Effects of rosiglitazone – peroxisome proliferators-activated receptor gamma (PPARγ) agonist on cell viability of human pituitary adenomas *in vitro*

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Abstract

OBJECTIVES: Rosiglitazone (RGZ) belongs to thiazolidinediones – new class of antidiabetic drugs which are PPARγ agonists. It was shown that tumoural tissue, including the pituitary adenomas, posses PPARγ receptors. The activation of PPARγ receptors inhibits tumour growth in rodents and induces the oncostatic effect on human cancer cell lines. The aim of the present study was to examine the anti-tumour effect of RGZ on human pituitary adenomas *in vitro*. **MATERIALS AND METHODS:** Cells of eight pituitary adenomas removed neurosurgically were used to our experiment. Before the operation, the hormonal secretion of the tumour was estimated. After the surgery, the histological diagnosis and immunohistochemical detection of pituitary hormones and PPARγ receptors were performed. The cells of pituitary tumours were exposed in the primary culture to RGZ at the concentrations of 10^{-9} – 10^{-4} M for 24 hours. To measure the cell growth the modified colorimetric Mossman method detecting the cells viability was applied. **RESULTS:** On the basis of the pre-operative diagnosis the 6 clinically non-functioning adenomas (CNFPA), one case of acromegaly and one case of Cushing's disease were recognized. In 5 out of 6 CNFPA the immunopositive reaction for different pituitary hormones such as: LH, HGH, PRL, FSH and α-subunit was detected. Expression of PPARγ was found in all examined tumours. Rosiglitazone decreased the cell viability of all CNFPA and corticotropinoma for 20% or more. In somatotropinoma inhibition of the cell growth was about 13%. There is no correlation between PPARγ expression and efficacy of rosiglitazone.
THE MAIN FINDING: The obtained results indicate that RZG exerts a suppressive effect on the cell viability in non-functioning pituitary adenomas. The lack of correlation between PPARγ expression and anti-tumoural effect of RZG suggests that the above-mentioned action of this compound is independent on PPARγ expression. **CONCLUSION:** Our data suggest that rosiglitazone may be useful in the treatment of non-functioning pituitary adenomas, but its efficacy in Cushing's disease and acromegaly requires further study.

ORIGINAL ARTICLE

INTRODUCTION

Peroxisome proliferators-activated receptors (PPAR) are nuclear receptors widely expressed in mammalian tissues. Their activation leads to the transcription of multiple genes, which determines their involvement in numerous physiological and pathological functions [Desvergne & Wahli, 1999; Kersten *et al*., 2002]. PPAR occurs in three subtypes, called alpha, beta and gamma. The last subtype is drawing particular attention because it seems to play the important roles in the regulation of metabolism, as well as of the cell growth and differentiation [Murphy *et al*., 2000]. The last abilities predict the role of PPARγ in carcinogenesis and as a target of anticancer therapy. PPARγ were found in a normal anterior pituitary gland as well as in pituitary adenomas [Heaney *et al*., 2002, 2003; Winczyk & Pawlikowski, 2005; Bogazzi *et al*., 2005]. In the majority of studies, the overexpression of PPARγ in pituitary adenomas versus the normal gland was observed. It was also shown that PPARγ agonists, rosiglitazone (RZG) and troglitazone, commonly used as antidiabetic drugs, suppressed the growth and hormone secretion of several cell lines from murine and rat pituitary tumours. Moreover, PPARγ agonists called frequently glitazones, inhibited also the growth of primary cultures of human pituitary adenomas [Heaney *et al*., 2002; 2003]. In our laboratory we showed that RGZ decreased the cell viability of the estrogen-induced rat prolactinoma cells *in vitro* [Gruszka *et al*. 2005]. Peroxisome proliferators-activated receptors gamma were suggested as a therapeutic target for pituitary tumours [Heaney *et al*. 2003]. Because of the promising results of RZG treatment of ACTH-secreting adenoma cell line *in vitro* and experimental murine corticotropinoma *in vivo* [Heaney *et al*. 2002; 2003] several clinical trials with glitazones (mainly rosiglitazone) in Cushing's disease were performed. However, the results were univocal. Only less than 40% of investigated patients responded to glitazone treatment by the reduction of cortisol levels [Heaney, 2004; Ambrosi *et al*., 2005; Hull *et al*., 2005; Emery *et al*., 2006; Morcos *et al*., 2007]. Because the action of RZG on pituitary adenomas other than corticotropinoma are less known and the study assessing the influence of glitazones on growth of human pituitary adenomas are very limited, we examined the effects of RZG *in vitro* on the viability of the tumoral cells isolated mainly from clinically nonfunctioning human pituitary adenomas.

MATERIAL AND METHODS

Tumours and patients

Eight pituitary adenomas were investigated. The data concerning the patient's sex, age and hormone immunopositivity are presented in table I. Six adenomas were diagnosed before the surgery as clinically nonfunctioning. Five but one (female patient ES) expressed the immunopositive reactions for different pituitary hormones such as: LH, HGH, PRL, FSH and α-subunit. One female patient (UP) was diagnosed before the surgery as acromegaly and one (AK) presented Cushing's disease. All but two (ZZ and KJ) were primary tumours. Patient ZZ had the first recurrence, and patient KJ the third recurrence of pituitary adenoma.

The tumour tissues were obtained surgically by the transphenoidal approach. A fragment of each tumour was fixed in Bouin-Hollande fixative and embedded in paraffin for morphological and immunohistochemical investigation. Another part was mechanically dispersed and digested with collagenase to isolate the tumour cells.

Immunohistochemistry

Each tumour was immunostained using primary polyclonal or monoclonal antibodies against to the pituitary hormones and alpha-subunit (alpha-SU). Additionally, the tumour samples were immunostained with polyclonal anti-PPAR-gamma antibody (Calbiochem, La Jolla, USA) in working dilution of 1:1000. The immunostaining was visualized by the means of biotin-streptavidin-peroxidase method with 3,3'-diaminobenzidine as chromogen, using a Strept/ABC Complex / HRP kit (Dako Cytomation, Denmark). The number of PPARgamma-positive cell nuclei was counted in 1000 randomly chosen cells of each tumour.

Table 1. The data of the initials sex, age, clinical diagnosis, hormone immunopositivity, PPARγ immunodetection of the investigated patients and the maximal inhibitory effect of rosiglitazone on the cells viability of examined pituitary tumours (maximal drop). CNFPA - clinically non-functioning adenomas; M - male; F - female.

Patient's initials	Sex	Age [yrs]	Clinical diagnosis	Hormonal phenotype	PPAR γ [%]	Maximal drop vs control [%]
WJ	M	71	CNFPA	PRL, GH, αSU	5.8	25.6
MB	F	64	CNFPA	PRL, GH, LH	6.4	70.5
RG	M	41	CNFPA	LH, GH	6.5	25.2
ZZ	M	52	CNFPA	αSU	7.9	89.4
KJ	F	59	CNFPA	αSU	10.6	19.9
ES	F	46	CNFPA	None	14.7	48.7
UP	F	46	Acromegaly	GH, PRL, LH	18.2	12.7
AK	F	26	Cushing	ACTH, PRL, αSU	7.6	20.4

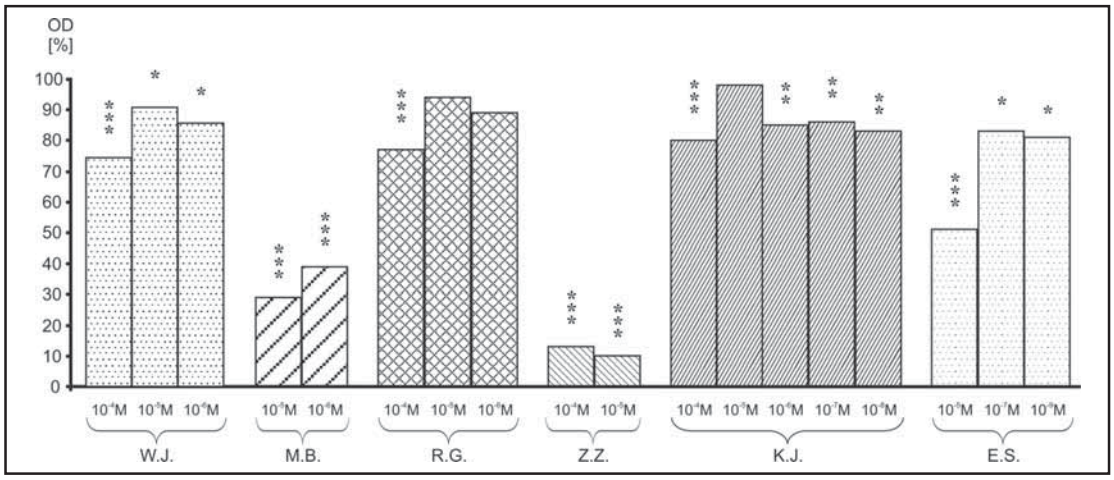

Fig. 1. The effects of rosiglitazone on the cells viability of clinically non-functioning adenomas. WJ, MB, RG, ZZ, KJ, ES, UP, AK – patient's initials; OD - optical density, * p< 0.05, ** p< 0.01, *** p< 0.001 vs control.

Cell cultures

The cells suspensions isolated from the tumour tissues were placed into multiwell culture dishes (Nunclon™ δ 96 MicroWell Plates) at a density of 200–500 thousands cells/well in RPMI 1640 medium supplemented with 10% Fetal Bovine Serum (FBS, Biochrom KG, Germany), 100 U/ml penicillin and 100 μg/ml streptomycin (Sigma) and incubated at 37°C in a humidified atmosphere of 95% air and 5% CO_2. The tumour cell suspensions were incubated for 24 hours with rosiglitazone (Alexis Biochemicals, San Diego, USA) at the concentrations of 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5} and 10^{-4} M. The quantity of the viable cells was measured using the EZ4U system, following the procedure recommended by the producer of the kit (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 into the culture medium were determined by the extinction measurement using the ELISA reader.

Statistical analysis

Statistical analysis of the data was performed using one-way ANOVA followed by Fisher's t-test (LSD – least significant difference) method according to Statgraphic Centurion XV computer program. Additionally, the Pearson linear correlation coefficient (r) between optical density – OD and PPARγ immunohistochemical detection was determined, in case of their statistically significance equation of regression was determined. The results are presented as mean ± SEM. Statistical difference between tested values was at a significance level of p<0.05

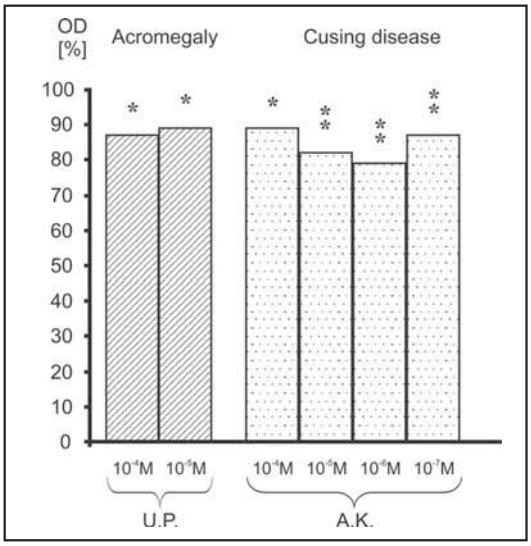

Fig. 2. The effects of rosiglitazone on the cells viability of ACTH-secreting pituitary adenoma (Cushing) and GH - secreting pituitary adenoma (acromegaly). OD - optical density, * p< 0.05, ** p< 0.01 vs control.

RESULTS

As it can be seen in table I, all the investigated adenomas exhibited the variable number of PPARγ-immunopositive cell nuclei. The exposure of adenoma cultures to rosiglitazone *in vitro* resulted in a statistically significant drop of the number of viable cells in all the samples. However, in one case (patient RG) a significant drop occurred only with the maximal concentration of rosiglitazone – 10^{-4} M (Fig. 1). In all but one tumour (patient UP, acromegaly) the maximal drop of the cell viability was equal or higher than 20% of the respective control. (Fig. 1 and 2). There is no correlation between the effect of RZG and the level of expression of PPARγ nuclear receptors (see Table I).

DISCUSSION

The data presented in this study are compatible with the earlier findings that PPAR agonist rosiglitazone suppresses the growth of human pituitary adenoma cells *in vitro* [Heaney *et al*., 2002; 2003]. This effect concerns also the clinically nonfunctioning pituitary adenomas (CNFPA) and these types of tumours seem to be a target for clinical trials of glitazone therapy. However, our material was too scarce to answer conclusively the question of possible link between of the tumour hormonal phenotype and the response to rosiglitazone. Several evidence showed that thiazolidinediones (TZDs) decrease the secretion of pituitary hormones in animals with various experimental pituitary adenomas [Heaney *et al*., 2002; 2003; Bogazzi *et al*., 2004; 2005]. The result of clinical study is not uniform. In patients with acromegaly treated for six weeks with RGZ, there is not observed the reduction of growth hormone and insulin-like growth factor-1 levels in plasma [Bastemir *et al*., 2007]. In patients with Cushing' disease the one- and two-days administration of this drug was also not effective [Cannavò *et al*., 2004; Pecori Giraldi *et al*., 2006]. The chronic therapy with RGZ caused a decrease of urinary cortisol level in persons with ACTH-secreting pituitary adenomas (about 40% cases), but did not modify plasma ACTH level [Ambrosi *et al*., 2005; Hull *et al*., 2005; Pecori Giraldi *et al*., 2006; Emery *et al*., 2006; Morcos *et al*., 2007]. These data suggest that glitazones do not influence the secretion of hormones such as GH and ACTH from human adenomas. However, our data indicate that RZG may be useful in therapy of CNFPA as an agent inhibiting the tumour growth. Although the RGZ belongs to PPARγ agonist, it is not clear whether its oncostatic action depends on the interaction with these type of receptors. We have found the lack of correlation between the inhibitory effect of this agent and the level of PPARγ expression as revealed by immunohistochemistry. Our results corroborate with the earlier findings published by Emery *et al*. [2005]. The quoted authors investigating the effects of RGZ and pioglitazone on growth of pituitary GH3 cell line showed that although both glitazones exerted the antiproliferative effect, the oncostatic action of these compounds was not reversed by a PPARγ antagonist. Moreover, Ambrosi and coworkers [2004] assessing the effect of rosiglitazone therapy on urinary cortisol level in four patients with Cushing's diseases showed that the expression of PPARγ in all removed ACTH-secreting pituitary adenomas was similar although only two patients responded to glitazone application. These evidences taken together suppose that RGZ exerts its oncostatic action on pituitary tumours independently of PPARγ via another, yet unknown mechanism.

Summing up, our obtained data suggest that RGZ may be useful in the treatment of non-functioning pituitary adenomas, but its efficacy in Cushing's disease and acromegaly requires further study.

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