Summary of PhD thesis of Magdalena Zdrowowicz

„Nucleobases derivatives as DNA sensitizers for electromagnetic radiation"

Radiotherapy is the most common modality for treating human cancers. However, oxygen levels in solid tumors are very low, and tumor cells become resistant to X-rays as a result. This fact calls for introducing radiosensitizers, i.e. chemical agents capable of radiosensitizing a cancer cells to the effects of high-energy radiation, and in consequence, effectively reducing the therapeutic dose of ionizing radiation, which is not neutral towards normal cells adjacent to the tumor. Modified nucleosides seem to be especially well suited for radiation-induced cell killing because of their specific features. The most unique property is the fact, that they can substitute (at least some of them) native nucleosides in DNA without affecting its structure and function. At the cellular level, these compounds should act like a „Trojan horse" – the lethal effects (DNA damage leading to cancer cells death) should be produced only as a result of interactions between radiation and nucleoside analogs incorporated into DNA. Structural modifications of nucleosides should rely on the introduction of suitable substituents to nucleobases that increases nucleosides' sensitivity to degradation induced by solvated electrons, one of the major products of water radiolysis under hypoxic conditions, to which native DNA is negligibly reactive. The modified nucleosides have also photosensitizing properties so they can be used in photodynamic anticancer therapies, based on the controlled destruction of the DNA molecule.

Despite many years of efforts, clinical use of this type sensitizers is not satisfactory and the range of radiosensitizers currently employed in the clinical practice is quite narrow. Additionally, the details of molecular mechanisms leading to their sensitizing action are still unclear. For this reason, the aim of current doctoral project was to explain the mechanism of

photosensitizing DNA by bromonucleosides and to investigate their reaction with the prototype of secondary organic radicals. Another purpose of this thesis was widening of the modest sensitizers range by proposing new nucleoside derivatives with confirmed radiosensitizing potential.

In the first step, the photoinduced degradation of DNA fragment labeled with 5-bromo-2'-deoxyuridine (BrdU)/5-bromo-2'deoxycytidine (BrdC) was studied. A double-stranded oligonucleotide, 80 base pairs in length, was multiply labeled with BrdC/BrdU using polymerase chain reaction (PCR). The modified oligonucleotide was irradiated with 300 nm photons. Using the LC-MS method, denaturing PAGE electrophoresis and tandem MS/MS analysis coupled with the enzymatic digestion, two types of DNA damage were discovered in UV-irradiated BrdU/BrdC-sensitized DNA: single strand breaks (related to the long-range photoinduced electron transfer from a distant guanine to the photoexcited BrU) and intrastrand crosslinking (formed in the cycloaddition reaction between the photoexcited BrU and the ground state pyrimidine nucleobase).

In the next step, the propensity of all bromonucleosides (BrdX) to damage induced by 2-hydroxypropyl radical ($OHisop^{\bullet}$) – a prototype of secondary organic radicals, generated in the cell as a result of exposure to radiation – was investigated. The analysis of photolytes and radiolytes revealed that two stable products, debrominated nucleosides (dX) and the adducts of $OHisop^{\bullet}$ and dX, were characteristic for the degradation of brominated pyrimidines. On the other hand, only the adduct was observed in the irradiated solutions of bromopurine nucleosides. The results of our investigations, confirm that adducts can be formed at ambient temperature due to interactions between $OHisop^{\bullet}$ and all BrdXs. The mechanism leading to dX is, however, completely different and is releted to electron transfer from $OHisop^{\bullet}$ to the brominated pyrimidines. The BrdX radical anion resulting from this process, swiftly

dissociates leaving behind a reactive pyrimidine nucleoside radical localized on the nucleobase. In a double-stranded DNA such radical abstracts a hydrogen atom from the sugar residue or interacts with the adjacent bases, leading to serious DNA damage as strand breaks and intra- or interstrand cross-links. The results of quantitative studies suggest that bromopyrimidines should be more effective sensitizers of radical DNA damage than bromopurines.

In other projects, a new, potential radiosensitizers – 5-tiocyanato-2'-deoxyuridine (SCNdU) and 5-selenocyanatouracil (SeCNU) were proposed, synthesized and examined. A combination of theoretical studies with negative ion photoelectron spectroscopy experiments demonstrated that SCNU possesses properties required for efficient radiosensitizers. The studies on mechanism of electron-induced degradation of SCNdU show that the electron attachment to SCNdU leads to two parallel reactions producing quite different products. In one path, the C5-S bond is broken and the secondary $U^{\bullet}$ is formed, giving dU as a stable product. On the other path, the S-CN bond cleavage in the thiocyanate substituent produces the $U\text{-}S^{\bullet}$ that ultimately forms a stable dimer (dU−S−S−dU). A similar approach was used for SeCNU. It has been proved, that $U{-}Se^{\bullet}$ as the primary product of dissociative electron attachment to SeCNU .The degradation of studied derivative caused by electron attachment, results in two major products: the U−Se−Se−U dimer and the adduct of the $^{\bullet}$OtBu radical to the $U{-}Se^{\bullet}$ radical, U−Se−OtBu. In addition, it has been shown that the tested derivatives are characterized by relatively low cytotoxicity. The research has established tio- and selenocyanatoderivatives of uracil as potential radiosensitizers that could cause very toxic DNA damage such as strand breaks, both intra- and interstrand DNA crosslinking as well as DNA-protein crosslinking.