SUMMARY

Cancer diseases are one of the major health concerns in clinical medicine and pharmacology nowadays. Acute myeloid leukemia is a malignant neoplasm with a rapid progression that, without treatment, can kill even within a few weeks. The method of therapy depends on the type of leukemia and the age of the patient and accompanying diseases. Despite significant advances in cancer chemotherapy, there are still difficulties with the treatment of acute myeloid leukemia, particularly in patients over 65, who make up a significant proportion of patients. The use of cytostatic drugs is often limited due to their toxic effect on healthy cells of the body and numerous undesirable effects, often posing a threat to the patient's life. One of these side effects is neutropenia, which can contribute to serious bacterial infections. Antibacterial antibiotics are used to prevent these types of complications. However, it should be taken into account that they may also cause side effects, thus worsening the patient's condition. The mechanisms of the interaction of many drug groups on individual cell structures and processes are very complex and still not fully understood. Currently, intensive researches are carried out to understand these interactions, reduce toxicity of cytostatic drugs and improve their therapeutic properties.

The aim of this work is the in vitro characterization of alterations of biomechanics in acute myeloid leukemia cells and nanostructural and mechanical changes in DNA strands under the influence of anticancer and antibacterial antibiotics. One of the main target of the interaction of cytostatic drugs is the DNA of the tumor cell, which due to the acquired mutations is significantly different from the normal genome. Therefore, there is a need to characterize the effect of cytostatic drugs on both nucleic acids and entire cells. As anticancer drugs, anthracycline antibiotics - doxorubicin, daunorubicin and epirubicin have been selected. In turn, antibacterial antibiotics were aminoglycosides (kanamycin, neomycin, tobramycin). The objects for the interaction of the examined drugs were purchased DNA fragments and myeloid blast cells isolated from bone marrow taken from AML patients of the Clinic of Heamatology, Blood Neoplasm and Bone Marrow Transplantation of the Medical University of Wroclaw. In addition, the penetration of anthracyclines into erythrocytes and their effect on the mechanics of red blood cells were examined.

The study of structural changes was carried out using nanoscopic techniques, such as atomic force microscopy (AFM) and transmission electron microscopy (TEM). In addition, molecular dynamics simulation of the interaction of drug molecules with DNA was performed. The experiment of biomechanical changes of nucleic acid and blast cells was carried out with

use of the modern optical tweezers technique. Studies on the intracellular localization of cytostatic drugs by means of confocal microscopy were also performed.

As a result of the conducted experiments, significant changes of DNA structure induced by the interaction of anthracycline antibiotics were observed. The intercalation of DNA by these drugs caused the elongation of the nucleic acids strands and the reduction of their height. These changes were dependent on drug concentration and the content of guanine and cytosine base pairs (GC) in the sequence of nucleotides. The results of the molecular dynamics simulation also showed the tendency of anthracyclines to extend the DNA strand. In addition, anthracyclines caused a reduction in the stiffness of the DNA double helix.

In the case of aminoglycoside antibiotics, significant differences between the mechanism of action on the DNA of first generation of aminoglycosides, such as kanamycin and neomycin, and the second generation antibiotic - tobramycin were found. Tobramycin caused ordered condensation of the DNA strand to various structures - especially rods, toroids and spheres. The molecular dynamic simulation of tobramycin-DNA interaction suggests that the observed condensation effect is the result of the interaction of tobramycin through both grooves of the

double helix in such a way that DNA molecule bends. In turn, kanamycin and neomycin caused direct damage to DNA, such as nucleic acid strand breaks.

Changes in the mechanical properties of blast cells after anthracycline interaction were also observed. In the initial phase of drug interaction, cell stiffness usually decreased, while after longer incubation with drugs it increased. Studies using a confocal microscope enabled the evaluation of the internalisation of drugs into cells and their internal location. There was a dependence between the presence of certain immunophenotypic markers, associated with a more aggressive course of the disease, and the weaker or lack of internalization of the drug to the cell nucleus, which is a characteristic feature of cells resistant to a given chemotherapy. Anthracyclines also penetrated into erythrocytes taken from the blood of AML patients and reduced their stiffness.

The results of this work may significantly influence the understanding of physiological and molecular changes of the cancer cells resulting from chemotherapy and enable the search for new strategies of cancer treatment. Above all such knowledge may help in determining an individual dose of the drug for patient and prevent side effects by minimizing the dose while maintaining the effectiveness of treatment.