Endocrinology & Diabetes Research

Research Article

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

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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.: Intraperitoneal; P.O.: Per Oral; LKB 1: Liver Kinase B1; NA: Nicotinamide; NF-kB: 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: Streptozotacin; TNF-α: Tumor Necrosis Factor α

Abstract

Background: Disturbances in mitochondrial complex1 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-kB) and increased the level of IL-10. The same effect observed in combined treatment of Nadcin® with antiheprglicemic drugs in ½ doses of its action in monotherapy. Treatment with metformin decease the level of IL-6, but not TNF-α and NF-kB 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® restores functioning of pancreases cells and inhibits proinflammatory cytokines releases that does not occurs under treatment with oral hypoglycemic agents.

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Background

The pathogenesis of diabetes mellitus (DM), as a complex metabolic heterogeneous disease in lipids, carbohydrates and proteins, involve 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 1 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 metabolic 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 electrons 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 deceasing 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 Ca^{2+}-regulated exocytosis of insulin granules while alterations in the NAD/NADH ration 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-imbalanced 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 is 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 aactivation 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 Interinsitutional (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 using combined treatment with streptozotocin (STZ)-nicotinamide(NA), as much closely similar pathophysiological situation to T2DM in human [3,10,20-22]. T2DM was induced by i.p. injection of 110 mg/kg body weight NA (Sigma, Saint Louis, MO, USA) 15 min before intravenously injection of 65 mg/kg body weight in 0.1M sodium citrate buffer, pH 4.5 STZ (Sigma, Saint Louis, MO, USA) in overnight fasted male rats in which food and water intake were closely monitored. The animals in control groups receive an equivalent amount of citrate buffer. NA is an antioxidant, which exerts protective effect on the cytotoxic action of STZ by scavenging free radicals and causes only minor damage to pancreatic beta cell mass and producing T2DM. Control, practically healthy animals received both vehicles. A week later, rats were fasted overnight and were treated intragastrically with 2 g glucose per kg body weight. After 30 min, blood samples were taken from the tail vein and glycaemia

was determined using a glucometer with a portable glucometer (Accu-Chek, Roche, Germany). STZ-NA-induced diabetic animals with moderate and stable non-fasting hyper glycaemia (blood glucose level ≥ 12.0 mmol/L) with simultaneously reducing blood insulin level no more than 43% and developed symptoms of T2DM such as polyphagia, polydipsia, polyuria, and weight loss were considered diabetic and included in the study. In the control rats, blood glucose 30 min after glucose load was about 7-8 mM. The selected cohort of rats with T2DM were randomized into 5 groups dependent of receive therapy: control II group, receiving 1 ml of 0.9% NaCl (n=9), main I group, receiving metformin 350 mg/kg in aqueous suspension orally (n=11), main II – receiving glibenclamide 0.6 mg/kg in aqueous suspension orally (n=7), main III- receiving Nadcin®, 16 mg/kg (n=9), main IV- receiving metformin 100 mg/kg +Nadcin* 16 mg/kg salved in 1 ml of water for injection, intraperitonially (n=8), and V main – receiving glibenclamide 0,3 mg/kg and Nadcin*, 16 mg/kg of body weight (n=9). Animals of all groups were treated 21 days after diabetes was reproduced. During experimental period, body weight, blood glucose and water consumption and physical examinations were determinate every week. At the end of the treatment period, the animals were fasted for at least 16 hours and euthanized by injection of 60 mg/kg pentobarbitone sodium i.p.

NADCIN®, lyophylizate for preparation salin for i/v and i/m injection, containing 0.5 mg oxidized form of NAD, inosine 80.0 mg and 10.0 mg sodium chloride, in 5 ml ampoule (patent WO 200789166 A1 Sukoyan G.V. Medicinal preparation for regulating a systemic inflammatory response syndrome. 2007-08-09), manufactured by "Biotechpharm GE", Ltd, Georgia

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) $CaCl_2$ 2.5 mmol/L, HEPES 25 mmol/L, and KCl 0.1 mmol/L solution and centrifuged at 9,000*g* 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 (ET_1) 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 characterists 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 as 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-α, NF-kB and increased the level of IL-10. The same effect observed in combined treatment of Nadcin® with antiheprglicemic 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/NADH (r=-0.71, p<0.001) and NADP/NADPH (r=-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-α 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-α and IL-1β in STZ-NA-induced diabetes rats were significantly increase, while the level of antiinflamatory IL-10 did not change (Table 2). In pancreas levels of TNF-α 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 decease the level of IL-6, but not TNF-α and NF-kB. Early TNF-α 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-κB pathway [14-16]. Furthermore, overproduction of TNF-α and IL-6 in pancreas could cause islet dysfunction and accelerate the progression of diabetes [12-14].

Table 1: General characteristics of animals with STZ-NA-induced diabetes and influence of different treatment

Group/parameters	Control 1	Diabetic type 2, treatment					
		Control II	+ M	+GC	+Nadcin®	+M +Nadcin®	+GC +Nadcin®
Body weight (BW), g	289 ± 10	261 ± 9	269 ± 10	271 ± 9	285 ± 11	286 ± 8	290 ± 10
Blood glucose, mmol/L	4.7 ± 1.0	9.4 ± 0.9***	6.9 ± 2.9***	6.2 ± 2.7**##	5.9 ± 2.1**##	5.8 ± 1.5 **##	6.9 ± 1.3**##
HbA1c, %	5.8 ± 1.0	11.1 ± 1.0***	8.9 ± 1.0*#	7.9 ± 0.9**#	6.2 ± 0.7**##	6.0 ± 0.6**##	6.0 ± 0.8**##
Plasma insulin, ng/ml	1.90 ± 0.07	1.32 ± 0.14	1.69 ± 0.10	1.60 ± 0.10	1.78 ± 0.09	1.83 ± 0.09	1.84 ± 0.09
Insulin pancreases, ng/mg wet weight of tissues	100 ± 9	76 ± 6***	87 ± 5**	86 ± 4**	94 ± 5##	95 ± 5##	92 ± 7##
Pancreases weight(PW), g PW/BWx10^2	1.49 ± 0.19 0.52 ± 0.04	1.14 ± 0.19* 0.43 ± 0.04*	1.21 ± 0.19* 0.45 ± 0.05	1.23 ± 0.19* 0.46 ± 0.03	1.43 ± 0.19*# 0.50 ± 0.02#	1.40 ± 0.19*# 0.49 ± 0.03#	1.42 ± 0.19*# 0.51 ± 0.04#

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.

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

Group/parameters	Control 1	Diabetic type 2					
		Control II	+ M	+GC	+Nadcin	+M +Nadcin	+GC +Nadcin
Amylase activity, U/mg wet weight	39 ± 10	18 ± 4	25 ± 5	29 ± 4	36 ± 3#	39 ± 4#	37 ± 6#
NAD, µMol/mg protein	4.9 ± 0.4	3.1 ± 0.6***	2.9 ± 0.4***	3.1 ± 0.4***	4.7 ± 0.7##	4.4 ± 0.4#	4.4 ± 0.5#
NADH, µMol/mg protein	4.7 ± 1.0	6.4 ± 0.9***	6.9 ± 0.7	6.2 ± 0.7	5.2 ± 0.7	5.0 ± 0.5	5.1 ± 0.3
NAD/NADH	1.05 ± 0.09	0.48 ± 0.08***	0.43 ± 0.07***	0.50 ± 0.09***	0.90 ± 0.07###	0.88 ± 0.08###	0.86 ± 0.08###
NADP, µMol/mg protein	5.20 ± 0.27	3.32 ± 0.14	3.69 ± 0.10	3.60 ± 0.10	5.18 ± 0.19	5.03 ± 0.16	5.04 ± 0.16
NADPH, µMol/mg protein	5.10 ± 0.19	6.8 ± 0.1***	6.84 ± 0.15**	6.82 ± 0.16**	5.4 ± 0.3##	5.5 ± 0.2##	5.2 ± 0.4##
NADP/NADPH	1.02 ± 0.08	0.49 ± 0.07***	0.54 ± 0.04***	0.53 ± 0.05***	0.96 ± 0.06##	0.91 ± 0.07##	0.96 ± 0.05##
NF-kB (p65), ng/mg protein	4.8 ± 0.2	5.9 ± 0.4**	6.1 ± 0.4**	6.0 ± 0.5**	4.8 ± 0.3#	4.7 ± 0.4#	4.7 ± 0.6#
TNF-α, pg/mg protein	10.6 ± 1.1	20.8 ± 1.9***	19.8 ± 1.1***	18.9 ± 1.3***	12.1 ± 1.3#	12.8 ± 1.0#	11.9 ± 1.9#
IL-1β, pg/mg protein	3.6 ± 1.1	7.8 ± 1.9***	4.9 ± 0.5#	4.8 ± 0.6#	4.8 ± 0.3#	4.7 ± 0.4#	4.7 ± 0.6#
IL-6, pg/mg protein	9.6 ± 1.2	17.8 ± 1.9***	18.6 ± 1.4***	16.9 ± 1.2***	10.4 ± 1.2##	10.0 ± 1.0##	11.1 ± 0.9##
IL-10, pg/mg protein	9.6 ± 1.2	4.8 ± 0.9***	3.86 ± 0.60***	4.12 ± 0.73***	11.6 ± 1.4##	10.6 ± 0.7##	9.9 ± 0.9##
ET-1, fg/mg protein	1.89 ± 0.11	4.16 ± 0.06**	4.06 ± 0.09**	3.93 ± 0.05**	1.93 ± 0.08##	1.97 ± 0.08##	± 1.97 ± 0.07##

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.

Discussion

Disturbances in mitochondrial complex1 functioning plays a crucial role in the pathogenesis of β cells dysfunction in diabetic pancreas [2-7,9]. Glucose metabolized and using as an energy source in pancreatic β cells and electrons 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. Pancreas characterized very low lactate dehydrogenase activity and ability for regenerate NAD in glycolytic pathways [2,4-9]. The polyol pathways reaction of sorbitol oxidation to form fructose, in which under diabetes metabolized about 30% of the glucose, also needs NAD and generates NADH [5-6,9]. Thus, redox-potential is a contributing factor to redox imbalance, pseudohypoxia development and leading factor in hyperproduction of ROS under diabetic state in pancreas [4-9]. The establishment of chronic pseudohypoxic condition under DM states could play triggers role for chronic inflammation in diabetic pancreas. The objective of this study was to assess the correlation between changes in redox-potential and inflammation response of blood and pancreases in STZ-NA induced DM rats and ability of various pharmacological agents to its correction. Experimental model of NA-STZ-induced DM in depending on the dosage of these two compounds and age of animals mimics only some but not all the symptoms of T2DM, and it may tend to express symptoms of insulin resistance or glucose intolerance more than T2DM features [20-22]. In STZ-induced diabetic rats, insulin deficiency or hypoinsulinemia develops as a consequence of irreversible destruction of the β-cells of the pancreas resulting in hyperglycemia. Furthermore, the current diabetic model did not fully follow metabolic changes of high fat diet-STZ induced diabetic rats; though the immunological behaviors of those obese type 2 diabetic rats have not completely investigated. Pretreatment with nicotinamide as a NAD+ precursor in practically healthy animals recruit the consumption of NAD in pancreatic β cell immediately after STZ injection and then mildly-moderate hyperglycemia develop. As shown our results, while NA pretreatment, the stable disturbances in the redox balance without alterations in the total pool of pyridine nucleotide in pancreatic cells occurs after 21 days of STZ administration. The content of oxidized form of NAD and NADP and the ratios of NAD/NADP/NADPH in pancreas did not improved after treatment with hypolipidemic agents, metformin and glibenclamide. Pancreatic cells has a very high sensitivity to the reduction of redox-potential NAD/NADH 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: first, reduces 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

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 Nadcin® 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 same about second intracellular mechanism of action of metformin in high concentrations (5 mmol/L) via the inhibition of NADH 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 Nadcin®. Moreover, the capacity of cells to produce TNF-α 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 states [14-17]. The positive effect of NAD on the cytokine profile strengthening in Nadcin® by inosine, which themselves can ability to prevent overproduction of proinflammatory cytokines, and enhance the production of the protective IL-10 via at least partially 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 kappa B, or elevation of intracellular cAMP 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 all of the other biochemical abnormalities, including activation of proinflammatory cytokines and suppress antiinflammatory component synthesis in pancreases and endothelin 1 synthesis in endotheliocytes in T2DM. We therefore hypothesized that Nadcin® 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.

Ethics Approval and Consent to Participate

The experimental protocols were approved by the Interinstitutional Animal Ethics Committee (IIAEC) (ICIBMCT/S/8/13) at International Centre of Introduction of New Biomedical Technology, Tbilisi, Georgia.

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