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EFFECTS OF C-PEPTIDE AND NICOTINAMIDE ON SERUM LH, FSH, TESTOSTERONE LEVELS AND SPERM COUNT IN NICOTINAMIDE/ STREPTOZOTOCIN-INDUCED-DIABETES IN MICE

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Abstract

Introduction. One of the diabetes complications in men is infertility. C-peptide and Nicotinamide have antidiabetic and antioxidant activity. So, the aim of this study was to investigate the effects of these drugs on male infertility caused by diabetes.

Methods. In this experimental study 40 adult male NMRI mice (20-25g) were obtained and randomly divided into 5groups: controls, diabetes model, diabetes+C-peptide, diabetes+Nicotinamide, diabetes+C-peptide+Nicotinamide. Diabetes induced was confirmed 3 days after administration of a single dose of streptozotocin (STZ) (65mg/kg) 15 min after an intraperitoneal injection of Nicotinamide (120mg/kg). Then C-peptide (25nmol/kg) and Nicotinamide (100mg/kg) were injected for 28 days. 24h after the last drugs injection serum samples, testes and cauda epididymis of animals were removed for hormonal, testis morphology and sperm count assessment.

Results. Diabetes induced could decrease serum testosterone level and sperm count significantly ($p<0.001$, $p<0.05$ respectively). Serum LH, testosterone levels and sperm count increased in Diabetes+C-peptide+Nicotinamide *versus* diabetes group ($p<0.05$, $p<0.001$, $p<0.01$ respectively). Also administration of Nicotinamide alone showed an increase in serum testosterone ($p<0.001$).

Conclusion. The results of this study demonstrated that combined administration of Nicotinamide and C-peptide improved diabetes induced male reproductive disorders by enhanced serum LH, testosterone levels and sperm count in diabetic mice.

Key words: diabetes, C-peptide, nicotinamide, sperm, mice.

INTRODUCTION

Male infertility is about 30-50% of all infertilities worldwide and almost 30-40% of them are sperm disorders (1). There are about 143 million people suffering from diabetes mellitus in the world. This number may be double in 2030 (2). Modern societies revealed

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that diabetes mellitus has been closely associated with infertility and sexual dysfunction in men and experimental animals due to decrease in serum gonadotropin (LH, FSH), testosterone levels and sperm count (3-4).

C-peptide is a bioactive small peptide of 31 amino acids with short half-life (approximately 30 minutes). Its secretion into the bloodstream is equimolar with insulin (5). C-peptide specifically binds to cell membranes then activates a G-protein coupled membrane receptor and Ca_2^+-dependent intracellular signaling pathways and leads to stimulation of Na^+/K^+-ATPase activities (6). Also this peptide could increase glucose utilized, muscle and skin blood flow, antioxidant enzymes activities and could decrease the reactive oxygen species (ROS) (7-8).

Nicotinamide is the amide form of water-soluble vitamin (vitamin B3). This vitamin is generated due to the hepatic conversion of nicotinic acid or through the hydrolysis of nicotinamide adenine dinucleotide (NAD) and it has been shown to exhibit antioxidant properties and metabolic improvements such as diabetes via inhibiting beta-cell dysfunction and apoptosis (9-10). Nicotinamide provides protection against free radicals and oxidative stress. Also this vitamin can improve infertility via its antioxidant activities (9). In the previous study it was demonstrated that administration of C-peptide and Nicotinamide could improve type 2 diabetes via increasing insulin sensitivity and decreasing serum insulin and glucose levels in Nicotinamide-STZ induced diabetic mice (11), but there was no study to investigate the effect of these drugs on the reproductive system in diabetic mice model. Therefore, the aim of this study was to determine the effects of C-peptide and Nicotinamide on serum levels of gonadotropin (LH, FSH), testosterone and sperm count in mice with Nicotinamide/STZ induced type2 diabetes mellitus.

MATERIALS AND METHODS

Animal preparation

In this experimental study, 40 adult male NMRI mice (20-25gr) were obtained from Ahvaz Jundishapur University of Medical Sciences (AJUMS) animal facility. Mice used in this study were treated in accordance with principles and guidelines on animals care of AJUMS and were housed in a separate room in cages at 20-24°C under 12-h darkness: 12-h light cycle with free access to tap water and commercial chow.

Experimental design

In the present study 3-month-old mice were used, because full maturity and growth have been formed at this age (12). After one week acclimatization to the laboratory, the animals were divided randomly into 5 groups (eight mice in each group): control (received saline 0.9%), diabetes model group, diabetes+C-peptide (25nmol/kg) (Bachem Company, Germany), diabetes+Nicotinamide (100mg/kg), diabetes+C-peptide+Nicotinamide (dissolved in saline 0.9%). For inducing diabetes, a single dose of STZ (65 mg/kg) (Sigma-Aldrich Co, USA) was injected 15 min after a

single intraperitoneal administration of Nicotinamide (120 mg/kg) (Sigma-aldrich Co, USA). Diabetes induced was confirmed by an elevation in blood glucose level more than 200mg/dL that was determined after 3 days (13). Also a single drop of tail blood was collected, and glucose level was measured by a glucometer device weekly. Moreover, for treatment animals after induced diabetes, the drugs were administered for a period of 28 days (14-15).

24 hours after the last drug injection, blood samples were collected by cardiac puncture under deep anesthesia and centrifuged at 3000 rpm for 15 minutes. Then serum samples were frozen at −70°C until the hormonal assays were performed.

Morphological analysis of testis

Testes of all animals were immediately removed and their weight, length, width and volume were analyzed. Also testes volume was calculated according to the following formula (volume= (D2/4×ϖ) L×K) (length (L), width (D), K=0.9, ϖ=3.14) (16).

Serum hormonal assessment

Serum concentration levels of LH, FSH and testosterone were measured by using ELISA assay kits (DRG Instruments GmbH, Germany). The sensitivity of hormone detection per assay tube for LH, FSH and testosterone were 1.27, 0.856 mIU/mL and 0.083 respectively.

Sperm count assessment

Cauda epididymis of all animals was dissected and teased in 3mL normal saline 0.9%. Then one drop of containing sperm normal saline solution was transferred into each chamber of Neubauer hemocytometer (HBG. Company, Germany) (Tiefe depth profondeur 0.100 mm and 0.0025 mm^2 area). At the end sperm number was manually counted by light microscopy (Olympus light microscope Tokyo, Japan) in white blood cell grids. Ultimately, data were expressed as the count of sperm/mL (17).

Statistical analysis

Data were statistically analyzed by SPSS software with one-way ANOVA followed by post hoc LSD tests. The values were expressed as means ± SEM and differences were considered significant at $p<0.05$.

RESULTS

Effect of Nicotinamide-STZ induced diabetes on testis morphology, serum LH, FSH, testosterone levels and sperm count in animals

As shown in Table 1, testis volume tends is to decrease in diabetes group but there was no significant differences between all groups in testis morphology. Diabetes could decrease serum testosterone level in comparison to control group ($p<0.001$) also there were no significant changes in serum LH and FSH levels in this group (Figs 1-3). Sperm count in diabetic mice model decreased significantly *versus* control group ($p<0.05$).

Effect of C-peptide, Nicotinamide and C-peptide+Nicotinamide on testis morphology and serum LH, FSH, testosterone levels and sperm count in animals

The results of administration

Table 1. Effect of Nicotinamide-STZ induced diabetes, C-peptide and Nicotinamide on testis morphology, (Mean±SEM)

Groups	Testis weight (mg)	Testis length (mm)	Testis width (mm)	Testis volume (mm^3)
Control	85.16±6.25	5.5±0.54	3.08±0.3	42.49±9.46
Diabetes	83.16±6.47	5.25±0.49	2.95±0.3	31.94±8.65
Diabetes+C-peptide	93.8±8.89	6.6±0.59	3.7±0.25	54.44±10.03
Diabetes+Nicotinamide	78.25±8.23	6±0.31	3.1±0.1	37.64±7.25
Diabetes+Nicotinamide+C-peptide	83.4±6.5	6.7±0.56	3.3±0.12	52.01±9.78

n=8 number of mice in each group, SEM: standard error mean.

of C-peptide and Nicotinamide did not show any significant variation in testis morphology (Table 1). Serum LH level in Diabetes+C-peptide+Nicotinamide when compared to control and diabetes groups increased significantly ($p<0.05$). Also there were no significant differences in FSH among groups. Diabetes induced reduction of testosterone was significantly recovered following treatment with Nicotinamide and C-peptide + Nicotinamide ($p< 0.001$). Also administration of C-peptide plus Nicotinamide improved D-galactose-induced decrease in sperm count of diabetic mice ($p< 0.01$).

DISCUSSION

The results of this study demonstrates that experimental diabetes could decrease sperm count as well as serum testosterone level reduction. La Vignera S. *et al.* (18) indicated that testosterone and sperm count decreased in type 2 diabetes patients. Previous studies revealed that combined administration of STZ with Nicotinamide leads to moderate

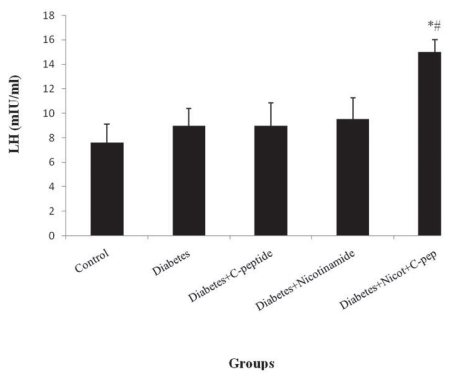

Figure 1. Effect of Nicotinamide-STZ induced diabetes, C-peptide and Nicotinamide on serum LH level. n=8 number of mice in each group, Mean±SEM, SEM: standard error mean, *P<0.05 Significant as compared to Diabetes group, #P<0.05 Significant as compared to Control group.

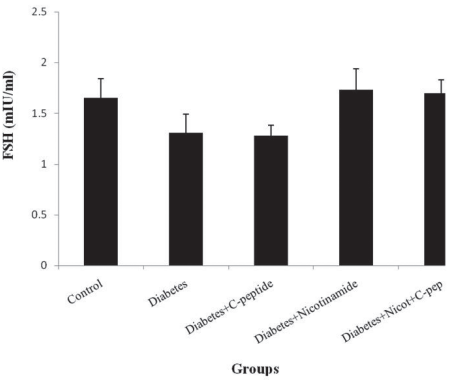

Figure 2. Effect of Nicotinamide-STZ induced diabetes, C-peptide and Nicotinamide on serum FSH level. n=8 number of mice in each group, Mean±SEM, SEM: standard error mean.

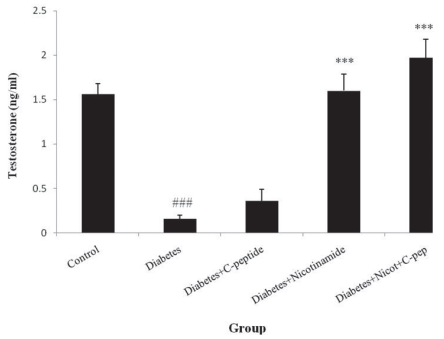

Figure 3. Effect of Nicotinamide-STZ induced diabetes, C-peptide and Nicotinamide on serum testosterone level. n=8 number of mice in each group, Mean±SEM, SEM: standard error mean, ***P<0.001 Significant as compared to Diabetes group, ###P<0.001 Significant as compared to Control group.

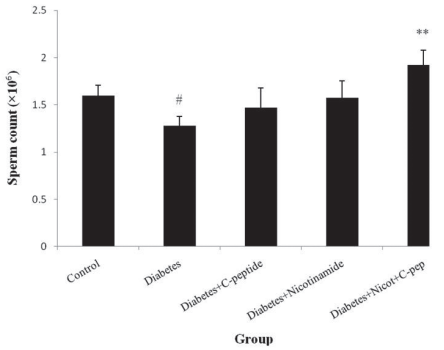

Figure 4. Effect of Nicotinamide-STZ induced diabetes, C-peptide and Nicotinamide on sperm count. n=8 number of mice in each group, Mean±SEM, SEM: standard error mean, **P<0.01 Significant as compared to Diabetes group, #P<0.05 Significant as compared to Control group.

hyperglycemia and chronic diabetes in animals. Nicotinamide preserved β-cells STZ-mediated cytotoxic damages via protecting the intra cellular pool of NAD by acting as a direct precursor or inhibiting of ADP-ribose synthetase activity (19). STZ made a rapid depletion in β-cells and results in insulin release reduction (20). Subsequent hyperglycemia causes oxidative damage due to over generation of ROS and leads to development of diabetic complications such as decreasing in testosterone and sperm count (21). ROS mainly interact with unsaturated fatty acids such as phospholipids, glycolipids, glycerides and sterols. Also, oxidation of these fatty acids results in the increase of the cellular membrane permeability. Thus, overproduction of free radicals can induce lipid peroxidation due to breakdown of polyunsaturated fatty acids in cell membrane. Since spermatozoa have a high concentration of polyunsaturated fatty acids which are involved in regulation of spermatogenesis and sperm maturation, it is so susceptible to peroxidative damage. This could destroy the structure of spermatozoa and decrease sperm count (22).

The results of Rabbani SI *et al.* (23) study demonstrated Nicotinamide-STZ induced diabetes could deplete sperm count and increased sperm abnormality followed by enhanced free radicals production and decrease of antioxidant enzymes activities. So, according to the diabetes group results of this study, it could be suggested that sperm count's decline occurred through ROS over production and decreased antioxidant enzymes activities.

Administration of Nicotinamide has shown protective effects on β-cells survival and function in animals due to its action as a free radical scavenger (24). The results of Nicotinamide diabetic mice injection in this study showed an

increase in serum level of testosterone. Furthermore, distinct advantage of Nicotinamide and C-peptide did not show any significant increase in sperm count but simultaneous utilization of these drugs indicated reproductive system improvement in diabetic mice by elevating in serum LH, testosterone levels and sperm count. The results of a previous study revealed, Nicotinamide and C-peptide could treat Nicotinamide-STZ induced diabetes due to decrease in glucose and insulin resistance (11). So, it could be suggested that administration of Nicotinamide and C-peptide leads to reduce over production of ROS caused by hyperglycemia. Antioxidants enzymes are compounds which scavenge and suppress the formation of free radicals, ROS and lipid peroxidation. Increase in antioxidant enzyme activities and expression could increase intratesticular testosterone and decrease spermatozoa peroxidative damage and germ cell apoptosis (25). The use of systemic antioxidants as a treatment for male infertility has been proved in some studies (26). Therefore according to the results of this research it could be suggested simultaneous administration of Nicotinamide and C-peptide improved reproductive dysfunction caused by diabetes via increase in antioxidant enzymes activities and decrease in ROS production.

In conclusion, the findings of this study indicated that Nicotinamide-STZ induced diabetes in mouse could produce reproductive disturbances by decline in sperm count and serum level of testosterone. Administration of Nicotinamide together with C-peptide improved this diabetic reproductive disorder via enhanced in serum LH, testosterone levels and sperm count.

Conflict of interest

We declare that there is no conflict of interest.

Acknowledgment

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