THE EFFECT OF PIOGLITAZONE ON NON-PREGNANT FEMALE GENITAL TRACT IN EXPERIMENTAL DIABETES MELLITUS

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THE EFFECT OF PIOGLITAZONE ON NON-PREGNANT FEMALE GENITAL TRACT IN EXPERIMENTAL DIABETES MELLITUS (Abstract)

Aim: The effects of pioglitazone, a very used drug in the treatment of non-insulin dependent diabetes mellitus, were tested at the level of ovary of non-pregnant female rats. Material and methods: The experiment was performed on three groups of adult non-pregnant female rats. group 1 was a control group (and did not receive any substance), group 2 received streptozotocin 60mg/kg i.p. (single administration), and group 3 received streptozotocin 60 mg/kg i.p. (single administration) and pioglitazone 5 mg/kg/day p.o., daily for 8 weeks. The plasma glucose, cholesterol and triglyceride levels were determined before drugs administration and during the experiment. After 8 weeks the animals were anesthetized and sacrificed. The ovaries were examined by optical microscopy. A morphometric evaluation was performed. The obtained data were statistically interpreted by ANOVA test. Results: After 8 weeks of treatment the plasma glucose and triglyceride levels were significantly lower in the pioglitazone treated group compared to the streptozotocin only group. In the pioglitazone group the number of primordial and primary ovarian follicles was significantly higher than in streptozotocin only group. Conclusions: The results showed a partial protective action of pioglitazone on ovary in non-pregnant diabetic female rats. Keywords: DIABETES MELLITUS, STREPTOZOTOCIN, PIOGLITAZONE, OVARY

Diabetes mellitus (DM) is one of the most important diseases in human clinic. It affects all body tissues but unequally. In the therapy of non-insulin dependent diabetes mellitus (NIDDM) a significant number of oral antidiabetic agents with different chemical structures and mechanisms of action are used. One of the major classes of antidiabetic drugs is the thiazolidinedione group.

Thiazolidinediones (such as rosiglitazone and pioglitazone) are peroxisome proliferator-activated receptor-γ (PPARγ) agonists and are used to improve insulin sensitivity in DM patients. Thiazolidinediones are drugs that reduce the plasma glucose level, haemoglobin A1C level and enhance tissue insulin sensitivity (1). There are many data on how diabetes mellitus affects the female reproductive organs but much less information on DM influence on non-pregnant female genital tract. The aim of this study was to highlight the action of pioglitazone on ovaries in non-pregnant female rats with experimentally induced diabetes.
MATERIAL AND METHODS

We worked on three groups of eight adult non-pregnant female Wistar rats each (eight weeks old, weighing 170-250 g) bred in normal laboratory conditions. The animals were housed in polycarbonate cages, at a temperature of 22±2°C. During the study the animals were fed with a commercial granular diet and water ad libitum. DM was induced in overnight fasted rats by a single intraperitoneal administration of 60 mg/kg streptozotocin (STZ) (Sigma®) dissolved in citrate buffer.

Group 1 of female rats was the control group and did not receive any substance.

Group 2 of female rats received STZ 60 mg/kg i.p.

Group 3 of female rats received STZ 60 mg/kg i.p. and pioglitazone 5 mg/kg/day p.o., daily for 8 weeks.

After 8 weeks all animals were anaesthetized and killed by decapitation and the ovaries were removed and examined by optic microscopy. Plasma glucose, cholesterol and triglyceride levels were determined at baseline (before STZ administration) and after 8 weeks of treatment in each group. The obtained data were statistically interpreted by ANOVA test. Morphometric analysis was performed on scanned images of histologic samples, using Zeiss Observer Z1, Tissue Gnostics 9, Tissue Faks system. The aquired images were analysed in order to determine the most representative fields; all the investigated parameters were identified in selected fields of view (FOV) from each distinct region of the resulted images.

The study protocol was approved by the Ethics Committee of the Iasi “Grigore. T Popa” University of Medicine and Pharmacy. All animal procedures were performed according to the European Union law on the “Care and Use of Animals for Scientific Purposes” and in accordance with the “Recommendations of the Declaration of Helsinki”.

RESULTS

After 8 weeks of treatment the plasma glucose and triglyceride levels were significantly lower in the pioglitazone group compared to STZ only group (mean plasma glucose levels of 203.83±14.61 mg/dl in pioglitazone group vs. 352.17±38.79 mg/dl in STZ group, and mean triglyceride levels of 191.3±12.04 mg/dl in pioglitazone group vs. 261.2±9.52 mg/dl in STZ group, p<0.05). The mean cholesterol level was 93.83±7 mg/dl in STZ group compared to 80±7.51 in pioglitazone treated female rats. In pioglitazone group the number of primordial and primary ovarian follicles was significantly higher than in STZ only group (p<0.05).

DISCUSSION

DM is associated with diabetic impairment of female reproductive function and the reduction of fertility (2). The STZ-induced diabetes mellitus is a classical model for experimental diabetes. Streptozotocin (60 mg/kg) is a substance known to destroy only a part of beta pancreatic cells (about 60% of all beta pancreatic cells). The plasma insulin level significantly decreased but with the preservation of an important mass of functionally beta cells (3, 4). After STZ administration the rat pancreas produces about 25-30% of the initial insulin secretion. The STZ-induced diabetes is characterized by the increase in the levels of fasting plasma glucose, lipid peroxidative products and a significant decrease of antioxidant enzymes (superoxide dismutase (SOD), catalase, glutathione peroxidase and non-enzymatic antioxidants (reduced glutathione) (5).
Thiazolidinediones have been extensively used as insulin-sensitizers in the treatment of NIDDM. Activation of PPARγ by this group of drugs leads to a lot of metabolic and anti-inflammatory effects (6).

PPAR-γ is a member of the nuclear hormone receptor super-family. Pioglitazone, a specific agonist for PPAR-γ, was implicated in the control of inflammatory processes and the modulation of various cytokines expression. PPAR-γ agonists inhibit the synthesis of pro-inflammatory cytokines, such as TNF-α and IL-6 (involved in the failure of female reproductive activity) (7), diminish the oxidative stress (8) and inhibit or prevent cell apoptosis. Pioglitazone also reduces the pro-inflammatory cytokines IL-6 and IL-8 synthesis in endometrial stromal cells by a PPAR-γ-independent mechanism (9) and has a favourable effect in NIDDM, decreasing the incidence and severity of some complications. This drug also showed...

** p<0,01 vs. Control group; 
◊ p<0,05 vs. STZ group.

**Fig. 1** The influence of pioglitazone on primordial follicles in STZ-induced DM

* p<0,05 vs. Control group; 
◊ p<0,05 vs. STZ group.

**Fig. 2** The influence of pioglitazone on primary follicles in STZ-induced DM

* p<0,05 vs. Control group; 
◊ p<0,05 vs. STZ group.

**Fig. 3** The influence of pioglitazone on atretic follicles in STZ-induced DM

* p<0,05 vs. Control group; 
◊ p<0,05 vs. STZ group.

**Fig. 4** The influence of pioglitazone on triglycerides in STZ-induced DM
The effect of pioglitazone on non-pregnant female genital tract in experimental diabetes mellitus

a protective effect in retinal ischemia/reperfusion injury in rats (10).

Suppression of IL-1β could be another mechanism in pioglitazone-induced protective effect on ovary and uterus in STZ diabetic rats (11).

In our study, the effects of pioglitazone on non-pregnant rat ovary were different in different follicle types. The protective effect was stronger in the primordial follicles. The results were obtained with pioglitazone doses usually used in rat experimental tests (3-20 mg/kg/day) (12, 13). There were not observed influences on genital female system and fertility in normal animals after long time (8 months) of pioglitazone administration (14).

The obtained data are in agreement with the results of other authors which observed a partial protective effect of pioglitazone in experimentally-induced gastric ulcer (11), streptozotocin-induced diabetic nephropathy (13, 15) and experimentally-induced liver fibrosis in rat (16). Besides increasing sensitivity to insulin, pioglitazone increases tissue glucose utilization and decreases the synthesis of glucose from the liver (17). Pioglitazone also exerts a protective effect on β-cell function. Thiazolidinediones promote beta-cell survival and regranulation as well as maintenance of beta-cell mass (18). In addition to its beneficial effects on glucose homeostasis, pioglitazone exerts a number of other pleiotropic effects. This drug reduces blood pressure, improves endothelial dysfunction, corrects diabetic dyslipidemia and decreases the circulating levels of inflammatory cytokines and others (19). PPAR γ agonist pioglitazone used to treat NIDDM has also been demonstrated to be an effective antioxidant substance in oxidative stress in the animal models (20). Pioglitazone improves adipose tissue and liver insulin sensitivity and increases insulin-stimulated inhibition of lipid oxidation (21).

The obtained data show that pioglitazone significantly reduces the number of atretic ovarian follicles in STZ diabetes and increases survival of the ovarian primordial follicles.

Our results are in agreement with data indicating that pioglitazone treatment in polycystic ovary syndrome is associated with improvements in insulin action and glucose homeostasis and ameliorated the hyperandrogenic ovarian response. The granulosa cell responsiveness to FSH was enhanced by insulin after improved insulin sensitivity induced by pioglitazone (22). This drug ameliorated endothelial dysfunction of NIDDM patients. Increased nitric oxide production could be associated with a protective action and with the improvement of endothelial dysfunction.

CONCLUSIONS

This study showed that in STZ-induced DM in non-pregnant rats, the treatment with pioglitazone increased the number of primordial and primary ovarian follicles and reduced the number of atretic follicles.

REFERENCES

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