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Effect of silver nanoparticles on the metabolic rate, hematological response, and survival of juvenile white shrimp *Litopenaeus vannamei*

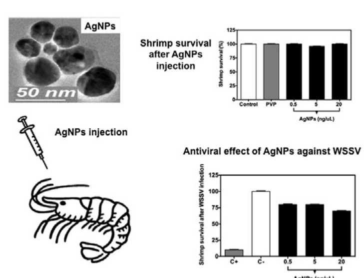
Karla Juarez-Moreno [a, b, *], Claudio Humberto Mejía-Ruiz [c], Fernando Díaz [d], Horacio Reyna-Verdugo [e], Ana Denisse Re [d], Edgar F. Vazquez-Felix [c], Edna Sánchez-Castrejón [d], Josué D. Mota-Morales [a, b], Alexey Pestryakov [f], Nina Bogdanchikova [a]

[a] *Center of Nanosciences and Nanotechnology, National Autonomous University of Mexico, Km. 107 Carretera Tijuana-Ensenada, Ensenada, Baja California, C.P. 22860, Mexico*
[b] *CONACYT Research Fellow at Center Nanosciences and Nanotechnology, National Autonomous University of Mexico, Km. 107 Carretera Tijuana-Ensenada, Ensenada, Baja California, C.P. 22860, Mexico*
[c] *The Northwestern Center of Biological Research (CIBNOR; Centro de Investigaciones Biológicas del Noroeste, S.C.), Mar Bermejo No. 195 Colonia Playa Palo de Santa Rita, C.P. 23090, La Paz, Baja California Sur, Mexico*
[d] *Marine Biotechnology Department, Ensenada Center for Scientific Research and Higher Education, Carretera Ensenada-Tijuana #3918, Ensenada, Baja California, Mexico*
[e] *Instituto Tecnológico del Valle del Yaqui, Block 611, Municipio Bacum, Sonora, Mexico*
[f] *Tomsk Polytechnic University, Tomsk, 634050, Russia*

HIGHLIGHTS

- Intramuscular injection of AgNPs and their stabilizer into *Litopenaeus vannamei* shrimps is not toxic.
- Shrimp survival after AgNPs injection was 98.7%.
- AgNPs injection does not affect metabolic rate or total hemocytes count.
- After virus infection, shrimps injected with AgNPs survived 80% for 96 h.
- Proof of principle of AgNPs to act as antiviral against the white spot syndrome virus.

GRAPHICAL ABSTRACT



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ABSTRACT

White spot syndrome virus (WSSV) is highly lethal and contagious in shrimps; its outbreaks causes an economic crisis for aquaculture. Several attempts have been made to treat this disease; however, to date, there is no effective cure. Because of their antimicrobial activities, silver nanoparticles (AgNPs) are the most studied nanomaterial. Although the antiviral properties of AgNPs have been studied, their antiviral effect against viral infection in aquaculture has not been reported. The AgNPs tested herein are coated with polyvinylpyrrolidone (PVP) and possess multiple international certifications for their use in veterinary and human applications.

The aim of this work was to evaluate the survival rate of juvenile white shrimps (*Litopenaeus vannamei*) after the intramuscular administration of AgNPs. For this, different concentrations of metallic

* Corresponding author. Centro de Nanociencias y Nanotecnología UNAM, Km. 107 Carretera Tijuana-Ensenada, Ensenada, Baja California, C.P. 22860, Mexico.
E-mail address: kjuarez@cnyn.unam.mx (K. Juarez-Moreno).

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AgNPs and PVP alone were injected into the organisms. After 96 h of administration, shrimp survival was more than 90% for all treatments. The oxygen consumption routine rate and total hemocyte count remained unaltered after AgNP injection, reflecting no stress caused. We evaluated whether AgNPs had an antiviral effect in shrimps infected with WSSV. The results revealed that the survival rate of WSSV-infected shrimps after AgNP administration was 80%, whereas the survival rate of untreated organisms was only 10% 96 h after infection. These results open up the possibility to explore the potential use of AgNPs as antiviral agents for the treatment of diseases in aquaculture organisms, particularly the WSSV in shrimp culture.

1. Introduction

Although shrimp farming has been growing rapidly in the past decades, viral infections such as white spot syndrome (WSS) have seriously affected this industry. White spot syndrome virus (WSSV) is highly virulent and can cause disease in 100% of organisms within 3–7 days of infection, resulting in huge economic losses for the aquaculture industry every year (Ramos-Carreño et al., 2014).

Different approaches have been explored to treat WSSV infection; however, to date, they have not fulfilled key parameters, e.g., stability and ease of handling. These include virus neutralization in the fleshy prawn shrimp *Penaeus chinensis* by egg yolk antibodies (IgY) (Kim et al., 2004), injection of shrimp lysozyme as a protective agent against WSSV infection in the blue shrimp *Litopenaeus stylirostris* (Mai and Wang, 2010), delivery of double-stranded RNA (ds-RNA) of the main viral proteins VP28 and VP26 against WSSV in *Litopenaeus vannamei* (Mejía-Ruíz et al., 2011), VP28 gene delivery by chitosan nanoparticles (Vimal et al., 2013), and RNA silencing with siRNA from VP28, VP6, or WSSV-DNA polymerase (Wu et al., 2007; Xu et al., 2007).

In the aquaculture industry, nanotechnology has been poorly applied, and it has been mainly restricted to that improvement of the quality of ingredients in food formulations, antifouling coatings, antibacterials for tanks and packaging of sea food products, and environmental remediation systems (Can et al., 2011; Corsi et al., 2014). Recently, the usage of virus-like nanoparticles (VLPs) from *Macrobrachium rosenbergii* nodavirus as nanocarriers to encapsulate ds-RNA from the major WSSV envelope protein VP28 was reported, and this resulted in a survival rate of 44.5% compared with the use of free ds-RNA (Jariyapong et al., 2015). To the best of our knowledge, this was the first approach of using nanotechnology for the treatment of WSSV infection. However, to date, no other nanomaterial has been used as an antiviral agent to overcome the WSSV infection. Therefore, there is an increasing need to find alternative treatments to overcome infections in shrimps and avoid the massive economic losses in the aquaculture field.

Silver nanoparticles (AgNPs) are one of the most widely used nanomaterials in commercial products because of their beneficial antibacterial, antifungal and antiviral properties (Chen and Schluesener, 2008; Marambio-Jones and Hoek, 2010; Galdiero et al., 2011; Ge et al., 2014; Podkopaev et al., 2014; Franci et al., 2015). Taking advantage of the well-known antiviral properties of AgNPs against a wide spectrum of virus, the purpose of this study was to investigate whether the injection of AgNPs affects the oxygen consumption rate, the total hemocyte counts (THCs), and the survival of juvenile white shrimps (*L. vannamei*). It was explored whether the treatment with safe doses of AgNPs may be useful as an antiviral agent against WSSV infection. For this, the percentage of viability in WSSV-infected shrimps untreated and treated with AgNPs was compared as a proof of concept for the evaluation of AgNPs aiming to reduce the infectivity of WSSV disease in juvenile white shrimps.

2. Materials and methods

2.1. Reagents

Polyvinylpyrrolidone (PVP) and all components to prepare phosphate-buffered saline (PBS) (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4 pH 7.0) were purchased from Sigma-Aldrich (St. Louis MO, USA). All other chemicals were obtained from commercial sources and were of analytical grade.

2.2. AgNPs suspension

An AgNPs solution named Argovit™ was kindly donated by Dr. Vasily Burmistrov from the Scientific and Production Center Vector-Vita (Russia). Argovit is a preparation of highly dispersed AgNPs with an overall concentration of 200 mg/mL (20%) of PVP-coated AgNPs in water. The content of metallic Ag in Argovit preparation is 12 mg/mL, stabilized with 188 mg/mL of PVP. AgNPs concentrations were calculated according to the metallic Ag content in Argovit preparation. Solutions were prepared in PBS and were kept at 4 °C in darkness.

After a comparison of different AgNPs commercially available, it was concluded that only Argovit preparation had multiple certificates for its usage in veterinary and human applications (Borrego et al., 2016).

2.3. AgNPs characterization

Size distribution and morphology of AgNPs were determined on the basis of the results obtained by high-resolution transmission electron microscopy (HRTEM) using a JEOL-JEM-2010 microscope. Hydrodynamic radius and zeta potential were measured by using dynamic light scattering (Malvern Instruments Zetasizer Nano NS model DTS 1060, UK) equipped with a green laser operating at $\lambda = 532$ nm at 25 °C. AgNPs were characterized by UV–Vis spectroscopy in the range of 200–900 nm using a Cary 60 UV–Vis spectrophotometer (Agilent Technologies, Santa Clara, CA, USA). Further characterization of lyophilized Argovit was performed with Fourier transform infrared spectroscopy-attenuated total reflectance (FTIR-ATR) analysis in the range of 400–4000/cm with a resolution of $2/cm^1$ on a universal diamond ATR top plate accessory (Perkin Elmer, USA); the sample spectrum was compared with that of standard solid PVP (Mw 100 kDa).

2.4. Shrimp culture

Juvenile white shrimps (*L. vannamei*) obtained from a farm (Aquasoles S.A. de C.V, Obregón Sonora, Mexico) were cultivated as previously described (Re et al., 2012). Briefly, postlarvae weighing 2 g were placed in 2000-L reservoirs with continuous flow of seawater at 35 psμ and maintained at a temperature of 28 ± 1 °C. Shrimps were maintained in these conditions until they reached 10