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Short Communication

# INVESTIGATION GENOTOXICITY OF NEW SILVER-BASED COMPLEXWITH ANTIMICROBIAL ACTIVITY

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**ABSTRACT**

**Objective:** Investigation of genotoxicity effects of the new antibacterial silver-based compound with of general formula $[Ag(CH_2)_6N_4]^+NO_3$.

**Methods:** The comet assay applied for assessment of genotoxic effects on leukocytes of mice blood. DNA damage was assessed on the percentage of DNA in a comet tail (tail DNA), under influence of silver complex in different concentrations.

**Results:** During experiments found a significant increase in the level of DNA damage in cells, at the maximum tested concentrations. At low doses, the drug has no significant genotoxic effect.

**Conclusion:** Tested silver complex did not cause DNA damage at low concentrations and could be considered as a base for new antibacterial drug.

**Keywords:** Geno toxicity, Comet assay, Silver-based complex, Antibacterial drug, Silver hexa-methylene tetramine.

Antibacterial activity of silver has been known since ancient times, and now different kinds of silver are widely used for medical purposes. In recent years, interest in preparations of silver emerged with renewed strength, due to wide spread of antibiotic-resistant strains of microorganisms. However, silver is heavy metal and quite toxic by the oral and intravenous route. Therefore, it is vital topic of creating new antibacterial drugs, which can be alternative antibiotics [1]. One of the possible mechanisms of silver action *in vivo* is that $Ag^+$enters the cell and intercalates between the purine and pyrimidine base pairs disrupting the hydrogen bonding between the two anti-parallel strands and denaturing the DNA molecule [2].

Various silver-based substances may have impressive useful properties, but the same properties can be potentially hazardous to human health and the normal functioning of biological systems [3]. Therefore, it is extremely important to assess the ability of nanoparticles to cause cyto-and genotoxic effects. Currently, the comet assay (or single cell gel electrophoresis) is a convenient technique among the methods for assessing the genotoxic properties, in view of rapidity and performance. This method is recommended by the World Health Organization for genotoxicity assessment. In recent years, comet essay is widely used in nano toxicology [4].

The purpose of this work is to study genotoxic properties of nitrate hexamethylenetetramine of silver, with formula $[Ag(CH_2)_6N_4]^+NO_3$. This compound possesses excellent antibacterial properties. For further development of the new drug on its base is necessary to evaluate toxicological properties.

Genotoxicity of perspective silver-based compound was assessed by the comet assay. Mouse blood leukocytes were used for experiments. Adult male BALB/c mice (2 months of age, 22–25 g in body weight) were obtained from the Laboratory Animal Breeding Facility (Branch of the Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Pushchino, Moscow Region, Russia) and used in all experiments. The mice were housed in an air-conditioned room with a controlled 12-h light–dark cycle and free access to standard chow and tap water. All manipulations with the animals were conducted in accordance with experimental protocols approved by the Local Animal Care and Use Committee (Branch of Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Pushchino, Moscow Region, Russia). Samples of peripheral blood were collected from the tail vein of mice to Eppendorf tubes contained phosphate buffered saline (PBS, 136.7 mM NaCl, 2.7 mM KCl, 8.1 mM $Na_2HPO_4$, and 1.5 mM $KH_2PO_4$; pH=7.2) with 1 mM EDTA as anti-coagulating agent. The blood was diluted 1:7 to achieve the final concentration of leukocytes of approximately $1\times10^6$cells/ml. Tested solution at final concentrations of 0.01, 0.03, 0.1, 0.3, and 1 mg/ml incubated in aliquots of diluted blood at 37 °C for 30 min with stirring according guidelines [5]. Incubation with distilled water in equivalent volume (1/5 of the sample) considered as a negative control. Treatment of cells with hydrogen peroxide at a concentration of 2 μM was used as a positive control. Analysis of the level of DNA damage in cells performed using an alkaline comet assay with some modifications [6]. The method based on analysis of the electrophoresis pattern of nucleoids of individual cells stained with a fluorescent DNA dye [7]. Microscope slides were prepared from three layers of 0.5% low-melting point agarose (Serva, Germany) with the cells immobilized in the middle layer. Three microscope slides were prepared for each sample. Ready slides subjected to the comet assay procedures according to the *in vitro* protocol [8].

Briefly, all slides were immersed in lysing solution (2.5 MNaCl, 100 mM EDTA, 1% sodium lauroylsarcosine, 10 mMTris-HCl, pH=10, and 1% Triton X-100) and left at 37 °C for 25 min in dark. Immediately after the lysis, the slides were exposed to an alkaline solution (0.3 MNaOH, 1 mM EDTA; pH>13.0) at 4 °C for 20 min in dark. Thereafter the slides were transferred into a SE-1/S-1N electrophoretic chamber (Helikon, Russia) and subjected to electrophoresis in a fresh portion of alkaline solution for 20 min at 4 °C (electric filed intensity 2 V/cm, amperage 300 mA). The slides were then washed twice in distilled water for 5 min and stained with ethidium bromide (1 μg/ml) in PBS at room temperature for at least 1 h in dark. After staining, all slides coded and passed to the next group of scientists who did the photometry. Each slide washed in distilled water for 5 min, and was cover slipped before analysis. All procedures were conducted under yellow light with minimal handling of samples to prevent the occurrence of additional DNA damage in the cells. The slides were examined using a Comet Expert System (Gene Expert, Russia). Routinely, about 50 cells per slide were registered and DNA damage was assessed on the percentage of DNA in a comet tail (tail DNA). All experiments were conducted utilizing the "blind" experimental protocol, when an investigator making the measurements did not know which treatments were made. All the experiments repeated three times. Mean values and standard errors of the mean (SEM) for each treatment were calculated from independent experiments (animals) (n = 3). Non-parametric Mann-Whitney U-test applied for pair-wise comparison of different groups of data.

Studies have shown that incubation of leukocytes in the presence silver compound leads to an increase the level of DNA damage in cells with increasing concentrations of substance (fig. 1). Significant differences from control were found at concentrations above 0.3 mg/ml. The level of DNA damage in these conditions was about 0.8%, which corresponds to the level of DNA damage by a known oxidative stress agent hydrogen peroxide in a concentration of 2 μM (0.76±0.18%).

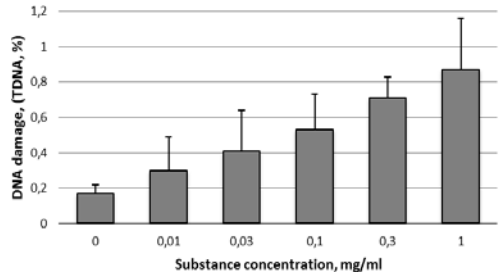


**Fig. 1: DNA damage in leucocytes under influence of the investigated substance**

As shown in fig. 1, increasing 3 times the concentration of the substance caused increasing damage only by 30%. It can be due to simultaneous direct and indirect mechanism of DNA damage in this case. Spontaneous mean level of DNA damage was 0.17%. Only high concentration of substance up to 1 mg/ml caused 5 times increase DNA damage level, compare to control.

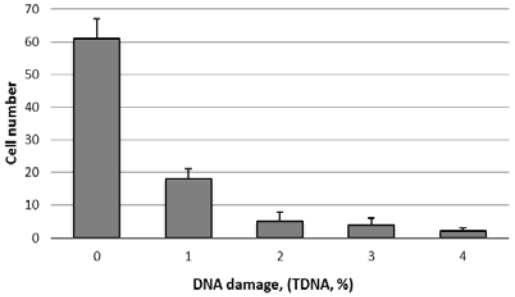


**Fig. 2: Cell distribution according to the percentage of DNA damage after incubation with the investigated substance at constant concentration of 0.3 mg/ml**

It should be noted quite heterogeneous character of cell distribution in the level of DNA damage (fig. 2). In the same sample could be seen intact nucleoids and ones with a high level of DNA damage. So, even at high concentration, about 60 cells on slide revealed a zero level of DNA damage, less than 20 cells revealed 1% DNA damage, 5 cells revealed 2% and about 10 cells revealed 3%, 4%, 5% and more damage. Visual and quantitative analysis of the data suggests that the result of contact with the substance is activation of phagocytosis processes in certain cell (neutrophils, monocytes), which leads to registration increased level of DNA damage in this cells.

This effect appears only at high concentrations, much higher antibacterial ones. These assumptions do not contradict the literature [4, 9]. Thus, the results of the comet-assay test indicate that the incubation of leukocytes with substance at concentrations above 0.3 mg/ml revealed a significant increase in DNA damage in cells, comparable with the action of hydrogen peroxide in a concentration of 2 μM.

Presumably, an increase in DNA damage associated with chemical inactivation of intracellular enzymes and activation effect of the complex on phagocytosis and subsequent activation of cells death. This mechanism can play the major role in local drug application.

Thus, new antibacterial complex did not cause significant DNA damage at low concentrations (0.01-0.03 mg/ml) and could be considered as a base for new antibacterial drug. Relevant level of genotoxicity observed only at high concentrations of 0.3 mg/ml and above.

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**CONFLICT OF INTERESTS**

Declared None

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