SILVER NANOPARTICLES APPLICABILITY AS ANTIMICROBIAL SUBSTANCE IN THE PREVENTION AND TREATMENT OF SEPTIC COMPLICATIONS

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Abstract

Septic complications are one of the most frequent complications and the leading cause of mortality in hospitals around the world. Its etiological structure is presented by a wide range of pathogens. Special significance today obtains the general spreading of microorganisms with multiple-drug resistance. These facts make topical the search for new, more efficient agents for effective prevention and treatment of septic complications. Metal nanoparticles, in particular silver nanoparticles, are possessed by significant potential in this way.

In order to search of the antimicrobial substance, high effective in the prevention and treatment of septic complications, 30 nm silver nanoparticles have been synthesized by the method of chemical condensation in aqueous medium. Synthesized nanoparticles have been characterized as biosafe and biocompatible using in vitro and in vivo markers.

Silver nanoparticles’ high antimicrobial activity has been revealed in vitro using test strains of Staphylococcus aureus MRSA ATCC 43300, Pseudomonas aeruginosa ATCC 27853, Escherichia coli ATCC 2592, Shigella sonnei, Salmonella typhimurium 144, Bacillus subtilis ATCC 6633 as well as clinical multiple resistant isolates of Klebsiella ozaenae 4348, Citrobacter freundii 4369, Escherichia coli 4358, Enterobacter aerogenes 2476, Proteus mirabilis 4363, Staphylococcus aureus 4312, Pseudomonas aeruginosa 283, Enterococcus faecalis 4305, Candida 4418 separated from the surgical patients.

The peculiarities of silver nanoparticles influence on the organism of model animals (outbred white mice, Wistar rats) have been revealed under the conditions of intravenous substance injection in concentrations 1.6 mg/kg, 8.0 mg/kg and 16.0 mg/kg.

Keywords: Silver nanoparticles, antimicrobial substance, multi-drug-resistant microorganisms, effectiveness

1. INTRODUCTION

Septic complications are one of the most frequent complications and one of the leading causes of death in hospitals among intensive care unit patients of all age groups. This problem affects millions of people around the world, killing more than 30 % of patients with this sort of complications. Increasing in incidence is observed each year. According to some statistics data 1% and 0.3% of all patients admitted to hospitals suffer, respectively, from bacteraemia alone and bacteraemia with severe sepsis [1-3].

Etiological structure of septic complications is presented by a wide range of pathogens. Among most commonly isolated microorganisms are Gram(+) Staphylococcus epidermidis MRSE, Staphylococcus aureus MRSA, non-fermenting Gram(-) Acinetobacter baumannii, Pseudomonas aeruginosa, etc. Special significance today obtains the general spreading of microorganisms-causative agents with multiple-drug resistance [4-6].
These facts make topical the search of new, more efficient agents for effective prevention and treatment of septic complications. Metal nanoparticles with antimicrobial properties, such as silver nanoparticles, are possessed by significant potential in this way.

The aim of present study was to estimate antimicrobial properties of silver nanoparticles in order to search of antimicrobial agents with high effectiveness in the prevention and treatment of septic complications.

2. MATERIALS AND METHODS

Spherical silver nanoparticles (AgNP) with average size 30 nm have been synthesized by the method of chemical condensation in aqueous medium in F.D. Ovcharenko Institute of Biocolloidal Chemistry according to the original protocol. The concentration of obtained AgNP was 8.0 mg/ml by metal. The passport of nanomaterial for the synthesized silver nanoparticles includes their characteristic in compliance with biosafety and biocompatibility parameters with using wide spectrum of in vitro and in vivo tests.

AgNP antimicrobial activity has been studied in vitro with using two methods. Method of serial dilutions in Muller-Hinton agar according to «Guidelines for Susceptibility Testing of Microorganisms to Antibacterial Agents» (4.2.1890-04) has been used for AgNP antimicrobial activity estimation concerning test strains: Staphylococcus aureus MRSA ATCC 43300, Pseudomonas aeruginosa ATCC 27853, Escherichia coli ATCC 2592, Shigella sonnei, Salmonella typhimurium 144, Bacillus subtilis ATCC 6633.

For estimation of AgNP antimicrobial activity concerning clinical isolates of microorganisms the original protocol of SI “Institute of Urology of NAMS of Ukraine” has been used. The AgNP drops have been applied on the agar (Muller-Hinton agar for bacteria and Sabouraud agar for Candida fungi) with seeded microorganisms in seed doses \(10^5\) and \(10^7\) CFU/ml. The terminal concentrations of AgNP in drops were 10 mcg and 20 mcg.

Clinical isolates with multiple drug resistance: Klebsiella ozaenae 4348, Citrobacter freundii 4369, Escherichia coli 4358, Enterobacter aerogenes 2476, Proteus mirabilis 4363, Staphylococcus aureus 4312, Pseudomonas aeruginosa 283, Enterococcus faecalis 4305, Candida 4418 have been used for AgNP antimicrobial effectiveness estimation. The clinical isolates of microorganisms have been separated from wounds, urine, scrape of cervical canal and fauces of surgical patients.

The peculiarities of silver nanoparticles influence on the organism have been studied with using model animals: outbred white mice and Wistar rats. The AgNP influence has been estimated under the conditions of 5 times and 10 times intravenous injections of the substance in concentrations 1.6 mg/kg, 8.0 mg/kg and 16.0 mg/kg per one time. The parameters of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), bilirubin and creatinine have been analyzed using Filicit-Diagnostics kits (Ukraine).

Experimental animals have been housed on the standard regime. All experiments with model animals have been carried out in compliance with “Guide for the Care and Use of Laboratory Animals”.

3. RESULTS AND DISCUSSION

Biosafety is one of the main criteria of metal nanoparticles eligibility for medical application. Experimental 30 nm spherical AgNP have been characterized as noncytotoxic, nongenotoxic, nonmutagenic and biosafe according to the main biochemical markers in accordance with their passport of nanomaterial. These data indicate the possibility of next study for the 30 nm AgNP with the goal of their medical application.

High bactericidal action concerning different species of microorganisms, including multiple drug resistant strains, is necessary condition for effective prevention and treatment of septic complications.

The estimation of 30 nm AgNP antimicrobial activity concerning test-strains S. aureus MRSA ATCC 43300, P. aeruginosa ATCC 27853, E. coli ATCC 2592, S. sonnei, S. typhimurium 144, B. subtilis ATCC 6633 indicated high bactericidal action of the substance concerning all investigated test strains. It has been
observed total inhibition of microorganisms’ growth under the AgNP terminal concentration in the determination medium 133.80 mcg/ml and 100.38 mcg/ml.

Total inhibition of S. aureus MRSA ATCC 43300, P. aeruginosa ATCC 27853, E. coli ATCC 2592, S. sonnei, S. typhimurium 144 test-strains’ growth has been also revealed under the conditions of 66.90 and 33.46 mcg/ml AgNP concentrations.

The growth of single colonies and intensive growth under the concentrations of AgNP 66.90 mcg/ml and 33.46 mcg/ml respectively has been observed only for B. subtilis ATCC 6633 test-strain.

High antimicrobial action of 30 nm AgNP has been also indicated concerning multidrug-resistant clinical isolates: K. ozaenae 4348, C. freundii 4369, E. coli 4358, E. aerogenes 2476, P. mirabilis 4363, S. aureus 4312, P. aeruginosa 283, E. faecalis 4305, Candida 4418, separated from wounds, urine, scrape of cervical canal and fauces of surgical patients.

It has been observed total inhibition of microorganisms’ growth for K. ozaenae 4348, C. freundii 4369, E. coli 4358, E. aerogenes 2476, P. mirabilis 4363, S. aureus 4312, P. aeruginosa 283 under the influence of both studied AgNP concentrations: 10 mcg and 20 mcg.

Along with the high antimicrobial action of the 30 nm AgNP, which has been shown in vitro, the biosafety of the substance has been shown in vivo under the conditions of AgNP intravenous introduction. The absence of silver nanoparticles’ toxic influence on the organism of outbred white mice and Wistar rats has been revealed according to the analysis of changes in blood serum parameters (ALT, AST, bilirubin and creatinine) under the conditions of the AgNP intravenous injections in all studied concentrations (1.6 mg/kg, 8.0 mg/kg and 16.0 mg/kg).

4. CONCLUSION

High bactericidal action of experimental 30 nm spherical silver nanoparticles has been revealed in vitro concerning wide spectrum of Gram(+) and Gram(-) microorganisms: test-strains and multidrug-resistant clinical isolates.

The results of antimicrobial activity along with the biosafety level according to the passport of nanomaterial as well as obtained in vivo data of the substance influence under the conditions of intravenous recurring injections indicate the great potential of 30 nm spherical AgNP as antimicrobial agent with high effectiveness in the prevention and treatment of septic complications.

LITERATURE


