Antituberculous effect of silver nanoparticles

G N Kreytsberg¹, I E Gracheva¹, B S Kibrik², I V Golikov³

¹Limited liability Company “Scientific and Production Association (NPO)“Likom”,
Russia, 150049, Yaroslavl, Magistralnaya str., 32.

²The Yaroslavl State Medical Academy
Russia, 150000, Yaroslavl, Revolutsionnaya str., 5

³The Yaroslavl State Technical University
Russia, 150023, Yaroslavl, Moskovskiy avenue, 88

e-mail: likomm@yaroslavl.ru

The in vitro experiment, involving 1164 strains of the tuberculosis mycobacteria, exhibited a potentiating effect of silver nanoparticles on known antituberculous preparations in respect of overcoming drug-resistance of the causative agent. The in vitro experiment, based on the model of resistant tuberculosis, was performed on 65 white mice. An evident antituberculous effect of the nanocomposite on the basis of silver nanoparticles and isoniazid was proved. Toxicological assessment of the of nanopreparations was carried out. The performed research scientifically establishes efficacy and safety of the nanocomposite application in combination therapy of patients suffering from drug-resistant tuberculosis.

Tuberculosis problem

The current trait of the problem is expressed in extremely low efficacy in treatment of patients, particularly, concerning those who are diagnosed on a result of their visit to a doctor. In many cases the efficacy falls below 30%, which proves low efficiency of treatment rate of the modern strategy in tuberculosis treatment [5, 11]. The main reason lies in drug resistance of the tuberculosis mycobacterium to the existing numerous drugs, which to a large degree have lost their potency [7]. In Russia milliards of roubles are spent on free treatment of tuberculosis every year. Due to drug resistance considerable part of the sponsored funds, before the treatment starts, can be regarded and planned as useless and futile expenses. Ineffective treatment promotes formation and occurrence of “new” tuberculosis, called chemo-resistant. The research objective is to evaluate efficacy, safety and tolerance of the silver nanoparticles in isolated form as well as in joint application with the antituberculous preparation isoniazid in vitro and in vivo research during treatments of experimental drug-resistance cattle tuberculosis.
Treatment and nanoparticles of silver

One of the ways out of the existing situation might be the search for the means to reverse the known antituberculosis preparations directed at overcoming the tuberculosis mycobacteria resistance during their interaction with the chemi-pharmacological agents. The nanoparticles of silver are discussed to be such an agent. The history of the scientific research of silver proves its pronounced antiseptic properties. The history of silver research studies established that the daily diet of a man should contain on average 80 micrograms of silver, since the latter is a microelement essential for maintaining normal metabolism as well as regular functioning of his immune system. The same research proved that the doses of silver in the volume of 50 – 250 mg per liter are physiological and during prolonged consumption have no negative effect on the human organism. The “Sanitary Regulation and Standards 2.1.4.1074-01”, existing in the Russian Federation, state that the Maximum Concentration Limit (PDK) of silver cations in drinking water should be within 0,05 mg per litre. According to a number of researches the doses of silver in volumes of 50 – 200 mg per litre eliminate possibility of argyria. The effect of silver exceeds similar concentrations of chlorine, chlorinated lime and sodium hypochlorite as well a number of other potent oxidizers. The research established bacteriostatic and bactericidal action of ions of silver on drug-resistant strains of staphylococcus, Proteus vulgaris, blue pus bacillus and colon bacillus. It was established that ions of silver are able to inactivate vaccine of variolovaccine, strains of the A-1 and B flue, of entero and adenoviruses. The dose-dependent action of ion solutions of silver towards a number of causative agents was proved. For instance, complete elimination of colon bacillus bacteriophage N 163, the Coxsackie virus of the serotype A-5, A-7, A-14, requires a higher concentration of silver (500 – 5000 mg per litre) than elimination of Escherichia, Salmonella, Shigella and causative agents of other enteric infections (100 – 200 mcg). [2, 8, 10].

Mechanism of bactericidal action of nanosilver

The main function of the silver nanoparticles is a continuous accumulation of silver ions in the vicinity of the affected area. This is to protect the silver ions from the interaction with the anions of salts in the blood, and thereby increase the therapeutic effect of application composition. The mechanism of antimicrobic action of silver has not been studied exhaustively. Adsorption theory focuses on derangement of electrostatic interactions, which occurs between the cells of bacteria, charged negatively, and positively charged ions of silver. Some researches explain the bactericidal action of silver by its catalytic properties, which trigger the “oxygen cascade” accompanied by destruction of bacteria protoplasm. Certain data is available, indicating that silver ions interact with the exterior peptidoglycanes of cell membrane, which promotes interruption of oxygen delivery to bacterial cell. A number of researchers suggest that the effect of the antimicrobic action of silver might be explained by the fact that it inhibits the transmembrane electrolyte transport. Other data draw attention to formation of complexes of nucleic acids with silver ions, which disturbs stability of the DNA and bacteria viability. Research of interaction of ions of silver with the cells of pro- and eukaryotic microorganisms indicated that the biocidic effect of silver substrates is determined by its attachment to the membrane-associated protein and lipid stroma of membranes, which leads to changes of the transmembrane potential and necrocytosis. [2, 9].

Technologies of nanosilver preparation have raised topicality of its application to a new level. [6, 12, 13, 14, 15, 16, 17]. Utilization of silver in its nanodimensional range makes it possible to reduce its concentration hundredfold and at the same time multiplying its biocidic properties. Study of bactericidal properties of silver nanoparticles indicated that they possess a more pronounced biocidic effect in comparison with ionic silver [1, 3, 4, 9]. Comparative study of antimicrobial properties in ionic and cluster form proved that application of silver nanoparticles on suspension of yeast cells
results in destruction of the membrane surfaces, but utilization of ionic silver is not accompanied by destruction of the cells. [4].

Materials and methods

For the first time Russian researchers demonstrated the antituberculous effect of silver nanoparticles with isoniazid. [18].

The water solution of particles of the silver, transferred to tests, is received by a method in detail described in the patent application [19], are characterized by the spectroscopy and electron microscopy method. Results are presented in the table and the figures 1, (conducted by the TsKP DMIS (Common User Facilities “Diagnostics of micro- and nanostructures”of Yaroslavl).

Table 1. Key characteristics of silver nanoparticles.

| Determined characteristics                           | Test results |
|-------------------------------------------------------|-------------|
| Silver nanoparticles size, nm                         | From 5 to 50|
| Number of silver nanoparticles in 1 mcm²              | 120-270     |
| Size of the stabilizer-formed membrane                | From 2 to 5 |

Figures 1. Distribution of the nanoparticles according to sizes

Water solutions of silver nanoparticles were analysed in concentration 2.5 mg/l, 5 mg/l, 25 mg/l, 50 mg/l and as antitubercular activity of a composition of silver nanoparticles in concentration 5 mg/l, 25 mg/l, 50 mg/l in a combination to 1 mg of an isoniazid is studied are taken [20].

Research has been performed according to the recommendations stated in «the Management on experimental to studying of new forms of pharmacological substances» [21].

Statistical data processing has been performed using the standard methods of the statistical analysis at medical and biological researches. Reliability level was estimated as sufficient at p>0.05.
Results and their discussion

We performed bacteriological research on 1164 clinical isolates of Koch's bacillus (MTB), bacteriological research, on 65 white mice, and evaluation of general toxicity on 83 non-linear white mice as well as on 146 white rats. We carried out comparative evaluation of antituberculous activity of the isolated nanoparticles in concentrations of 5, 25 and 50 mg per l and the nanocomposite, which contained the specified concentration of nanosilver in combination with antituberculous preparations that inhibited growth of the MTB strains, featuring initial resistance to the used preparations [20].

The efficacy study of various silver nanoparticles concentration in isolated mode of application (figures 2) indicated that at concentration of 5 mg/l complete growth inhibition of the MTB occurred 3.5 times more often, than at concentration of 25 mg/l and 7 times more often than at concentration of 50 mg/l.

![Figure 2](image1.jpg)

**Figures 2.** Inhibition of the MTB growth by the nanoparticles of silver solutions of various concentrations in isolated mode of application.

Results of the research, performed to establish efficacy of the silver nanoparticles in concentration of 2.5, 5, 25 и 50 mg/l in combination with individual antituberculous preparations, is represented in the chart of the figures 3.

![Figure 3](image2.jpg)

**Figures 3.** Inhibition of the MTB growth by the nanocomposites with various concentrations of the silver nanoparticles.
Efficacy of combined application of the nanoparticles with chemopreparations significantly exceeded isolated mode of the nanoparticles application. It is worth noting that maximal inhibiting efficacy was exhibited at concentration within the range of 2.5 – 5.0 mg/l.

The research data as obtained in vitro indicate the possibility of potentiating effect of silver nanoparticles on the total spectrum of known antituberculous chemopreparations in respect of overcoming drug-resistance of the Koch’s bacilli. The highest bacterial growth-inhibitory activity was displayed by the silver nanoparticles with isoniazid 95.4%, with rifampicin – 93.3%, ethionamide – 100%, levofloxacin - 100% and ofloxacin - 100%. The lowest efficacy was observed (61.2%) in case the silver nanoparticles were used in combination with kanamycin. (figures 4).

![Figures 4. Bacterial growth-inhibitory activity of nanocomposites on the basis of silver nanoparticles and chemopreparations (H - isoniazid, R-rifampicin, S – streptomycin, K – kanamycin, E - ethambutol, Ea - ethionamide, Lft – levofloxacin, Ofl – ofloxacin, Cs - cycloserine.)](image)

The results of the bacteriological research in vitro significantly indicate existence of potentiating bacteriostatic and bactericidal effect of silver nanoparticles towards the drug-resistant MBT strains that reaches 100 % in case of reserve preparations (drugs).

Research of the chemotherapeutic activity was performed in the in vivo experiment on 65 white mice. The laboratory animals were infected with a two-week virulent culture of the clinical M. tuberculosis strain, isolated from a tuberculous patient, resistant to traditional antituberculous drugs. The infective dose of the white mice amounted to 5x10⁶ CFU (colony-forming units), which was contained in 0.5 ml of the obtained suspension. The experimental animals were infected via the intravenous route, using the aforementioned dose in the area of retroorbital sinus.

When survival rate of the animals was estimated, it was revealed that at the moment of death of all 100% of the mice from the control group that didn’t receive the treatment, the survival rate of the animals that were treated with nanoparticles in combination with isoniazid, amounted to 95%. Survival rate of the animals that received isolated nanoparticles of silver amounted to 35% (figures 5).
Conclusions

The data, obtained in the course of the in vivo experiment made it possible to conclude with statistical validity that the antituberculous activity of the silver nanoparticles in isolated variant as well as used jointly with isoniazid possess a dose-dependent character. The maximal antituberculous activity on the model of the experimental drug-resistant tuberculosis was observed when the silver nanoparticles where used in the dose of 25 mg/kg and isoniazid in the dose of 50mg/kg.

The results of the therapeutic action of the silver nanoparticles on the models of generated tuberculosis of the white mice in terms of clinical doses of the preparation for a man, obtained in the course of the preclinical research, allow establishing a minimal therapeutic dose that equals 2 mg/kg.

The results of the performed research revealed a significant potentiating effect of silver nanoparticles on known antituberculous preparations (isoniazid, rifampicin, streptomycin, kanamycin, ethambutol, ethionamide, levofloxacin, ofloxacin, cycloserine) with possibility to overcome the drug resistance of the pathogenic agent.

List of bibliography

[1] Baranova E, Revina A, Voyno L and Gorbatiuk V 2003 Comparison of effect of silver ions and silver nanoparticles on the yeast cells and colon bacillus (E Cili) Moscow: Materials of the 1st Russian Scientific Methodological Workshop 53-60
[2] Blagitko E, Burmistrov V, Kolesnikov A, Mikhailov Y and Rodionov P 2004 Silver in Medicine Novosibirsk: Nauka-Center 254
[3] Gmoshinski I, Khotimchenko S and Vernikov V 2009 Nanoparticles of Silver in nature, industry, packing materials, intended for food products: characteristics of possible risks Issues of Nutrition 6 13-20
[4] Yegorova E, Revina A, Rostovschikova T and Kisiliova O 2001 Bactericidal and catalytic properties of stable metallic nanoparticles in inverted micelle Moscow University Newsletter series 2: chemistry 42 332 – 338
[5] Kibrik B and Chelnokova O 2005 Acute progressive destructive forms of pulmonary tuberculosis Yaroslavl 191
[6] Kuzmin S, Privalov L, Katsnelson B, Nikolaeva E, Degtariyova T, Minigalieve I, Valamina I, Kireeva E, Sutunkova M, Shur V, Yereminko O, Khodos M, Glazirina Y, Kozitsina A, Malakhova N and Shishkin E 2010 Experimental data to evaluation of pulmonary toxicity and resopitive toxicity of magnetite particles (Fe3O4) of nano- and micrometer range Toxicological bulletin 2 17 – 24
[7] Mishin V 2009 Pulmonary tuberculosis with drug resistant of the causative agent Moscow: GEOTAR-Media 208
[8] Odegova G, Burmistrova V and Rodionov P 2004 *Study of silver state in silver-containing antibacterial preparations argovit and argogel* Novosibirsk: Collection of scientific papers, connected with research and practice conference “New chemical systems and processes in medicine” 58 – 63

[9] Parkhomenko N, Yurkova I and Riabushko V 2008 *Antibacterial and antifungal effect of water-soluble silver-based nano-bio formulations and sea biopolymers* Works of Taurida National University named after V.I. Vernadskiy. Biology and Chemistry Series 21 106 – 112

[10] Blagitko E 2004 *Applications of silver preparations in medicine* Novosibirsk: Collection of scientific papers, connected with research and practice conference “New chemical systems and processes in medicine” 115

[11] Shilova M and Khruliova E  2005 *Efficacy of tuberculosis patients treatment at the present stage* Problems of tuberculosis and lung disease 3 3-11

[12] Samsonova M 2008 *Nanomedicine: modern approach to diagnostics and treatments of diseases, problems of safety* Pulmonology 5 5 – 13

[13] Shvetsh V, Gelperina C, Maksimenko O, Vanchugova L, Bondarenko G, Shipulo E and Shandruk G 2010 *Influence of toxicological parameters on physicochemical properties of poli-lactide nanoparticles, containing rifampicin* Yaroslavl 42 – 47

[14] Sahoo S, Parveen S and Panda J 2007 *The present and future of nanotechnology in human health care* Nanomedicine/ Nanotechnology/ Biology and Medicine 3 20 -31

[15] Alt V, Bechert Th, Steinrücke P, Wagener M, Seidel P, Dingeldein E, Domann E and Schnettler R 2004 *An in vitro assessment of the antibacterial properties and cytotoxicity of nanoparticulate silver bone cement* Moscow: Biomaterials 25 4383-4391

[16] *Safety evaluation of nanomaterials* 2008 Moscow: Federal Service on Surveillance for Consumer rights protection and human well-being, approved on 12 oct 2008 280

[17] Shahverdy A, Fakhimi A and Minaian S 2007 *Synthesis and effect of silver nanoparceles on the antibacterial activity of different antibiotics against Staphylococcus and Escherichia coli* Nanomedicine: Nanotechnology/ Biology and Medicine 3 168–171

[18] Russian Agency for Patents and Trademarks Registration № 2008142461 of 28 oct 2008, decision of grant of 22 apr 2010, № 151410

[19] Russian Agency for Patents and Trademarks patent № 2390344 for the invention *Ways of generation of silver nanoparticles in aqueous medium*

[20] Kibrik B, Zaharov A and Kreytsberg G 2010 *Nanocomposite “isoniasidi+Ag” in the treatment of tuberculosis in vitro, in vivo* Yaroslavl: “YarMediaGrup” 97 – 195

[21] Habriev R 2005 *Management on experimental to studying of new pharmacological substances* Moscow