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Changes in the transcriptional activity of the entero-insular axis genes in streptozotocin-induced diabetes and after the administration of TNF-α non-selective blockers

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Objective. The aim of the present study was to investigate the transcriptional activity of the GLP-1R, DPP-4, SGLT-1, INSR, and IGF-1R genes in GALT cells of rats with streptozotocin-induced diabetes in both untreated and treated with pentoxifylline, as a non-specific blocker of TNF-α.

Methods. The expression of GLP-1R, DPP-4, SGLT-1, INSR, and IGF-1R genes in GALT cells of rats was studied by real time quantitative polymerase chain reaction.

Results. It was shown that the development of diabetes was accompanied by the decrease of GLP-1R and an increase of DPP-4 genes expression in rat ileum. The administration of pentoxifylline to diabetic animals led to an increase in the transcriptional activity of GLP-1R on the 4th week and decrease in transcriptional activity of DPP-4 on the 2nd and 4th weeks of the experiment. An increase in the normalized expression of SGLT-1 on the 4th week of the experimental diabetes was also noted, while the administration of pentoxifylline to diabetic animals did not lead to significant changes in this index. The transcriptional activity of the INSR and IGF-1R genes was reduced in diabetic rats and the administration of the non-specific TNF-α blocker – pentoxifylline led to a significant increase only for INSR gene in animals on the 4th week of the experimental diabetes.

Conclusions. The expression of incretins, glucose transporters, and pro-inflammatory cytokines (e.g. TNF-α) in immune cells may be used as markers of several autoimmune pathologies progression such as type 1 diabetes due to their effect on the balance of pro- and anti-inflammatory factors.

Key words: entero-insular axis, mRNA, diabetes mellitus, pentoxifylline

Diabetes mellitus (DM) is a multifactorial metabolic disorder, characterized by chronic hyperglycemia leading to significant physiological, biochemical, and histological changes in the affected organisms (Guzyk et al. 2017; Krynytska and Marushchak 2018). Development of type 1 diabetes (T1D) can be triggered by genetic predisposition as well as changes occurring in the gut-associated lymphoid tissue (GALT) combined with an imbalance in the composition of the intestinal microbiome. These changes are associated with the development of chronic inflammation as a result of activation of both the innate and adaptive parts of the immune response (Fasano 2012; Khaleghi et al. 2016; Koval et al. 2018). Dysregulation of proinflammatory cytokines is a key feature in the development of chronic inflammatory disorders (Zherebiatiev and Kamyshnyi 2016).

Intestinal epithelial structures are increasingly being recognized as key players in maintaining metabolic and immune homeostasis, which relies on the

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ability of epithelial structures to efficiently absorb nutrients, maintain interaction with the external environment while supporting an effective barrier function and affecting immune response (Clavel and Haller 2007; Topol et al. 2014). At the same time, metabolic disorders are implicated in the development of pathophysiological changes in GALT in T1D. Another current venue of research is the relationship between the production of gastrointestinal hormones and their role in the initiation and progression of T1D, accompanying metabolic disorders and changes to GALT immunoreactivity. After stimulation, L-cells of the intestinal mucosa secrete various peptide hormones, including incretin glucagon-like peptide-1 (GLP-1), a transcription product of the proglucagon gene, which also encodes GLP-2 and other factors. It is predominantly expressed in the ileum and colon. GLP-1 has endocrine activity on the peripheral blood flow and also paracrine effects: for example, it stimulates vagus afferent neurons. The latter mediates signal transmission from the intestine to the brain with anorexigenic effect, while its hepatoportal signaling can affect metabolic function of the liver (Zietek and Daniel 2015). GLP-2 has been shown to be important for gut epithelial function by stimulating epithelial cell regeneration and improving barrier function in the intestine (Greiner and Backhed 2016).

Dipeptidyl peptidase-4 (DPP-4), also known as cluster of differentiation 26 (CD26), is a ubiquitously expressed glycoprotein (epithelial cells, fibroblasts, and white blood cells). It is a type II transmembrane protein that can cleave from the membrane and dissolve into the peripheral blood stream. DPP-4 plays a key role in the differentiation and activation of T cells and performs regulatory functions (Klemann et al. 2016). The soluble form of DPP-4 is characterized as an adipokine (Lamers et al. 2011), and its levels correlate with the extent of metabolic syndrome (Sell et al. 2013). As a serine protease, DPP-4 cleaves various substrates, suggesting a complex nature of its action.

There are two main classes of glucose transporters that mediate glucose transport to and from cells: sodium-dependent glucose co-transporters (SGLT) and facilitating glucose transporters (GLUT). SGLT-1 has a high affinity for glucose and galactose, but not fructose (Wright et al. 2011). In the small intestine, SGLT-1 is expressed on the apical membrane of enterocytes and is responsible for the transport of glucose and galactose controlled by Na^+ gradient created by the Na^+/K^+ ATPase, while GLUT-2 mediates subsequent glucose transport through the basolateral membrane to interstitium, and, into circulation (Gorboulev et al. 2012; Roder et al. 2014). SGLT-1-mediated glucose uptake modulates the secretion of intestinal hormones and primarily GLP-1 by the intestinal L-cells. Stable postprandial secretion of GLP-1 can be reduced in T2D, which can be secondary to the increased SGLT-1 expression in the proximal intestine, thereby limiting glucose delivery to the more distal intestine.

Insulin receptor (INSR) and insulin-like growth factor-1 (IGF-1) contribute to the finely-tuned balance of pro-inflammatory and anti-inflammatory signaling implicated in autoimmune pathology, including T1D. Activation of naive T cells in response to antigens shifts the metabolic processes towards aerobic glycolysis (Pearce 2010; Chang et al. 2013). This increases glucose uptake, which is thought to be associated with the induction of INSR expression. Therefore, it is likely that INSRs play a crucial role in T cell function and adaptive immunity (Stentz and Kitabchi 2003; Frauwirth and Thompson 2004). IGF-1, in turn, also exerts a regulatory effect on the immune cells through endocrine, paracrine, autocrine, and possibly intracrine loops, and, therefore, plays a role in the pathogenesis of autoimmune diseases. It was shown that IGF-1 and insulin-like growth factor-1 receptor (IGF-1R) affect T cells function through cell proliferation, chemotaxis, activation and apoptosis (Smith 2010). IGF-1 can act as a pro-inflammatory factor by stimulating pro-inflammatory cytokines and chemokines such as tumor necrosis factor alpha (TNF-α) and interleukin-8 (IL-8), respectively (Kooijman et al. 2003). The role of IGF-1 in enhancing adhesion of monocytes and inhibition of neutrophil apoptosis is well understood (Kooijman et al. 2002). At the same time, IGF-1 can also produce anti-inflammatory effects by stimulating the expression and secretion of interleukin-10 (IL-10) and inhibiting Th1-mediated cellular immune responses (Kooijman and Coppens 2004).

Since chronic and immune-mediated diseases, such as types 1 and 2 diabetes mellitus, insulin resistance, obesity, and inflammatory bowel diseases, are associated with an increase in the levels of pro-inflammatory cytokines including TNF-α and interleukin 6 (IL-6) (Brestoff and Artis 2015), as well as with intestinal microbiome changes (Clavel and Haller 2007; Clavel et al. 2014), this study aimed to investigate the transcriptional activity of the GLP-1R, DPP-4, SGLT-1, INSR and IGF-1R genes in GALT cells of rats with streptozotocin-induced diabetes, both untreated and treated with pentoxifylline as non-specific blocker of TNF-α.

Materials and methods

Animals and study design. The experimental animals, white matured male Wistar rats (n=80) obtained from the nursery of Veterinary Medicine Association Ltd. "Biomodelservis" (Kyiv) were housed under standard conditions, with proper diet and water ad libitum at the animal facility of Zaporizhzhia State Medical University. Animal treatment and all experimental procedures were performed in compliance with the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes. The study was approved by the Ethical Committee of Zaporizhzhia State Medical University.

Experimental study design comprised five groups: control (group 1; n=16); animals with experimental diabetes mellitus (EDM), 14 days after streptozotocin (STZ) administration (group 2; n=16); animals with EDM, 28 days after STZ administration (group 3; n=16); animals with EDM, 14 days after STZ administration, and treated with pentoxifylline (PTX) (group 4; n=16); animals with EDM, 28 days after STZ administration, and treated with PTX (group 5; n=16).

EDM was induced by a single intraperitoneal administration of STZ (Sigma Chemical, USA) at a dose of 50 mg/kg body weight (b.w.). Immediately prior to the administration, STZ was dissolved in 0.1 M citrate buffer (pH 4.5). The time period from STZ administration to termination of the experiment was interpreted as the duration of EDM. The control group received a corresponding amount of citrate buffer.

The mechanism of EDM induction by STZ: STZ contains a glucose molecule (in deoxy form) that is linked to a highly reactive methylnitrosourea moiety that is thought to exert STZ's cytotoxic effects, while the glucose moiety directs the chemical to the pancreatic beta cells. STZ recognizes the GLUT2 receptor that is abundant on beta cell plasma membranes. Therefore, pancreatic beta cells are specific targets of STZ which results in their autoimmune destruction with emergence of clinical diabetes within 2–4 days (Wu and Yan 2015).

PTX was administrated orally at a dose of 9 mg/kg b.w. for 2 or 4 weeks from the 1st day of EDM induction. We used PTX, a methylxanthine derivative and a non-selective phosphodiesterase inhibitor, because it has been reported that it might also influence the function of immune cells and the production of cytokines. In particular, PTX was shown to inhibit efficiently TNF-α transcription in various *in vitro* and *in vivo* systems (Nguyen-Chi et al 2017).

Blood glucose determination. Blood glucose concentration was determined using the glucose oxidase method with BIONIME Rightest TM GM 110 glucometer (Switzerland) 12 hours and then on the days 1, 2, 3, 5, 7, 10, 14 and 28 after STZ administration. Blood samples were taken from the tail vein. Animals with fasting glucose level of >8.0 mmol/l were selected for the study. Glucose concentration was determined after 6 h of starvation on the 3rd day after STZ administration.

Real-time RT-PCR analysis. Real-time reverse transcription polymerase chain reaction (RT-PCR) was used to analyze expression of the genes. Tissue samples (ileum with isolated lymphoid follicles) embedded in paraffin were cut with a microtome (slice thickness of 15 μm) and placed in Eppendorf tubes (Eppendorf AG, Germany). The tissue samples were dewaxed by incubation in xylene twice for 5 min, then in 100% ethanol twice for 5 min. Isolation of total RNA from rat tissues was performed with Trizol RNA Prep 100 Kit (IZOGEN, RF) according to the manufacturer's protocol.

The concentration and quality of isolated total RNA were determined on a Libra S32PC spectrophotometer (Biochrom ltd., England). For the subsequent reverse transcription procedure, RNA samples with the ratio of A260/A280 within the range of 1.8–2.2 were selected.

Reverse transcription (cDNA synthesis) was performed using the Reagent Kit for Reverse Transcription (SINTOL, RF). The reaction mix with a final volume of 25 μl contained 10 μl of the 2.5X reaction mixture, 11 μl of deionized H_2O, 1 μl of random hexonucleotide primers, 1 μl of reverse transcriptase and 2 μg of RNA. Reverse transcription was performed at 45 °C for 45 min, followed by heating at 92 °C for 5 min. Amplification was performed in a CFX96™ Real-Time PCR Detection System (Bio-Rad Laboratories, Inc., USA) with Maxima SYBR Green/ROX qPCR Master Mix (2X) (Thermo Scientific). The reaction mixture for amplification included SYBR Green dye, Maxima HotStartTaq DNA Polymerase, 0.2 μl of specific forward and reverse primers, and 1 μl of template (cDNA), with a total volume brought to 25 μl with deionized H_2O. Specific primer pairs (5'–3') for the analysis of the studied and reference genes were designed using the PrimerBlast software (www.ncbi.nlm.nih.gov/tools/primer-blast) and manufactured by Metabion (Germany) (Table 1).

After initial denaturation for 10 min at 95 °C, amplification consisted of 45 cycles and was carried out under the following conditions: denaturation –95 °C for 15 s, annealing –58–61 °C for 30–60 s,

elongation –72 °C for 30 s. The glyceraldehyde-3-phosphate dehydrogenase gene (GAPDH) was used as a reference gene to determine relative change in the expression level of the studied genes. Relative normalized amount of cDNA of a targeted gene was determined by the ΔΔCt method. Statistical analysis of PCR data was performed using CFX Manager™ software (Bio-Rad, USA). The following negative controls were included in the experiment: no cDNA matrix in the PCR reaction, no mRNA matrix in cDNA synthesis, and no enzyme in cDNA synthesis. All amplification reactions were performed on individual samples in triplicate.

Statistical analysis. The experimental data were processed and analyzed using MS Office 2016 EXCEL (Microsoft Corp., USA) and STATISTICA 13 (TIBCO Software Inc., 2018). The results were expressed as a mean ± SEM. The difference between the groups was determined using the Student's t-test. A probability level (p-value) of less than 0.05 was considered to be statistically significant. The 95% confidence interval (95% CI) was calculated.

Results

Transcriptional activity of the GLP-1R gene in rat ileum cells showed a 3.1-fold decrease in mRNA concentration on the 2nd week of EDM, and a 4.2-fold decrease on the 4th week, compared to the control group of animals (Figure 1A). Normalized expression of mRNA DPP-4 increased against the background of EDM progression. On the 28th day of the EDM, this index increased six times compared to the control group (Figure 1D).

PTX administration to the experimental animals led to a 2.1-fold increase in the level of GLP-1R expression on the 4th week of EDM compared to the diabetic untreated animals (Figure 1C), and to significant decrease in the DPP-4 mRNA levels: 16.3-fold on the 2nd week of EDM (Figure 1E), and 5-fold on the 4th week compared to the diabetic untreated animals from corresponding cohorts (Figure 1F).

Relative normalized expression of the SGLT-1 gene in rat ileum cells on the 4th week after the onset of EDM showed 6-fold increase in mRNA concentration compared to the control group of animals (Figure 2A). Diabetic animals of both groups that were administered PTX did not show significant changes to the transcriptional activity of SGLT-1 gene compared to the groups of untreated EDM-induced rats (Figures 2B, C).

The level of relative normalized expression of INSR mRNA in the ileum cells of rats two weeks after the onset of EDM decreased 2.4 times compared to the control. However, there were no significant changes to the expression of this gene in the rats 4 weeks after EDM induction (Figure 3A). In contrast, the expression level of IGF-1R mRNA, showed significant changes only on the 28th day after EDM onset, decreasing by 6.8 times compared to the control group (Figure 3D).

PTX administration to the EDM animals led to a significant 2.2-fold increase in the transcriptional activity of the INSR gene in the group of animals with a four week-long EDM of compared to the untreated group with the same EDM duration (Figure 3C).

Transcriptional activity of the IGF-1R gene does not show significant changes after pentoxifylline administration to EDM animals both on the day 14 and on day 28 after onset of the pathology compared to the respective cohorts of untreated EDM animals (Figures 3E, F).

Discussion

Recently, the mechanisms of cascading pathogenetic changes taking place during the development of autoimmune pathology, including T1D, received considerable attention. Immune disorders lead to the development of T1D; simultaneously hyperglycemia increases the autoimmune response, leading to development of a "vicious" circle (Putilin and Kamyshnyi 2016).

The entero-insular axis a promising area of research of the link between the gut and beta cells of the pancreatic islets; it encompasses nutrient, hormonal and neural signals affecting these two structures. The key signal transductors are gastrointestinal hormones, collectively known as incretins (Catoi et al. 2015; de Laat et al. 2016). Incretins have a multi-layered effect on the balance of pro-and anti-inflammatory signals.

GLP-1, an intestinal peptide that acts as an incretin hormone, secreted in response to nutrient uptake, enhances the glucose-dependent stimulation of insulin secretion after oral nutrient uptake, and also controls blood glucose levels by inhibiting glucagon secretion and gastric emptying. Long-term treatment with GLP-1R agonists reduces food intake and contributes to weight loss (Drucker and Nauck 2006). GLP-1 acts on a specific receptor, GLP-1R, which was first detected in the β-cells and then in extrapancreatic tissues (including the liver, lungs, kidneys, heart, brain and intestines). GLP-1R transcripts are expressed in the spleen, thymus, and lymph nodes of nonobese diabetic (NOD)

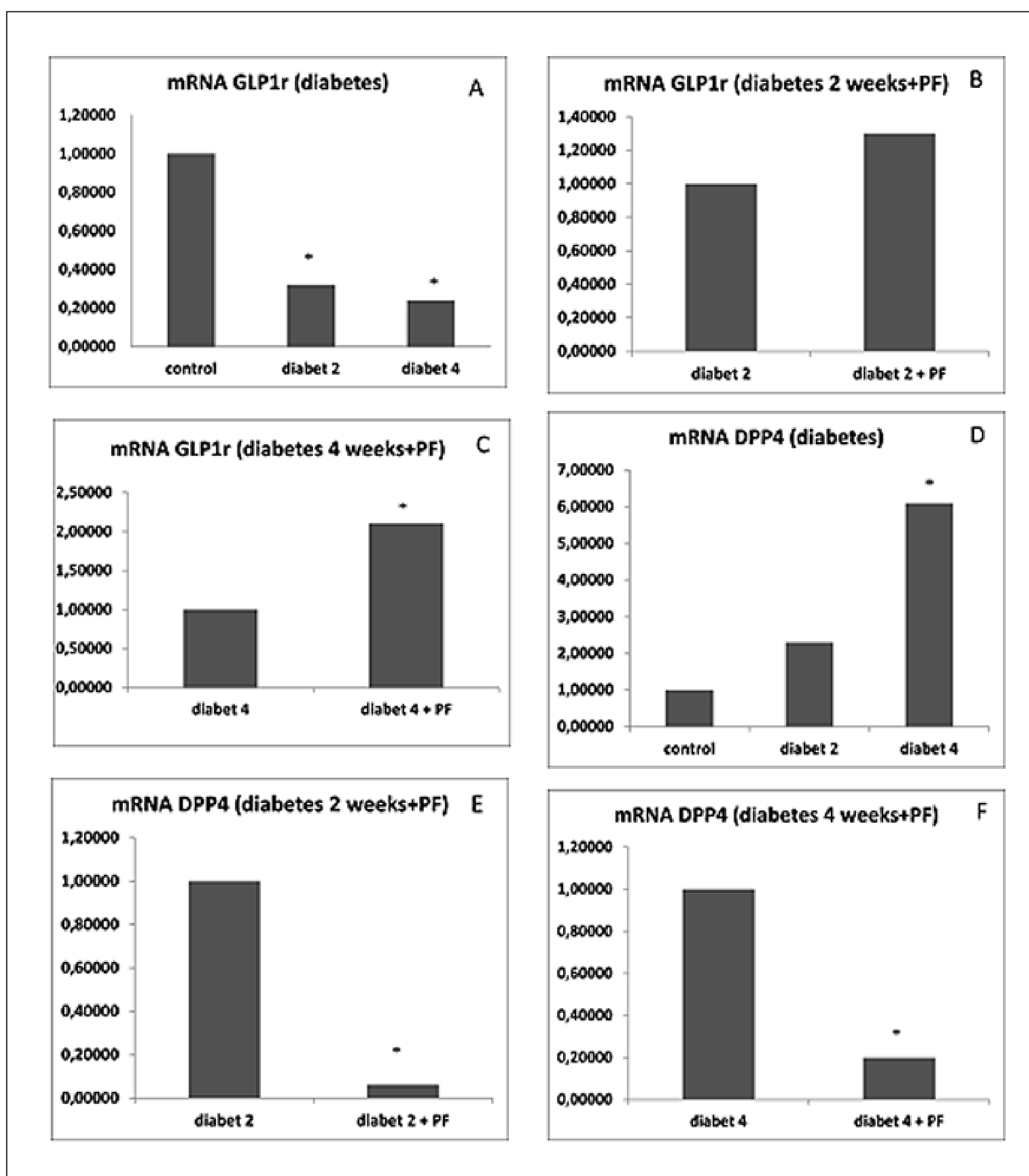

Figure 1. Relative normalized mRNA number of *GLP-1R* and *DPP-4* genes in the rat ileum cells. Normalization using the ΔΔCt method with *GAPDH* as a reference gene. d2, d4: 2nd and 4th week of EDM, respectively; d2 + pentoxifylline, d4 + pentoxifylline: after administration of pentoxifylline to diabetic animals.

and C57BL/6 mice (Hadjiyanni et al. 2008). Some subpopulations of immature lymphocytes express GLP-1R transcripts in the thymus and bone marrow. In particular, bone marrow $CD19^+$ B cells of female C57 mice are positive for GLP-1R transcripts. Regulatory T cells (Tregs), especially naturally arising $CD4^+CD25^+$ Tregs, in which expression of the transcription factor fork head box p3 ($FOXP3^+$) occurs in the thymus, actively engage in the maintenance of immunological self-tolerance and immune homeostasis (Wing et al. 2019). The lymph nodes of male $GLP\text{-}1R^{-/-}$ knockout mice show significant decrease in the number of $CD4^+CD25^+FOXP3^+$ regulatory T cells (Tregs), thus suggesting that GLP-1R signaling may play a role in the maintenance and functioning of Tregs. In addition, GLP-1R is also

active on invariant natural killer T (iNKT) cells, leading to cAMP increase and phosphorylation of cAMP-response element binding protein (CREB) transcriptional factor (Liu et al. 2008), which regulates the expression of pro-inflammatory genes (e.g. IL-10). *In vitro* experiments showed that GLP-1 and its analogues modulated the production of IFN-γ and IL-4, but did not change the killer activity of iNKT cells. This indicates an immunoregulatory rather than immunosuppressive effect of GLP-1 (Hogan et al. 2011).

The mechanisms that regulate gene transcription and enzymatic activity of DPP-4 are not yet fully understood and may depend on the type of cells being studied. However, it is known that DPP-4 promoter region contains consensus sites for various transcription factors, such as nuclear factor κB (NF-κB), specificity protein 1 (SP-1), epidermal growth factor receptor (EGFR) and activating protein-1 (AP-1)/NF-κB. Inhibition of DPP-4/CD26 has an anti-inflammatory effect on the immune cells, mediated by chemokines and anti-inflammatory cytokines such as IL-10 and transforming growth factor β (TGF-β) (Yazbeck et al. 2009). Thus, most of the effects of DPP-4 in the immune cells can be attributed to its non-enzymatic activity. However, DPP-4 inhibitors are capable of modulating innate and adaptive immunity and suppressing inflammation via NOD-, LRR- and pyrin domain-containing protein 3 (NLRP3), a multiprotein complex involved in the activation of caspase-1 and therefore affecting the maturation of pro-inflammatory cytokines, Toll-like receptor 4 (TLR4) and IL-1β in the macrophages (Aroor et al. 2013; Dai et al. 2014). Circulating DPP-4 increased *in vitro* after TNF-α and insulin stimulation. In activated lymphocytes, IL-12 promotes DPP-4 translation, while TNF-α reduces their expression on the cell surface, which may be associated with an increased release of sDPP-4 (Salgado et al. 2000). This is associated with inhibition of NF-κB-mediated production of TNF-α and IL-6 and simultaneous increase in the levels of anti-inflammatory IL-10 (El-Sahar et al. 2015).

The glucose transporter SGLT-1, which induces incretin synthesis, also has an indirect effect on the balance of pro- and anti-inflammatory signals, demonstrated on the SGLT-1$^{-/-}$ knockout mice (Roder et al. 2014). Inhibition of SGLT1 expression in the intestine decreases glucose uptake and suppresses synthesis of incretins (primarily GLP-1). However, STZ-induced diabetes induction in mice and rats increased the transcriptional activity of SGLT1 mRNA and SGLT1 expression on cell membrane, intensifying glucose uptake and increasing its levels in the blood (Powell et al. 2013; Ogata et al. 2014). Moreover, in activated T cells the increased glucose uptake promotes glycolysis, which may be associated with intensified INSR expression. This process is more

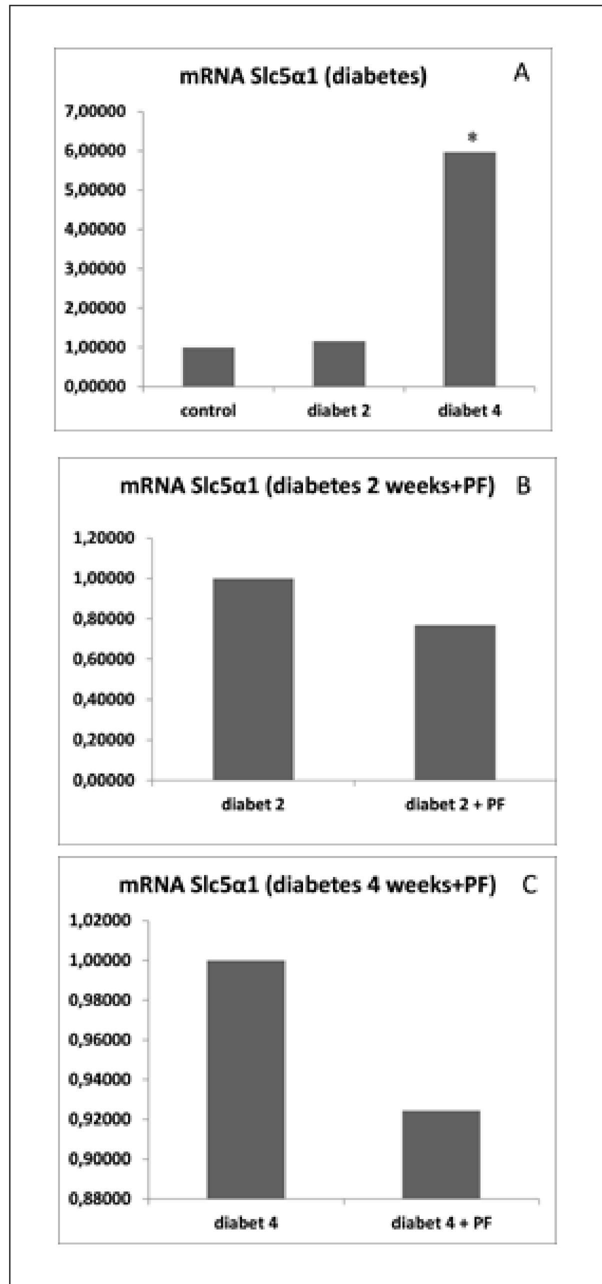

Figure 2. Relative normalized mRNA number of *SGLT-1* gene in the rat ileum cells. Normalization using the ΔΔCt method with *GAPDH* as a reference gene. d2, d4: 2nd and 4th week of EDM, respectively; d2 + pentoxifylline, d4 + pentoxifylline: after administration of pentoxifylline to diabetic animals.

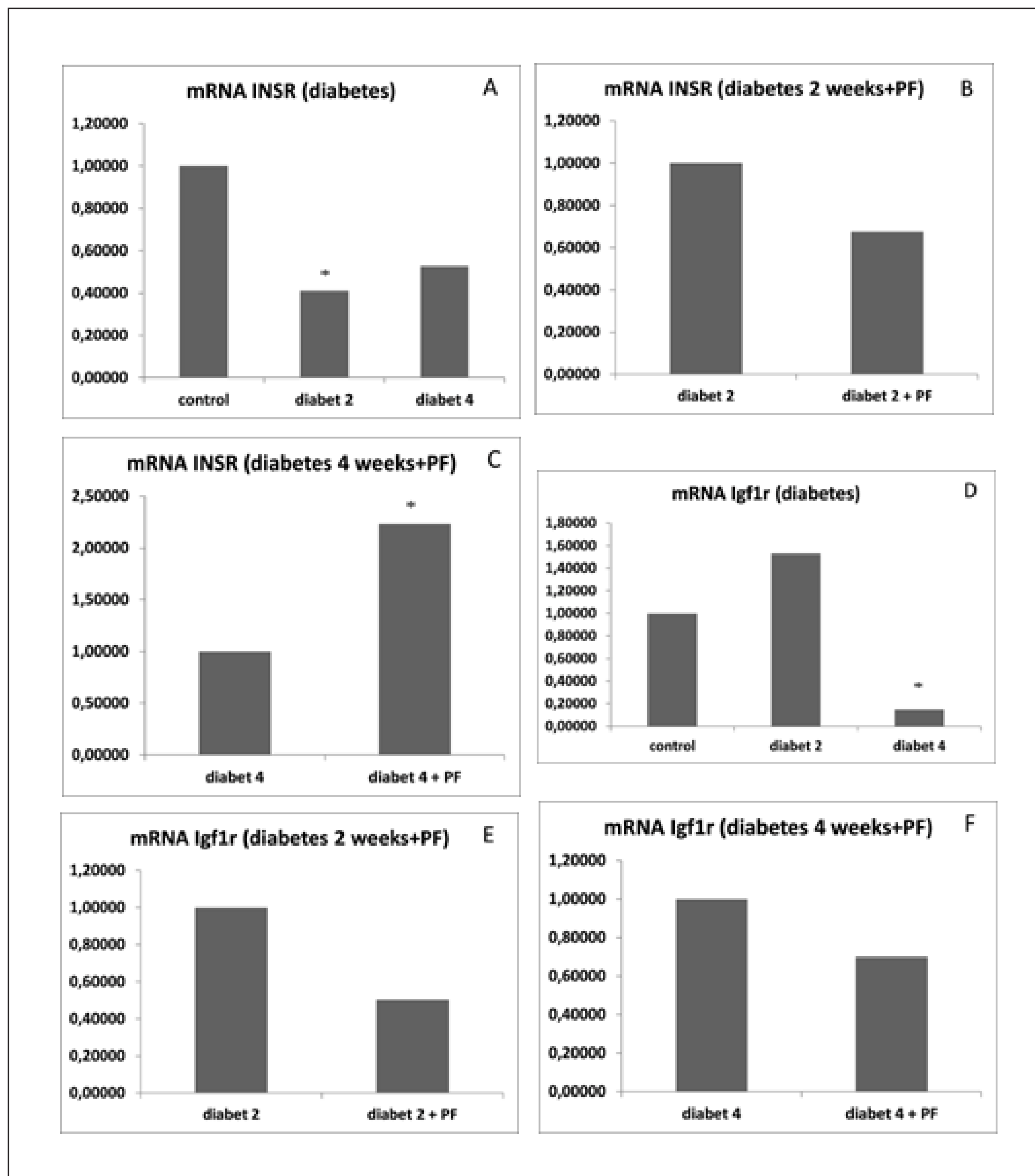

Figure 3. Relative normalized mRNA number of *INSR* and *IGF-1R* genes in the rat ileum cells. Normalization using the ΔΔCt method with *GAPDH* as a reference gene. d2, d4: 2nd and 4th week of EDM, respectively; d2 + pentoxifylline, d4 + pentoxifylline: after administration of pentoxifylline to diabetic animals.

often observed in pro-inflammatory subpopulations of T lymphocytes, since regulatory T cells are more dependent on the oxidation of fatty acids rather than on glycolysis. This can be explained by the activation of cluster of differentiation 28 (CD28) and membrane recruitment of Akt; inhibition of these pathways results in decreased expression of IL-17 (Michalek et al. 2011; Shi et al. 2011; Yang and Chi 2014). Thus, an increased glucose uptake, a typical sign of inflammatory diseases, is required for the full activation of effector T cells, which suggesting an important role of INSR in the adaptive immune response.

IGF-1 is another crucial player in maintaining the balance of pro- and anti-inflammatory signals. Most

of the effects of IGF-1 are mediated by the surface tyrosine kinase receptor IGF1R, which is expressed by peripheral mononuclear cells and human anti-CD3-activated T lymphocytes, as well as natural killer cells and CD4-positive T helper cells (Poppi et al. 2002). A number of cytokines can directly affect the synthesis of IGF-1. Since both TNF-α and prostaglandin E2 (PGE2) stimulate the synthesis of IGF-1 in macrophages, the Th2 cytokines IL-4 and IL-13 produce similar effects. In contrast, INF-γ is produced by Th1 inhibiting IGF-1 expression in macrophages with the participation of signal transducer and activator of transcription 4 (STAT4). In turn, circulating monocytes usually do not express mRNA or IGF-1 peptide, but can acquire this property under the influence of inflammatory mediators, including IL-1 and TNF-α (Wynes and Riches 2003). IGF-1 has anti-apoptotic properties against myeloid through the activation of PI 3-kinase, and inhibition of FAS expression. Thus, the IGF-1/IGF-1R system is a powerful mediator of cell transformation, adhesion and survival, acting by endocrine, autocrine and paracrine signaling (Tu et al. 2000). Studies show that treating human Treg cells with commercial recombinant human insulin-like growth factor (rhIGF) preparations, leads to increased proliferation and expression activation of FOXP3.These findings were also confirmed in Treg model mice: rhIGF-1-stimulated cells retained the ability to inhibit the proliferation of T effector lymphocytes, while at the same time the anti-apoptotic effect of rhIGF-1 was not observed (Liu et al. 1997). The proliferative effect of rhIGF-1 is mediated through surface markers of activation of Treg cells associated with the canonical PI3K signaling pathway (Smith 2010), CD71, CD44, and the homing receptor (Fisson et al. 2003). Notably, rhIGF-1 does not have a stimulating effect on CD4+CD25+ cells (Th0) or on polarized pro-inflammatory IL-17^{+} (Th17) and IFN-c-secreting (Th1) subpopulations in vitro, underscoring its power to shift the balance of regulatory/pro-inflammatory subpopulations of lymphocytes towards the anti-inflammatory side. Moreover, this action affects both circulating Treg cells and infiltrating tissues. Current biochemical studies are largely in agreement that disruption of the homeostatic balance between auto-aggressive cells and Treg cells is one of the triggers for the development of type 1 diabetes. In C57/Bl6J mice with STZ-induced type 1 diabetes, the administration of rhIGF-1 led to a gradual restoration of glycemic balance, as well as conservation of mass and micro-architectonics of glucose-sensitive insulin-producing pancreatic islets (LeRoith and Yakar 2007). However, in non-diabetic animals, the administration of rhIGF-1 did not affect glucose levels. Early administration of rhIGF-1 to NOD mice prone to a spontaneous development of type 1 diabetes at the age of 9–12 weeks led to a delay in the development of this pathology. In diabetic animals of the same line, rhIGF-1 therapy led to a decrease in glycaemia, and, ultimately, mortality.

It has been found that the ratios of CD4+CD25^{hi}Treg/Th17 cells and CD4^{+}CD25^{hi}Treg/Th1 cells were significantly decreased in T2DM patients (Zeng et al. 2012). Qiao et al. (2016) found that the patients with T2DM had increased serum levels of IL-6, TGF-β, and TNF-α, but decreased percentage of peripheral CD4^{+}CD25^{+}Foxp3^{+}Treg and serum IL-10 level. TNF-α, through increasing the activities of the NF-κB transcriptional factor, protein kinase C, amino terminal kinase and inhibitor kinase, could cause serine/threonine phosphorylation of the insulin receptor substrate, interfere with normal phosphorylation of tyrosine, and weaken signal transduction of insulin, resulting in insulin resistance, and thus participates in the pathogenesis of T2DM and obesity (Yuan et al. 2010). On the other hand, TNF-α may be result in the destruction of pancreatic beta cells and lead to the development of T1DM (Qiao et al. 2017). Lechleitner et al. (2000) have demonstrated TNF-α levels were elevated in T1DM which was correlated positively with HbA1c.

One of the promising areas of diabetes therapy is the use of TNF-α inhibitors. PTX is a non-selective phosphodiesterase inhibitor and has a special effect on immune responses through TNF-α suppression (Hosseini et al. 2019). Experimental studies have shown that its administration causes immune modulation in a dose-dependent manner. This is exemplified by increased leukocyte deformability and chemotaxis, decreased endothelial leukocyte adhesion, neutrophil degranulation, TNF-α production and NK cell activity (Brie et al. 2016; Chen et al. 2017).

Garcia et al. (2015) showed that PTX reduces inflammatory factors such as TNF-α, interleukin-6, and inducible nitric oxide synthase, which can improve diabetic neuropathy by reducing inflammation. On the other hand, Han et al. (2015) did not observe any change in serum TNF-α associated with PTX treatment. A meta-analysis of PTX for treating nonalcoholic fatty liver disease also failed to show changes in serum TNF-α levels (Zeng et al. 2014). The reasons for these inconsistent results are unclear.

Our results showed that development of diabetes was accompanied by the decrease of GLP-1R and

an increase of DPP-4 genes expression in rat ileum. Administration of PTX to diabetic animals led to increasing in transcriptional activity of GLP-1R on the 4th week and decreasing in transcriptional activity of DPP-4 on the 2nd and 4th weeks of the experiment. An increase in the normalized expression of SGLT-1 on the 4th week of the experimental diabetes was also noted in case of PTX treatment. The transcriptional activity of the INSR and IGF-1R genes was reduced in diabetic rats and the administration PTX led to a significant increase only for INSR gene in animals on the 4th week of the experiment. It indicates that PTX is a potential therapeutic alternative for treatment of type 1 diabetes mellitus and further research is required to provide its effectiveness.

Conclusions

The results of this investigation demonstrate that the expression of incretins, glucose transporters, and pro-inflammatory cytokines (e.g. TNF-α) in immune cells may be used as markers of several autoimmune pathologies progression such as type 1 diabetes mellitus due to their effect on the balance of pro- and anti-inflammatory factors, including the level of cytokines, modulation of cell differentiation of both the adaptive and innate immunity, and homing of immunocompetent cells. Pentoxifylline is a potential therapeutic alternative for treatment of type 1 diabetes mellitus or other autoimmune pathology characterized by excessive production of proinflammatory cytokines.

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