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Research Article

Investigation of Bismuth Nanoparticles Antimicrobial Activity against High Pathogen Microorganisms

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Abstract

Spherical bismuth nanoparticles with average size 40 nm have been synthesized by the colloidal-chemical method in water medium. 100% of Bi content has been revealed in the nanoparticles by the method of energy-dispersive X-ray spectroscopy. Synthesized bismuth nanoparticles have been characterized as biosafe and biocompatible according to the Guidelines «Safety assessment of medical nanopreparations». Bismuth nanoparticles' antimicrobial activity *in vitro* has been estimated against wide spectra of high pathogen microorganisms - ***potential causative agents of human and animals' diseases***. Bismuth nanoparticles' high bactericidal action against all investigated test strains: *Campylobacter jejuni* Pl – 09.c; *Listeria monocytogenes* ATCC 19112; *Yersinia enterocolitica* 12/15-08; *Salmonella typhimurium* №16; *Escherichia coli* №4; *Mycoplasma arginini* G 230; *Acholeplasma laidlawii* ATCC 23206; *Bacillus anthracis* M-71; *Leptospira* Pomona has been revealed.

Keywords: Bismuth nanoparticles; Synthesis; Antimicrobial activity; Pathogens

Abbreviations

BiNP: Bismuth Nanoparticles

Introduction

Despite of the common efforts in biodefense around the world the problem of high dangerous pathogens distribution, especially under the threat of terrorist attacks, is still urgent. Among the biotoxins that could be used for dispersion, leading experts identify large number of high pathogen microorganisms of category A and B, such as well-known pathogens: *Bacillus anthracis*, *Salmonella*, *Escherichia etc.*[1-3].

The most important problem in the struggle against biohazard is high effective defense of civil population. Such effective defense can be provided using safe drugs, which are effective against wide range of biotoxins.

Modern antimicrobial preparations and vaccines don't possess by wide range of activity against different classes of pathogens. So, the search and development of new high effective antimicrobial substances with high effectiveness against wide spectra of high pathogen microorganisms - causative agents of most dangerous human and animals' diseases is topical problem today. Metal nanoparticles are possessed by high potential in this area.

The main goal of presented work was antimicrobial activity investigation of the synthesized bismuth nanoparticles against wide spectra of pathogen microorganisms - potential causative agents of high dangerous human and animals' diseases.

Materials and Methods

Spherical Bismuth Nanoparticles (BiNP) have been synthesized by the method of chemical condensation in water medium. The concentration of obtained BiNP was 77.5 mg/ml by metal. The shape and size of synthesized BiNP have been defined by the method of Transmission Electron Microscopy (TEM) (JEM-1230 «JEOL LTD», Japan). The method of energy-dispersive X-ray spectroscopy has been used for chemical composition's X-ray microanalysis of the synthesized nanoparticles (IETEM 250 with detector X-Max 80, Oxford Instruments Analytical, UK for JEM-1230).

BiNP biosafety level has been estimated *in vitro* using parameters of cytotoxicity, genotoxicity, mutagenicity and biochemical markers according to the Guidelines «Safety assessment of medical nanopreparations» [4].

High pathogen strains - causative agents of most dangerous human and animals' diseases: *Campylobacter jejuni* Pl – 09.c; *Listeria monocytogenes* ATCC 19112; *Yersinia enterocolitica* 12/15-08; *Salmonella typhimurium* №16; *Escherichia coli* №4; *Mycoplasma arginini* G 230; *Acholeplasma laidlawii* ATCC 23206; *Bacillus anthracis* M-71; *Leptospira* Pomona from the Collection of The State Scientific

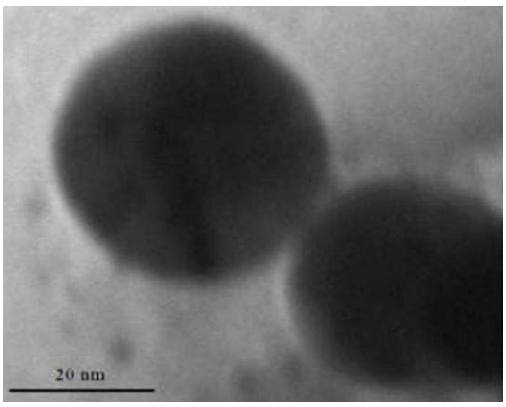

Figure 1: Transmission electron microscope image of synthesized spherical bismuth nanoparticles with average particles' size 40 nm.

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Table 1: Bismuth nanoparticles antimicrobial activity against wide spectra of test pathogens.

Test pathogen	Seed-dose of test strain, CFU/cm^3	Bismuth nanoparticles (C=77.5 mg/ml by metal) Terminal concentration of bismuth nanoparticles in determination medium, mg/ml by metal		Control of test strain growth
		6.50	12.90	
Campylobacter jejuni Pl – 09.c	10^3	I	I	++++
	10^4	I	I	++++
	10^5	I	I	++++
	10^6	I	I	++++
Listeria monocytogenes ATCC 19112	10^3	I	I	++++
	10^4	I	I	++++
	10^5	I	I	++++
	10^6	I	I	++++
Yersinia enterocolitica 12/15-08	10^3	I	I	++++
	10^4	I	I	++++
	10^5	I	I	++++
	10^6	I	I	++++
Salmonella typhimurium №16	10^3	I	I	++++
	10^4	I	I	++++
	10^5	I	I	++++
	10^6	I	I	++++
Escherichia coli №4	10^3	I	I	++++
	10^4	I	I	++++
	10^5	I	I	++++
	10^6	I	I	++++
Mycoplasma arginini G 230	10^5	I	I	++++
Acholeplasma laidlawii ATCC 23206	10^5	I	I	++++

«I» - total inhibition of microorganisms growth;
«++++» - intensive growth of microorganisms;
CFU: colony-forming unit.

Control Institute of Biotechnology and Strains of Microorganisms (Kyiv, Ukraine) have been used for BiNP antimicrobial activity estimation *in vitro*.

«Method of serial dilutions in agar» according to Guidelines for Susceptibility Testing of Microorganisms to Antibacterial Agents (4.2.1890-04) has been used for BiNP antimicrobial activity estimation against test-strains *Campylobacter jejuni* Pl – 09.c; *Listeria monocytogenes* ATCC 19112; *Yersinia enterocolitica* 12/15-08; *Salmonella typhimurium* №16; *Escherichia coli* №4, *Mycoplasma arginini* G 230; *Acholeplasma laidlawii* ATCC 23206; *Bacillus anthracis* M-71 [5].

Muller-Hinton agar has been used in the study of BiNP antimicrobial activity against *Campylobacter jejuni* Pl – 09.c; *Listeria monocytogenes* ATCC 19112; *Yersinia enterocolitic* 12/15-08; *Salmonella typhimurium* №16; *Escherichia coli* №4.

Agar medium for *Mycoplasma* and *Acholeplasma* cultivation, which has been developed and patented in The State Scientific Control Institute of Biotechnology and Strains of Microorganisms (Kyiv, Ukraine) has been used in the study of BiNP antimicrobial activity against *Mycoplasma arginini* G 230 and *Acholeplasma laidlawii* ATCC 23206.

Hottinger's agar (pH 7.2) has been used in the study of BiNP antimicrobial activity concerning *Bacillus anthracis* M-71.

«Method of serial dilutions in broth» according to Guidelines for Susceptibility Testing of Microorganisms to Antibacterial Agents (4.2.1890-04) [5] has been used in the study of BiNP antimicrobial activity against *Leptospira* Pomona test-strain. Korthof medium with 10% rabbit blood serum addition (pH 7.2-7.4), which provide optimal growth of *Leptospira* cultures, has been used.

The test tubes with working solutions of BiNP' certain concentrations in the determination medium (1 ml per test tube) were prepared by the method of serial dilution according to the Guidelines 4.2.1890-04. 1 ml of the medium with *Leptospira* cultures has been added in the each test tube with 1 ml (per test tube) of the working solutions of bismuth nanoparticles in the medium. On the next stage the test tubes with *Leptospira* cultures and bismuth nanoparticles have been incubated 24 hours at 29 ^{0}C. The control of *Leptospira* cultures growth has been provided in the medium without BiNP adding.

After 24 hours incubation 1 ml of *Leptospira* culture has been

Table 2: Bismuth nanoparticles antimicrobial activity against test pathogen *Bacillus anthracis* M-71.

Seed-dose of test strain *Bacillus anthracis* M-71, CFU	Bismuth nanoparticles (C=77.5 mg/ml by metal)				Control of test strain growth
	Terminal concentration of bismuth nanoparticles in determination medium, mg/ml by metal				
	0.32	3.20	6.50	12.90	
10^7	I	I	I	I	++++

«I» - total inhibition of microorganisms growth;
«++++» - intensive growth of microorganisms.
CFU - colony-forming unit.

Table 3: Bismuth nanoparticles antimicrobial activity against test pathogen *Leptospira* Pomona.

	Bismuth nanoparticles (C=77.5 mg/ml by metal)																				
	Terminal concentration of bismuth nanoparticles in determination medium, mg/ml by metal																				
	0.00004	0.00007	0.00014	0.0003	0.0006	0.0012	0.0024	0.005	0.01	0.02	0.04	0.08	0.15	0.3	0.6	1.2	2.4	4.8	9.7	19.4	Control of test strain growth
strain *Leptospira* Pomona, cells quantity	80	80	80	80	80	80	80	80	80	80	80	80	70	70	60	40	20	I	I	I	80

«I»: total inhibition of microorganisms growth.

reseeded from the each test tube with certain concentration of the bismuth nanoparticles into the test tube with 10 ml of the medium without nanoparticles. After 10-14 days the calculation of results has been provided.

Results and Discussion

Water dispersion of BiNP has been synthesized with the aim of its antimicrobial activity estimation. By the method of transmission electron microscopy it has been determined that synthesized nanoparticles have spherical form and average particles' size 40 nm (Figure 1).

Chemical composition's X-ray microanalysis of the synthesized bismuth nanoparticles specified by the method of energy-dispersive X-ray spectroscopy has shown 100% of Bi content in the nanoparticles. The presence of oxygen in the particles' structure has not been fixed. This is an indication that the synthesized nanoparticles are particles of zero-valent Bi (Bi^0NP).

On the next step BiNP biosafety level has been estimated *in vitro* using wide spectrum of biosafety tests. Such characteristic is necessary condition in the case of their potential medical application [4]. The synthesized BiNP have been characterized as noncytotoxic, nongenotoxic, nonmutagenic and biosafe according to the main biochemical markers.

On the modern level high bactericidal activity against wide spectra of pathogens - potential causative agents of high dangerous human and animals' diseases is necessary condition in the development of new antimicrobial preparations.

The investigation of 40 nm BiNP antimicrobial activity against test pathogens *Campylobacter jejuni* Pl – 09.c, *Listeria monocytogenes* ATCC 19112, *Yersinia enterocolitica* 12/15-08, *Salmonella typhimurium* №16, *Escherichia coli* №4, *Mycoplasma arginini* G 230 and *Acholeplasma laidlawii* ATCC 23206 has shown high bactericidal action of the nanoparticles against all analyzed strains (Table 1).

Total inhibition of the microorganisms' growth has been observed in both terminal concentrations of bismuth nanoparticles in determination medium: 12.9 and 6.5 mg/ml by metal.

BiNP high bactericidal action has been obtained against test strain *Bacillus anthracis* M-71 - anthrax causative agent (Table 2).

In this case total inhibition of the microorganisms' growth has been revealed under the influence of BiNP in concentration range 12.9 - 0.32 mg/ml by metal. Such results indicate high potential of BiNP application in the development of new drugs for anthrax effective prophylaxis and treatment.

The experimental data of BiNP antimicrobial activity investigation against test strain *Leptospira* Pomona are presented in the Table 3.

For this pathogen it has been revealed total inhibition of the microorganisms' growth under the influence of BiNP concentration range 4.8 – 19.4 mg/ ml by the metal in the medium. Three successive passages on the medium for *Leptospira* cultivation have been provided for the quality control of the bismuth nanoparticles' activity.

Conclusion

1. Spherical BiNP with average size 40 nm have been synthesized by the colloidal-chemical method in water medium.

2. Synthesized bismuth nanoparticles have been characterized as noncytotoxic, nongenotoxic, nonmutagenic and biosafe according to the main biochemical markers according to the Guidelines «Safety assessment of medical nanopreparations», that indicate the possibility of their potential medical application.

3. BiNP high antimicrobial activity concerning all investigated test pathogens - potential causative agents of human and animals' diseases *Campylobacter jejuni* Pl – 09.c; *Listeria monocytogenes* ATCC 19112; *Yersinia enterocolitica* 12/15-08; *Salmonella typhimurium* №16; *Escherichia coli* №4; *Mycoplasma arginini* G 230; *Acholeplasma laidlawii* ATCC 23206; *Bacillus anthracis* M-71; *Leptospira* Pomona has been revealed under the *in vitro* conditions.

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