International Journal of Veterinary Science and Medicine xxx (2017) xxx–xxx

HOSTED BY

Contents lists available at ScienceDirect

International Journal of Veterinary Science and Medicine

journal homepage: www.elsevier.com/locate/ijvsm
www.vet.cu.edu.eg

Full Length Article

Antibacterial effect of gold nanoparticles against *Corynebacterium pseudotuberculosis*

Marwah M. Mohamed [a], Shereen A. Fouad [a], Hisham A. Elshoky [b], Gina M. Mohammed [c], Taher A. Salaheldin [b,d,*]

[a] Department of Bacterial Diagnostic Products, Veterinary Serum and Vaccine Research Institute, Egypt
[b] Nanotechnology and Advanced Material Central Lab, Agriculture Research Center, Egypt
[c] Central Laboratory for Evaluation Veterinary Biologics, Agriculture Research Center, Egypt
[d] Mostafa Elsayed Nanotechnology Research Centre, British University in Egypt, Egypt

ARTICLE INFO

Article history:
Received 4 January 2017
Revised 3 February 2017
Accepted 13 February 2017
Available online xxxx

Keywords:
C. pseudotuberculosis
Gold nanoparticles
Laser
Antibacterial agent
Caseous lymphadenitis
Sheep and goats

ABSTRACT

Corynebacterium pseudotuberculosis is the etiological agent of chronic caseous lymphadenitis. The bacterium infects goats and sheep causing great economic loss worldwide annually. The present work aims to evaluate the efficiency of gold nanoparticles (AuNPs) and AuNPs – laser combined therapy as antibacterial approaches against *C. pseudotuberculosis* bacteria *in vitro*. Gold nanoparticles 25 nm were synthesized by co-precipitation method and characterized by different techniques including; Transmission Electron Microscope (TEM), X-ray Diffraction and Dynamic Light Scattering. Three concentrations of AuNPs (50, 100 and 200 μg/ml) were utilized for estimating the bacterial growth rate and the Minimum Inhibitory Concentration (MIC). The mechanism of interaction between AuNPs and bacteria was evaluated by transmission electron microscopic image analysis. Confocal Laser Scanning Microscopic technique was used to study the cytotoxic action of AuNPs and laser against *C. psudotuberculosis*. Results revealed that MIC of AuNPs and AuNPs – laser combined therapy were 200 μg/ml and 100 μg/ml respectively. TEM image analysis illustrated that gold nanoparticles penetrated the thick wall of *C. psudotuberculosis* and accumulated as intracellular agglomerates. Laser light enhanced the antimicrobial activity of gold nanoparticles by at least one fold due to its photo thermal combined effect that might be used as an effective antibacterial approach against *C. pseudotuberculosis*.

1. Introduction

Caseous lymphadenitis (CLA) is one of the most sporadic chronic bacterial origin disease, infects mainly goats and sheep caused by *C. pseudotuberculosis.* This organism is gram positive, non-spore and facultative anaerobic rod shaped bacteria, several of which are pathogenic for man and animals. As long as the animal becomes infected with *C. pseudotuberculosis*, it survives and replicates within cells of the immune system that normally be the defense system against it and once the disease established, CLA is difficult to eradicate [1–3].

Most of the therapeutic routs are futile even the chemotherapeutic one that is because of the thick capsule that surrounds the abscesses of *C. pseudotuberculosis* [4]. Although the vaccination against *C. pseudotuberculosis* with dead bacteria or with an excreted proteins provides limited protection, the need for antigenic compounds that can activate both humeral and cellular arms of the immune system is still the demand [5]. However, such methods are hard to achieve and hence limited effectiveness.

Nanomaterials became a promising and efficient candidate that can replace conventional materials with most applications in all fields of science and technology. As a result of the ultra-small size of nanomaterials that have higher surface to volume ratio and increased number of active atoms at their outer surfaces [6]. Some metallurgic nanomaterials have been approved as bactericidal and bacteriostatic agents, among those used are silver, gold and zinc, each with different properties and spectrum activities [7,8]. Gold nanoparticles (AuNPs) are widely used in enormous biological applications mainly in medical and gene therapy and biosensors for diagnosis. AuNPs are easy to prepare by co-precipitation

Peer review under responsibility of Faculty of Veterinary Medicine, Cairo University.
* Corresponding author at: Mostafa Elsayed Nanotechnology Research Centre, British University in Egypt, El Sherouk City, and Suez Desert Road, Cairo 11837, P.O. Box 43, Egypt.
E-mail addresses: Taher.salah@bue.edu.eg, T1salah@hotmail.com (T.A. Salaheldin).
URL: http://www.bue.edu.eg (T.A. Salaheldin).

http://dx.doi.org/10.1016/j.ijvsm.2017.02.003
2314-4599/

Please cite this article in press as: Mohamed MM et al. Antibacterial effect of gold nanoparticles against *Corynebacterium pseudotuberculosis*. Int J of Vet Sci Med (2017), http://dx.doi.org/10.1016/j.ijvsm.2017.02.003

approach and may have lower toxicity compared to other metallic nanomaterials such as silver nanoparticles [9]. The problem of antibiotic resistant bacteria and their emphasis on health care costs, that encourages researchers to innovate new approaches to develop more effective antimicrobial agents to overcome the bacterial resistance and reduce their cost [10]. One approach being tested is photodynamic therapy (PDT), which uses light absorbing dyes to generate toxic oxygen radicals to kill the bacteria. However, this treatment might not be effective for infections in hypoxic environments. Another promising approach is to use metal nanoparticles, and laser energy to physically damage the bacteria through Photo Thermal Therapy (PTT) [11]. The optical properties of conductive nanoparticles (NPs), such as those made of gold have been associated with the Surface Plasmon Resonance (SPR), which when confined to small colloids, is referred to as the localized surface Plasmon resonance (LSPR). This phenomenon, in which the surface electrons oscillate collectively when irradiated with particular light energy resonated with its LSPR, causes wavelength dependent photo thermal effect. When gold NPs absorb resonating light energy, they release heat in accordance, making them useful in photo thermal therapy applications such as targeting cancer and bacterial cells. Laser-induced photothermal phenomena induce physical disruption of the bacterial cells leading to death [12]. The purpose of this study is to evaluate the efficiency and mechanism of the antimicrobial activity of gold nanoparticles (AuNPs) and AuNPs–laser combined therapeutic approach against *C. pseudotuberculosis* bacteria.

2. Materials and methods

2.1. Synthesis of gold nanoparticles (AuNPs)

Gold nanoparticles colloidal solution (25 ± 5 nm) was synthesized by co-precipitation protocol through the reduction of Gold Chloride hydrate ($HAuCl_4$) (99.99%, Aldrich, USA) with Sodium citrate tribasic dihydrate (99%, Aldrich, USA) Sunder boiling conditions [13]. All glass wears were cleaned and sterilized by aqua regia and dried in oven dryer at 120°C. DNA free deionized water (Millipore, USA) was used for preparation and dilutions. Fifty mL (0.03 mM) $HAuCl_4$, in 250 mL beaker, was brought to boil under stirring for 5 min. Sodium citrate tribasic dihydrate (0.5 mL) 1% solution was added at once under continuous stirring. The solution color turned bright red forming gold nanoparticles colloid, then left to cool and proceed for physiochemical characterization.

2.2. Characterization of gold nanoparticles

The characteristic SPR of AuNPs was recorded by absorption spectroscopic technique using a double beam UV–Vis-NIR spectrophotometer (Cary 5000, Agilent, UK) within the scanning range of 200–800 nm. Actual morphology of the prepared gold nanoparticles was imaged by High Resolution Transmission Electron Microscope (HR-TEM) operating at an accelerating voltage of 200 kV (Tecnai G2, FEI, Netherlands). Diluted colloidal gold nanoparticles solution was ultra-sonicated for 5 min to reduce the particles aggregation. Using micropipette, three drops from the sonicated solution were deposited on carbon coated-copper grid and left to dry at room temperature. HR-TEM images of the gold nanoparticles that deposited on the grid were captures for morphological evaluation. Dynamic Light Scattering (DLS) technique was utilized to estimate the average particle size distribution that was measured by zeta sizer (Malvern, ZS Nano, UK). The chemical structure of prepared gold nanoparticles was assessed using X- ray Diffraction (XRD) technique. Colloidal gold solution was centrifuge at 18,000 rpm for 30 min using cooling centrifuge, the precipitated reddish brown pellet was dried in vacuum oven for 2 h then grinded into fine powder to be bombarded by X-ray for phase analysis. The corresponding XRD pattern was recorded in the scanning mode (X'pert PRO, PAN analytical, Netherlands) operated by Cu K radiation tube (=1.54 A°) at 40 kV and 30 mA. The obtained diffraction pattern was interpreted by the standard ICCD library installed in PDF4 software. Qualitative and quantitative measurements of the applied gold nanoparticles concentrations were determined by Inductivity Coupled Plasma (ICP) technique (PerkinElmer ICP-OES: Optima 2000, Germany). Synthesis and characterization of gold nanoparticles were performed in Nanotechnology & Advanced Materials Central Laboratory, Agriculture Research Center, Egypt.

2.3. Strain and growth culture

For study the antibacterial activity of Au NPs on *C. pseudotuberculosis*, a local strain was isolated from lymph nodes of a native sheep suffered from caseous lymphadenitis, it was identified morphologically, biochemically and biologically at Bacterial Diagnostic Products, Veterinary Serum and Vaccine Research Institute, Cairo, Egypt. Brain heart infusion broth (DIFCO, Detroit, Mich., USA) and Brain heart infusion agar (DIFCO, Detroit, Mich., USA) were used as culture media [14,15]. The growth culture was performed using single colony of fully identified *C. pseudotuberculosis* bacteria field strain, it was picked up and inoculated into 100 mL of brain heart infusion broth then incubated at 37 °C for 48 h. Serial dilutions were carried out using broth media to give a final organism density of 10^5 CFU/mL.

2.4. The antimicrobial activity of AuNPs

The Minimum Inhibitory Concentration (MIC) of three different concentrations of AuNPs (50, 100 and 200 μg/mL was determined using the plate count method [16,17]. Twenty mL from the previously prepared culture medium (containing 10^5 CFU/mL of bacterial cells) was a liquated into four tubes (5 mL each), then the Au NPs were added in each tubes in the following concentration 0, 50, 100 and 200 μg/mL, respectively. The tubes were incubated in a shaking incubator at 37°C for 24 h. After incubation, 50 μL from each tube was spread onto brain heart infusion agar and incubated at 37°C for 24 h; the numbers of colonies growing on agar were estimated. Measurements of the optical density (O.D.) at 600 nm of the treated bacteria were graphed to estimate the growth curves [10].

2.5. Laser induced gold nanoparticles antibacterial effects

The MIC for 3 groups was performed from a mixture of culture media and Au NPs (0, 50, 100 and 200 μg/mL) [16,17], the culture tubes were incubated in a shaking incubator at 37°C for 24 h. Incubation tubes were subjected to 520 nm laser light, 20 mW, at different exposure time (5, 10 and 20 min.) for each group, respectively. Fifty μL from each culture tube were spread onto brain heart infusion agar and incubated at 37°C for 24 h; the numbers of colonies growing on agar were estimated. Measurements of the O.D. at 600 nm of the treated bacteria were graphed to estimate the growth curves [10].

2.6. AuNPs antibacterial mode of action

Electron microscope imaging and EDX analysis were performed to study the effect of AuNPs on the bacterial cell integrity and to elucidate whether the mode of action either extra or intra cellular. Culture treated with different concentrations of AuNPs and non-treated one of *C. pseudotuberculosis* were proceeded for fixation

Please cite this article in press as: Mohamed MM et al. Antibacterial effect of gold nanoparticles against *Corynebacterium pseudotuberculosis*. Int J of Vet Sci Med (2017), http://dx.doi.org/10.1016/j.ijvsm.2017.02.003