ORIGINAL ARTICLE

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 (ZnONPs) and silver (SNPs) nanoparticles to ameliorate the oxidative stress resulting from diabetes in diabetic rats.*

Methods: *Fifty male albino rats were randomly used: 10 served as a control and 40 rats were injected with single dose (100 mg/kg) of streptozotocin (STZ) intraperitoneally. They were subdivided into untreated diabetic, + ZnONPs, diabetic + SNPs, and diabetic + insulin. The activities and messenger RNA (mRNA) expression levels of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione reductase (GRD) were determined in the brain tissues. Malondialdehyde, 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. Malondialdehyde levels were significantly decreased where there was a significant increase in the Zn, Ag and TAC in the brain of ZnONP- and SNP-treated rats, when compared with diabetic or diabetic + insulin and their control.*

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

Keywords: Oxidative stress, antioxidants, diabetic brain, nanoparticles.

INTRODUCTION

Diabetes mellitus refers to a group of metabolic impairments characterisation by high blood glucose levels (1). The greatest 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 oxidative stress, leading to apoptosis (4). The key mechanism is illustrated as an increase in glucose metabolism due to hyperglycaemia that promotes mitochondrial respiration, resulting in a 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-signalling pathway. Thus, it improves glucose utilisation (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. It also enhances the structural integrity of insulin (9).

Decreased zinc in the pancreas may reduce the ability of the islet β-cells to synthesise and lead insulin into the blood (10). Furthermore, knowing zinc's antioxidant role (11), reduced zinc may exacerbate the oxidative stress-mediated complications of diabetes.

In recent years, there has been a great development of nanotechnology in the field of science and technology. Metallic nanoparticles, such as gold, silver, zinc, and metal oxide nanoparticles, have shown great challenges in the field of medicine (12).

In a previous study, we proved the antidiabetic effect of zinc oxide (ZnONPs) and silver (SNPs) nanoparticles

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as a novel agent to control diabetes mellitus in rats (13). The aim of this study is to test the ability of ZnONPs and SNPs to reduce the oxidative stress induced by diabetes mellitus in the brain tissues of diabetic rats.

MATERIALS AND METHODS

Animals

Fifty male albino rats, weighing 120 ± 20 g at the beginning of the experiment, were selected. All rats were placed into five groups. The first—the control (N = 10)—was not subjected to any treatment. The other groups included the remaining 40 animals which were induced to be diabetic through the injection of a single dose (100 mg/kg) of streptozotocin (STZ) intraperitoneally (Sigma-Aldrich, Catalog No S0130, Seelze, Germany). The diabetic rats were 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 a subcutaneous dose of insulin (0.6 units/50 g) for 30 consecutive days (13).

Ethical statement

All experimental procedures were performed in agreement with the Saudi Arabian laws and university guidelines for experimental animal care and rights.

Sampling protocol

One gram of brain tissue was collected in liquid nitrogen, and then divided into different aliquots which were preserved at –80°C until they were used in biochemical and molecular biological investigations.

Biochemical assay

Brain Zn and Ag concentrations were analysed with an inductively coupled plasma–atomic emission spectroscopy, using an Ultima 2 apparatus (Horiba Jobin, Yvon, France).

Brain homogenate was used for determination of CAT, GPx and GRD activity using a kit (Cat. No. NWK-CAT01, NWK-GPX01 and NWK-GR01) purchased from Northwest Life Science Specialties (NWLSSTM), Vancouver, Canada. Superoxide dismutase (SOD) activity was determined by using Cayman SOD diagnostic kit (Cat. No. 706002, Cayman, USA). Malondialdehyde (MDA) was analysed 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 2512, Giza, Egypt).

Molecular analysis

Brain SOD, CAT, GPx and GRD gene expression were quantified using real-time polymerase chain reaction (PCR). Total RNA was isolated from tissue samples using the RNeasy Mini Kit Qiagen (Cat. No.74104), and 0.5 µg of total RNA was used for production of cDNA, using Qiagen Long Range 2 Step RT-PCR Kit (Cat. No.205920). Approximately 5 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 housekeeping gene, β-actin, was used as a constitutive control for normalisation. 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 gene sequences (Table 1) of NCBI database; all primers were

Table 1: Primers oligonucleotide sequences of SOD, CAT, GPx, GRD and β-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′		
β-actin	F	5′-TCACTATCGGCAATGTGCGG-3′	260	NM_007393
	R	GCTCAGGAGGAGCAATGATG-3′ 5′-		

SOD = superoxide dismutase, CAT = catalase, GPx = glutathione peroxidase, GRD = glutathione reductase.

provided by Sigma Aldrich (Sigma-Aldrich Chemie GmbH, Steinheim, Germany). Polymerase chain reaction (PCR) reactions were carried out in an AbiPrism 7300 (Applied Biosystems, Waltham, MA, USA). The RNA concentration in each sample was determined from the threshold cycle (Ct) values. The messenger RNA (mRNA) expression levels were calculated relative to β-actin gene mRNA levels using the $2^{-DD\,CT}$ method.

Statistical analysis

The obtained data were analysed using SPSS version 20 (IBM 1 New Orchard Road Armonk, NY, USA). 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 were shown in rats treated with ZONPs and SNPs, as compared with the diabetic rats (Tables 2 and 3). Malondialdehyde levels were significantly decreased where there was a significant increase in the Zn, Ag and TAC in the brain of ZnONP- and SNP-treated rats when compared with the diabetic or diabetic + insulin groups and the control (Tables 2 and 4).

DISCUSSION

In the present study, we examined the ameliorative effect of both ZnONPs and SNPs on the oxidative stress generated in brain cells of the diabetic rats. Both Zn and Ag particles were increased significantly in brain tissues of diabetic rats subjected to treatment with ZnONPs and SNPs. Zinc levels tended to be 32 ± 3 and 21 ± 5 µg/g and 7.3 ± 1.5 and 13 ± 3 µg/g for silver, when compared with their control: 22 ± 4 µg/g for Zn and 8 ± 1 µg/g for silver (Table 2). Long circulation of nanoparticles leads to an increased 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. Administration of ZnONPs and SNPs in diabetic rats resulted in the prevention of the decrease in the activity and mRNA expression levels of SOD, CAT, GRD and GPx.

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 decomposed/ mg P/min)	900 ± 20	500 ± 15***	790 ± 10$^{*\#\#}$	710 ± 20$^{*\#}$	550 ± 23**
GPx (µmol/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, SOD = superoxide dismutase, CAT = catalase, GPx = glutathione peroxidase, GRD = glutathione reductase, SNP = silver nanoparticle, ZnONP = zinc oxide nanoparticle.
Results are tabulated as means ± SD of 10 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: Messenger RNA 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$^{*\#}$

SOD = superoxide dismutase, CAT = catalase, GPx = glutathione peroxidase, GRD = glutathione reductase, SNP = silver nanoparticle, ZnONP = zinc oxide nanoparticle.
Results are tabulated as mean ± SD of 10 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*#

MDA= malondialdehyde TAC= total antioxidant capacity, SNP = silver nanoparticle, ZnONP = zinc oxide nanoparticle. Results are tabulated as mean ± SD of 10 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.

We attributed the high activities and expression levels of antioxidant enzymes in the brains of ZnONP- and SNPs-administered rats to the effect of these nanoparticles improving 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 effects 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 over production of superoxide free radicals is eliminated by the overexpression of antioxidant enzymes (18). Malondialdehyde 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 therefore increased their levels in tissues and blood in diabetic models (20). Our results showed a significant increase in MDA levels in the brains of diabetic rats. We attribute 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 nanoparticle-administered rats may be due to the activity of these nanoparticles to improve the insulin secretion (13) and the increase in the SOD, CAT, GRD and GPx activities and mRNA expression. To demonstrate the TAC of the brain in diabetics 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 (Table 4). We attribute the high TAC in nanoparticle-administered rats to the ability of these particles to improve the antioxidant power of the cells proved recently by Kunjiappan *et al.* (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 in those treated with insulin. Up till now, we have not had a clear explanation about the action of these nanoparticles. But the speed at which these nanoparticles were 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

Both 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.

AUTHORS' NOTE

The authors declare that they have no conflict of interest.

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