Journal of Taibah University Medical Sciences (2014) 9(3), 182–186

Taibah University

Journal of Taibah University Medical Sciences

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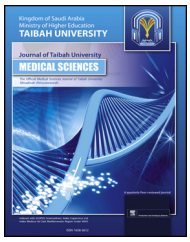

Experimental Article

Antioxidant status of rats administered silver nanoparticles orally

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Received 3 December 2013; revised 11 March 2014; accepted 12 March 2014
Available online 11 July 2014

الملخص

تستخدم الفضة النانوية بصورة متزايدة لأغراض الطب الحيوي بسبب إحتمال وجود خاصية مضادتها للميكروبات. ومع ذلك فإن تأثيرها على الأنظمة الخلوية لم يذكر إلا في عدد قليل من الدراسات البحثية. قمنا في بحثنا هذا بتقييم آثار هذه الجسيمات النانوية على حالة الفئران المضادة للأكسدة على 6 مجموعات من ذكور الفئران ويستار كل مجموعة تتكون من خمس فئران. وتم اعطائهم 100 و 1000 أو 5000 ملغ / كيلوغرام يوميا من الفضة النانوية من خلال قارورة عن طريق الفم لمدة 7 أو 14 أيام، وتلقت مجموعة واحدة 5000 ملغ/ كغ لمدة 21 يوما، وتلقت مجموعة مراقبة الماء المقطر فقط. تم ذبح الحيوانات بعد24 ساعة من نهاية تلقيهم العلاج، وأخذت عينات من مصل وأنسجة الفئران للدراسة. أثرت الفضة النانوية معنويا ($P < 0.05$) على زيادة تركيزات بعض الإنزيمات وخفض البعض. وهذه النتائج تشير إلى أن جزيئات الفضة قد تسبب أكسدة الدهون وتغيير الوضع المضادة للأكسدة الأنظمة الخلوية للفئران.

الكلمات المفتاحية: المضادة للمضادات الأكسدة; تأكسد الدهون; جسيمات معدنية متناهية الصغر طب النانوي; السمية

Abstract

Silver nanoparticles are being used increasingly for biomedical purposes because of their broad antimicrobial potential. Their effects on cellular systems, however, have been addressed in only a few studies. We evaluated the effects of these nanoparticles on the antioxidant status of groups of five male Wistar rats. Six groups of rats were given 100, 1000 or 5000 mg/kg daily through an oral cannula for 7 or 14 days, one group received 5000 mg/kg for 21 days, and a control group received distilled water. The animals were sacrificed 24 h after the end of treatment, and serum and tissue homogenates were prepared. Silver nanoparticles significantly ($p < 0.05$) increased the concentrations of malondialdehyde and superoxide dismutase but decreased the levels of reduced glutathione, glutathione *S*-transferase and catalase. These results indicate that silver nanoparticles may cause lipid peroxidation and alter antioxidant status in a manner that may cause oxidative stress.

Keywords: Antioxidant; Lipid peroxidation; Metal nanoparticle; Nanomedicine; Toxicity

Introduction

Nanoparticles are employed in constructing micro and nano devices used in electronics, in applications including adhesion, lubrication, stabilization and controlled flocculation of colloidal dispersions and in several other applications, including cellular delivery and imaging.[1,2] Owing to their small size and large ratio of surface area to volume, nanoparticles can interact with biomolecules and penetrate cell and nuclear membranes, causing indirect oxidative damage to DNA, inducing an inflammatory response and oxidative stress.[3] Potential risks may therefore be associated with their use. For instance, nanoparticles have been implicated in cellular injury[4] because of their ability to produce reactive oxygen species directly or indirectly.[5]

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Peer review under responsibility of Taibah University.

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http://dx.doi.org/10.1016/j.jtumed.2014.03.002

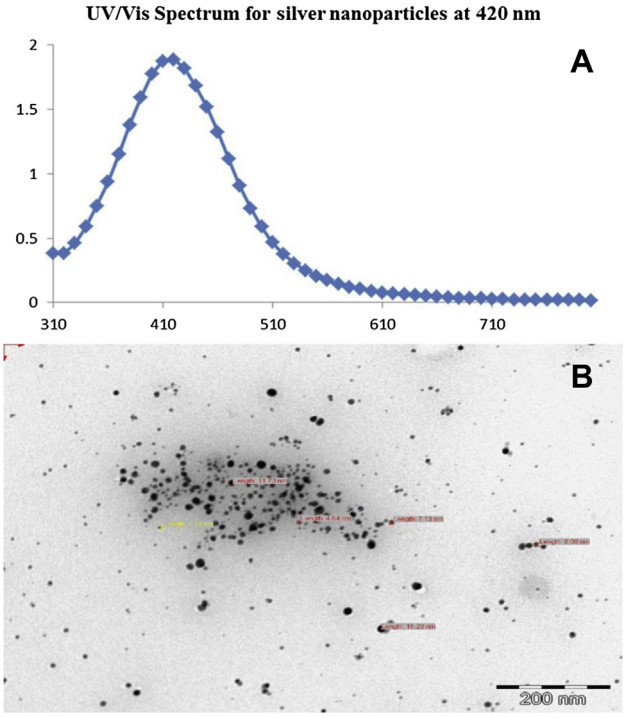

Figure 1: UV/Vis spectrum at 430 nm and transmission electron microscopy characterization of silver nanoparticles.

Silver nanoparticles have the broadest commercial applications of all nanomaterials because of their antibacterial properties.[3,6,7] Although they can act as free radical scavengers or reducing agents and quenchers of singlet oxygen formation,[8] they can also interact with cellular proteins and enzymes, with toxic effects.[9–11] However, there are no comprehensive safety or toxicity profiles of silver nanoparticles. The aim of this study was to investigate the effects of repeated administration of these nanoparticles on the antioxidant status of Wistar rats.

Materials and Methods

Preparation of nanoparticles

Silver nanoparticles were synthesized according to established protocols, with little modification. First, 100 mmol/L silver nitrate were added to a 1% (w/v) tannic acid solution (pH adjusted to 8 by addition of 150 mmol/L potassium carbonate) of polyvinylpyrrolidone with stirring.[12] A pale yellow colour revealed the presence of silver nanoparticles. The particles were filtered through a 0.22-μm filter and characterized by ultraviolet–visible (UV/Vis) spectrophotometry (Biotek Epoch, USA), inductively coupled plasma optical emission spectrometry (Cambridge, United Kingdom) and transmission electron microscopy (TEM, Brno, Czech Republic). All reagents were of analytical grade and prepared in distilled water unless otherwise stated.

Animals and treatments

Male Wistar rats weighing 190–220 g were obtained from the University of Ibadan, Ibadan, Nigeria, and housed in a well-ventilated, hygienic experimental animal house for 1 week to acclimatize them before the start of the experiment. They were fed a constant weight of commercial rat pellets and clean water.

Forty rats were grouped randomly into eight groups of five animals. One served as the control group and received distilled water. Three groups were treated with 100, 1000 or 5000 mg/kg silver nanoparticles for 7 days, and three further groups received the same concentrations for 14 days, while the eighth group received 5000 mg/kg for 21 days.

The doses were determined on the basis of a recent study in which the LD_{50} for silver nanoparticles was reported to be of >5000 mg/kg.[13] The nanoparticles were administered daily to the rats by means of a cannula. The rats were weighed daily to determine the effects of the nanoparticles on their weight and were sacrificed 24 h after cessation of treatments. The study was carried out in accordance with Institutional guidelines on the handling of animals as approved for scientific research.

Necroscopy

Animals were fasted overnight and sacrificed under slight chloroform anaesthesia 24 h after the last dose of nanoparticles. Blood samples were obtained by cardiac puncture, transferred to clean test bottles and centrifuged at 4000 × *g* for 5 min to obtain serum. The heart, liver and kidneys of each animal were removed and weighed immediately, then homogenized in ice-cold 0.25 mol/L sucrose (1:5 w/v) in a Teflon homogeniser (Sigma–Aldrich Chemie GmbH, Munich, Germany). The homogenates were then centrifuged at 4000 × *g* for 5 min (Heraeus Labofuge 300, Thermo Scientific, Hampshire, United Kingdom) to remove unbroken particulates. Tissue homogenates and serum were kept frozen until analysis.

Biochemical assays

Extracts of serum, kidney, liver and heart were analysed in a UV/Vis spectrophotometer (Shimadzu, Kyoto, Japan) for reduced glutathione (GSH), catalase, superoxide dismutase, malondialdehyde and glutathione *S*-transferase (GST). GSH was determined by the protocol described by Ellman[14] as previously reported,[15] catalase by the method described by Singha,[16] superoxide dismutase by the method described by Misra and Fridovich,[17] as previously reported by Adeyemi et al.,[15] malondialdehyde by the protocol described by Niehaus and Samuelson[18] and GST by the method described by Habig et al.[19]

Data analysis

Data were analysed on Graphpad Prism 3 (GraphPad Software Inc., San Diego, California) with one-way analysis of variance (ANOVA). Post-hoc tests were conducted with the Tukey test. Data are reported as means ± standard error of mean. Values of $p < 0.05$ were considered significant.

Table 1: Effects of silver nanoparticles on levels of reduced glutathione (GSH), malondialdehyde (MDA), superoxide dismuatse (SOD), catalase (CAT) and glutathione −s− transferase (GST) in rat serum, after daily oral exposure.

	GSH	MDA	SOD	CAT	GST
7 days					
Control (0 mg/kg)	0.14 ± 0.01	0.05 ± 0.00	6.06 ± 0.39	0.45 ± 0.02	3.73 ± 0.14
100 mg/kg	0.15 ± 0.01	0.17 ± 0.00*	8.23 ± 0.07*	0.13 ± 0.03*	0.13 ± 0.01*
1000 mg/kg	0.16 ± 0.00	0.19 ± 0.01*	12. 46 ± 0.34*	0.18 ± 0.02*	0.22 ± 0.00*
5000 mg/kg	0.16 ± 0.02	0.29 ± 0.00*	12.56 ± 0.21*	0.25 ± 0.02*	0.65 ± 0.02*
14 days					
Control (0 mg/kg)	0.14 ± 0.01	0.05 ± 0.00	6.06 ± 0.39	0.45 ± 0.02	3.73 ± 0.14
100 mg/kg	0.12 ± 0.00	0.14 ± 0.03*	8.67 ± 0.67*	0.13 ± 0.02*	0.44 ± 0.00*
1000 mg/kg	0.01 ± 0.00*$^{\alpha}$	0.29 ± 0.00*	7.00 ± 0.28$^{\#}$	0.47 ± 0.03	1.23 ± 0.11*
5000 mg/kg	0.01 ± 0.00$^{\alpha}$	0.30 ± 0.00*	9.10 ± 0.23*	0.26 ± 0.02*	0.38 ± 0.20*
21 days					
Control (0 mg/kg)	0.14 ± 0.01	0.05 ± 0.00	6.06 ± 0.39	0.45 ± 0.02	3.73 ± 0.14
5000 mg/kg	0.01 ± 0.00$^{\alpha}$	1.00 ± 0.00*$^{\beta}$	10.06 ± 0.27*	0.36 ± 0.02$^{\beta}$	0.34 ± 0.01*

Values are mean ± SEM ($n = 5$). Values with different superscripts in the same column are significantly different at $p < 0.05$. * is significant relative to control group; α is significant relative to groups receiving 100 mg/kg; β is significant relative to groups receiving 100 and 1000 mg/kg.

Results

The aqueous solution of silver nanoparticles had a characteristic brownish-yellow colour (Figure 1). TEM images showed that the diameter of the nanoparticles was 8−20 nm.

Daily administration of silver nanoparticles to rats significantly decreased the levels of GSH relative to the control group in serum (), liver, kidney and heart, while it significantly increased the levels of malondialdehyde (Tables 1−4), depending on the concentration and duration of treatment. Silver nanoparticles inconsistently increased the serum level of superoxide dismutase relative to the control group (Tables 1−4) but significantly increased the levels in liver, kidney and heart. Inconsistent alterations were observed in the levels of catalase relative to controls (Tables 1−4). The nanoparticles decreased the serum and tissue levels of GST at all concentrations (Tables 1−4).

Discussion

Exposure to silver nanoparticles significantly decreased the levels of GSH in rat serum and tissues. GSH is an antioxidant that can quench free radicals or serve as a substrate for other antioxidant enzymes, such as glutathione peroxidase and glutathione reductase. The decreased levels of GSH after exposure to silver nanoparticles may be due to complexing of silver nanoparticles with thiol groups[20,21] or to increasing use of GSH to downplay the effect of free radicals after exposure to of the nanoparticles.[22] These nanoparticles have a strong affinity for thiol groups[20] and may therefore predispose to a decrease in GSH content, thereby leading to the formation of complexes between radical species and cellular proteins or other biomolecules.

The levels of malondialdehyde were elevated in serum and tissues after exposure to silver nanoparticles, which may

Table 2: Effects of silver nanoparticles on levels of reduced glutathione (GSH), malondialdehyde (MDA), superoxide dismuatse (SOD), catalase (CAT) and glutathione −s− transferase (GST) in rat liver, after daily oral exposure.

	GSH	MDA	SOD	CAT	GST
7 days					
Control (0 mg/kg)	0.16 ± 0.00	0.03 ± 0.00	2.21 ± 0.01	1.41 ± 0.03	3.17 ± 0.11
100 mg/kg	0.07 ± 0.00*	0.08 ± 0.00*	5. 85 ± 0.20*	1.88 ± 0.10*	2.19 ± 0.02*
1000 mg/kg	0.07 ± 0.00*	0.11 ± 0.01*	4. 92 ± 0.25*$^{\alpha}$	1.87 ± 0.08*	0.46 ± 0.00*
5000 mg/kg	0.06 ± 0.00*	0.13 ± 0.01*	5.79 ± 0.17*	1.13 ± 0.10	0.67 ± 0.02*$^{\beta}$
14 days					
Control (0 mg/kg)	0.16 ± 0.00	0.03 ± 0.00	2.21 ± 0.01	1.41 ± 0.03	3.17 ± 0.11
100 mg/kg	0.04 ± 0.00*	0.08 ± 0.00*	3.27 ± 0.09*	0.42 ± 0.02*	2.28 ± 0.07*
1000 mg/kg	0.04 ± 0.00*	0.20 ± 0.00*	3.18 ± 0.11*$^{\alpha}$	0.38 ± 0.01*	2.23 ± 0.11*
5000 mg/kg	0.06 ± 0.00*	0.14 ± 0.02*	3.77 ± 0.10*	0.62 ± 0.05*$^{\beta}$	1.15 ± 0.03*$^{\beta}$
21 days					
Control (0 mg/kg)	0.16 ± 0.00	0.03 ± 0.00	2.21 ± 0.01	1.41 ± 0.03	3.17 ± 0.11
5000 mg/kg	0.01 ± 0.00*	0.33 ± 0.01*	6.10 ± 0.40*	0.51 ± 0.03*	0.93 ± 0.10*$^{\beta}$

Values are mean ± SEM ($n = 5$). Values with different superscripts in the same column are significantly different at $p < 0.05$. * is significant relative to control group; α is significant relative to groups receiving 100 and 5000 mg/kg for 7 and 21 days; β is significant relative to groups receiving 100 and 1000 mg/kg.

Table 3: Effects of silver nanoparticles on levels of reduced glutathione (GSH), malondialdehyde (MDA), superoxide dismuatse (SOD), catalase (CAT) and glutathione −s− transferase (GST) in rat kidney, after daily oral exposure.

	GSH	MDA	SOD	CAT	GST
7 days					
Control (0 mg/kg)	0.16 ± 0.01*	0.06 ± 0.03	5.85 ± 0.45	0.53 ± 0.06	1.69 ± 0.05
100 mg/kg	0.04 ± 0.00*	0.07 ± 0.02	9.77 ± 0.44*	0.27 ± 0.05*	0.69 ± 0.06*
1000 mg/kg	0.06 ± 0.00*	0.10 ± 0.04*	17.38 ± 0.79*	0.11 ± 0.01*	0.55 ± 0.03*
5000 mg/kg	0.07 ± 0.01*	0.10 ± 0.00*	14.68 ± 1.12*	0.28 ± 0.02*	0.27 ± 0.05*
14 days					
Control (0 mg/kg)	0.16 ± 0.01	0.06 ± 0.03	5.85 ± 0.45	0.53 ± 0.06	1.69 ± 0.05
100 mg/kg	0.01 ± 0.00*	0.21 ± 0.02*	10.65 ± 0.33*	0.31 ± 0.06	0.66 ± 0.08*
1000 mg/kg	0.04 ± 0.00*	0.10 ± 0.04*	6.73 ± 0.06$^{\beta}$	0.40 ± 0.04	0.93 ± 0.06*
5000 mg/kg	0.06 ± 0.01*	0.10 ± 0.01*	17.71 ± 0.84*	0.31 ± 0.06	0.82 ± 0.05*
21 days					
Control (0 mg/kg)	0.16 ± 0.01	0.06 ± 0.03	5.85 ± 0.45	0.53 ± 0.06	1.69 ± 0.05
5000 mg/kg	0.07 ± 0.00*	0.26 ± 0.10*$^{\alpha}$	14.74 ± 0.29*	0.34 ± 0.05	0.91 ± 0.06*

Values are mean ± SEM ($n = 5$). Values with different superscripts in the same column are significantly different at $p < 0.05$. * is significant relative to control group; α is significant relative to groups receiving 100 and 1000 mg/kg; β is significant relative to groups receiving 100 and 5000 mg/kg.

Table 4: Effects of silver nanoparticles on levels of reduced glutathione (GSH), malondialdehyde (MDA), superoxide dismuatse (SOD), catalase (CAT) and glutathione −s− transferase (GST) in rat heart, after daily oral exposure.

	GSH	MDA	SOD	CAT	GST
7 days					
Control (0 mg/kg)	0.19 ± 0.001	0.04 ± 0.00	4.72 ± 0.36	1.10 ± 0.02	2.23 ± 0.04
100 mg/kg	0.17 ± 0.003$^{\alpha}$	0.06 ± 0.00	7.17 ± 0.50*	0.87 ± 0.05	0.23 ± 0.01*
1000 mg/kg	0.01 ± 0.001*	0.06 ± 0.00	8.80 ± 0.50*	0.83 ± 0.05	1.06 ± 0.04*
5000 mg/kg	0.01 ± 0.001*	0.07 ± 0.00	10.96 ± 0.32*	0.81 ± 0.07	0.81 ± 0.01*
14 days					
Control (0 mg/kg)	0.19 ± 0.001	0.04 ± 0.00	4.72 ± 0.36	1.10 ± 0.02	2.23 ± 0.04
100 mg/kg	0.10 ± 0.002*	0.89 ± 0.01*	5.39 ± 0.07	0.78 ± 0.03	0.10 ± 0.00*
1000 mg/kg	0.13 ± 0.002	0.74 ± 0.01*	10.90 ± 0.28*	0.47 ± 0.03*	0.15 ± 0.00*
5000 mg/kg	0.10 ± 0.001*	0.84 ± 0.01*	6.99 ± 0.32	0.26 ± 0.03*	0.13 ± 0.01*
21 days					
Control (0 mg/kg)	0.19 ± 0.001	0.04 ± 0.00	4.72 ± 0.36	1.10 ± 0.02	2.23 ± 0.04
5000 mg/kg	0.01 ± 0.001*	1.28 ± 0.02*$^{¥}$	8.76 ± 0.43*	0.36 ± 0.02*	0.34 ± 0.01*

Values are mean ± SEM ($n = 5$). Values with different superscripts in the same column are significantly different at $p < 0.05$. * is significant relative to control group; α is significant relative to groups receiving 1000 and 5000 mg/kg; ¥ is significant relative to groups receiving 100 and 1000 mg/kg.

indicate oxidative stress. A similar result was found previously in zebrafish.[15] We also found that superoxide dismutase levels were inconsistently elevated after exposure to the nanoparticles. This is an inducible enzyme, and elevated levels may indicate the presence of reactive species; a previous report linked elevated superoxide dismutase levels to the presence of oxidative stress.[15] Catalase levels were inconsistently altered after exposure, as observed previously in broilers.[23] Conversely, silver nanoparticles caused inconsistent reductions in the levels of GST in serum and tissues, perhaps because the nanoparticles have an affinity for thiol groups.[20,21] The alterations in the levels of these enzymes may represent an adaptive mechanism to offset the stress of exposure.

Our findings are not completely in accordance with those of an earlier report,[13] in which the LD_{50} of silver nanoparticles in mice was >5000 mg/kg. Other studies showed that exposure of rats to silver nanoparticles can alter biochemical processes[24,25] independently of dose,[26] and several studies have demonstrated that these nanoparticles can sequester and accumulate in vital organs, including the kidney, liver, testes and brain.[24–26] This may explain why the biochemical alterations caused by silver nanoparticles became more pronounced with longer exposure and indicates that caution should be exercised in their use for biomedical purposes.

Conclusions

Silver nanoparticles caused lipid peroxidation and altered the levels of GSH, superoxide dismutase and catalase in Wistar rats. Further in-depth studies of the interactions between metal nanoparticles and cellular materials are required to increase our understanding of their mechanisms of action.

Conflicts of interest

The authors have no conflict of interest to declare.

Authors' contribution

AOS conceived the study, designed the experimental protocol, analysed the results and drafted the manuscript. FTO collected the experimental data.

Acknowledgements

We thank the National Research Foundation of South Africa and the Department of Biochemistry, Rhodes University, South Africa.

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