Ameliorative effect of zinc oxide and silver nanoparticles on antioxidant system in the brain of diabetic rats.

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ABSTRACT

Objective: The present study tests the ability of both zinc oxide (ZnONPs) and silver (SNPs) nanoparticles to ameliorate the oxidative stress resulted from diabetes in diabetic rats was tested in the present study.

Methods: Fifty male albino rats are randomly used; ten of them are served as a control and forty rats injected with single dose (100 mg/kg) of streptozotocin intraperitoneal. They were subdivided into, diabetic + ZnONPs, diabetic + SNPs and diabetic + insulin. The activities and mRNA expression levels of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GRD) were determined in brain tissues. Malondialdehyde (MDA), total antioxidant capacity (TAC), zinc (Zn) and silver (Ag) concentrations were estimated in the brain tissues of all rats.

Results: A significant increase in the activities and mRNA expression levels of SOD, CAT, GPx and GRD was shown. MDA levels were significantly decreased where there is a significant increase in the Zn, Ag and TAC in brain of ZnONPs and SNPs treated rats when compared with diabetic or diabetic + insulin and their control.

Conclusion: ZnONPs and SNPs can be used to ameliorate the oxidative stress in brain resulted from diabetes mellitus.

Introduction

Diabetes mellitus is referring to a group of metabolic impairments characterized by highly raised blood glucose levels(1). The most consequence of diabetes mellitus is the generation of reactive oxygen species (ROS) (2). The ROS can induce B-cell failure and develop insulin resistance (3). Diabetes mellitus can directly affect brain cells, through the generation of an oxidative stress leading to apoptosis (4). The key mechanism is illustrated as an increase in glucose metabolism due to hyperglycemia that promotes mitochondrial respiration, resulting in release of superoxide and other reactive oxygen or nitrogen species into the cytoplasm (5). Zinc has been reported to play a direct role in glucose homeostasis through enhancing hepatic glycogenesis through its actions on the insulin signaling pathway and thus improves glucose utilization (6), it inhibits intestinal glucose absorption (7) and increases glucose uptake in skeletal muscle and adipose tissue (6). Moreover, zinc is reported to inhibit glucagon secretion (8), thus reducing gluconeogenesis and glycogenolysis, it also enhances the structural integrity of insulin (9). Decreased zinc in the pancreas may reduce the ability of the islet β-cells to synthesize and lead insulin in to blood (10). Furthermore, knowing zinc's antioxidant role (11), reduced zinc may exacerbate the oxidative stress-mediated complications of diabetes. In recent years there is a great development of nanotechnology in the field of science and technology, metallic nanoparticles, like gold, silver, zinc and metal oxides nanoparticles, have shown great challenges in the field of medicine (12). In a previous study, we proved the antidiabetic effect of ZnONPs and SNPs as a novel agent to control diabetes mellitus in rats (13). The aim of the study is to test the ability of ZnONPs and SNPs to reduce the oxidative stress induced by diabetes mellitus in brain tissues of diabetic rats.

Materials and Methods

Animals

Fifty male albino rats, are weighting 120 ± 20 grams at the beginning of the experiment. All rats were grouped into five groups; the first one is control (N=10); it was not subjected to any treatment. The second group includes the remaining forty animals, they were induced to be diabetic through injection of single dose (100 mg/kg) of streptozotocin intra peritoneal (STZ) (Sigma-Aldrich, Catalog No S0130 SIGMA, Seelze, Germany). The diabetic rats are further divided into four groups; diabetic rats (N-10); served as a positive control with no treatment, diabetic + ZnONPs; were administered a daily dose of ZnONPs per-Os (Sigma-Aldrich, Catalog No.721077, Seelze Germany) of 10 mg/kg, diabetic + SNPs group; were administered a daily dose of SNPs Per-Os (Sigma-Aldrich, Catalog No. 730793, Seelze, Germany) of 10 mg/kg and diabetic + insulin group; were injected with subcutaneous dose of insulin (0.6 units/50 g) for 30 constitutive days (13).

Ethical Statement

All experimental procedures were performed in agreements with the Saudi Arabian laws and University guidelines for experimental animals care and rights.

Sampling Protocol

One gram of brain tissue was collected on liquid nitrogen then divided into different aliquots which were preserved at -80 ^{o}C until their use in biochemical and molecular biological investigations.

Biochemical assay

Brain Zn and Ag concentrations were analyzed using an inductively coupled plasma–atomic emission spectroscopy (ICP-AES) using an Ultima 2 apparatus (Horiba Jobin, Yvon, France). Brain homogenate was used for determination of CAT, GPx and GR activity using a kit (Cat. No. NWK-CAT01, NWK-GPX01 and NWK-GR01) purchased from Northwest Life Science Specialties (NWLSSTM), Vancouver, Canada. SOD activity was determined by using Cayman SOD diagnostic kit (Cat. No. 706002, Cayman, USA). Malondialdehyde (MDA) was

analyzed by measuring the production of thiobarbituric acid reactive substances (TBARS) using TBARS assay kit (Cat. No.10009055, Cayman, USA). Total antioxidant capacity (TAC) was determined by using a kit supplied by Bio-diagnostic (CAT. NO. TA 25 12, Giza, Egypt).

Molecular analysis

Brain SOD, CAT, GPx, GRD genes expression were quantified using real time PCR. Total RNA was isolated from tissue samples using the RNeasy Mini Kit Qiagen (Cat. No.74104). 0.5µg of total RNA, was used for production of cDNA using Qiagen Long Range 2 Step RT-PCR Kit, (Cat. No.205920). Five µL of total cDNA was mixed with 12.5 µL of 2x SYBR® Green PCR mix with ROX from BioRad and 10 pmol/µL of each forward and reverse primer for the measured genes. The house keeping gene β-actin was used as a constitutive control for normalization. Primers were designed using Primer3 software (The Whitehead Institute, http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi) as per the published rat SOD, CAT, GPx, GRD and β-actin genes sequences (Table 1) of NCBI database all primers were provided by Sigma Aldrich (Sigma-Aldrich Chemie GmbH, Steinheim, Germany). PCR reactions were carried out in an AbiPrism 7300 (Applied Biosystems, USA). The RNA concentration in each sample was determined from the threshold cycle (Ct) values. The mRNA expression levels were calculated relative to ß-actin gene mRNA levels using the $2^{-DD\ CT}$ method.

Statistical analysis.

The obtained data were analyzed using SPSS version 20. (IBM 1 New Orchard Road Armonk, New York 10504-1722 United States) . Data were presented as a mean ± SD,(N = 10). Student's t-test was used to calculate the differences between groups at $P < 0.05$.

Results

A significant increase in the activities and mRNA expression levels of SOD, CAT, GPx and GRD was shown in rats treated with ZONPs and SNPs as compared with the diabetic rats

(Table 2 and 3). MDA levels were significantly decreased where there is a significant increase in the Zn, Ag and TAC in brain of ZnONPs and SNPs treated rats when compared with diabetic or diabetic + insulin and their control (Table 2 and 4)

Discussion

In the present study we tended to examine the ameliorative effect of both ZnONPs and SNPs on the oxidative stress generated in brain cells of the diabetic rats. In the present study Zn and Ag particles were increased significantly in brain tissues of diabetic rats subjected to treatment with ZnONPs and SNPs, Zn levels tended to be (32±3 and 21±5 µg/g) and (7.3±1.5 and 13±3µg/g) for silver when compared with their control (22±4µg/g) for Zn and (8±1µg/g) for silver (Table 2). Long circulation of nanoparticles leads to increase the chance of their passage to tissues, and hence higher cellular uptake (14). Once taken up in cells, particles encounter an increasingly acidic environment as they move from early to late endosomes and finally to lysosomes, resulting in their dissolution (15). Such phenomena may have contributed to the observed increases in tissue Zn and Ag levels in our study. ZnONPs and SNPs administration to diabetic rats resulted in prevention of the decrease in the activity and mRNA expression levels of SOD, CAT, GRD and GPx. We tended the high activities and expression levels of antioxidants enzymes in brains of ZnONPs and SNPs administered rats to the effect of these nanoparticles to improve the insulin secretion and amelioration of the oxidative stress induced due to impairment of glucose homeostasis (13). These enzymes play a pivotal role in the elimination of free radicals from the tissues (16) so their high activities and expression levels in brain cells protect them from the effect of oxidative stress. Diabetes mellitus is always associated with the generation of ROS (17), and this is clear in our study proved by a reduction in both activities and expression of the examined antioxidant enzymes in diabetic untreated rats. In the same line of our study, the overproductions of superoxide free radicals are eliminated by over expression of antioxidant enzymes (18). MDA was used as a potent marker for oxidative stress (19). Many authors have cited that diabetes mellitus is

usually associated with an increase in MDA production and so increased their levels in tissues and blood in diabetic models (20). Our results showed a significant increase in MDA levels in the brain of diabetic rats. We tended this increase to the oxidative stress generated due to high glucose levels as mentioned before. In ZnONPs and SNPs the levels of MDA were decreased in the brain tissues. The reduction of MDA levels in the brain of nanoparticles administered rats may be due to the activity of these nanoparticles to improve the insulin secretion (13) and the increase the SOD, CAT, GRD and GPx activities and mRNA expression . To demonstrate the total antioxidant capacity of the brain in diabetic and diabetic rats administered ZnONPs and SNPs, the TAC was measured in the brain of all rats. The highest capacity was observed in diabetic rats administered ZnONPs followed by diabetic rats administered SNPs when compared to diabetic rats and control (Tale 4). We tended the high TAC in nanoparticles administered rats due to the ability of these particles to improve the antioxidant power of the cells proved recently by Kunjiappan (21). In all examined rats the effect of ZnNPs and SNPs was more obvious than the insulin in diabetic rats. There is more improvement in total antioxidant power in the brain of diabetic rats administered nanoparticles than that treated with insulin. Up till now we have not a clear explanation about the action of these nanoparticles. But the speed by which these nanoparticles was up taken by the cells (14, 15) and its ability to induce endogenous insulin secretion (13) may be the usual cause of their ability to improve the antioxidant capacity in the brain cells.

Conclusion

ZnONPs and SNPs are potent agents for improvement of the antioxidant power of brain cells. They have the ability to protect brain cells from the oxidative stress generated in diabetes mellitus.

Conflict of interest The authors declare no conflict of interest.

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Table1. Primers oligonucleotide sequences of SOD, CAT, GPx, GR and ß-actin genes.

Gene	Oligonucleotides sequences		Size (bp)	Gene ID
SOD	F	5'-ATGGGGACAATACACAAGGC-3'	225	Z21917.1
	R	5'-TCATCTTGTTTCTCGTGGAC-3'		
CAT	F	5'-GTCCGATTCTCCACAGTCGC-3'	272	AH004967.1
	R	5'-CGCTGAACAAGAAAGTAACCTG-3'		
GPx	F	5'-CACAGTCCACCGTGTATGCC-3'	292	S50336.1
	R	5'-AAGTTGGGCTCGAACCCACC-3'		
GRD	F	5'-CCATGTGGTTACTGCACTTCC-3'	171	NM_053906
	R	5'-GTTCCTTTCCTTCTCCTGAGC-3'		
ß-actin	F	5'-TCACTATCGGCAATGTGCGG-3'	260	NM_007393
	R	GCTCAGGAGGAGCAATGATG-3' 5'-		

Table 2. Biochemical investigations in experimental rats administered zinc oxide and silver nanoparticles.

Parameters	**Control**	**Diabetic**	**Diabetic + ZnONPs**	**Diabetic + SNP**	**Diabetic + insulin**
Zn (µg/g)	22±4	14±2**	32±3$^{*\#\#}$	21±5$^{\#}$	22.5±3$^{\#}$
Ag (µg/g)	8±1	7±1.3	7.3±1.5	13±3$^{*\#\#}$	7.5±2
SOD (µmol/ mg wt.w / min)	32 ± 2	8.2 ± 2***	25 ± 3$^{*\#\#\#}$	18.7 ± 1.5$^{*\#}$	10.3±1**
CAT (µmol/ H_2O_2	900 ± 20	500 ±	790 ± 10$^{*\#\#}$	710 ± 20$^{*\#}$	550±23**

decomposed/ mg P/ min)		15***			
GPx (μ mol/ mg P/ min)	130 ± 13	48.3 ± 7.6***	100 ± 10*###	90 ± 3*##	50±4***
GRD (U/ mg P)	6.5 ± 0.5	2.8 ± 0.3***	6.3 ± 0.3###	4.4 ± 0.4*##	3±0.5***

wt.w; wet weight tissue. Results are tabulated as means ± SD of ten animals. Statistical analyses were performed using two tail Student's t-test *p < 0.05 ** P < 0.01 *** P < 0.001 comparing to control and #p < 0.05 ## P < 0.01 ### P <0.001 comparing to the diabetic rats.

Table 3**.** mRNA expression profile (relative expression to β-actin) of antioxidant genes in diabetic rats administered zinc oxide and silver nanoparticles.

Genes	**Control**	**Diabetic**	**Diabetic + ZnONPs**	**Diabetic + SNP**	**Diabetic + insulin**
SOD	1.1 ± 0.001	0.2 ± 0.003**	0.9 ±0.0 1*##	0.7 ± 0.002*#	0.7±0.09*#
CAT	1.3 ± 0.002	0.3± 0.001***	1 ± 0.02*##	0.8 ± 0.01*#	0.6±0.01*#
GPx	0.96± 0.06	0.44 ±0.0 3**	0.9 ± 0.04##	0.72 ±0.0 2*##	0.6±0.05*#
GRD	0.9 ± 0.05	0.5 ± 0.002**	1.7 ± 0.3*###	1 ± 0.04##	0.7±0.03*#

Results are tabulated as mean ± SD of ten animals. Statistical analyses were performed using two tail Student's t-test *p < 0.05 ** P < 0.01 *** P < 0.001 comparing to control and #p < 0.05 ## P < 0.01 ### P <0.001 comparing to the diabetic rats.

Table 4**.** Biochemical effect of zinc oxide, silver nanoparticles on malondialdehyde and total antioxidant capacity in brain tissue of diabetic rats.

Parameters	**Control**	**Diabetic**	**Diabetic + ZnONPs**	**Diabetic + SNP**	**Diabetic + insulin**
TAC (μMg^{-1} wt. w)	6.1 ± 0.66	2.8 ± 0.3***	6.6 ± 0.6###	5.7 ± 0.6##	4±0.9*#
MDA ($nmolg^{-1}$wt.w)	0.95 ± 0.1	4.3 ± 0.3***	1.6 ± 0.3###	1.1 ± 0.4##	2.5±0.05*#

Results are tabulated as mean ± SD of ten animals. Statistical analyses were performed using two tail Student's t-test *p < 0.05 ** P < 0.01 *** P < 0.001 comparing to control and $^{\#}$p < 0.05 $^{\#\#}$ P < 0.01 $^{\#\#\#}$ P <0.001 comparing to the diabetic rats.