

Submitted: 11/04/2018

Accepted: 21/09/2018

Published: 15/10/2018

Comparative survey on scolicial effects of selenium and silver nanoparticles on protoscolices of hydatid cyst

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Abstract

Hydatidosis is a zoonotic diseases which affects human and livestock animals. The surgical treatment of hydatid diseases in man is still the most effective approach, but spillage of protoscolices is the risk of surgery. Therefore, it is essential to develop alternative approaches. Injection of protoscolicidal agents into the cysts preoperatively is an alternative method. The aim of the present study was the determination and comparison of lethal effects of silver nanoparticles (Ag-NPs) and selenium nanoparticles (Se-NPs) on protoscolices of hydatid cyst. Livers and lungs of sheep, which was naturally infected by hydatid cyst were collected from Tabriz slaughterhouse and transferred to the parasitology laboratory. Initially, the surface of cysts was disinfected by 70% Alcohol. Then, 25 ml of each cyst fluid were aspirated by sterile syringe and transferred to Erlenmeyer flask. After 30 minutes, the supernatant was discarded and protoscolices were washed with phosphate-buffered saline and tested by 1% eosin to assess their viability. Next, the samples of protoscolices, which were over 90%, were selected and the effect of 50, 125, 250 and 500 ($\mu\text{g/ml}$) of Se-NPs and Ag-NPs in the exposure times of 10, 20, 30 and 60 min were evaluated. Data were analysed by SAS software and analyzed by Duncan's comparison test. The significant differences were considered to be $p < 0.0001$. The difference between the scolicial effects of Se-NPs was statistically significant for all examined concentrations and exposure times compared to the control group ($p < 0.0001$). On the other hand, the scolicial effect of Ag-NPs in 10 and 20 min exposer was not significant in comparison to the control group. Overall scolicial effect of Se-NPs was higher than Ag-NPs and the difference was significant ($p < 0.0001$). The results of the present research indicated that it is possible to use Se-NPs as an effective scolicial treatment. It is suggested the study of employing this compound in vivo or in combination with other procedures in treating hydatid cyst in further studies.

Keywords: Ag-NPs, Hydatid cyst, Nanoparticle, Se-NPs.

Introduction

Echinococcosis, which is commonly called hydatidosis, is a zoonotic disease. This disease caused by larval stages (metacestodes) of the tapeworm *Echinococcus granulosus* and results in significant financial losses in livestock animals. Besides, it has led to a serious health problem in human in a number of countries (Eckert *et al.*, 2001). Human cystic hydatidosis is a chronic and potentially life-threatening infection with the larval cystic stage (hydatid) of the dog taeniid tapeworm *E. granulosus*. Adult tapeworms can measure up to 2-7mm long. Adult worms attach themselves to the walls of the small intestine of infected dogs and its eggs passed from feces of dogs and infested the environment. Following ingestion by a suitable intermediate host, e.g. human, sheep, and other livestock animals, the eggs hatch in the upper small intestine and hydatid cyst is formed in intermediate host's viscera, including the liver, and lungs, and less frequently in the muscle, kidney, bone, spleen, and other organs (Eckert and Deplazes, 2004). The surgical treatment of hydatid diseases (percutaneous aspiration,

injection, and respiration-PAIR) is still the most effective approach, which can be done successfully in a large number of patients if a cyst does not have a risky location. However the operation is risky when the cyst is either in multiple organs or in risky locations including in brain or other vital organs. In the other hand to reduce the risk of inoperative spillage protoscolices of cysts and formation of secondary cysts, alternative and low risky methods can be replace surgery. Injection of protoscolicidal agents into the cysts preoperatively is an alternative method. These agents such as Ag-nitrate, cetrimide, hypertonic saline, and ethanol are employed to treat hydatid cyst but they have side effects such as toxemia, liver necrosis, and sclerosane colangitis (Rahimi *et al.*, 2015). Therefore, it is essential to develop new scolicial materials with more efficacies and low side effects, which can be implemented during surgery. It has currently been demonstrated that nanoparticles (NPs) have various unique properties due to their large surface-volume ratio. NPs are also able to enter cells more frequently than other particles. Among several metal NPs, which

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have been evaluated, one can name copper (Cu), magnesium (Mg), gold (Au), zinc (Zn), titanium (Ti), and silver (Ag) (Retchkiman-Schabes *et al.*, 2006; Khan *et al.*, 2017). The synthesized silver NPs (Ag-Nps) were introduced as the most effective one with large capacity in direct contact with their environment and it has acceptable antimicrobial efficacy against parasites, viruses, and bacteria (Mahmoudvand *et al.*, 2014). Few studies have shown that selenium NPs (Se-NPs) can effectively inhibit the growth of some bacteria such as *Staphylococcus aureus* and pathogenic *Escherichia coli* (Yang *et al.*, 2009; Tran and Webster, 2011). The aim of the study was to explore and compare the scolicidal effects of in vitro Ag-NPs and Se-NPs.

Material and Methods

Collection of protoscolices

Livers and lungs of sheep, which were infected naturally by hydatid cyst, were collected from Tabriz slaughterhouse, northwest of Iran. In the laboratory, the hydatid fluid was aspirated by a 20 ml syringe and aseptically transferred into an Erlen Meyer flask. After 30 min, the supernatant was discarded and the protoscolices were washed three times with phosphate-buffered saline (pH7.2) solution. The viability of the protoscolices was tested using eosin solution (0.1%) under a light microscope. The concentration of protoscolices per ml of the hydatid fluid in normal saline solution (0.9% NaCl) was detected. Samples containing 5000 ml protoscolices with more than 90% viability were used.

Characterization of the Se-NPs and Ag-NPs

Se-NPs and Ag-NPs were purchased from *Iranian Nanomaterials Pioneers Company*, NANOSANY (Mashhad, Iran). The size of NPs was estimated to be 5-8 nm in diameter, metal basis and spherical. Deionized water was used to prepare desirable solutions.

Scolicidal effects of the Se-NPs and Ag-NPs

In the present study, different concentrations of Ag-NPs and Se-NPs including 50, 125, 250, and 500 ($\mu\text{g/ml}$) were used for different exposure times including 10, 30 and 60 minutes. Initially, 0.5 ml of the protoscolices solution was placed in test tubes. Then 0.5 ml of various Se-NPs and Ag-NPs concentrations were added to each test tube. The tubes were incubated at 37 °C for 10, 20, 30, and 60 minutes. At the end of each incubation, the supernatant was removed. Next, 50 μl of 0.1% Eosin stain was added to each tube. After 5 minutes, protoscolices were smeared on a glass slide, were covered with a cover glass, and examined under a light microscope.

Viability

Stained protoscolices were considered as dead and unstained protoscolices were recorded as alive. The control group included non-treated protoscolices with the NPs.

Statistical analysis

Data were analysed by SAS software and means compared with *Duncan's* comparison test. The differences were considered significant at least $p < 0.05$.

Results

The scolicidal effect of various Se-NPs concentrations for different exposure times was shown in Fig 1. The difference between the scolicidal effects of Se-NPs was statistically significant for all examined concentrations and exposure times in comparison to the control group ($p < 0.0001$). Scolicidal effect of various Ag-NPs concentrations for a different exposed time was shown in Fig. 2. As seen in Figs. 1 and 2, the scolicidal effect of Ag-NPs in 10 and 20 min exposure was not significant in comparison to the control group. Overall scolicidal effect of Se-NPs was higher than Ag-NPs and these difference was significant ($p < 0.0001$) (Fig. 3).

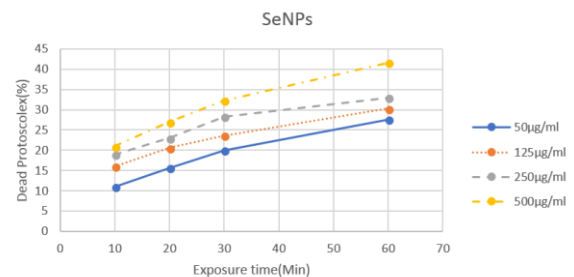


Fig. 1. Scolicidal effects of selenium nanoparticles (Se-NPs) against protoscolices of *Echinococcus granulosus* at the various concentrations following various exposure times.

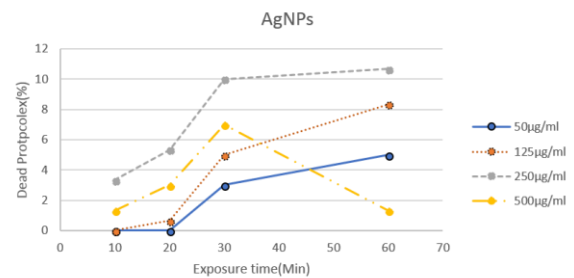


Fig. 2. Scolicidal effects of silver nanoparticles (Ag-NPs) against protoscolices of *Echinococcus granulosus* at the various concentrations following various exposure times.

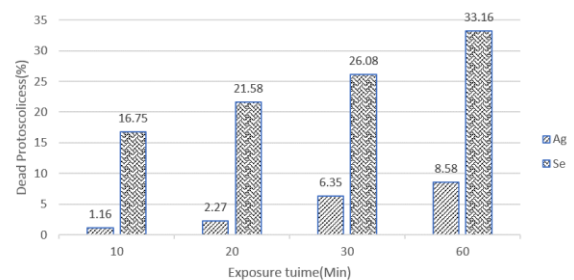


Fig. 3. Comparison on scolicidal effect of silver nanoparticles (Ag-NPs) and selenium nanoparticles (Se-NPs).

Discussion

In this study, scolicidal effect of various dilutions (50, 125, 250, and 500 µg/ml) of in vitro Ag-NPs and Se-NPs in different exposure times (10, 20, 30, and 60 min) were compared. Overall scolicidal effect of Se-NPs was higher than Ag-NPs.

By considering the side effects of surgical approaches in the treatment of CHD, such as the formation of new cysts or death due to spillage of the cyst contents, injection, and respiration of scolicidal agents are suggested for replacing routines surgical methods (Yang *et al.*, 2009). Many studies were performed on the scolicidal effect of chemical compounds such as hypertonic saline, silver nitrate, cetrimide, ethyl alcohol, H₂O₂, povidone iodine, Albendazole, and chlorohexidine gluconate. However, the majority of these compounds had side effects that limit their use in the treatment of in vivo hydatid cyst (Rouhani *et al.*, 2013). Also in other studies, the scolicidal effect of plant extracts such as *peganum harmala* and *Alium sativum* (garlic), *Zataria multiflora*, *Olea Europaea*, *Pistacia Atlantica* and *ephedra major* was proved (Yetim *et al.*, 2005; Nematollahi and Ghazi, 2014; Mahmoudvand *et al.*, 2016; Zibaei *et al.*, 2017). Recently many studies showed that NPs have potent scolicidal effect against protoscolices of hydatid cyst.

NPs are the wide class of materials that include particulate substances, which have one dimension less than 100 nm at least (Laurent *et al.*, 2008). NPs have drawn increasing interest from every branch of medicine because of their ability to deliver drugs in the optimum dosage range, which often results in an increased therapeutic efficacy of the drugs, weakened side effects and improved patient compliance (Alexis *et al.*, 2008).

The implication of NPs against parasites and other microorganism was shown in previous studies. Beheshti *et al.* (2013) reported the efficacy of biogenic Se-NPs against *Leishmania major* in vitro and in vivo. They showed that Se-NPs could inhibit the proliferation of the promastigote and amastigote forms of *L. major* and limit localized cutaneous leishmaniasis in an animal model (Beheshti *et al.*, 2013). Mahmoudvand *et al.* (2014) used biogenic Se-NPs against protoscolices and proved potent scolicidal effects especially at concentrations of 500 and 250 mg/mL. They showed that the scolicidal activity was 100% after 10 and 20 minutes of application, respectively (Mahmoudvand *et al.*, 2014). Rahimi *et al.* (2015) used Ag-NPs against protoscolices and showed that the at all concentrations had high scolicidal effects. Besides, they emphasized that the concentrations of 0.1 and 0.15 mg/mL after 120 min of exposure times showed 83% and 90% mortality rate, respectively (Rahimi *et al.*, 2015).

In the present study, both Se and Ag-NPs were used against protoscolices in vitro and the scolicidal effects

of them were compared. The results of this study were proved that scolicidal effect of Se-NPs was higher than Ag-NPs. Besides, the results revealed that scolicidal effect at concentration 500 µg/ml was comparable with scolicidal effects of chemical compounds and plants extracts as previously described.

Although the silver compounds such as Ag-nitrate was reported as one of the most effective scolicidal agents, Lashkarzadeh *et al.* (2015) reported that Ag-NPs was less effective against protoscolices of hydatid cyst (Lashkarzadeh *et al.*, 2015). A similar finding of Ag-NPs was reported by other researchers. They exhibited that Ag-NPs were not as effective as Ag ions in killing *Escherichia coli* bacteria (Choi *et al.*, 2008).

The results of present study showed a high effect of Se-NPs on protoscolices of hydatid cyst. This result was similar to that of Beheshti *et al.* (2013), who proved that biogenic Se-NPs had toxic effect against *Leishmania major* in vitro (Beheshti *et al.*, 2013). On the other hand, the safety of Se-NPs for living organisms was proved. For example, Shakibaie *et al.* (2013) showed this by oral administration of 10 mg/kg of Se-NPs to male mice (Shakibaie *et al.*, 2013).

In conclusion, it is suggested the study of employing this compound in vivo or in combination with other procedures in treating hydatid cyst in further studies.

Acknowledgment

This work was financially supported by University of Tabriz.

Conflict of interest

The authors declare that there is no conflict of interest.

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