH-secreting pituitary adenomas [3]. In in vitro experiments on ACTH-secreting pituitary tumor cells, troglitazone and RGZ induced G0/G1 cell-cycle arrest and decreased the number of cells in the S phase [3].

Table 1. Thiazolidinedione treatment in patients with Cushing's disease

Reference	Patients	Treatment (mg/day)	Period	Response	Note
Alevizaki et al. [4]	5	RGZ 8	16 weeks	4/5	
Hull et al. [5]	2	RGZ 8	20–33 days	1/2	metyrapone co-treatment
Ambrosi et al. [6]	14	RGZ 8	1–7 months	6/14	
Suri et al. [7]	5	PGZ 45	30 days	0/5	
Barbaro et al. [8]	1	PGZ 45	8 months	1/1	
Mullan et al. [9]	7	RGZ 8	12 weeks	0/7	adrenalectomized patients plasma ACTH
Pecori Giraldi et al. [10]	10	RGZ 4–16	1–8 months	3/10	
Munir et al. [11]	6	RGZ 12	14 weeks	0/6	Nelson's syndrome

In mouse corticotropic pituitary tumor (AtT20) cells, RGZ was shown to dose-dependently stimulate apoptosis by a decreased expression of the anti-apoptotic protein Bcl-2, an increase in the expression of the pro-apoptotic proteins Bax and p53 and a 4-fold increase in cleaved caspase-3.

In addition to its antiproliferative effect, RGZ also induced an inhibition of pro-opiomelanocortin *(Pomc)* mRNA, whose encoded precursor peptide can be processed to ACTH in corticotropic cells.

In the same paper, using an in vivo mouse xenograft model subcutaneously inoculated with AtT20 cells, the administration of very high doses of RGZ (150 mg/kg/day) delayed tumor growth and caused a 75% reduction of ACTH and a 96% reduction of serum corticosterone levels.

The results raised great interest and led to the use of TDZ in the treatment of Cushing's disease [4–11]. A summary of the clinical studies using TDZ in Cushing's disease is reported in table 1.

PPAR-γ and Adrenocortical Cancer

PPAR-γ has been shown to be expressed in normal and tumoral adrenocortical cells as well as in the human adrenocortical cancer (ACC) cell lines H295R and SW13.

TZD have been shown to inhibit the proliferation of H295R cells in a dose-dependent manner [12]. In fact, thymidine incorporation was reduced by 60% by 20 μM of both RGZ and PIO. TDZ also increased the number of cells in the G0/G1 phase and decreased their number in the S phase increasing the expression of the cell-cycle inhibitors p21 and p27 and decreasing the expression of cyclin D1.

In the same paper [12], it was also shown that 20 μM of both RGZ and PIO reduced H295R invasiveness through matrigel by about 85% and that metalloproteinase-2 secretion was inhibited in a dose-dependent manner.

Similar results on the antiproliferative effects of TDZ were obtained by Betz et al. [13] who also documented a pro-differentiating effect of RGZ on H295R cells.

In an attempt to investigate the mechanisms involved in the anti-neoplastic effects of TDZ in ACC cells, Cantini et al. [14] evaluated RGZ interactions with the signaling pathways of the activated IGF-I receptor which is overexpressed and hyperactivated by IGF-II in human ACC [15, 16]. We demonstrated that RGZ inhibits ACC cell proliferation by interfering with the PI3K/Akt and ERK1/2 signaling pathways downstream of the activated IGF-I receptor.

In a preclinical study in a xenograft mouse model injected subcutaneously with H295R cells and treated with placebo or 5 mg/kg/day oral RGZ, we confirmed the antitumoral effects of TDZ in vivo [17]. A significant reduction of tumor growth in RGZ versus the control group was observed. Tumor histological evaluation revealed characteristics of invasiveness, richness in small vessels and mitotic figures in the control group, while RGZ group tumors presented noninfiltrating borders, few vessels and many apoptotic bodies. Tumor immunohistochemistry showed that Ki-67 was significantly reduced in the RGZ versus the control group. Quantitative real-time RT-PCR demonstrated a significant reduction in the expression of angiogenic (VEGF), vascular (CD31), proliferation (BMI-1) and anti-apoptotic (Bcl-2) genes in RGZ versus control group tumors.

In spite of these encouraging preclinical results, at present no clinical data are available on TDZ administration in patients with ACC.

Discussion

Preclinical studies have largely demonstrated the antitumoral properties of TZD, but in spite of their antiproliferative, proapoptotic and differentiating effects, TZD use in clinical trials planned for the treatment of limited cohorts of patients affected by different types of solid tumors has given controversial and, on the whole, not encouraging results [18]. Only a few studies found TZD to exert some positive therapeutic effect, particularly if combined with chemotherapeutic and angiostatic agents [18, 19].

However, all the clinical studies reported so far have enrolled a limited number of patients and are too heterogeneous in terms of type and stage of tumors, patients' age and sex and type of treatment (TZD doses, combined therapy, duration) to allow a definitive judgment on TZD anticancer effects.

The difference in results obtained using RGZ in clinical and preclinical studies may be due to several factors such as the duration of treatment and the doses of RGZ. In particular, in preclinical studies, RGZ has been used at concentrations far higher than those used in clinical trials, where the doses are in the antidiabetic therapeutic range and might consequently not be effective for antitumor results. Moreover, we have to consider that PPAR-γ agonists not only act on gene expression through a direct transcription and an indirect transrepression activity but also exert rapid nongenomic activity affecting post-translational modifications of the extranuclear protein involved in cell signaling (fig. 1) [20]. Whether these different actions also depend on the TZD concentrations is still to be evaluated. Finally, although different TZDs have been demonstrated to be effective in tumor cells, the role of PPAR-γ in mediating the anticancer effects of its ligands is still controversial.

Acknowledgments

This work was supported by Tuscany REgional Study Of Rosiglitazone (TRESOR), Istituto Toscano Tumori (ITT) and Cassa di Risparmio di Firenze (CRF).

Disclosure Statement

The authors of this paper do not have any relationships to disclose.

References

1 Lehrke M, Lazar MA: The many faces of PPARgamma. Cell 2005;123:993–999.
2 Panigrahy D, Huang S, Kieran MW, Kaipainen A: PPARgamma as a therapeutic target for tumor angiogenesis and metastasis. Cancer Biol Ther 2005;4:687–693.
3 Heaney AP, Fernando M, Yong WH, Melmed S: Functional PPAR-gamma receptor is a novel therapeutic target for ACTH-secreting pituitary adenomas. Nat Med 2002;8:1281–1287.
4 Alevizaki M, Philippou G, Zapanti L, Alevizaki CC, Anastasiou E, Mavrikakis M: Significant improvement of recurrent pituitary-dependent Cushing's syndrome after administration of a PPAR-gamma agonist (abstract). The Endocrine Society's 86th Annual Meeting, New Orleans, 2004, pp 2–453.
5 Hull SSA, Sheridan B, Atkinson AB: Pre-operative medical therapy with rosiglitazone in two patients with newly diagnosed pituitary-dependent Cushing's syndrome. Clin Endocrinol (Oxf) 2005;62:259–261.
6 Ambrosi B, Dall'Asta C, Cannavo S, Libe R, Vigo T, Epaminonda P, et al: Effects of chronic administration of PPAR-gamma ligand rosiglitazone in Cushing's disease. Eur J Endocrinol 2004;151:173–178.
7 Suri D, Weiss RE: Effect of pioglitazone on adrenocorticotropic hormone and cortisol secretion in Cushing's disease. J Clin Endocrinol Metab 2005;90:1340–1346.
8 Barbaro D, Lapi P, Orsini P, Pasquini C, Ciaccio S: Pioglitazone treatment in Cushing's disease (letter). J Endocrinol Invest 2005;28:388–389.
9 Mullan KR, Leslie H, McCance DR, Sheridan B, Atkinson AB: The PPAR-gamma activator rosiglitazone fails to lower plasma ACTH levels in patients with Nelson's syndrome. Clin Endocrinol (Oxf) 2006;64:519–522.
10 Giraldi FP, Scaroni C, Arvat E, Martin M, Giordano R, Albiger N, et al: Effect of protracted treatment with rosiglitazone, a PPARgamma agonist, in patients with Cushing's disease. Clin Endocrinol (Oxf) 2006;64:219–224.
11 Munir A, Song F, Ince P, Walters SJ, Ross R, Newell-Price J: Ineffectiveness of rosiglitazone therapy in Nelson's syndrome. J Clin Endocrinol Metab 2007;92:1758–1763.
12 Ferruzzi P, Ceni E, Tarocchi M, Grappone C, Milani S, Galli A, Fiorelli G, Serio M, Mannelli M: Thiazolidinediones inhibit growth and invasiveness of the human adrenocortical cancer cell line H295R. J Clin Endocrinol Metab 2005;90:1332–1339.
13 Betz MJ, Shapiro I, Fassnacht M, Hahner S, Reincke M, Beuschlein F, for the German and Austrian Adrenal Network: Peroxisome proliferator-activated receptor-gamma agonists suppress adrenocortical tumor cell proliferation and induce differentiation. J Clin Endocrinol Metab 2005;90:3886–3896.

14 Cantini G, Lombardi A, Piscitelli E, Poli G, Ceni E, Marchiani S, Ercolino T, Galli A, Serio M, Mannelli M, Luconi M: Rosiglitazone inhibits adrenocortical cancer cell proliferation by interfering with the IGF-IR intracellular signaling. PPAR Res 2008;2008:904041.
15 Boulle N, Logi´e A, Gicquel C, Perin L, Le Bouc Y: Increased levels of insulin-like growth factor II (IGF-II) and IGF-binding protein-2 are associated with malignancy in sporadic adrenocortical tumors. J Clin Endocrinol Metab 1998;83:1713–1720.
16 Barlaskar FM, Spalding AC, Heaton JH, Kuick R, Kim AC, Thomas DG, Giordano TJ, Ben-Josef E, Hammer GD: Preclinical targeting of the type I insulin-like growth factor receptor in adrenocortical carcinoma. J Clin Endocrinol Metab 2009;94:204–212.
17 Luconi M, Mangoni M, Gelmini S, Poli G, Nesi G, Francalanci M, Pratesi N, Cantini G, Lombardi A, Pepi M, Ercolino T, Serio M, Orlando C, Mannelli M: Rosiglitazone impairs proliferation of human adrenocortical cancer: preclinical study in a xenograft mouse model. Endocr Relat Cancer 2010;17:169–177.
18 Read WL, Baggstrom MQ, Fracasso PM, Govindan R: A phase I study of bexarotene and rosiglitazone in patients with refractory cancers. Chemotherapy 2008;54:236–241.
19 Reichle A, Bross K, Vogt T, Bataille F, Wild P, Berand A, Krause SW, Andreesen R: Pioglitazone and rofecoxib combined with angiostatically scheduled trofosfamide in the treatment of far-advanced melanoma and soft tissue sarcoma. Cancer 2004;101:2247–2256.
20 Luconi M, Cantini G, Serio M: Peroxisome proliferator-activated receptor gamma (PPAR-γ): is genomic activity the only answer? Steroids 2009;75:585–594.