3-bromopuryvate (3-BP) is an intensively studied compound exhibiting anticancer activity. This activity is concerned with inhibition of biological system of produce energy exhibited by cancer cells (Warburg effect). Warburg effect is intensive glycolysis which also occurs in the presence of oxygen. The studies performed on animal and human cells reveal high anticancer activity of 3-BP. What is important, the concentration of 3-BP which is active on tumor cells is approx. 3 - fold lower than the lethal concentration for healthy cells. It is connected not only with the unusual metabolism represented by tumor cells, but also with the transport of 3-BP in a tumor cells. The 3-BP is transported into the cell by MCT1 transporter which in tumor cells occurs in larger amount then in normal cells. In the human body there are cells, which also have numerous of MCT1 transporters what could result in the accumulation of 3-BP also in these cells during therapy. Despite of a numerous studies, the exact mechanism of 3-BP action, is still not known. However it is known, that this compound is highly reactive. It alkylates proteins (probably the glycolytic and mitochondrial enzymes) and might induce oxidative stress. The main goal of proposed project is to study, if 3-BP is a genotoxic compound in the model eukaryotic organism, budding yeast Saccharomyces cerevisiae. Mutagenic activity of 3-BP will be examined for both the nuclear and the mitochondrial genome. The intracellular processes, like cell response to the DNA damage (activation of cell cycle checkpoint, the DNA repair mechanisms) are remarkably similar between the yeast cells and higher eukaryotes. S. cerevisiae is useful model for study many molecular mechanism, as well as activity of novel chemotherapeutics and the cell response for their presence. The advantage of choosing the eukaryotic yeast as a model to study is not only their significant similarity to higher eukaryotes, but also the fact that they are easy and cheap to breeding. Molecular biology has many methods, which allow in a relatively short time obtain many results. So far application of yeast model significantly expanded the knowledge of eukaryotic cell molecular biology. To determine whether the 3-BP has genotoxic activity we plan to perform a series of experiments in which we verify, if in the presence of 3-BP DNA damage are generated and if cel response to DNA damage is activated. My preliminary studies allowed me to select mutants without proteins important for correct DNA damage response which are sensitive to 3-BP. To verify, if genotoxicity is one of the mechanism of 3-BP cytotoxicity, next experiments will be focused on the sensitivity of another mutants lacking the proteins important for cell cycle progression and involved in the response to DNA damage. In the next step we determine whether in response to the presence of 3-BP the cell cycle checkpoints are activated. In the early phase of response to DNA double strand break and disorders in replication, phosphorylation of histone H2A occurs. This process is associated with the activation of cell cycle checkpoints. By using western blot method phosphorylation of histone H2A in the presence of 3-BP will be detected. In response to the DNA damage also Rad53 kinase is phosphorylated what in turn leads to the activation of cell cycle checkpoints. Level of Rad53 phosphorylation in the presence of 3-BP will be also determined. Detection the phosphorylation of kinase Rad53 and histon H2A, as a molecular markers of single-stranded and double-stranded breaks, inform us not only about existence of DNA damage but also about activation of checkpoints in response to the DNA damage. Strain in which Rad52 protein, mediator of homologous recombination, is expressed together with a yellow fluorescent protein will be used to check, if in the response to the 3-BP DNA damage occurs which are repaired using homologous recombination. To achieve this goal the fluorescence microscopy will be used to observe if the DNA repair protein aggregates in the presence of 3-BP. This foci correspond to a functional DNA repair centers. Increase in the number of cells in which Rad52 foci are observed as a response to the 3-BP, indicate the presence of DNA damage, which repair involves homologous recombination. In order to determine whether the test compound induce double strand breaks the pulsed-field gel electrophoresis (PFGE) will be used. This method allows to separate of whole chromosomes in the gel and visualization as distinct, separated bands, each of which presents at least one yeast chromosome. The disappearance of bands and accumulation of low molecular weight "smear" indicates the fragmentation of chromosomes. In response to the DNA damage cell cycle checkpoints are activated what resulting in the cell cycle slowing or stopping. To determine if exposure to 3-BP resulting in disturbed cell cycle kinetics, we decided to analyze the cell cycle process in response to the 3-BP using flow cytometry. To obtain a complete picture of the 3-BP activity on the DNA of eukaryotic cells , the influence of 3-BP on mitochondrial genome will be also examined. We will also check, if 3-BP causes an genomic instability using methods concern the determination of specifying minimal recombination frequencies in the region of a transcriptionally highly active rDNA.

Due to the increase cases of cancer, high cost of treatment and often observed lack of effectiveness of the therapy the new methods of treatment are constantly searching. Fully reasoned seem to be studies about a comprehensive characterization of a new drug, what can result to using an innovative methods in therapy. The complete characterization of the mechanism of 3-BP action may result in the effectiveness of the therapy. If the 3-BP induces DNA damage, this process might be unrecognized so far, a new mechanism of action (mutagenicity), by which the cancer cells are destroyed, because in those cell the repair mechanisms may not be sufficiently effective. The results obtained under this project may be a prelude to confirmation the mechanism of action of 3-BP on higher eucaryotes. The realization of this project will also provide new and basic information on the cellular response to stress.