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 GH3, LH-secreting LβT2 and α-T3 cells). TZDs have been demonstrated to induce G0–G1 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.
Introduction

Peroxisome proliferator-activated receptors γ (PPAR-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 GH3, LH-secreting LβT2 and α-T3 cells [9]). TZDs have been demonstrated to induce G0–G1 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 (NunclonTM ∆ 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}\text{M} at 37°C in the humidified atmosphere of 95% air and 5% CO\text{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}\text{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 G0–G1 cell-cycle arrest and apoptosis in ACTH-secreting AtT20 cells [8], PRL- and GH-secreting GH3 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...
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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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