RADIATION AND BIOLOGICAL STUDIES OF URACYL DERIVATIVES IN THE CONTEXT OF RADIOTHERAPY SUPPORTING

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Cancer is one of the main causes of death worldwide. Traditional therapies, such as: radiotherapy, chemotherapy or surgery are often ineffective. What is more, the use of these modalities can lead to dangerous and onerous side effects, which reduce quality of patient life during and after the treatment, and even to death in extreme cases.

Almost 50% of cancer patients are treated with radiation therapy (are exposed to certain doses of ionizing radiation). Ionizing radiation (IR) can damage the DNA of living cells directly by ionizing the biomolecule, or indirectly by water radiolysis products. The most genotoxic product of water radiolysis are hydroxyl radicals ($^{\bullet}$OH). It is worth emphasizing that cells forming solid tumors are characterized by reduced oxygen concentration, which contributes to their reduced sensitivity to IR (by up to 2 – 3 times compared to the well-oxygenated cells). In addition, under hypoxia hydrated electrons (also formed by water radiolysis) are generated in the amount equal to that of the $^{\bullet}$OH radicals.

Introduction to anticancer therapy sensitizers of DNA damage induced by IR seems to be a promising alternative to traditional radiotherapy. This PhD work is devoted to a group of radiosensitizers operating in hypoxic conditions. This group comprises modified nucleosides (MNs) whose mechanism of action is based on the concept of "Trojan Horse". Ideally, a modified nucleoside becomes incorporated into DNA (the enzyme machinery used for DNA biosynthesis should not distinguish between a nucleoside derivative and its native form) without interfering with the biopolymer function. Only the

action of ionizing radiation initiates a cascade of events, which eventually leads to the cell death.

Modification of the chemical structure of the nucleoside is aimed at increasing the electrophilic properties of the compound, making it an electron trap. The attachment of hydrated electron (produced as a result of water radiolysis) to the modified nucleoside incorporated into DNA should lead to the formation of an unstable anion, which by dissociation generates a reactive radical inside the DNA molecule. The latter, abstracting a hydrogen atom from the sugar moiety of neighboring nucleoside may lead to DNA damage such as single strand break, double strand break, inter-strand crosslink or purine cyclization.

It should be emphasized that the number of modified nucleosides used in anticancer therapy is surprisingly low, which may be related to the fact that the mechanism of their action has still not been fully understood. Taking into account a potential of modified nucleosides and growing demand for new, effective therapies search for MNs with sensitizing properties becomes increasingly important.

This work presents the results of studies on the radiosensitizing properties of four uracil derivatives: 5-bromo-4-thio-2'-deoxyuridine (BrSdU), 5-iodo-4-thio-2'-deoxyuridine (ISdU), 5-(*N*-trifluoromethylcarboxy)-aminouracil (CF_3CONHU) and uracil-5-yl *O*-sulfamate (SU).

Radiolytic experiments carried out for aqueous solutions of ISdU and BrSdU confirm that both compounds undergo degradation induced by ionizing radiation. However, only in the case of iodine derivative the product of dissociative electron attachment, which is responsible for DNA damage, was identified. These results were mirrored in the cellular assays. Treating MCF-7 cells with ISdU in the clonogenic assay results in a decrease of viability even at the lowest radiation dose – 0.5 Gy. Moreover, the cytometric analysis

of histone H2A.X phosphorylation, which is a marker of double strand breaks, confirms the increased sensitivity of cells to the action of ionizing radiation, which at least partially is associated with the generation of this type of damage. Treating tumor cells with ISdU also increases the number of early-apoptotic cells, which further confirms its radiosensitizing properties.

In case of the second derivative, BrSdU, no differences were observed in γH2A.X positive cells or early-apoptotic cells between the treated and untreated cells. The activation barrier in the DEA process, which is 12.6 and 26 kJ · mol^{-1} (at the B3LYP (PCM) / DGDZVP ++ level), for BrSdU and ISdU, respectively, seems to be responsible for the differences in the properties of ISdU and BrSdU. A significantly higher activation barrier calculated for BrSdU probably makes the life-time of the $BrSdU^{\bullet-}$ anion radical sufficiently long for protonation reactions to take place which prevents the dissociation process and deprives BrSdU of its sensitizing properties.

Degradation leading to the formation of DEA product as a result of stationary radiolysis in an aqueous solution was observed for 5-(*N*-trifluoromethylcarboxy)-aminouracil (CF_3CONHU) as well .The clonogenic assay also indicates decrease of the viability of PC3 cells treated concomitantly with CF_3CONHU and ionizing radiation. This result confirms the radiosensitive potential of the studied compound.

The last derivative – uracil-5-yl *O*-sulfamate (SU), has been characterized by a promising DEA profile at the M06-2X/6-31++G(d,p) level. The dissociative electron attachment process in the gas phase is complicated and depends on the energy of low-energy electrons. It leads to many degradation products of the substituent and uracil ring. The stationary radiolysis of the aqueous solution of SU indicates that this derivative is resistant to the action of hydrated electrons. Neither the change in pH nor the use of higher doses of IR

affects the results of the experiment. The reason of the lack of correlation between the experiment and calculations was traced back to the inaccuracy of the DFT model – in particular, to the error in the description of the dissociation of the S–O bond. SU is an example of MN which suggests that more precise theoretical methods such as extrapolation models (e.g. G2) should be used to verify the radiosensitizing properties of nucleosides.

The current study shows two analogues, ISdU and CF_3CONHU, to be potential radiosensitizers. The results obtained for the former nucleoside were so promising that its use as a radiosensitizer has been claimed for a patent application. The present investigations also explain several mechanistic details of the DEA process and improve theoretical model verifying the radiosensitive properties of modified nucleosides. Further research into these derivatives, extending the theoretical procedure used so far to account for the hydrolytic stability of the proposed structural modifications, the ease of their enzymatic phosphorylation, the acceptance of proposed MN triphosphates by DNA polymerases, and finally, the efficiency of the DEA process, should lead to the proposal of nucleoside derivative(s) which will substantially improve the efficacy of radiotherapy.