Rosiglitazone, PPAR-γ receptor ligand, decreases the viability of rat prolactin-secreting pituitary tumor cells *in vitro*

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Abstract

OBJECTIVES: PPAR-γ is a member of the nuclear receptor superfamily. PPAR-γ activation is associated with glucose metabolism regulation, adipocyte differentiation, inhibition of macrophage and monocyte activation and anti-angiogenesis. PPAR-γ ligands thiazolidinediones (TZDs) have been shown to inhibit the growth and secretory activity of several rat and murine pituitary tumors *in vivo* as well as *in vitro* (ACTH-secreting AtT20, PRL- and GH-secreting GH_3, LH-secreting LβT2 and α-T3 cells). TZDs have been demonstrated to induce G_0–G_1 cell-cycle arrest and apoptosis in human, rat somatolactotroph, murine corticotroph and gonadotroph pituitary tumor cells.

In the present study we have investigated for the first time the effects of PPAR-γ receptor ligand rosiglitazone on the rat estrogens-induced, PRL-secreting pituitary tumor cells *in vitro.*

MATERIAL AND METHODS: Four weeks old male Fischer 344 rats were used in the experiment. Pituitary tumors were induced by subcutaneous implantation of capsules containing diethylstilboestrol (DES).

Eight weeks after the implantation of capsules the rats were sacrificed and pituitary tumors were collected. Tumorous cells were isolated and exposed in the primary culture to rosiglitazone at the concentrations 10^{-10} – 10^{-4}M for 24 hours. The cell growth was estimated by the measurement of the cells metabolic activity using the EZ4U system.

RESULTS: We have demonstrated that rosiglitazone at the concentrations 10^{-10} – 10^{-4}M significantly decreases the number of viable rat PRL-secreting pituitary tumor cells *in vitro.*

CONCLUSION: These results suggest that PPAR-γ receptor agonists thiazolidinediones may be useful in the medical treatment of pituitary tumors.

ORIGINAL ARTICLE

Abbreviations

ACTH	–adrenocorticotrophic hormone
CNFPAs	–clinically nonfunctioning pituitary adenomas
DES	–diethylstilboestrol
ELISA	–enzyme-linked immunosorbent assay
FBS	–Fetal Bovine Serum
GH	–growth hormone
LH	–luteinizing hormone
OD	–optical density
PPAR-γ	–peroxisome proliferator-activated receptor γ
PRL	–prolactin
TZDs	–thiazolidinediones

Introduction

Peroxisome proliferator-activated receptors γ (PPARs-γ) are members of the nuclear receptor superfamily, and thus, are ligand-activated transcription factors [11, 23]. PPAR-γ activation is associated with glucose metabolism regulation, adipocyte differentiation [24], inhibition of macrophage and monocyte activation [21, 12].

The recent evidence has shown that PPAR-γ ligands suppress the growth of thyroid, breast, prostate, gastric, pancreatic and colonic carcinoma cell lines [18, 16, 6, 13, 15, 17, 22] and are potent inhibitors of angiogenesis both *in vitro* and *in vivo* [27, 19]. PPAR-γ ligands thiazolidinediones (TZDs) have been also shown to inhibit the growth and secretory activity of several rat and murine pituitary tumors *in vivo* and *in vitro* (ACTH-secreting AtT20 [8], PRL- and GH-secreting GH_3, LH-secreting LβT2 and α-T3 cells [9]). TZDs have been demonstrated to induce G_0–G_1 cell-cycle arrest and apoptosis in human, rat somatolactotroph, murine corticotroph and gonadotroph pituitary tumor cells [8, 9, 4]. PPAR-γ is abundantly expressed in human pituitary adenomas of different subtypes compared with normal pituitary tissue [9] with the strongest expression observed in *prolactinomas* [26].

These data indicate that PPAR-γ is an important molecular target for treating patients with pituitary adenomas, especially GH- and PRL-secreting tumors which are unresponsive to dopamine agonists or somatostatin receptor analogues and ACTH-secreting and clinically nonfunctioning pituitary tumors, for which no efficient drug therapies are currently available [10]. Preliminary results of clinical studies in small groups support the potential usefulness of PPAR-γ agonists in some patients with Cushing's disease [1, 2].

In the present study we have investigated for the first time the effects of PPAR-γ receptor ligand rosiglitazone on the estrogens-induced, PRL-secreting rat pituitary tumor *in vitro.*

Material and methods

Four weeks old male Fischer 344 rats were used in the experiment. The animals were housed in a room with controlled illumination (L:D 12:12) and temperature (23±2°C), with constant access to tap water and standard laboratory food. Pituitary tumors were induced by the subcutaneous implantation of capsules containing 10 mg of diethylstilboestrol (DES, Sigma-Aldrich, Germany).

Eight weeks after the implantation of the capsules the rats were sacrificed and pituitary tumors were aseptically removed. The procedure of induction and collection of the pituitary tumors was approved by the Local Ethical Committee for Animal Experimentation (decision Nr Ł/BD/142, October 28, 2002). Sliced tumor tissue fragments were mechanically dispersed using 70 μm Nylon Cell Strainer (Falcon, Becton Dickinson Labware, New Jersey, USA). The monodispersed tumor cell suspension in RPMI 1640 medium containing 10% Fetal Bovine Serum (FBS, Biochrom KG, Germany), 100 U/ml penicillin and 100 μg/ml streptomycin solution (Sigma), was placed into multiwell culture dishes (Nunclon™ Δ 96 MicroWell Plates, Nalge Nunc International Corp., USA) at the density of 5×10^5 cells/well. The cells were incubated for 24 h in the presence of rosiglitazone at the concentrations 10^{-10} – 10^{-4}M at 37°C in the humidified atmosphere of 95% air and 5% CO_2. The group with the addition of solvent only served as control. The rosiglitazone (Rosiglitazone maleate, GlaxoSmithCline Beecham Pharmaceuticals, Worthing, West Sussex, UK) was dissolved in ethanol and further diluted with 0,01N acetic acid containing 0,1% FBS. The final maximal concentration of ethanol was 0,05% in experimental medium and control.

The cell viability was estimated by the measurement of the metabolic activity of the cells using the EZ4U system (Easy for You, the 4th Generation Non Radioactive Cell Proliferation & Cytotoxicity Assay, Biomedica GmbH, Austria). The assay is based on the transformation of tetrazolium salt into coloured soluble formazans as a result of the mitochondrial activity of the viable cells. The red soluble formazans released to the culture medium, were determined by the extinction measurement using the ELISA reader.

Statistical analysis was performed using ANOVA followed by LSD test. $P<0.05$ was considered to indicate significance.

Results

Rosiglitazone at the concentrations 10^{-10} – 10^{-4}M significantly decreased the viability of the rat PRL-secreting pituitary tumor cells *in vitro* (Figure 1).

Discussion

Thiazolidinediones (TZDs), potent oral antidiabetic compounds that activate peroxisome proliferator-activated receptors γ (PPARs-γ), have been shown to have antitumor activity in several experimental cancer models [18, 16, 6, 13, 15, 17, 22], including human and rat pituitary tumor cells [8, 9, 4]. TZDs induced G_0–G_1 cell-cycle arrest and apoptosis in ACTH-secreting AtT20 cells [8], PRL- and GH-secreting GH_3 cells, LH-secreting LβT2 cells and α-T3 cells [9] and suppressed pituitary hormone secretion *in vitro*. *In vivo* the growth of murine somatolactotroph, corticotroph and gonadotroph tumors, generated by subcutaneous implantation

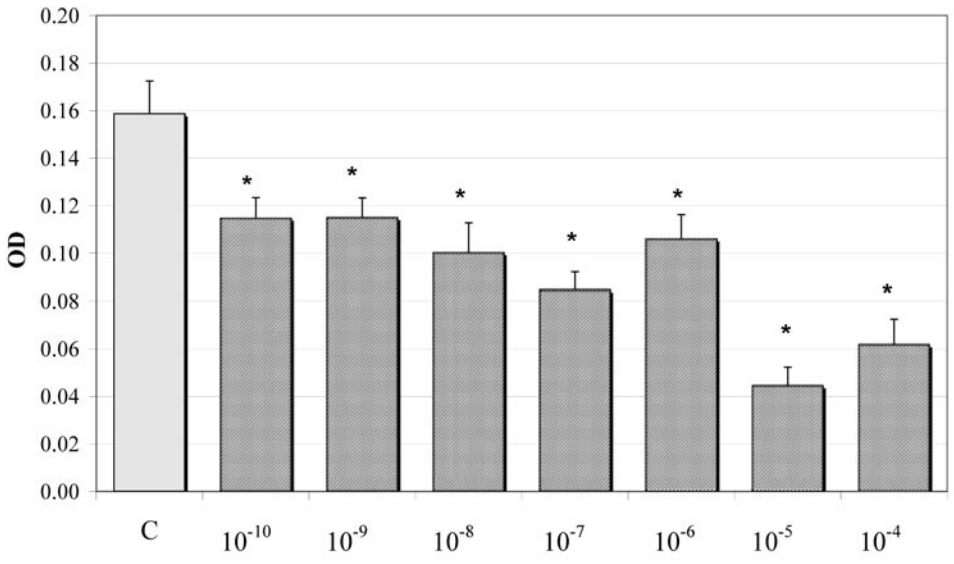

Figure 1. Effect of rosiglitazone on the viability of the rat *prolactinoma* cells *in vitro*. Bars represent the means ± SEM, * P<0.05 vs control (C); OD – optical density.

of GH3, AtT20, LβT2 and α-T3 cells, respectively, was suppressed in TZDs-treated mice and serum GH, PRL, ACTH and LH levels were significantly lower as compared with vehicle-treated tumor-bearing animals [9].

Here we report that rosiglitazone, a TZD compound, inhibits the viability of estrogen-induced, PRL-secreting rat pituitary tumor cells *in vitro*. Rosiglitazone was effective at the concentrations from 10^{-10} to 10^{-4}M. Further studies are required to evaluate the mechanism of rosiglitazone action in rat *prolactinoma* cells. The results of experiments concerning other pituitary tumor models have demonstrated cell-cycle arrest and increased apoptosis after TZDs treatment [4, 10, 7]. Apoptosis has been also shown to play a role in antitumor activity of PPAR-γ receptor agonists in thyroid carcinoma, colon carcinoma and glioblastoma cell lines [18, 3, 25].

As the recent studies have shown abundant PPAR-γ expression in human pituitary tumors compared with normal pituitary tissue [9, 26], PPAR-γ receptor ligands, such as rosiglitazone, may be potential therapeutic agents for treating the patients with pituitary adenomas. TZDs might be especially useful in the patients with PRL- and GH-secreting tumors that fail to respond to dopamine agonists and/or somatostatin analogs or are intolerant of these drugs. Other candidates for the treatment with PPAR-γ receptor agonists are patients with clinically nonfunctioning pituitary adenomas (CNFPAs). CNFPAs constitute about 30% of surgically removed pituitary macroadenomas. However, because of considerable size and growth potential, the effects of surgical treatment of these tumors are not satisfactory and no effective drug therapies for these tumors are available. To our knowledge, so far the clinical studies have concerned TZDs administration only in patients with Cushing's disease [1, 2, 5, 20]. These pilot studies, except for one [20], have shown that chronic rosiglitazone therapy seems able to normalize cortisol secretion in some patients, at least for short periods.

Rosiglitazone is a potent oral antidiabetic drug already used by millions of patients for the treatment of type 2 diabetes. Rosiglitazone has been shown to have greater affinity for PPAR-γ than other thiazolidinedione compounds, pioglitazone and troglitazone. Unlike troglitazone, which has been associated with idiosyncratic hepatotoxicity, rosiglitazone is safe and well-tolerated. Moreover, new PPAR-γ receptor ligands with potent antitumor activity, like RWJ-241947 [14] are still being developed and might prove effective in the medical treatment of pituitary tumors. Our findings, taken together with earlier studies, justify further clinical trials of the treatment of pituitary tumors with PPAR-γ agonists.

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