Redox-Potential and Immune-Endothelial Axis States of Pancreases in Type 2 Diabetes Mellitus in Experiments

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Abstract

Background: Disturbances in mitochondrial complex I functioning plays a crucial role in the pathogenesis of β-cells dysfunction in pancreas in diabetic mellitus (DM), and the redox-potential is a contributing factor to redox imbalance, pseudo hypoxia and chronic inflammation. The objective of this study was to assess the correlation between changes in redox-potential and inflammation response of blood and pancreases in streptozotocin (STZ)-nicotinamide (NA) induced DM in rats and ability of various pharmacological agents to its correction.

Materials and methods: In randomized controlled study in rats with DM type 2 (T2DM) induced by i.p. injection of 110 mg/kg NA 15 min before intravenously injection of 65 mg/kg of STZ the redox-immune axis disturbances and efficacy of various pharmacological agents to its correction was study. The selected cohort of T2DM animals were randomized into 5 groups dependent of 21 days receiving therapy: control II - 1 ml of 0.9% NaCl, main I - metformin 350 mg/kg, main II – glibenclamide 0.6 mg/kg, main III- Nadcin® 16 mg/kg, main IV- metformin, 100 mg/kg +Nadcin® 16 mg/kg, and V main- glibenclamide 0.3 mg/kg and Nadcin®, 16 mg/kg.

Results: Treatment with glibenclamide, metformin, Nadcin® or its combination significantly decreased the glucose and increased insulin levels. Nadcin® alone or in combination brought towards normal levels of HbA1c and endothelin-1 (ET-1), fully restored the pool of oxidized NAD(P) and the level of redox-potentials. Changes in the level of ET-1 correlated with deterioration of redox-potential NAD/NADH and NADP/NADPH in pancreatic cells. Treatment with Nadcin® decreased the level of TNF-α, nuclear factor kappa B (NF-κB) and increased the level of IL-10. The same effect observed in combined treatment of Nadcin® with antihypoglicemic drugs in ½ doses of its action in monotherapy. Treatment with metformin dease the level of IL-6, but not TNF-α and NF-κB activity.

Conclusion: Redox potential imbalance represents a therapeutic target for T2DM and trigger for disturbances in innate immunity system activity. Course treatment with NAD-containing drug, Nadcin®; Redox-potential; Pancreas; Cytokine; Metformin; Glybenclamide

Keywords

Experimental type 2 diabetes mellitus model; NAD-containing drug; Nadcin®; Redox-potential; Pancreas; Cytokine; Metformin; Glybenclamide

Abbreviations: AMPK: AMP-Activated Protein Kinase; CD38: Cluster Of Differentiation 38 (Cyclic ADP Ribose Hydrolase); DM : Diabetes Mellitus; ET : Endothelin; IL: Interleukin; Foxp3-Transcriptional Factor; JNK: C-Jun N-Terminal Kinase; I.P.: Intraperitonal; LKB 1: Liver Kinase B1; NA: Nicotinamide; NF-κB: Nuclear Factor Kappa B; P38: Activating Transcription Factor-2; P42/44: Mitogen-Activated Protein Kinase; PARP: Poly-(ADP-Ribose) Polymerase; ROS: Reactive Oxygen Species; Sirt – Sirtuin; STZ: Streptozotacitin; TNF-α: Tumor Necrosis Factor α

Background

The pathogenesis of diabetes mellitus (DM), as a complex metabolic heterogeneous disease in lipids, carbohydrates and proteins, involves a defective secretion of insulin in pancreatic β cells with the following progressive development of circulatory hyperglycemia and insulin resistance. According to statistics, there will be 8 billion individuals suffering from type II diabetes mellitus (T2DM) in 2030 [1]. Insulin resistance, developing in T2DM, initially compensated by increased insulin secretion, and blood glucose levels are near normal or moderately increased. One of the key problem is that persistent glucose overload state leads to decrease reserve ability of the redox-homeostasis maintenance systems, which provide electrons for respiratory chain activation and ATP synthesis in mitochondria’s or in glycolysis pathways in cytoplasm’s. Disturbances in mitochondrial complex I functioning play a crucial role in the pathogenesis of β cells dysfunction in diabetic pancreas [2,3]. Oversupply of NADH and decreasing redox-potential NAD/NADH play key trigger point for started the vicious cycle of both conventional glucose metabolism and polyol pathways activities deterioration in the pathogenesis of diabetes and its complications [2-8]. Glucose metabolized and using as an energy source in pancreatic β cells and cells derived from glucose metabolism are stored in NADH and FADH2 which then recycled by mitochondrial complex I and maintaining NAD/ NADH redox balance, glucose sensing, activity of NAD-dependent enzymes such as sirtuins, CD38 and polyADP ribose polymerase 1 (PARP-1) [9]. Pancreas characterized very low lactate dehydrogenase activity and ability for regenerate NAD for glycolysis. The polyol pathways reaction of sorbitol oxidation, in which under diabetes metabolized about 30% of the glucose also used NAD, and decreasing redox-potential NAD/NADH. Thus redox-potential, NAD/NADH became the contributing factor to pseudohypoxia development, hyperproduction of reactive oxygen species (ROS), oxidative damage of DNA and lipids, membrane permeability transition pore opening and induced downregulation activities of NAD-dependent enzymes [5-9]. On the other hand, decreasing of redox-potential and overactivation of PARP-1 under oxidative stress formation secondly diminish or depleted the level of cellular NAD that potentially will be trigger for disturbances of functioning of sirtuins (Sirt) and innate...
immunity system [7,9]. Imbalance in the second redox potential NADP+/NADPH ratio in pancreatic β-cells in response to rises of glucose in extracellular medium directly stimulated Ca²⁺-regulated exocytosis of insulin granules whereas alterations in the NAD/NADH ratio do not have such an effect. Effects of NADPH on exocytosis are proposed to be mediated by the redox proteins glutaredoxin (GRX, expression of GRX mRNA is very high in β-cells) and thioredoxin (TRX) which are localized in distinct micro domains in the cytosol of β-cells [8]. Microinjection of recombinant GRX potentiated effects of NADP/NADPH imbalance effect on exocytosis, whereas TRX antagonized the redox-imbalance effect [5,7-9]. The establishment of chronic pseudo hypoxic condition under T2DM states could play triggers role for chronic inflammation in diabetic pancreas via redox potential dependent activation of TNF-α/NF-kB signaling pathways [10-17]. Intracellular NAD concentration regulates TNF-α synthesis includes action at post-transcriptional step in Sirt dependent manner [14-17]. In fact, the pancreatic cells are produced TNF-α in both normal rat pancreas and is characterized by hyper production of endogenous TNF-α, IL-1β and IL-6 in the pancreatic acini under T2DM [9] and is causally involved in the development of insulin resistance [8-11]. The first steps in TNF-α signaling activation is elevation the activity of the nuclear transcription factor kappa B (NF-kB) with following stimulation of NF-kB translocation into the nuclei and potentiation apoptotic cell death in acinar pancreatic cells [18,19]. In according with above mentioned, in this study we investigate the dependence between changes in redox-potential and TNF-α/NF-kB signaling pathways in the pancreatic and blood on the model of T2DM in experiments, and possibility of its pharmacological correction.

**Materials and Methods**

**Experimental design**

In the cohort controlled randomized study were included 65 male Wistar rats, weighing 270-320 g. Animals received humane care in compliance with “Guide for the Care and Use of Laboratory animals” (National Institutes of Health publication 86-23, Revised 1996) and was performed with approval of the local Interinstitutional (International Scientific Centre of Introduction of New Biomedical Technology, Department of Pharmacology, Faculty of Medicine, I.Javakhishvili Tbilisi State University and Department of Medical Pharmacology and Pharmacotherapy, Tbilisi State Medical University, Tbilisi) Ethics Committee. The animals were acclimatized to the animal room condition for at least a week at 25 ± 2°C with 12-hour light/12-hour dark cycles prior to the experiment. All animals were supplied with commercial pellet food and water ad libitum. Induction of diabetes type 2 in experiments, and possibility of its pharmacological correction.

**Organs weighting and preparation of tissues homogenate**

Immediately after euthanization and pancreases were dissected, rinsed in ice-cold isotonic saline, weighted, then fragmented, and stored at -70°C. For measurement of pyridine nucleotide of pancreases and plasma tissues were frozen and homogenized in liquid nitrogen, before the experiments homogenate rapidly placed in modified Krebs’ solution [22]. After weighing, each of tissues were minced and homogenate using Teflon homogenizer were prepared with 10% (w/v) of 1% Triton X-100 phosphate buffer (pH 7.4) containing (mmol/L) CaCl₂, 2.5 mmol/L, HEPES 25 mmol/L, and KCl 0.1 mmol/L solution and centrifuged at 9,000g for 30 min at 4°C. The supernatant was pooled and used for the estimations. The Bradford method (Coomassie brilliant blue G-250 staining) was used to detect protein content and calculate the protein excretion at 24 h.

**Determination of pancreatic pyridine nucleotide and cytokine**

Pyridine nucleotides, cytokines and level of Endothelin-1 (ET₁) determination in tissue homogenates and plasma were determinate as described [23-25].

**Amylase in blood and pancreas**

Pancreatic Amylase content in blood and pancreatic lobules were determined according to a method described [26-27].

**Blood parameters**

Plasma glucose, insulin and glycosinated and blood glycosylated hemoglobin was estimated using commercial kits. Serum alkaline phosphatase (ALP) was determined by methods described [28].
Statistical analysis

All values were expressed as the mean ± standard deviation. Analyses were performed using SPSS software, version 21.0 (SPSS, Inc., Chicago, IL, USA). Statistical analysis for comparison between different groups of animals was assessed by two-way unpaired Student t-test. P<0.05 was considered to indicate a statistically significant difference.

Results

Body weight, food intake and general characteristics and efficacy of therapy

The body weight in STZ-NA-induced diabetic rats during 4 weeks of experiment significantly reduced in comparison to practically healthy rats, which accompanied with a marked increase in plasma glucose levels by 75% and decrease in insulin level by 43% than in control group. Neither glibenclamide nor metformin prevented weight loss in diabetic rats, while including of Nadcin® significantly improved body weight (p<0.05) as alone in combination with glibenclamide or metformin (Table 1). The diabetic control rats consumed significantly more pellets than the non-diabetic control rats. Although the amount of pellets consumed by the T2DM rats treated with glibenclamide, metformin, Nadcin®, or their combinations was less compared to that consumed by the diabetic control rats, there was no significant difference.

Blood parameters

Treatment with glibenclamide, metformin, or Nadcin® significantly decreased the glucose levels by 34, 35 and 39% respectively and increased insulin levels by 33, 32 and 78% respectively. Treatment with combination of glibenclamide or metformin with Nadcin® reduced the glucose concentrations (5.8 ± 1.5 or 6.9 ± 1.3 mmol/L, respectively) and increased insulin levels (0.53 ± 0.04 or 0.50 ± 0.05 mmol/L, respectively). The HbA1c increased significantly in diabetic rats and after treatment with Nadcin®, and combined therapy were brought towards normal levels.

Efficacy of various algorithm of treatment on the pancreatic tissue

Plasma and pancreatic amylase activity decrease after 4 weeks of STZ-NA-induced diabetic and did not changes significantly under monotherapy with hypoglicemic drugs. Including in the therapy Nadcin® fully restored the pool of oxidized NAD(P) and the level of redox-potentials in pancreases tissues and as a results abolished the STZ-induced beta-cell deterioration, improved insulin secretion and amylase activity. Treatment with combined therapy Nadcin® and glibenclamide or metformin fully reverse the decreasing of insulin secretion in pancreatic tissue and relative weight of pancreatic which associated with increased the level of NAD or NADP and redox-potential, but the effect did not exceed the action of Nadcin® in monotherapy. The level of insulin secretion in pancreatic positive correlated with redox-potential NADP/NADPH (r=-0.74, p<0.001) and NAD/NADH (r=-0.69, p<0.01). Glibenclimide and metformin decrease the level of IL-6 by 22% and 21% and did not influence on the level of TNF and antiinflammatory IL-10. Treatment with Nadcin® decreased the level of TNF-a, NF-kB and increased the level of IL-10. The same effect observed in combined treatment of Nadcin® with antihyperglycemic drugs in ½ doses of its action in monotherapy. Metformin or glibenclamide also did not significantly improved level of vasoconstrictory component of endothelial system, and changes of levels of ET-1 strongly linear correlated with deterioration of redox-potential NAD/NADPH (-0.71, p<0.001) and NADP/NADPH (-0.81, p<0.0001). It is necessary to pay attention that the blood redox-potential decreasing though is unidirectional with changes in a pancreatic tissue, but is considerably less expressed. Reliable change of level of TNF-a in plasma doesn’t observed, and the level of IL-6 and IL-1β increased by 50% and 30% respectively while in pancreatic in more than two fold for both cytokines. Plasma levels of TNF-a and IL-1β in STZ-NA-induced diabetes rats were significantly increase, while the level of antiinflammatory IL-10 did not change (Table 2). In pancreas levels of TNF-a and IL-6 in STZ-NA-induced diabetes rats increased more than on, periphery and level of IL-10 significantly decrease. The rises of level of TNF- associated with the increase level of NF-kB (65). Treatment with metformin decrease the level of IL-6, but not TNF-a and NF-kB. Early TNF-a and IL-6 were important mediators of insulin resistance, as they could induce serine phosphorylation of insulin receptor substrate (IRS) through activation of JNK or NF-kB pathway [14-16]. Furthermore, overproduction of TNF-a and IL-6 in pancreas could cause islet dysfunction and accelerate the progression of diabetes [12-14].
Discussion

Disturbances in mitochondrial complex I functioning plays a crucial role in the pathogenesis of β cells dysfunction in diabetic pancreas [2-7,9]. Glycolysis metabolized about 30% of the glucose, also needs NAD and NADP/NADPH, because the activity of lactate dehydrogenase is very lower and mitochondrial NAD(P)-dependent glycerol-3-phosphate dehydrogenase (GPDH, family of this enzymes represents the N-terminal NAD-binding domain) is 40–70 times higher in comparison to other tissues [26,29]. Glibenclamide, metformin significantly reduced blood glucose concentrations in our study which is similar to findings from previous studies [30,31].

Glibenclamide is a second-generation sulfonylurea that reduces blood glucose level and hepatic glucose production by stimulation of insulin secretion in pancreatic cells via the antagonizes ATP-dependent potassium channels [30]. Protective mechanism of glibenclamide's also included two main anti-inflammatory pathways: reducing IL-1β production and attenuation IL-8 production and neutrophilic and monocytic influx into the lung, and the second, glibenclamide’s also included two main anti-inflammatory pathways: reducing IL-1β production and attenuation IL-8 production and neutrophilic and monocytic influx into the lung, and the second, reducing systemic vasodilatation and maintaining normal peripheral vascular resistance [30,31]. However, the use of glibenclamide is limited due to prolonged hypoglycemia, high secondary failure rate and other adverse events [32]. Metformin is an oral hypoglycaemic agent that exhibits an antihyperglycemic effect devoid of insulin

Table 2: Pancreatic redox-immune-endothelial axis disturbances in animals with STZ-NA-induced diabetes and influence of different treatment.

<table>
<thead>
<tr>
<th>Group/parameters</th>
<th>Control 1</th>
<th>Diabetic type 2</th>
<th>+ M</th>
<th>+GC</th>
<th>+Nadcin</th>
<th>+M +Nadcin</th>
<th>+GC +Nadcin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amylase activity, U/mg wet weight</td>
<td>39 ± 10</td>
<td>18 ± 4</td>
<td>25 ± 5</td>
<td>29 ± 4</td>
<td>36 ± 3*</td>
<td>39 ± 4*</td>
<td>37 ± 6*</td>
</tr>
<tr>
<td>NAD, µMol/mg protein</td>
<td>4.9 ± 0.4</td>
<td>3.1 ± 0.6</td>
<td>2.9 ± 0.4</td>
<td>3.1 ± 0.4</td>
<td>4.7 ± 0.7**</td>
<td>4.4 ± 0.4</td>
<td>4.4 ± 0.5*</td>
</tr>
<tr>
<td>NADH, µMol/mg protein</td>
<td>4.7 ± 1.0</td>
<td>6.4 ± 0.9*</td>
<td>6.9 ± 0.7</td>
<td>6.2 ± 0.7</td>
<td>5.2 ± 0.7</td>
<td>5.0 ± 0.5</td>
<td>5.1 ± 0.3</td>
</tr>
<tr>
<td>NAD/NADH</td>
<td>1.05 ± 0.09</td>
<td>0.48 ± 0.08**</td>
<td>0.43 ± 0.07**</td>
<td>0.50 ± 0.09**</td>
<td>0.90 ± 0.07***</td>
<td>0.88 ± 0.09***</td>
<td>0.86 ± 0.08***</td>
</tr>
<tr>
<td>NADP/NADPH</td>
<td>5.20 ± 0.27</td>
<td>3.32 ± 0.14</td>
<td>3.69 ± 0.10</td>
<td>3.60 ± 0.10</td>
<td>5.18 ± 0.19</td>
<td>5.03 ± 0.19</td>
<td>5.04 ± 0.16</td>
</tr>
<tr>
<td>NADPH, µMol/mg protein</td>
<td>5.10 ± 0.19</td>
<td>6.8 ± 0.11**</td>
<td>6.84 ± 0.15**</td>
<td>6.82 ± 0.16**</td>
<td>5.4 ± 0.3**</td>
<td>5.5 ± 0.2**</td>
<td>5.2 ± 0.4**</td>
</tr>
<tr>
<td>NADP/NADPH</td>
<td>1.02 ± 0.08</td>
<td>0.49 ± 0.07**</td>
<td>0.54 ± 0.04**</td>
<td>0.53 ± 0.05**</td>
<td>0.96 ± 0.06**</td>
<td>0.91 ± 0.07**</td>
<td>0.96 ± 0.05**</td>
</tr>
<tr>
<td>NF-kB (p65), ng/mg protein</td>
<td>4.8 ± 0.2</td>
<td>5.9 ± 0.4</td>
<td>6.1 ± 0.4</td>
<td>6.0 ± 0.5</td>
<td>4.8 ± 0.3</td>
<td>4.7 ± 0.4</td>
<td>4.7 ± 0.6</td>
</tr>
<tr>
<td>TFN-α, pg/mg protein</td>
<td>10.0 ± 1.1</td>
<td>20.8 ± 1.9**</td>
<td>19.8 ± 1.1**</td>
<td>18.9 ± 1.3**</td>
<td>12.1 ± 1.0</td>
<td>12.8 ± 1.0</td>
<td>11.9 ± 1.9**</td>
</tr>
<tr>
<td>IL-1β, pg/mg protein</td>
<td>3.6 ± 1.1</td>
<td>7.8 ± 1.9**</td>
<td>4.9 ± 0.5</td>
<td>4.8 ± 0.6</td>
<td>4.8 ± 0.3</td>
<td>4.7 ± 0.4</td>
<td>4.7 ± 0.6</td>
</tr>
<tr>
<td>IL-6, pg/mg protein</td>
<td>9.6 ± 1.2</td>
<td>17.8 ± 1.9**</td>
<td>18.6 ± 1.4**</td>
<td>16.9 ± 1.2**</td>
<td>10.4 ± 1.2**</td>
<td>10.0 ± 1.0**</td>
<td>11.1 ± 0.9**</td>
</tr>
<tr>
<td>IL-10, pg/mg protein</td>
<td>9.6 ± 1.2</td>
<td>4.8 ± 0.9</td>
<td>3.86 ± 0.60**</td>
<td>4.12 ± 0.73**</td>
<td>11.6 ± 1.4**</td>
<td>10.6 ± 0.7**</td>
<td>9.9 ± 0.9**</td>
</tr>
<tr>
<td>ET-1, fg/mg protein</td>
<td>1.89 ± 0.11</td>
<td>4.16 ± 0.06*</td>
<td>4.06 ± 0.09*</td>
<td>3.93 ± 0.05*</td>
<td>1.93 ± 0.08**</td>
<td>1.97 ± 0.08**</td>
<td>± 1.97 ± 0.07**</td>
</tr>
</tbody>
</table>

Note: each value remains mean standard deviation, symbols - significance of difference between the group, “*” - with control, “#” - with the STZ-NA-induced diabetes; one symbol - p<0.05, two - p<0.01; three - p<0.001.
release [33-35]. After being delivered to the liver from the intestines, metformin can inhibit gluconeogenesis by activating hepatic AMPK through liver-kinase B1 (LKB1) and decreased energy charge. Early, it was shown that exogenous NAD and Nadin® as NAD containing drug, inhibited cardiac hypertrophy progression and transition in maladaptive form throughout direct positive action on the SIRT-LKB1 signaling pathways and both of enzymes is NAD-dependent [24,36]. The intracellular target of action of metformin in high concentrations (5 mM/L) via the inhibition of NAD(H) coenzyme Q oxidoreductase (complex I) in the mitochondrial electron transport chain which also is fully dependent of redox-potential NAD(P)/NAD(P)H. The third intracellular target of metformin is mitochondrial glycerol phosphate dehydrogenase (mG3PDH), will affect transport of NADH from the cytoplasm into mitochondrion, suppressing gluconeogenesis process from lactate, and is much closely related to action of Nadin®. Moreover, the capacity of cells to produce TFN-a appears to be directly correlated with intracellular NAD levels, and at the same time NAD, as a cofactor of PARP-1, has been shown to act as a transcriptional modulator of NF-kB [25,37]. Despite the reduced number of regulatory T cells and an increased number of proinflammatory T helper 17 cells under immune homeostasis disturbances in various diseases, NAD able to promote an impressive allograft survival through a robust systemic IL-10 production independent of CD4+CD25+Foxp3 cells. This effect is mediated via adenosine receptors and main at the posttranscriptional and did not involve interference with the activation of p38, p42/44, c-Jun N-terminal protein kinase, degradation of inhibitor of kapa B, or elevation of intracellular AMP levels [37].

Conclusion

Reverse the ability of pancreases cells to maintenance polyol pathway-induced redox changes decreased redox potential NAD/NADH and NADP/NADPH or hyperglycemia-induced other mechanism of ROS hyperproduction leads in the basis of improvement for the other biochemical abnormalities, including activation of proinflammatory cytokines and suppress antiinflammatory component synthesis in pancreases and endothelium 1 synthesis in endotheliocytes in T2DM. We therefore hypothesized that Nadin® may possess therapeutic effects on diabetes and diabetes-associated nephritis. In particular, it was assessed how oxidative factors and inflammatory mediators were affected during model establishment and drug administration.

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Ethics Approval and Consent to Participate

The experimental protocols were approved by the Interinstitutional Animal Ethics Committee (IIAEC) (ICIBMCT/S/9/13) at International Centre of Introduction of New Biomedical Technology, Tbilisi, Georgia.

References


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