Antioxidant and anti-apoptotic effects of selenium nanoparticles against murine eimeriosis

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Abstract: Eimeriosis is caused by a protozoan parasite of the genus Eimeria and infection affecting most domestic animal species. The aim of this research was to comprehend the impact of selenium nanoparticles (SeNPs) on eimeriosis induced by Eimeria papillata in mouse jejunum, and how they work as antioxidants and anti-apoptotic agents against eimeriosis. The numbers of meronts, gamonts, and developing oocysts of E. papillata reduced after the infected mice were treated with the SeNPs. The levels of malondialdehyde (MDA), nitric oxide (NO), and other oxidative stress-related molecules, such as glutathione (GSH), catalase (CAT), and superoxide dismutase (SOD), were assayed. E. papillata was able to change the redox status of the jejunal cells; this was confirmed by the elevation of the MDA and NO levels, and the decrease of the GSH levels and the activities of the antioxidant enzymes CAT and SOD. SeNP treatment significantly reversed this disturbance of the redox status. The expression levels of the apoptotic markers Bax and caspase-3 in the jejunal samples were evaluated using qRT-PCR. The SeNPs decreased the Bax and caspase-3 expression after being administered to the E. papillata-infected mice. Collectively, the SeNPs demonstrated antioxidant and anti-apoptotic activities against murine eimeriosis.

Key words: selenium nanoparticles, eimeriosis, oxidative stress, apoptosis, mice.

INTRODUCTION

Eimeriosis is one of the most dangerous poultry diseases around the world (Ola-Fadunsin & Ademola 2013). This parasitic disease, which is caused by coccidian parasites belonging to the genus Eimeria, induces problems for the poultry industry (Lawal et al. 2016). Reduced weight, diarrhea, and dehydration are the common clinical signs after the infection (Mehlhorn 2014). This disease has led to a global annual loss of about $3 billion USD for the poultry industry (Blake & Tomley 2014).
agent with low toxicity (Hu et al. 2012, Gangadoo et al. 2018).

During eimeriosis, there was an imbalance in the antioxidant status, where free radicals were highly produced (Dkhil et al. 2013). Additionally, the parasite stimulates the formation of reactive oxygen species, to induce injuries to the intestinal epithelium, resulting in an overload of free radicals and oxidative stress (Georgieva et al. 2006). Several studies confirmed that NS could efficiently produce favorable antioxidant effects (Bai et al. 2017). In addition, Alkhudhayri et al. (2018) reported that selenium nanoparticles (SeNPs) are considered to be efficient antioxidants.

Our previous work demonstrated the anti-inflammatory effects of NS against *E. papillata* infection. These effects occurred via the downregulation of the gene expression of pro-inflammatory cytokines (Alkhudhayri et al. 2018). This is the first study to our understanding that examines the impacts of SeNPs on eimeriosis induced apoptosis. The efficient dose of the SeNPs used in this research was the same as the dose used in our prior research (0.5 mg/kg); thus, we investigated the role of SeNPs against apoptotic changes in mouse jejunum and the oxidative damage caused by *E. papillata* infection.

**MATERIALS AND METHODS**

**Characterization of SeNPs**

NS with a particle size of about 5-50 nm was purchased from the Nanocs Inc (Boston, MA, USA). The nanoparticles were re-dispersed in aqueous medium by sonication. Transmission electron microscopy (TEM) was performed using a JEOL JEM-2100 microscope (JEOL Ltd., Tokyo, Japan) with an accelerating voltage of 200 kV to characterize the shape and size of SeNPs (Jiang et al. 2008). The composition or finger print of the NS was characterized by Fourier Transform Infrared (FTIR) spectroscopy in the range of 4000-400 cm⁻¹.

**Animals**

Thirty adult male C57BL/6 mice (20-23 g, 10-12 weeks old) were obtained from the animal housing facilities at the King Saud University. A normal diet and water ad libitum were fed to the mice. All animal procedures and tests in this research have been endorsed by the committee of the Department of Zoology, King Saud University (Number: DGS 1438/6/20) and performed in accordance with animal experimentation rules.

**Infection and treatment**

To prepare the parasite culture (sporulated *Eimeria papillata* oocysts) for the infection of the mice, unsporulated oocysts were collected from mice feces and allowed to sporulate in 2.5% (w/v) potassium dichromate, counted, and then expressed as the number of oocysts per gram of feces (Schito et al. 1996).

The animals were divided into three groups (10 mice per group): non-infected control group, comprising mice that orally gavaged with 100 μl of phosphate buffer, infected group, comprising mice infected with 1000 sporulated oocysts of *E. papillata*, and the infected-treated group, in which after being infected with 1000 oocysts, the mice were daily inoculated with 0.5 mg/kg SeNPs for 5 days. The dose was chosen depending on our previous work (Alkhudhayri et al. 2018). All animals were killed by dislocation of the cervix.

**Sample collection**

On day 5 post-infection (p.i.), fresh feces were collected from the mice and the oocyst output per gram of feces was calculated (Esch & Petersen 2013). Additionally, the suppression of oocyst shedding was calculated as follows:

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100 - (oocysts output in treated group/oocysts output in the infected group) × 100.

**Parasitic stages**

To evaluate the number of *E. papillata* cells from different parasitic stages in the mouse jejunum samples, fresh jejuna were collected after the dissection of the mice on day 5 p.i. and fixed in 10% buffered formalin. Paraffin sections were prepared and stained with eosin and hematoxylin (Adam & Caihak 1964). Under the light microscope, the number of meronts, gamonts, and developing oocysts per 10 crypt-villus units were counted.

**Oxidative stress markers and antioxidant enzymes**

A part of the jejunal samples was homogenized immediately, to prepare a 10% (w/v) homogenate in ice-cold medium containing 50 mM Tris-HCl and 300 mM sucrose (Tsakiris et al. 2004). For 10 min, the homogenate was centrifuged at 1500 g. For the different biochemical determinations, the supernatant (10 percent) was used. Ellman’s (1959) method has been used to determine glutathione (GSH). Lipid peroxidation was determined using the method described by Ohkawa et al. (1979). The nitric oxide (NO) level was measured by the determination of the total nitrate and nitrite concentrations in the samples using the method described by Berkels et al. (2004). Catalase (CAT) and superoxide dismutase (SOD) activities were assayed according to the protocols described by Aebi (1984) and Nishikimi et al. (1972), respectively.

**Apoptotic changes**

To examine the apoptotic changes induced by *E. papillata* infection, the mRNA expression of Bcl-2-associated X protein (Bax) and cysteine-aspartic acid protease-3 (Caspase-3) was assayed using quantitative real-time polymerase chain reaction (RT-PCR). Using Trizol (Thermo Fisher Scientific, Waltham, MA, USA), total RNA was isolated from the jejunal samples of the mice. QuantiTect® Reverse Transcription kit (Qiagen, Hilden, Germany) was used to generate the cDNA. RT-PCR was performed using the QuantiTectTM SYBR® Green PCR kit (Qiagen). The 2−ΔΔCt method described by Livak & Schmittgen (2001) was used to evaluate the differences between the mean expression levels of the apoptotic genes and the reference gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Livak & Schmittgen 2001). The following primers, which were purchased from Qiagen (Hilden, Germany), were used: CASP3 (Mm_Casp3_1_SG, QT00260169), BAX (Mm_Bax_1_SG, Cat. No. QT00102536), and GAPDH (Mm_Gapdh_3_SG, Cat. No. QT01658692) (Krücken et al. 2009).

**Statistical analysis**

ANOVA analysis was performed in one way and statistical comparisons between groups were performed using Duncan’s test. Values were expressed as the mean ± SD, at a significance level of p≤ 0.05.

**RESULTS**

To evaluate the detailed morphology of the nanoparticles, the samples were analyzed using TEM. The morphology of the SeNPs is presented in Figure 1a. The image shows that the NPs are very small and fine, with a clear spherical and cubic-shaped morphology. The size of each nanoparticle is about 20 nm, with a spherical shape. Figure 1b shows the high-magnification TEM image of the NPs; the lattice distance between two fringes is ~0.235 nm, which corresponds to the lattice constant of the SeNPs. Additionally, FTIR spectroscopy
was utilized to identify the functional groups present in aqueous SeNPs (Figure 2). For the analysis of FTIR measurement, the liquid sample comprising the SeNPs was drop casted onto the KBr plate and fixed to the holder. The FTIR spectrum shows the representative asymmetric and symmetric stretching vibration bands of the SeNPs. A broad peak was observed in the range of 3200-3500 cm⁻¹, which is closely related to the amide (–N–H) group, whereas the peak at 1635 cm⁻¹ represents the C=O group (Kalishwaralal et al. 2014). The peak at 791 cm⁻¹ corresponds to the stretching mode of the metal peaks of selenium.

Jejunal sections from mice infected with *E. papillata* on day 5 p.i. (Figure 3) showed different the developmental stages of the parasite, i.e. meronts, gamonts and developing oocysts. The number of meronts, gamonts, and developing oocysts of the parasite decreased from 14.2 ± 0.7, 22 ± 0.9, 30.2 ± 1.2 in the mice from the infected group to 10.2 ± 0.1, 11 ± 0.3, 10.6 ± 0.2 in those from the infected-treated group, respectively (Table I). The SeNPs were able to suppress the proportion of meronts, gamonts, and developing oocysts by 28.2, 50, and 64.7%, respectively (Table I).

To investigate the antioxidant effects of the SeNPs, the GSH, malondialdehyde, NO, CAT, and SOD levels were examined (Table II). Compared to the non-infected control group, the infected jejunal level of GSH (0.422±0.05), malondialdehyde (97.32±7.76) and NO (1.74±0.21) were changed. Also, the same for the activity of CAT (43.78±5.95) and SOD (11.5±2.44), they were significantly decreased.

The disturbances in the levels of these markers due to *E. papillata* infection were significantly ameliorated, and the oxidative stress in the jejunum was inhibited after the treatment with the SeNPs.

There was a significant change in the expression of apoptotic genes after the treatment of infected mice with the SeNPs. *E. papillata* infection induced the upregulation of the gene
expression of Bax and Caspase-3 (Figure 4). The SeNPs induced the downregulation of Bax and Caspase-3 gene expression. The Caspase-3 gene expression decreased significantly from 2.01 to 1.2 fold, and the Bax gene expression was downregulated from 2.9 to 2.3 fold after treatment with the SeNPs (Figure 4).

**DISCUSSION**

Nanoparticles are now offer new opportunities for novel medical treatments to counter infection by parasites (Alkhudhayri et al. 2018). For example, nano-selenium was used against schistosomiasis (Dkhil et al. 2016) and leishmaniasis (Beheshti et al. 2013).

This study demonstrated that SeNPs show anti-coccidial, antioxidant, and anti-apoptotic activities during *E. papillata* infection. The imbalance of the antioxidant defense system due to *Eimeria* infection leads to harmful cellular effects (Esch & Petersen 2013). To control the oxidative stress induced by the infection, the parasite increases the levels of antioxidant enzymes such as CAT (Dkhil 2013). This enzyme catalyzes the conversion of hydrogen peroxide into water and oxygen. Thus, the level of H₂O₂ is diminished (Bosch et al. 2015). Additionally, the level of the biological marker malondialdehyde increases during infection with *Eimeria* (Dkhil et al. 2013). Treatment of the infected mice with SeNPs significantly reduced the oxidative damage caused by *Eimeria* infection. In our previous study, we proved that only the glutathione peroxidase activity increased in the jejuna of *E. papillata*-infected mice after treatment with NS (Alkhudhayri et al. 2018).
The oxidative damage induced in the jejuna of the infected mice activates molecular pathways to drive jejunal inflammation. Our previous study reported that NS could significantly improve the histopathological changes caused in the jejunum due to *E. papillata* infection, and also increase the glutathione peroxidase level in the jejunum of infected mice (Alkhudhayri et al. 2018).

In this study, we focused extensively on the oxidant/antioxidant status during *Eimeria* infection. The results showed that SeNPs work as excellent antioxidants. This was evidenced by the increase in lipid peroxidation, and the increase in SOD activity due to the enhanced scavenging of O$_2$ after the treatment of the infected mice with the SeNPs.

The anti-eimerial effect of the SeNPs has been proven via two ways. The first way involves the decreased oocyst output in the feces from mice in the treated group. The second way involves the ability of the SeNPs to significantly (p<0.01) decrease the number of meronts, gamonts, and developed oocysts in the jejunum from *E. papillata*-infected mice. Dkhil et al. (2013) proved the same effect, but by adding elemental selenium to the diet of the *E. papillata*-infected mice. Suradji et al. (2001) reported that selenium has a negative effect on the development of the pathogenic malarial parasite *P. falciparum*. Since the development of the parasite is affected by the intestinal microbiota, selenium could alter this effect (Hrdina et al. 2009, Kasaikina et al. 2012).
In a variety of intracellular parasitic infections, apoptosis could regulate the host response to parasites and could help in the elimination of injured or infected cells (Lüder et al. 2001, Balamurugan et al. 2002). Tissue homeostasis is maintained by apoptosis (Vaux & Strasser 1996), and the relationship between the developmental stages of *E. papillata* and host apoptosis has not yet been investigated intensively. In this study, the death of mouse jejunal cells was evidenced by a significant upregulation of the pro-apoptotic genes, Bax and caspase-3. Rossé et al. (1998) reported that Bax has been shown to induce cytochrome c release and caspase activation, which in turn, leads to cell death. Our group reported the increase in the number of apoptotic cells in the jejuna from *E. papillata*-infected mice (Dkhil 2013, Metwaly et al. 2014). SeNPs could improve the eimeriosis-induced apoptotic changes in jejunal cells. The anti-apoptotic activity of SeNPs during myringosclerosis has also been documented by Görür et al. (2002).

Our results demonstrate that SeNPs possess an antioxidant and anti-apoptotic activity against murine eimeriosis. Further studies are required to elucidate the mechanism underlying their effects on the host and parasite.

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