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Wpływ 5',8-cyklo-2'-deoksypuryn na mechanizmy naprawy uszkodzeń zespolonych DNA w ekstraktach mitochondrialnych

Effect of 5',8-cyclo-2'-deoxypurines on the mechanism of repair of clustered DNA damage in mitochondrial extracts

mgr inż. Karolina Boguszewska

Promotor: prof. dr hab. n. farm. Bolesław T. Karwowski

Zakład Bromatologii

Uniwersytetu Medycznego w Łodzi

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Streszczenie w języku angielskim

Each living cell is constantly exposed to many factors, both external and internal, that interact with its genetic material. Ionizing radiation, chemotherapeutics, food contamination, but also products of cellular metabolism, and reactive oxygen species (ROS) are factors that potentially cause DNA damage. DNA lesions may block gene expression or lead to mutagenesis. Mutations can subsequently lead to disturbances in the correctness and stability of genetic information. DNA repair systems must be constantly active, able to detect and eliminate the effects of DNA damage before the incorrect genetic information is passed on to the next generations. The efficiency of repair mechanisms depends on many factors, including the type of cell and the occurring type of DNA damage. When the number of DNA lesions in the genome exceeds its repair efficiency threshold or when DNA repair systems do not function properly, the cell may enter the path of controlled death or the path of uncontrolled cellular divisions leading to e.g., the development of cancer.

Clustered DNA damage (CDL), defined as the presence of at least 2 lesions within 1-2 turns of the DNA helix, may contain various types of single DNA lesions and consequences for the cell may differ according to the spatial arrangement of lesions.

In my doctoral research, I have focused on the base excision repair (BER) system which is the most conservative repair system in most cell types, from bacterial to human. Specific enzymes recognize and remove damaged nucleotides/nucleosides. It is followed by a series of enzymatic reactions leading to the insertion of the correct nucleotide or longer fragment of 2-10 nucleotides. It is assumed that the BER system is responsible for the repair of most DNA damage in the mitochondria.

5',8-cyclo-2'-deoxypurines (cdPu) are formed as a result of the ionizing radiation (generating •OH) impact on the cell. It is a complex case of DNA damage containing more than one modification within a single nucleotide. The presence of cdPu in the DNA structure may lead to impaired processes of DNA repair. When CDL contains cdPu, the efficiency of DNA repair mechanisms is impaired, especially in the case of the BER system. Moreover, when the CDL contains DNA lesions that are repaired by different mechanisms, the repair efficiency of the "simpler" lesion can be significantly reduced. As part of this dissertation, I used a CDL model containing an apurinic/apyrimidinic site (AP site) and 5′,8-cyclo-2′-deoxypurines.

Mitochondria are a key element in keeping the cell alive and providing it with an adequate level of energy. Mitochondria are crucial for cellular processes such as energy production, homeostasis, and cell survival. They carry out the processes of aerobic respiration and the production of adenosine triphosphate (ATP) in eukaryotes. However, oxidative phosphorylation (OXPHOS) is also a source of ROS, which are important and dangerous to the cell at the same time.

Mitochondria are an interesting element of human cells also due to their origin. It is assumed that mitochondria are a remnant of a bacterial symbiont absorbed by a larger cell, which enabled the development of the first primitive organisms in the aerobic conditions that were developing on the planet at that time. There are many arguments in favor of the endosymbiotic theory. One of the arguments is the fact that human mitochondria contain a separate genetic material in the form of mitochondrial DNA. It is particularly vulnerable to DNA damage generation caused by ROS activity (such as $O_2^{\bullet -}$, H_2O_2, $^{\bullet}OH$) formed during cellular respiration. For this reason, properly functioning mtDNA repair systems, the main one being BER, are crucial for maintaining the function of the mitochondria, and thus the entire cell. Mitochondria are an extremely interesting subject of research in the context of complex DNA damage and their impact on mtDNA repair. Mutagenesis of the mitochondrial genome can result in pathological conditions such as mitochondrial, neurodegenerative, and/or cardiovascular diseases, premature aging, and cancer. In addition, in recent years, mitochondria have been the subject of research in terms of their participation in civilization diseases, e.g., in the field of metabolizing nutrients such as fats (e.g., ketogenic diet) or their participation in the body's reactions to exposure to cold practiced by an increasing number of people to prevent the above-mentioned pathological conditions. Despite this, studies that consider complex DNA damage containing cdPu and their impact on mtDNA repair processes are scarce. Researching this area is crucial to fully understand these processes and to develop new therapies for diseases linked to mitochondrial DNA damage at the molecular level.

This dissertation focuses on clustered DNA damage. In addition, the dissertation presents a holistic view of the similarities between human mitochondria and bacteria (in the context of the BER excision repair system) and points out that bacteria might be an interesting research model for mitochondrial diseases. Changes in the overall activity of proteins involved in the BER system were investigated based on three eukaryotic cell lines: xrs5 - a cell line of X-ray sensitive epithelial cells derived from Chinese hamster ovary, BJ - a primary cell line of human normal fibroblasts used as a control with efficient repair systems, and XPC –human fibroblasts obtained from a patient suffering from type C Xeroderma Pigmentosum. The performance level of the BER repair system was determined relative to a single- and double-stranded CDL. The model oligonucleotides used in this study contained an AP site and one of cdPu ((5′S)-5′,8-cyclo-2′-deoxyadenosine (ScdA), (5′R)-5′,8-cyclo-2′-deoxyadenosine (RcdA), (5'S)-5',8-cyclo-2'-deoxyguanosine (ScdG) or (5'R)-5',8-cyclo-2'-deoxyguanosine (RcdG)) located at different interlesion distances on same or opposite dsDNA strand. I have analyzed whether the presence of cdPu and their relative distance from a single lesion (AP site) on the opposite or the same DNA strand affect the ability to effectively repair the latter. The type of cdPu, its diastereomeric form, and the mutual distance between lesions in the range of up to 10 bp were taken under consideration.

To determine the baseline level of the repair system activity, I optimized and investigated the repair of CDL by the BER nuclear repair system. Next, I performed an analysis at the cytoplasmic level to determine whether the proteins involved in nuclear and mitochondrial DNA repair present enzymatic activity even before translocation to the target site in the cell and how the presence of complex damage such as cdPu affects the overall protein activity. I have performed the analysis of the activity of proteins involved in the initial stages of the BER system in the mitochondrial extracts of xrs5 cells. Moreover, I have investigated the mutagenic potential of the model CDL lesions and assessed trend correlations between the procaryotic and eukaryotic models of DNA repair.

The results of the study presented in this dissertation may be the basis for further research in the context of improving existing therapies that cause DNA damage, such as radiotherapy, and/or chemotherapy, but also developing modern therapies (e.g., synthetic therapeutic oligonucleotides) as well as due to their diagnostic potential (cdPu as biomarkers of oxidative DNA damage). Understanding the impact of cdPu occurrence in DNA is crucial for its potential applications in medicine, pharmacy, and food science.