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

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ABSTRACT

Objective: To test the ability of both zinc oxide nanoparticles (ZnONPs) and silver nanoparticles (SNPs) to ameliorate the oxidative stress resulted from diabetes in diabetic rats.

Methods: Fifty male albino rats were used; ten of them were served as control group and forty, as the experiment group, were injected with streptozotocin at the single intraperitoneal dose of 100 mg/kg. Then, the experiment group was subdivided into, diabetic, diabetic + ZnONPs, diabetic + SNPs and diabetic + insulin groups. The activities and mRNA expression levels of superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase were determined in brain tissues. Malondialdehyde, total antioxidant capacity, zinc and silver concentrations were estimated in the brain tissues of all rats.

Results: A significant increase in the activities and mRNA expression levels of superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase was shown. Malondialdehyde levels were significantly decreased while there was a significant increase in the zinc, silver concentrations and total antioxidant capacity in brain of ZnONPs and SNPs treated rats, compared with diabetic or diabetic + insulin group and their control group.

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

1. 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 β-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 by enhancing hepatic glycogenesis through its actions on the insulin signaling pathway and thus improves glucose utilization [6], 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, and 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 into blood [10]. Furthermore, knowing zinc's antioxidant role, reduced zinc may exacerbate the oxidative stress-mediated complications of diabetes. The pivotal role of zinc in diabetes mellitus was discovered by supplementation studies in diabetic rats [11]. In recent years, there is a great development of nanotechnology in the field of science and technology; and metallic nanoparticles, like gold, silver nanoparticles (SNPs), zinc oxide nanoparticles (ZnONPs) and metal oxide 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

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the study was to test the ability of ZnONPs and SNPs to reduce the oxidative stress induced by diabetes mellitus in brain tissues of diabetic rats.

2. Materials and methods

2.1. Animals

Fifty male albino rats weighed (120 ± 20) g at the beginning of the experiment. All the rats were first grouped into two groups: (1) control group (n = 10) without any treatments; (2) experiment group, including the remaining forty animals, which were induced to be diabetic through intraperitoneal injection of single dose (100 mg/kg) of streptozotocin (Sigma–Aldrich, Catalog No. S0130 SIGMA, Seelze, Germany). Then, the experiment group was further divided into four groups: (1) diabetic group (n = 10), which served as a positive control with no treatment; (2) diabetic + ZnONPs group (n = 10), which was administered with daily ZnONPs *per os* (Sigma–Aldrich, Catalog No. 721077, Seelze, Germany) at a dose of 10 mg/kg; (3) diabetic + SNPs group (n = 10), which was administered with SNPs *per os* (Sigma–Aldrich, Catalog No. 730793, Seelze, Germany) at a daily dose of 10 mg/kg; (4) diabetic + insulin group (n = 10), which was injected with subcutaneous dose of insulin (0.6 units/50 g) for 30 constitutive days [13].

2.2. Ethical statement

All experimental procedures were performed in agreement with the Saudi Arabian laws and University Guidelines for the care and rights of experimental animals.

2.3. Sampling protocol

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

2.4. Biochemical assay

Brain zinc and silver concentrations were analyzed by using an inductively coupled plasma-atomic emission spectroscopy with an ULTIMA 2 apparatus (Horiba Jobin Yvon, France).

Brain homogenate was used for determination of catalase (CAT), glutathione peroxidase (GPx) and glutathione reductase (GRD) activity by using a kit (Catalog No. NWK-CAT01, NWK-GPX01 and NWK-GR01) purchased from Northwest Life Science Specialties, Vancouver, Canada. SOD activity was determined by using Cayman SOD diagnostic kit (Catalog No. 706002, Cayman, USA). Malondialdehyde (MDA) was analyzed by measuring the production of thiobarbituric acid reactive substances by using TBARS assay kit (Catalog No. 10009055, Cayman, USA). Total antioxidant capacity (TAC) was determined by using a kit supplied by Bio-diagnostic (Catalog No. TA 25 12, Giza, Egypt).

2.5. Molecular analysis

Brain SOD, CAT, GPx, GRD gene expressions were quantified by using real-time PCR. Total RNA was isolated from tissue samples by using the Qiagen RNeasy Mini kit (Catalog No. 74104). About 0.5 μg of total RNA, was used for production of cDNA by using QIAGEN LongRange 2Step RT-PCR kit (Catalog No. 205920). About 5 μL of total cDNA was mixed with 12.5 μL of 2× SYBR® Green PCR mix with ROX from Bio-Rad 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 by using Primer 3 software (http://bioinfo.ut.ee/primer3/) as per the published gene sequences of SOD, CAT, GPx, GRD and β-actin in rats (Table 1) from NCBI database where all primers were provided by Sigma Aldrich (Sigma–Aldrich Chemie GmbH, Steinheim, Germany). PCR reactions were carried out in an Abi Prism 7300 (Applied Biosystems, USA). The RNA concentration in each sample was determined from the cycle threshold values. The mRNA expression levels were calculated relative to β-actin gene mRNA levels by using the $2^{-DD\ CT}$ method.

2.6. Statistical analysis

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

3. Results

3.1. Effect of ZnONPs and SNPs on zinc levels in brain tissue

Zinc levels in the brain tissue decreased significantly in diabetic group, compared with control group. Both SNPs and insulin treatment protected the brain tissue against the bad effect of diabetes. The ZnONPs carried the ability to increase zinc levels in brain tissue in ZnONPs treated rats, compared with the control group (Table 2).

Table 1
Oligonucleotide primer sequences of SOD, CAT, GPx, GR and β-actin genes.

Gene	Oligonucleotide 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′		
β-actin	F	5′-TCACTATCGGCAATGTGCGG-3′	260	NM_007393
	R	5′-GCTCAGGAGGAGCAATGATG-3′		

Table 2
Biochemical investigations in all groups.

Parameters	Control	Diabetic	Diabetic + ZnONPs	Diabetic + SNPs	Diabetic + insulin
Zinc (μg/g)	22.0 ± 4.0	14.0 ± 2.0**	32.0 ± 3.0$^{*\#\#}$	21.0 ± 5.0$^{\#}$	22.5 ± 3.0$^{\#}$
Silver (μg/g)	8.0 ± 1.0	7.0 ± 1.3	7.3 ± 1.5	13.0 ± 3.0$^{*\#\#}$	7.5 ± 2.0
SOD (μmol/mg wt.w/min)	32.0 ± 2.0	8.2 ± 2.0***	25.0 ± 3.0$^{*\#\#\#}$	18.7 ± 1.5$^{*\#}$	10.3 ± 1.0**
CAT (μmol/H_2O_2 decomposed/mg P/min)	900.0 ± 20.0	500.0 ± 15.0***	790.0 ± 10.0$^{*\#\#}$	710.0 ± 20.0$^{*\#}$	550.0 ± 2.03**
GPx (μmol/mg P/min)	130.0 ± 13.0	48.3 ± 7.6***	100.0 ± 10.0$^{*\#\#\#}$	90.0 ± 3.0$^{*\#\#}$	50.0 ± 4.0***
GRD (U/mg P)	6.5 ± 0.5	2.8 ± 0.3***	6.3 ± 0.3$^{\#\#\#}$	4.4 ± 0.4$^{*\#\#}$	3.0 ± 0.5***

wt.w: Wet weight tissue. *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$, comparing to control group; $^{\#}$: $P < 0.05$, $^{\#\#}$: $P < 0.01$, $^{\#\#\#}$: $P < 0.001$, comparing to diabetic group.

Table 3
Profile of mRNA expression (relative expression to β-actin) of antioxidant genes in all groups.

Genes	Control	Diabetic	Diabetic + ZnONPs	Diabetic + SNPs	Diabetic + insulin
SOD	1.100 ± 0.001	0.200 ± 0.003**	0.90 ± 0.01$^{*\#\#}$	0.700 ± 0.002$^{*\#}$	0.70 ± 0.09$^{*\#}$
CAT	1.300 ± 0.002	0.300 ± 0.001***	1.00 ± 0.02$^{*\#\#}$	0.800 ± 0.010$^{*\#}$	0.60 ± 0.01$^{*\#}$
GPx	0.960 ± 0.060	0.440 ± 0.030**	0.90 ± 0.04$^{\#\#}$	0.720 ± 0.020$^{*\#\#}$	0.60 ± 0.05$^{*\#}$
GRD	0.900 ± 0.050	0.500 ± 0.002**	1.70 ± 0.30$^{*\#\#\#}$	1.000 ± 0.040$^{\#\#}$	0.70 ± 0.03$^{*\#}$

*: $P < 0.05$,**: $P < 0.01$,***: $P < 0.001$, comparing to control group; $^{\#}$: $P < 0.05$, $^{\#\#}$: $P < 0.01$, $^{\#\#\#}$: $P < 0.001$, comparing to diabetic group.

Table 4
Biochemical effect of ZnONPs, SNPs on MDA and TAC in brain tissue of diabetic rats.

Parameters	Control	Diabetic	Diabetic + ZnONPs	Diabetic + SNPs	Diabetic + insulin
TAC (μmol/L/g wt. w)	6.10 ± 0.66	2.8 ± 0.3***	6.6 ± 0.6$^{\#\#\#}$	5.7 ± 0.6$^{\#\#}$	4.00 ± 0.90$^{*\#}$
MDA (nmol/g wt.w)	0.95 ± 0.10	4.3 ± 0.3***	1.6 ± 0.3$^{\#\#\#}$	1.1 ± 0.4$^{\#\#}$	2.50 ± 0.05$^{*\#}$

wt.w: Wet weight tissue. *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$, comparing to control group; $^{\#}$: $P < 0.05$, $^{\#\#}$: $P < 0.01$, $^{\#\#\#}$: $P < 0.001$, comparing to diabetic group.

3.2. Effect of ZnONPs and SNPs on silver levels in brain tissue

The silver levels in the brain tissue were not changed in all treated groups, except that SNPs treated rats showed a significant increase, compared with the other groups (Table 2).

3.3. Effect of ZnONPs and SNPs on antioxidant enzyme activities in brain tissue

Both diabetic and diabetic + insulin treated rats showed a significant decrease in the activities of SOD, CAT, GPx and GRD, compared with the control group. Either ZnONPs or SNPs treatment had the ability to protect the antioxidant enzymes in brain tissue against the bad effect of diabetes (Tables 2 and 3).

3.4. Effect of ZnONPs and SNPs on mRNA expression levels of antioxidant genes in brain tissue

A significant increase in mRNA expression levels of SOD, CAT, GPx and GRD was shown in rats treated with ZnONPs and SNPs, compared with the diabetic group (Tables 2 and 3).

3.5. Effect of ZnONPs and SNPs on MDA and TAC levels in brain tissue

MDA levels were significantly decreased while there was a significant increase in the TAC in the brain of ZnONPs and SNPs treated rats when compared with diabetic or diabetic + insulin group and control group (Table 4).

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, zinc and silver particles were increased significantly in the brain tissues of diabetic rats subjected to treatment with ZnONPs and SNPs, with zinc levels of [(32 ± 3) and (21 ± 5) μg/g] and silver levels of [(7.3 ± 1.5) and (13.0 ± 3.0) μg/g], compared with control group containing zinc level of (22 ± 4) μg/g and silver level of (8 ± 1) μg/g (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 of zinc and silver levels in brain tissue 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 antioxidant 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 by impairment of glucose homeostasis [13]. These enzymes play a pivotal role in the elimination of free radicals from the tissues so their high activities and expression levels in brain cells protect them from the effect of oxidative stress [16]. Diabetes mellitus is always associated with the generation of ROS [2], and this is clear in our study proved by

a reduction in both activities and expression of the examined antioxidant enzymes in untreated diabetic rats. In the same line of our study, the overproductions of superoxide free radicals are eliminated by overexpression of antioxidant enzymes [17]. Many authors have cited that diabetes mellitus is usually associated with an increase in MDA production and thus, increases the MDA levels in tissues and blood in diabetic models [18]. 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 groups, the levels of MDA were decreased in the brain tissues. The reduction of MDA levels in the brain of rats administered with nanoparticles may be due to the activity of these nanoparticles to improve the insulin secretion and to increase the SOD, CAT, GRD and GPx activities and mRNA expression [13]. To demonstrate the TAC levels of the brain in diabetic rats and diabetic rats administered with ZnONPs and SNPs, the TAC was measured in the brains of all rats. The highest capacity was observed in diabetic rats administered with ZnONPs followed by diabetic rats administered with SNPs, compared to diabetic group and control group (Table 4). We contended that the high TAC in nanoparticles administered rats was due to the ability of these particles to improve the antioxidant power of the cells proved recently by Kunjiappan *et al*. [19]. In all examined rats, the effect of ZnNPs and SNPs was more obvious than the insulin in diabetic rats. There are more improvements in total antioxidant power in the brain of diabetic rats administered with nanoparticles than those treated with insulin. Up till now, we have not a clear explanation about the action of these nanoparticles. Yet, the speed by which these nanoparticles were uptaken 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. 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 statement

We declare that we have no conflict of interest.

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