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Original research

Scolicidal effects of biogenic selenium nanoparticles against protoscolices of hydatid cysts

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ABSTRACT

Cystic echinococcosis (hydatid cyst, CE) as a zoonotic parasitic infection caused by the larval stage of the dog tapeworm *Echinococcus granulosus* is still an important economic and public health concern in the world. One of the treatment options for CE is surgical removal of the cysts combined with chemotherapy using albendazole and/or mebendazole before and after surgery. Currently, many scolicidal agents, which have some complications, have been used for inactivation of the cyst contents. Therefore the development of new scolicidal agents with low side effects and more efficacies is an urgent need for surgeons. The present study was aimed to investigate the *in vitro* scolicidal effect of selenium nanoparticles biosynthesized by a newly isolated marine bacterial strain *Bacillus* sp. MSh-1 against protoscoleces of *E. granulosus*. Protoscolices were aseptically aspirated from sheep livers having hydatid cysts. Various concentrations (50–500 μg/ml) of Se NPs (in size range of about 80–220 nm) were used for 10–60 min. Viability of protoscoleces was confirmed by 0.1% eosin staining. The results indicated that biogenic Se NPs at all concentrations have potent scolicidal effects especially at concentrations 500 and 250 μg/ml after 10 and 20 min of application, respectively. In conclusion, the findings of present study proven that Se NPs have potent scolicidal effects, therefore may be used in CE surgery. However, the *in vivo* efficacy of these NPs remains to be explored.

1. Introduction

Cystic echinococcosis (CE) or hydatid disease as a zoonotic parasitic infection caused by the larval stage of the dog tapeworm *Echinococcus granulosus* is still an important economic and public health concern in many countries of the world, such as Iran. The disease affects humans as well as domestic livestock including cattle, sheep, camels, pigs, horses and others [1,2]. The final host is the dog, in which adult tapeworms attached to the intestinal epithelium undergo sexual reproduction, leading to the development of eggs. These eggs are shed into the environment with the feces. The eggs contain an oncosphere are ingested by a suitable intermediate host. Oncospheres released from the eggs penetrate the intestinal mucosa and via the portal system, are disseminated in the liver, lungs, muscle or other organs, where the hydatid cysts grow up [3].

Surgery is the preferred treatment for particular WHO stage disease. Chemotherapy with benzimidazoles and PAIR (puncture, aspiration, injection and reaspiration) are recommended as alternative treatments to surgery, especially for the patients who cannot tolerate surgery [4]. However, albendazole and mebendazole used in treatment of hydatid cysts showed different adverse effects such as hepatotoxicity, severe leucopenia, thrombocytopenia and alopecia [5]. In the other hand to reduce the risk of intraoperative spillage of the cyst contents (scoleces) and subsequently recurrence of CE and secondary infection, which is observed in nearly 10% of the postoperative cases, the use of effective scolicidal agents are obligatory [5,6]. The common scolicidal agents, hypertonic saline, Ag-nitrate, cetrimide, and ethanol, which used for inactivation of the cyst contents present different dangerous side effects such as sclerosane colangititis (biliary tract fibrosis), liver necrosis and methaemoglobinaemia [7,8]. Therefore the development of new

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scolicidal agents with low side effects and more efficacies is an urgent need for surgeons [9].

Selenium (Se) is an essential micronutrient element with fundamental roles in human health [10]. So far, it has been used in different medical therapies such as cancer prevention including lung, esophagus, prostate and gastric-cardiac cancers, antiviral activities and antioxidant effects [11–13]. In addition, Se has additional important health activities related to the immune response and successful reproduction [13,14]. It has currently been demonstrated that nanoparticles (NPs) due to their large surface-volume ratio showed various unique properties. NPs are also able to enter cells more frequently than other particles [15]. Few studies have shown that NPs particularly Se NPs can effectively inhibit the growth of some bacteria such as *Staphylococcus aureus* and pathogenic *Escherichia coli* [15,16]. The mechanism of Se against micro organism remains unclear but there is some studies showed that the inorganic forms of selenium can react with membrane peroxidases to generate oxygen free radicals, such as superoxide anion (O^{-2}) [17]. Furthermore, the ability of biogenic Se NPs to induce apoptosis in another form of eukaryotic cell, the *Leishmania major* promastigotes, has been previously reported [18].

However, to the best of our knowledge and according to a survey of the literature, the effect of biogenic Se NPs on *E. granulosus* protoscoleces remains mainly unexplored. The aim of this *in vitro* study was to evaluate the scolicidal effects of biogenic Se NPs against protoscoleces of *E. granulosus*.

2. Materials and methods

2.1. Chemicals

Eosin powder was purchased from Sigma–Aldrich, St Louis, MO, USA. Selenium dioxide (SeO_2), nutrient broth, nutrient agar, *n*-octyl alcohol, sodium dodecyl sulfate (SDS) and Tris base were purchased from Merck Chemicals (Germany). All other chemicals and solvents were of analytical grade. The micro-organism used in this study was identified as *Bacillus* sp. MSh-1 by the methods described earlier [10]. The organism was continuously conserved on nutrient agar plates complemented with 1.26 mM SeO_2 using continuous sub-culturing every 14 days.

2.2. Biosynthesis and characterization of the Se NPs

Se NPs were prepared according to the method described elsewhere [10]. Briefly, a sterile nutrient broth (NB) medium was supplemented with the Se^{+4} ions (100 mg/L; equal to 1.26 mM SeO_2 solution) and 100 ml of this medium was transferring to a 500-ml Erlenmeyer flask. The medium was inoculated with 1 ml of the fresh inoculums (OD_{600}, 0.1) of *Bacillus* sp. MSh-1 and was incubated aerobically at 30 °C in a shaker incubator (150 rpm). After 14 h, the bacterial cells and Se NPs were removed from the culture medium by using centrifugation at 4000 *g* (10 min). The pellets were washed with 0.9% NaCl solution using centrifugation, transferred to a mortar and then it was frozen by adding liquid nitrogen and was then disrupted by a pestle. The resulting slurry was ultrasonicated at 100 W for 5 min and washed three times by sequential centrifugation (10,000 *g*, 5 min), with a 1.5 M Tris–HCl buffer (pH 8.3) containing 1% SDS and deionized water. The next step involved extracting and purifying the Se NPs through an organic-aqueous partitioning system (n-octyl alcohol-water). For transmission electron microscopy, an aqueous suspension containing the Se NPs was dispersed ultrasonically, and a drop of the suspension was placed on carbon-coated copper TEM (transmission electron microscope) grids and dried under an IR lamp. The crystalline structure of the Se NPs was evaluated by the X-ray diffraction (XRD) technique using an X-ray diffractometer (Philips PW1710) with CuKα radiation ($\lambda = 1.5405$ Å) over a scanning range of Bragg angles from 20° to 80 °C.

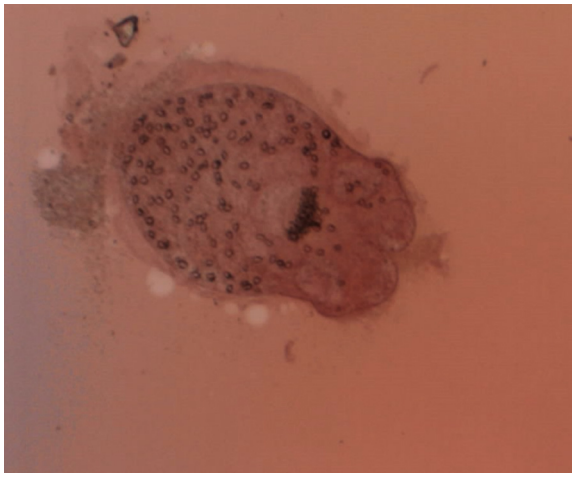

Fig. 1. Dead protoscolice after exposure to biogenic Se NPs and staining with 0.1% eosin.

2.3. Collection of protoscoleces

Protoscoleces of *E. granulosus* were collected from the naturally infected livers of sheep and goats slaughtered at Kerman abattoir, southeastern Iran and carried to the Parasitology Laboratory at the Department of Parasitology and Mycology, Kerman University of Medical Sciences, Kerman, Iran. The hydatid fluid aspirated by a 20 ml syringe and aseptically transferred into an Erlen Meyer flask was left to set for 30 min for protoscoleces to settle down. The supernatant was discarded and the protoscoleces were washed two times with PBS (pH 7.2) solution. The viability of the protoscoleces was confirmed by their flame cell motility and impermeability to eosin solution (0.1%) under a light microscope. For further use, the concentration of protoscoleces was confirmed as 2×10^3 protoscoleces in 1 ml of saline solution (0.9%) with more than 90% viability.

2.4. Scolicidal effects of Se NPs

To investigate the scolicidal effects of Se NPs against protoscoleces of hydatid cysts, four concentrations of the Se NPs (50, 125, 250 and 500 μg/ml) were used with different exposure times (10, 20, 30 and 60 min). Initially, 0.5 ml of the protoscoleces (2×10^3/ml) solution was placed in test tubes. Then 0.5 ml of various concentrations of Se NPs was added to each test tube. The contents of the tubes were gently mixed and then incubated at 37 °C for 10, 20, 30 and 60 min. At the end of each incubation time the upper phase was carefully removed so as not to interrupt the protoscoleces. Fifty μl of 0.1% eosin stain was then added to the remaining settled protoscoleces and mixed gently again. The upper portion of the solution was discarded after 10 min of incubation. The remaining pellet of protoscoleces was then smeared on a glass slide, covered with a cover glass and examined under a light microscope. The percentages of dead protoscoleces were determined by counting 300 protoscoleces. In addition, normal saline were used as control group and all experiments were performed in three replicates.

2.5. Viability test

In order to evaluate the viability of protoscoleces, eosin solution with a concentration of 0.1% (1 g of eosin powder in 1000 ml