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Study of the Antibacterial Action of Metal Nanoparticles on Clinical Strains of Gram-Negative Bacteria

I.A. Mamonova

Department of Fundamental and Clinical Experimental Investigations, FGBU
"Saratov Research Institute of Traumatology and Orthopaedics"
of the RF Ministry of Public Health, Saratov, Russia

Abstract: *Escherichia coli* is among the most frequently encountered germs of purulent septic infections of various localizations. The *E.coli* strains tend to develop multiple resistance caused by the production of extended spectrum beta-lactamases (ESBLs) and carbapenemases. This dictates the necessity to search for new antimicrobial agents. In this respect, metal nanoparticles are promising candidates for the development of a new class of antibacterial agents. Metal nanoparticles are less toxic than ions, behave as biotics, have prolonged action and stimulate the micronutrient regulation mechanism and the activity of antioxidant enzymes. We studied experimentally the antibacterial action of nickel and titanium nanoparticles against clinical strains of *E.coli*. A clear-cut bactericidal action of ultradispersed nickel powder was identified. The dependences of the antimicrobial action of nickel nanoparticles on their concentration and exposure time were elucidated. Weak antibacterial activity of titanium nanoparticles with respect to multi-antibiotic resistant strains of *E. coli* was established. The antibacterial action of titanium nanoparticles does not depend on their concentration or exposure time. The obtained results provide prospects for further investigation of the antibacterial action of nickel nanoparticles to be used as antibacterial agent.

Key words: Nanoparticles · Nickel · Titanium · Antibiotic resistance

INTRODUCTION

Despite the considerable progress in microbiology, the problem of infectious complications in humans still remains topical. The advent of new-generation antibacterial agents exhibiting activities against a broad range of microorganisms and able to fight the developed antibiotic resistance markedly accelerated the appearance of new resistant microbial strains. Of special significance are highly virulent and opportunistic hospital strains with multiple antibiotic resistance.

Escherichia coli is among the most frequently encountered germs of purulent septic diseases of various localizations. The *Escherichia coli* strains tend to develop multiple resistance caused by the production of extended spectrum beta-lactamases (ESBLs). This type of resistance induces immunity of microorganisms to generation I to IV cephalosporins. The main types of *E. coli* ESBL are TEM, SHV and CTX-M [1, 2].

In addition, *E. coli* strains may develop resistance to fluoroquinolones. The resistance to this group of antibacterial agents is provided by single nucleotide polymorphism in the *gyrA* and *parC* genes and activation of the efflux system [3, 4]. Moreover, this germ produces carbapenemases of various classes (class B MBL, class A KPC type and ÎÕÀ type, which hydrolyze class D beta-lactamase carbapenems, etc.), which forms resistance to carbapenems [5, 6].

All the foregoing dictates the necessity to look for new alternative antibacterial agents. In this respect, nanoparticles are the key candidates for the development of this class of agents. Metal nanoparticles are less toxic than ions, behave as biotics, have prolonged action and stimulate the micronutrient regulation mechanism and activity of antioxidant enzymes.

Corresponding Author: Mamonova, Department of Fundamental and Clinical Experimental Investigations, FGBU "Saratov Research Institute of Traumatology and Orthopaedics" of the RF Ministry of Public Health, 148, Chernyshevsky street, Saratov, Russia, 610002.

The antibacterial action of metal nanoparticles has not been fully studied. There are few publications dealing with the effect of silver, copper, nickel and titanium dioxide nanopowders on some microflora species [7-9]. For example, K. Yoonetal investigated the antimicrobial activity of nickel and silver nanoparticles on the standard strains of *E. coli* and *B. subtilis* and found high antibacterial activity for nickel nanoparticles [8]. Nickel nanoparticles have oligodynamic action as they exhibit bactericidal properties in very low concentrations [9]. Thus, further investigation of the antibacterial action of metal nanoparticles for their use as antibacterial agents appears promising.

MATERIALS AND METHODS

Highly dispersed nickel and titanium nanopowders to be used in the work were prepared from bulk metal samples by the plasma technique based on metal vaporization in a plasma stream at a temperature of 5000-60000 K to give ultradispersed particles of the desired size followed by vapor condensation. Metal nanoparticle suspensions in an isotonic salt solution in concentrations of 0.01, 0.05, 0.1, 0.5 and 1 mg/mL were used.

The investigation objects were 20 multi-antibiotic resistant strains of *E. coli*, isolated from trauma and orthopedic patients under treatment at the Saratov Research Institute of Traumatology and Orthopaedics (SarNIITO). A standard microbial suspension of the germs in a concentration of 30000 CFU/mL was used for inoculation.

The nanoparticle samples were incubated with the microbes for 30, 60, 90 and 120 min at room temperature. After that, 100 μL of each sample was inoculated on a solid medium and placed into a thermostat (37°C) for 24 hours. Statistical treatment of the results included determination of average values (M), root-mean-square errors (m) and confidence level (p).

RESULTS

The antibacterial action was estimated for metal nanoparticles in concentrations from 0.01 to 1 mg/mL for exposure times of 30 to 120 min. The results on antibacterial action of nickel nanoparticles are summarized in Table 1.

As can be seen from Table 1, the number of microbes grown on the solid nutrient medium after the action of ultradispersed nickel powder was lower in all tests than in the control. It was shown by the experiments that the action of nanoparticles in concentration of 0.01 mg/mL at exposure time of 60 min did not induce any change in the number of colonies. The exposure times of 30, 90 and 120 min decreased the percentages of viable microorganisms to 78.53±1.79%, 67.27±2.93% and 69.57±2.62%, respectively (at $p<0.001$).

The increase in the concentration of the nickel nanopowder to 0.05 mg/mL resulted in enhancement of the antibacterial activity. The percentage of surviving microorganisms in the case of 30-min exposure was 69.16±5.65%; for 60 min, this was 70.0±3.2%, that for 90 min was 53.37±2.78 and that for 120 min was 47.38±3.07% (at $p<0.001$).

Exposure to nickel nanoparticles in the concentration of 0.1 mg/mL induced further decrease in the fraction of viable microorganism, in particular, down to 61.02±5.41% upon 30-min exposure, 58.587±5.18% upon 60-min exposure, 43.74±2.86% upon 90-min exposure and 36.3±3.49% upon 120-min exposure (at $p<0.001$).

The concentration of 0.5 mg/mL with exposure times of 30, 60, 90 and 120 min resulted in a decrease in the bacterial cells down to 52.89±4.49%, 50.89±3.18%, 32.44±3.95% and 17.44±2.89%, respectively (at $p<0.001$).

The 1 mg/mL concentration at exposure times of 30, 60 and 90 min produced higher antimicrobial activity of the nanoparticles. In this case, the percentages of the surviving microorganisms were

Table 1: Antibacterial action of nickel nanoparticles on *E.coli* strains

	Number of colonies on solid nutrient media, M±m				
		Test groups			
		1	2	3	4	5
Exposure time, min	Control group (n=20)	0.01 mg/mL (n=20)	0.05 mg/mL (n=20)	0.1 mg/mL (n=20)	0.5 mg/mL(n=20)	1 mg/mL(n=20)
30	1366,2±58.03	1077.85±55.06***	918±39.87***	809±42.9***	722.75±71.18***	561.3±50.9***
60	1224±57.54	1065.25±57.54	838.7±31.56***	697.4±37.94***	621.2±45.14***	503.85±49.1***
90	1235,55±66.46	818.85±4.42***	646.9±36.27***	521.5±36.21***	396.3±43.46***	208.2±39.8***
120	1176,55±67.46	808.2±33.98***	544.75±26.78***	407.2±30.69***	199.8±25.98***	94.15±21.58***

Note: ***$p<0.001$ with respect to the control group

Table 2: Antibacterial action of titanium nanoparticles on *E.coli* strains

	Number of colonies on solid nutrient media, M±m					
		Test groups				
		1	2	3	4	5
Exposure time, min	Control group (n=20)	0.01 mg/mL (n=20)	0.05 mg/mL (n=20)	0.1 mg/mL(n=20)	0.5 mg/mL(n=20)	1 mg/mL(n=20)
30	1049.25±16.74***	923.8±19.65***	940.35±34.1**	1005.9±27.9	758.7±33.42***	826.95±57.6***
60	1060.3±31.43***	906.7±23.13***	858.9±21.08***	746.45±26.66***	845.9±18.85***	808.95±33.67***
90	1056.3±18.79***	948.0±21.02***	766.25±17.79***	748.6±20.58***	819.65±19.91***	895.55±12.89***
120	1076.3±21.7***	905.25±15.81***	925.1±22.38***	806.55±21.58***	782.0±24.18***	761.65±20.27***

Note: **p<0.01; ***p<0.001 with respect to the control group

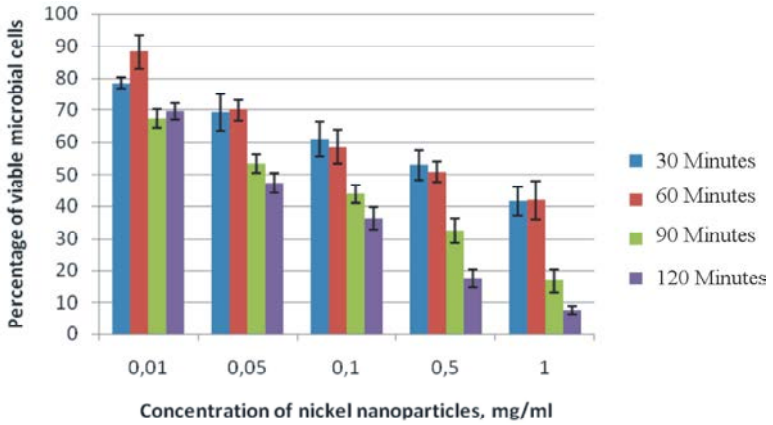

Fig. 1: Antibacterial activity of nickel nanoparticles against the antibiotic resistant strains of *E.coli* as a function of the concentration and exposure time

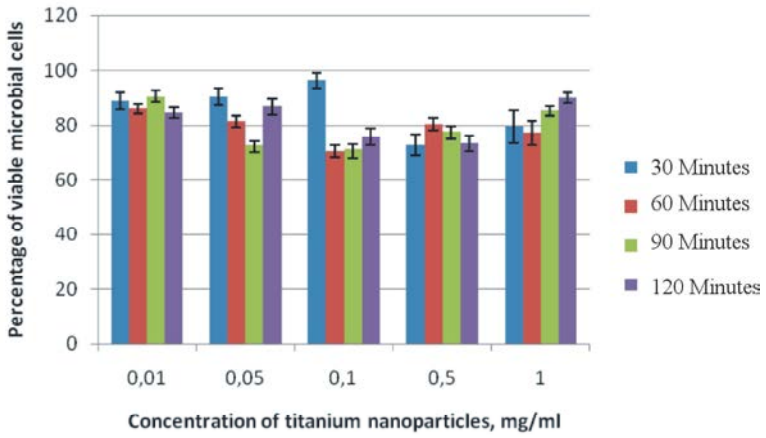

Fig. 2: Antibacterial activity of titanium nanoparticles against the antibiotic resistant strains of *E.coli* as a function of the concentration and exposure time

41.68±4.51%, 41.89±5.97% and 16.72±3.74, respectively (at p<0.001). Further increase in the incubation time to 120 min resulted in almost complete destruction of the microorganisms. The percentage of the bacterial cells was 7.77±1.27% (at p<0.001) (Fig. 1).

The results on antibacterial action of titanium nanoparticles are summarized in Table 2.

It can be seen from Table 2 that the number of microorganisms grown on the solid nutrient medium is always lower after exposure to the ultradispersed titanium powder than in the control group. The experiments showed that a 30-min exposure to nanoparticles in 0.1 mg/mL concentration did not induce a statistically valid change in the number of colonies grown on solid nutrient medium.

The exposure to titanium nanoparticles present in the concentration of 0.01 mg/mL caused a minor antibacterial effect. The percentage of viable microorganisms after 30 min exposure was 88.68±3.09%; after 60-min exposure, it was 85.95±1.86%; that for 90 min was 90.25±2.27% and that for 120 min was 84.62±1.98% (at $p<0.001$).

The increase in the concentration of titanium nanoparticles to 0.05 mg/mL resulted in slight increase in the antibacterial effect for exposure times of 60 and 90 min, the percentages of microbial cells being 81.47±2.08% and 72.79±1.71% (at $p<0.001$), respectively. Exposure times of 30 and 120 min caused some mitigation of the antimicrobial action, the percentages of microbial cells being 90.22±3.11% (at $p<0.05$) and 86.73±2.7 (at $p<0.001$), respectively.

The concentration of 0.1 mg/mL provided a somewhat higher antibacterial action. When the exposure times were 60, 90 and 120 min, the percentages of *E.coli* cells were 70.54±2.16%, 71.1±1.61% and 75.83±3.2% (at $p<0.001$), respectively. The percentage of viable cells upon a 30-min exposure was 75.83±3.2% (at $p<0.001$).

The increase in the titanium nanoparticle concentration to 0.5 mg/mL did not induce a marked increase in the antibacterial action either; the percentage of microorganisms for a 30 min exposure was 72.79±3.7%; for 60 min, it was 80.43±2.32%; for 90 min, it was 77.91±1.61%; and for 120 min, it was 73.42±2.91% (at $p<0.001$).

No significant changes were observed in the numbers of microbial colonies grown on solid nutrient media after exposure to titanium nanoparticles in concentrations of 1 mg/mL. After 30 min, 79.48±5.84% of microbial bodies were retained; after 60 min, this quantity was 77.2±4.45%; after 90 min, it was 85.21±1.71%; and after 120 min, it was 71.07±1.9% (at $p<0.001$) (Fig. 2).

CONCLUSION

The performed study demonstrated high antibacterial activity of nickel nanoparticles with respect to milti-antibiotic resistant strains of *E. coli*. The antimicrobial action of nickel nanoparticles depends on their concentration and exposure time. Titanium nanoparticles exhibit weak antibacterial activity with respect to milti-antibiotic resistant strains of *E. coli*. This does not depend on their concentration or exposure time.

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