ORIGINAL ARTICLE

ZINC AND PIOGLITAZONE EFFECTS ON OVARIES AND ENDOMETRIUM IN DIABETES

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Abstract

The effects of pioglitazone and zinc on ovary and endometrium of female non-pregnant rats during experimental-induced diabetes mellitus were tested. Adult female non-pregnant rats were divided into five groups. Group 1 served as control group receiving no substance. Group 2 received streptozotocin (STZ) 60 mg/kg bw i.p. (single dose). Groups 3, 4, and 5 received the same dose of STZ but also $ZnCl_2$ (5 mg/kg bw/day i.p.), pioglitazone (5 mg/kg bw/day p.o.), and $ZnCl_2$ (5 mg/kg bw/day i.p.) + pioglitazone (5 mg/kg bw/day p.o.), respectively, for 8 weeks. The plasma levels of glucose and the total antioxidant capacity were determined before STZ administration and at the end of the experiment. After 8 weeks, all animal were killed after anaesthesia and the ovaries and uterus from each animal were examined using optical microscopy and a program for morphometric. Zinc and pioglitazone both significantly reduced the number of atretic follicles. The endometrium thickness was reduced by STZ and improved by pioglitazone (13.53 ± 0.05 μm STZ group *vs.* 17.72 ± 0.34 μm in STZ + pioglitazone group $p < 0.01$). Pioglitazone and zinc reduced both the endometrial and ovarian damages in STZ – induced diabetes non-pregnant female rats.

Rezumat

Au fost testate efectele pioglitazonei şi zincului asupra ovarului şi endometrului femelelor de şobolan non-gestante cu diabet experimental indus. Femelele de şobolan Wistar au fost incluse în patru grupuri. Grupul 1 a fost group de control şi nu a primit nici o substanţă. Grupul 2 a primit streptozotocină (STZ) 60mg/kgc ip. (doză unică). Grupurile 3, 4 şi 5 au primit aceiaşi doză de STZ, dar au primit de asemenea $ZnCl_2$ 5mg/kgc/zi, pioglitazonă 5mg/kgc/zi *per os* şi respectiv $ZnCl_2$ 5mg/kgc/zi + pioglitazonă 5mg/kgc/zi *per os* 8 săptămâni. Nivelele plasmatice ale glucozei si capacitatea antioxidantă totală (TAS) au fost determinate înaintea administrării STZ şi la sfîrşitul experimentului. După 8 săptămîni, toate animalele au fost sacrificate după anestezie şi ovarele şi uterul au fost examinate prin microscopie optică şi deasemenea s-a utilizat un program computerizat de morfometrie. Zincul şi pioglitazona au redus ambele în mod semnificativ numărul de foliculi ovarieni atretici. Grosimea endometrului a fost redusă de către STZ, dar a fost crescută de către pioglitazonă (13,53 ± 0,05 μm in grupul cu STZ faţă de 17,72 ± 0,34 μm în grupul STZ + pioglitazonă $p < 0,01$). Pioglitazona şi zincul reduc ambele leziunile ovariene şi endometriale în diabetul indus cu STZ la femelele de şobolan non-gestante.

Keywords: diabetes, zinc, pioglitazone, endometrium

Introduction

Diabetes mellitus (DM) is a chronic disease that significantly affects the health**.** Zinc is an essential trace element very important for the activity of more than 300 enzymes, for protein synthesis and intracellular signalling [5, 17]. It is involved in the human body general homeostasis, in immune responses, in oxidative stress, in apoptosis and in ageing [21]. The zinc levels were lower in diabetic patients compared with healthy people [9].

Pioglitazone is a very used drug in the treatment of type 2 diabetes mellitus. It is a peroxisome proliferator activated receptor (PPAR) - gamma agonist that reduces insulin resistance [19]. The aim of the study was to show the influence of zinc and pioglitazone on the ovaries and endometrium of non-pregnant female rats in experimental-induced diabetes mellitus.

Materials and Methods

The experiment was conducted on five groups of eight adult non-pregnant female Wistar rats each (eight weeks old and 170-250 g weight) bred in normal laboratory conditions. The animals were housed in polycarbonate cages, at a temperature of 22 ± 2°C. During the study period the animals were fed with granulated diet and water *ad libidum*. The diabetes mellitus was induced in overnight fasted rats by a single intraperitoneal administration of 60 mg/kg bw streptozotocin (STZ) (FLUKA Chemika

Co Ltd Switzerland) dissolved in citrate buffer. The first group of female rats was the control group and did not receive any substance. The second group of female rats received only STZ 60 mg/kg bw i.p. (unique dose). The third group received STZ 60 mg/kg bw i.p. (unique dose) and pioglitazone 5 mg/kg bw/day p.o. daily, for 8 weeks, the next group received STZ 60 mg/kg bw i.p (unique dose) and $ZnCl_2$ 5 mg/kg bw/day i.p. daily, for 8 weeks and the last group received STZ 60 mg/kg bw (single dose) i.p. and daily pioglitazone 5 mg/kg bw/day daily p.o. + $ZnCl_2$ 5 mg/kg bw/day, i.p., daily for 8 weeks.

After 8 weeks all the animals were anesthetized and killed by carotid section and the uterus and ovaries were removed, fixed in 10 % formalin for 48 hours, cut into 5-10 mm transversal slices, embedded in paraffin blocks and examined using an optic microscopy. Before the histological examination, paraffin embedded fragments were cut at 5 μm and haematoxylin and eosin staining was performed on tissue samples.

For each of the groups, there were determined initially (before STZ administration) and after eight weeks of treatment the plasma levels of glucose. The total antioxidant status was measured in serum as Trolox Equivalent Antioxidant Capacity (TEAC), Total antioxidant status (TAS) was assayed with a slightly modified chemiluminometric method with luminol–horseradish peroxidase system using Berthold Lumat 9507 chemiluminometer (Berthold, Bad Wildbad Germany). In this method, constant light emission results from luminol degradation in the presence of a catalyst (horseradish peroxidase) with an enhancer (p-iodo-phenol) and is kinetically recorded. Calibration was made with Trolox (hydro-soluble vitamin E) (Sigma Aldrich, St. Louis, USA) and final results are expressed as Trolox equivalents. The prooxidant system which generates light, was brought to five millions relative units of light (RLU) and serum samples were used at a dilution of 1/10 [11]. Blood samples were taken from the tail. Zinc serum concentrations were determined before administration and 8 weeks after the first $ZnCl_2$ solution administration by atomic absorption spectrophotometry (using a spectrophotometer AAS1N Carl Zeiss Jena, Germany).

Morphometric analysis was performed on scanned images of histology samples, using Zeiss Observer Z1, TissueGnostics 9, TissueFax system (Tissue Gnostics GmbH, Vienna, Austria). The registered images were analysed in order to determine the most representative fields; all the investigated parameters were identified in selected FOV (fields of view) from each distinct region of the resulted images. The obtained data were statistically interpreted using ANOVA test. The number of normal follicles was determined by summing all types of follicles (primordial, primary, secondary and tertiary follicles).

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

Results and Discussion

In all animals STZ administration produced a significant increase of plasma glucose levels (Table I).

Table I

Plasma glucose concentration. The results are means ± SD

GROUP	Glucose (mMol/L)				
	Initially (Before STZ)	After 48h	p *vs.* STZ group	After 8 weeks	p *vs.* STZ group
Control	5.10 ± 0.59	5.27 ± 0.42	< 0.001	5.45 ± 0.27	< 0.01
STZ	5.16 ± 0.25	19.32 ± 0.30		19.69 ± 0.78	
STZ + Zn	5.36 ± 0.50	18.04 ± 0.24	NS	13.84 ± 0.27	< 0.01
STZ + Pioglitazone	5.19 ± 0.32	13.49 ± 0.29	< 0.01	11.72 ± 0.35	< 0.01
STZ + Zn + Pioglitazone	4.93 ± 0.46	12.42 ± 0.37	< 0.01	12.06 ± 0.33	< 0.01

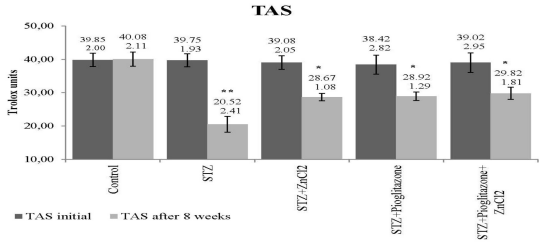

Figure 1.

Pioglitazone and zinc influences on TAS values in STZ induced diabetes in rats. The results are expressed as mean ± SD.

* p < 0.05 vs. initial; ** p < 0.01 *vs.* initial; ◊ p < 0.05 *vs.* STZ groups

The TAS values decreased from 39.75 ± 1.93 Trolox units before STZ to 20.52 ± 2.41 Trolox units in STZ group p < 0.01. Zinc and pioglitazone both improved the TAS in STZ treated rats (Figure 1).

The plasma zinc concentration significantly increased from 1.24 ± 0.15 mg/L before $ZnCl_2$ administration to 2.66 ± 0.11 mg/L after 8 weeks p < 0.01. The number of normal follicles was reduced

by STZ and increased by pioglitazone, but not by zinc (Figure 2).

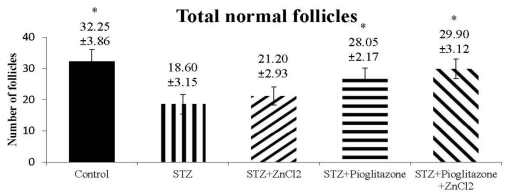

Figure 2.

Pioglitazone and zinc influences on total normal follicles in STZ induced diabetes in rats. The results are expressed as mean ± SD.

** p < 0.01 for comparison with STZ group

The number of atretic follicles significantly increased after STZ. Pioglitazone and zinc, the both, significantly reduced the number of atretic follicles in STZ treated animals (Figure 3).

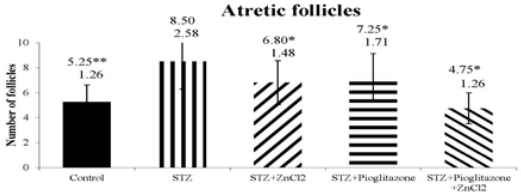

Figure 3.

Pioglitazone and zinc influences on atretic follicles number in STZ induced diabetes in rats. The results are expressed as mean ± SD.

* p < 0.05 for comparison with STZ group; ** p < 0.01 for comparison with STZ group; ◊ p < 0.05 between STZ + pioglitazone group *vs.* STZ + Pioglitazone + $ZnCl_2$ group

In STZ group, the endometrium thickness was significantly reduced compared to control group (29.04 ± 0.10 µm in control group *vs.* 13.53 ± 0.05 µm in STZ group p < 0.01). This effect was partially diminished by pioglitazone. Zinc addition to pioglitazone significantly improved the pioglitazone effect on the endometrium thickness (Figure 4).

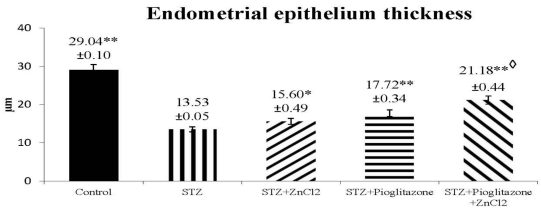

Figure 4.

Pioglitazone and zinc influences on endometrial epithelium thickness in STZ induced diabetes in rats. The results are expressed as mean ± SD.

* p<0.05 for comparison with STZ group; ** p<0.01 for comparison with STZ group; ◊ p < 0.05 between STZ + pioglitazone group *vs.* STZ + Pioglitazone + $ZnCl_2$ group

It has been reported that diabetes induced by STZ is the best characterized experimental type of induced diabetes and the most commonly used model for the screening of oral hypoglycaemic drugs effects [2]. During this type of experimental induced diabetes, the beta pancreatic islets are destroyed only in part [25]. For this reason, the STZ –induces diabetes is considered a mixed type of diabetes (type 1/type 2). The hyperglycaemia from diabetes mellitus increases the oxidative stress. The TAS levels are reduced in humans and also in experimental induced DM.

Zinc is a trace element important for reproduction in both sexes. In females, prolonged zinc deficiency causes developmental problems during pregnancy and the lack of *corpus luteum* development [8]. This cation plays also a role in oocyte development in periovulatory period. *In vivo*, zinc deficiency impairs oocyte maturation, and ovulation [22]. This cation concentration also decreased in experimental induced diabetes in rats [15]. The effect of zinc supplementation in the treatment of diabetes mellitus is controversial [28]. In our study, zinc clearly reduced the number of atretic follicles in STZ experimental induced diabetes. Authors consider that the most important mechanisms for zinc protective action are the reduction of oxidative stress induced by hyperglycaemia and directly by STZ. This toxic substance increases the free radicals formation and decreases the antioxidant system activity. In this study the zinc plasma concentration significantly increased after $ZnCl_2$ administration and improved the TAS (20.52 ± 2.41 Trolox units in STZ group compared to 28.67 ± 1.08 Trolox units in STZ + $ZnCl_2$ group p < 0.05). There are data that show that zinc significantly elevated total antioxidant status (TAS), reduced oxidative stress and histopathological damages in tissues of animals receiving various substances that increase oxidative stress (ethanol, alloxan and others) [13]. Other studies found that zinc prevents the follicles atresia induced by cadmium [14]. The oxidative stress is increased and the antioxidant status is decreased in erythrocytes of rats fed with zinc-deficient diet [23]. Another mechanism for zinc effect could be the reduction of apoptosis. Increased oxidative stress promotes apoptosis. Low Zn concentrations increase DNA damage and apoptosis. Zinc protects the integrity of DNA molecule and diminishes the percentage of apoptotic cells [1]. The results of this study were according to data which showed that zinc content was lower in the follicular fluid and granulosa cells of atretic follicles than the normal follicles [4]. The obtained data showed that zinc administration for 8 weeks significantly reduced the plasma glucose level and triglycerides concentration but not cholesterol level.

Pioglitazone activates the nuclear receptor peroxisome proliferator-activated receptor-γ (PPAR γ) and improves glucose plasma level control in patients with type 2 diabetes by increasing insulin sensitivity in the liver, adipose tissue and skeletal muscle. This medicine increases peripheral glucose uptake and reduces hepatic glucose synthesis [27]. Pioglitazone improves also the pancreatic β-cell activity [12] and reduces post meal glucose level. This antidiabetics drug decreases the beta cells apoptosis and increases glucose stimulated insulin secretion [10]. Pioglitazone has the ability to enhance regeneration of endogenous islet beta-cells [26]. PPAR γ are expressed in different tissues of the female genital tract and influence gametogenesis, ovulation, and the implantation process [24]. Oxidative stress is a common mechanism involved in all tissue damages in diabetes mellitus. In hyperglycaemic states, generation of free oxygen radicals is accelerated, and antioxidant defence systems are weakened [20]. There are data that showed that pioglitazone reduced to some extent the oxidative stress enhanced by chronic hyperglycaemia [6]. Besides reducing the free radicals formation, another possible mechanism by which pioglitazone produces partial protection could be the inhibition of some cytokines synthesis. The proinflammatory cytokine synthesis by monocytes, (such as TNF-*a*, IL-6 and others) was reduced by PPAR- γ agonists [7]. The obtained results in this study are in agreement with previous researches that proved administration of pioglitazone to women with polycystic ovarian syndrome restored ovulation [16]. This drug has also favourable effects in type 2 diabetic nephropathy [3] and in hepatic diabetic damages [18].

Conclusions

Our data indicated that pioglitazone and zinc reduced both the endometrial and ovarian damages in STZ – induced diabetes non-pregnant female rats. Zinc addition to pioglitazone significantly increased the pioglitazone improving effect on endometrial epithelium thickness and also the effect of pioglitazone in reducing the number of atretic follicles, but did not significantly change the hypoglycaemic effect of pioglitazone, nor its effect on the numbers of normal follicles.

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