

Short Communication

INVESTIGATION OF CYTOSTATIC ACTIVITY OF THE METHENAMINE SILVER *IN VITRO* AND *IN VIVO*

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ABSTRACT

Objective: Developing of novel pharmaceutical agents is an actual topic for chemotherapy. Heavy metals possess significant cytotoxic properties and some of them are widely introduced in medical practice, e. g. cisplatin.

Methods: Silver salt of methenamine was suggested as prospective substance and tested was tested on black-motley cattle with bovine leukemia to reveal influence on haematological parameters. The preparation was applied in form of 0.5% solution in different doses and was administered as a single intravenous injection. Blood parameters were determined by triple haematological monitoring. Investigation of cytostatic activity was conducted on the leukemic cells of HUT-102 line *in vitro* in the concentration range of 0.03-3.0 mg/ml.

Results: The results showed normalization of blood parameters in a dose-dependent manner after intravenous injection of methenamine silver salt. It was revealed a pronounced inhibitory effect of the silver-based drug on the leukemic cells both *in vitro* and *in vivo*. Control blood test one month after experimental therapy confirmed the stability of the achieved results.

Conclusion: The tested drug showed a moderate cytostatic effect. Methenamine silver nitrate can be considered as prospective pharmacological agent. Bovine leukaemia was shown as a convenient model for the development of new treatment of blood diseases. Continuation of the work is of interest for both veterinary and medical pharmacotherapy.

Keywords: Methenamine silver nitrate, HUT 102 cells, Lymphocytes, Leucosis, Cytostatic activity, Bovine leukemia, Silver hexamethylenetetramine

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Designing of novel cytostatic agents for leucosis treatment is a challenging task for modern pharmacology and medicine. Especially acute problem is a resistant form of disease [1-2]. A lot of modern approaches are used now for development delivery systems and screening of novel agents, e. g. virtual ligand screening [3-4]. Due to drug-resistivity, recently interest revived in the metal preparations of a new generation with cytostatic, antiviral and antibacterial activities [5-8]. Malignant and infectious diseases is a huge problem in veterinary practice as well, e. g. bovine leucosis causing enormous economic losses, and required new eradication approaches [9-10]. Currently, there are well-developed methods of laboratory diagnosis of bovine leukemia [11], but methods of treatment are not developed at all. Specific prophylaxis is only developed, but not introduced [12]. Bovine leukemia virus (BLV) is a retrovirus closely related to the human T-lymphotropic virus type 1 HTLV-I. Bovine leukemia virus (BLV) is a valuable model system for investigation of human lymphotropic viruses [13]. Here we considered viral bovine leukemia as a very convenient model for testing of promising antiviral and cytostatic agents for the medical practice, where leukemia is still a major problem [14]. The aim of this work is to study the cytostatic activity of stabilized solution of methenamine silver nitrate under *in vitro* and *in vivo* conditions. Previously, it was shown a wide range of anti-infective activity of methenamine silver nitrate [15].

Investigation of antiproliferative activity of methenamine silver nitrate was carried out on cattle with hematologically and serologically

(radial immune-diffusion test) confirmed the diagnosis of bovine leukemia. All cows were black-motley breed, age 2-6 y, the weight of 450-670 kg, the standard diet. Group of healthy cattle served as a control group (20 heads). All cows with bovine leucosis were randomly distributed in 2 experimental groups (15 animals each), depending on the dose of administration. The test preparation (methenamine silver nitrate) in 0.5% stabilized aqueous solution injected intravenously into the jugular vein at the dosage range of 0.3 mg/kg (first experimental group) and 0.9 mg/kg (second experimental group). The rates of the experimental dosages were chosen based on preliminary data to determine the acute and chronic toxicity in small laboratory animals; i.e. the value of the experimental doses about 1% LD₅₀. Blood parameters were determined by triple haematological monitoring (immediately before injection, during the week after and 1 mo after injection). Investigation of the drug cytostatic activity conducted on leukemic cells lines HUT-102 in the concentration range of 0.03-3.0 mg/ml. Cells were cultured in RPMI-1640 medium with 15% fetal calf serum according to standard procedures. After 24 h incubation, the percentage of viable cells was determined by flow cytometer. All results here are presented as mean±standard error of mean (SEM). Different groups of data were compared by Mann-Whitney U-test.

Results of *in vitro* testing of the drug at varying concentrations (table 1) showed a pronounced inhibitory effect on the leukemic cells with dose-dependent cytopathic effect.

Table 1: *In vitro* influence of methenamine silver nitrate on HUT 102 leukemic cells line, n=3 (mean±SEM)

The percentage of viable cells of HUT-102 line, (%)			
0.03 mg/ml	0.3 mg/ml	3 mg/ml	Control
43±6	0	0	92±3

Single intravenous administration of the experimental dose of 0.3 mg/kg, resulted in a slight decrease in the number of white blood cells to 14.4±1.1 ths/μl (13.7% recession) and the absolute number of

lymphocytes to 11.4±0.7 ths/μl (13.6% recession). Injection of single high dose (0.9 mg/kg) of the drug accompanied by more pronounced changes in the hematologic parameters, as shown in fig. 1.

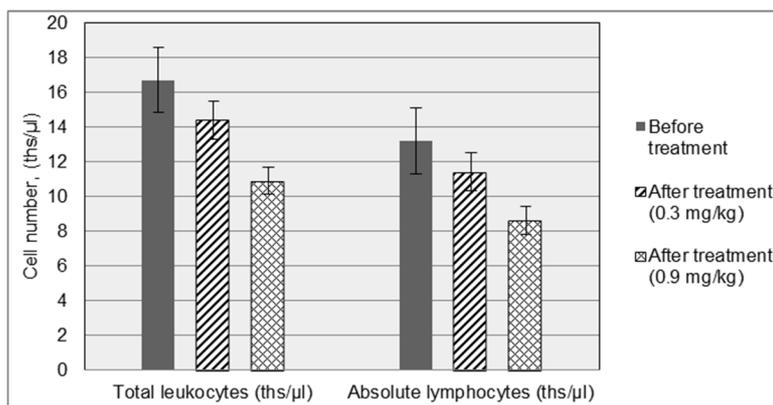


Fig. 1: *In vivo* influence of stabilized solution of methenamine silver nitrate on the white blood cells of cattle with leukemia, n=15 (bars represent the standard errors of the mean)

The drug contributes to the normalization blood parameters, reducing the number of white blood cells to 10.9 ± 0.8 ths/ μ l and the absolute lymphocyte count to 8.6 ± 0.5 ths/ μ l as well as the disappearance of leukemic cells. However, these parameters were different from healthy control group, where the total number of white blood cells was 7.7 ± 0.6 ths/ μ l, the absolute number of lymphocytes was 5.4 ± 0.3 ths/ μ l. Additional hematologic monitoring one month after treatment confirmed the stability of the achieved results of experimental therapy.

Thus, tested silver-based drug revealed the moderate cytostatic effects with a tendency to normalization of the haematological parameters in experimental animals. It is important that bovine leukemia can be considered as a promising model for pharmacotherapy of blood malignant diseases.

AUTHOR CONTRIBUTION

All authors took part in experiments. Dr. Evgenii Plotnikov made a work concept, provide a chemical synthesis of preparation and wrote the manuscript. Dr. Vladimir Plotnikov drafted the manuscript and provide statistical analyses.

CONFLICT OF INTERESTS

Declared none

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