Linear Energy Transfer Effects on Cystamine's Radioprotective Activity

Subjects: Biochemical Research Methods

Contributor: Samafou Penabeï, Esteban Sepulveda, Abdullah Muhammad Zakaria, Jintana Meesungnoen, Jean-Paul Jay-Gerin

Radioprotective agents are increasingly drawing attention for their potential uses in various critical fields. These include radiotherapy, which is crucial in cancer treatment, as well as public health medicine to safeguard against the health impacts of radiation. Moreover, they are vital in emergency scenarios involving massive accidental radiation exposure or impending radiological crises. Among these agents, cystamine, an organic diamino–disulfide compound, is particularly noted for its dual capabilities: it acts as a shield against radiation damage and also functions as a potent antioxidant.

Keywords: cystamine ; linear energy transfer (LET) ; radiolysis

1. Introduction

Cancer remains a pressing global health issue, accounting for a substantial number of deaths worldwide. Yet, recent progress in its detection and treatment has resulted in improved outcomes for many patients. Of the different treatment options available, radiation therapy (RT) has proven to be a particularly effective approach for treating cancer [1]. Approximately 50% of all cancer patients undergo antitumor radiotherapy at some point during their treatment, which accounts for about 40% of curative cancer interventions [2]. The primary objective of radiation therapy is to inhibit the ability of cancer cells to multiply, leading to their eventual elimination. To optimize tumor control, it is crucial to deliver the maximum radiation dose while protecting the surrounding healthy tissue from radiation-induced damage. The use of radioprotective agents, which operate via a variety of mechanisms, has been advocated to lessen both acute and delayed radiation toxicities to normal tissues, subsequently decreasing patient morbidity and mortality [3][4][5][6][7]. Radioprotectors here aim to protect normal tissues without markedly affecting tumor cells. As such, they play a significant role in clinical radiotherapy as well as in nuclear medicine practices [8][9][10]. Radioprotective drugs may also serve to protect large populations during wide-spread radiation exposure events, including nuclear power plant accidents, nuclear weapon deployment, radiological terrorism, or astronauts on long-distance space exploration missions. Moreover, these drugs can benefit workers involved in the decontamination of fallout regions or radioactive accident sites [11][12].

In light of this, gaining a thorough understanding of the molecular mechanisms driving the actions of cytoprotective compounds is essential to more effectively control and optimize their biological impacts. This becomes particularly vital considering that transient radiation-induced free radicals are precursors of radiobiological damage in the intricate pathways that ultimately lead to cellular and tissue changes after irradiation. For instance, many potent radioprotectors effectively scavenge water-derived free radicals, thereby reducing their concentration in the medium and consequently safeguarding vital biological molecules like DNA, proteins, and membrane lipids [13][14][15].

2. Radiolysis of Water: Formation of Primary Radical and Molecular Products and Influence of the Quality of the Radiation

Aqueous systems have attracted substantial interest in radiobiology applications. Given that water is by far the most abundant component in cells and tissues (accounting for around 70–80% of their mass), the reactive species produced from its radiolysis play a major role in radiation-induced damage [16][17][18]. In the absence of oxygen, these include the hydrated electron (e^-_{aq}), the hydroxyl radical ($^\bullet OH$), the hydrogen radical ($H^\bullet$), the molecular hydrogen (H_2), the hydrogen peroxide (H_2O_2), the hydronium ion (H_3O^+), the hydroxide ion (OH^-), the oxygen atom O(^1D) and $^\bullet O^\bullet$(^3P) in both its singlet ^1D excited and triplet ^3P ground states, etc. (e.g., see [19][20][21][22][23]). Among these, H_3O^+, $^\bullet OH$, and e^-_{aq} are produced in the highest concentrations [24]. Notably, $^\bullet OH$ is considered to be the primary species responsible for radiation damage to DNA through the indirect effect [16].

In the presence of oxygen, e^-_{aq} and $H^\bullet$ atoms are rapidly converted to superoxide anion/hydroperoxyl ($O_2^{\bullet-}/HO_2^\bullet$) radicals, where $O_2^{\bullet-}$ exists in a pH-dependent equilibrium with its conjugate acid (pK_a = 4.8 at 25 °C) [25]. Under normal

irradiation conditions, where dose-rate effects are absent, individual radiation tracks essentially do not overlap and develop independently over time [26]. In this case, the quality of the radiation (i.e., the type and energy of the radiation used), a measure of which is given by the “linear energy transfer” (or LET, also referred to as “stopping power” by physicists and expressed in keV/μm) [27][28], is then considered the main determinant of the yields (or *G* values) of the various chemical species created and their initial, spatially nonhomogeneous geometrical distributions [29][30]. For low-LET radiation (e.g., Compton electrons produced by ^{60}Co γ-rays, fast (e.g., MeV) electrons, or a few hundred MeV protons with typical LET values of ~0.3 keV/μm), the tracks are initially made up of strings of small, well-separated Magee-type “spurs” (“clusters” of reactive species, of spherical shape) [31][32] that develop in time without interference from the adjacent spurs. Under these conditions, the predominant effect of radiolysis is the generation of radicals. In the case of radiations of high LET, however, the average separation distance between neighboring spurs becomes so small that the string of spurs forms a dense, continuous, and axially homogeneous column (of cylindrical shape) of the species [29][30][33]. This allows more radicals to form in close proximity, promoting radical–radical combination or recombination reactions in the diffusing tracks. It follows that densely ionizing radiation results in the increased production of molecular products or the reformation of water, while reducing the yields of free radicals.

Depending on diffusion, the different radiolytic products may react within the spurs or tracks as they evolve over time, or they might escape and disperse into the bulk of the medium. In water at 25 °C, for low-LET radiations, the spur/track expansion is essentially complete by ~0.2 μs [34]. At this point in time, which marks the transition from nonhomogeneous track kinetics to homogeneous kinetics within the bulk solution, the radiation “track structure” no longer exists. Consequently, species that have escaped from spur/track reactions are now homogeneously distributed throughout the entire system [35][36]. The main reactive species remaining after the dissipation of spurs/tracks include e^-_{aq}, $H^\bullet$, and $^\bullet OH$ (the “radical” products), along with H_2 and H_2O_2 (the “molecular” products). While commonly referred to as “primary” species, this designation is not entirely accurate [26]. Nevertheless, the yields of these species are frequently termed “primary” or “escape” yields, symbolized by $g(e^-_{aq})$, $g(H^\bullet)$, $g(^\bullet OH)$, $g(H_2)$, and $g(H_2O_2)$. (It is worth noting that the lower-case ‘*g*’ denotes these primary yields; experimentally measured or final yields are always represented as $G(X)$ [20]). Once homogeneity is reached, these species become available to react with the dissolved solutes that were present in either low or moderate concentrations during the irradiation process.

3. Employing the Aqueous Ferrous Sulfate (Fricke) Dosimeter as an Indicator of Cystamine’s Radioprotective and Antioxidant Properties in the Context of Irradiations by Fast Carbon Ions in the Energy Range of 6–500 MeV per Nucleon

At the molecular level, chemical (i.e., nonbiological) radioprotectors for low- and high-LET ionizing radiation exert their protective effects in cellular systems through diverse mechanisms. Of particular significance are the suppression of indirect radiation damage through water-derived free-radical scavenging and the repair of direct or indirect damage through $H^\bullet$ atom transfer or donation (e.g., see [4][7][10][16]). In the first mechanism, radioprotector compounds remove or “scavenge” the highly reactive intermediates produced by water radiolysis before they can interact with and damage target biomolecules, especially DNA, thereby mitigating the harmful effects of radiation. In the second mechanism, radioprotectors with sulfhydryl (–SH) groups, known for their labile hydrogen atoms, can also provide cytoprotective action. They do so by donating $H^\bullet$ atoms, chemically repairing both direct and indirect molecular lesions in target macromolecules. This repair occurs after lesion formation but before the damage becomes irreversible due to the addition of O_2 and the subsequent formation of peroxyl radicals, which prevent the regeneration of the original compound. In this latter scenario, sulfhydryl molecules effectively compete with oxygen for interactions with DNA free radicals, thereby minimizing DNA damage and enhancing cell viability [37].

The majority of chemical radioprotective agents that have been developed and tested are aminothiols. Cystamine (RSSR, where R = NH_2–CH_2–CH_2) is the disulfide form of cysteamine (also known as 2-mercaptoethylamine or 2-aminoethanethiol, RSH), a member of the same aminothiol family, renowned for its radioprotective properties [38][39]. Depending on the local redox environment within cells, this disulfide undergoes in vivo reduction, resulting in the formation of two cysteamine molecules following the cleavage of its highly unstable disulfide bond [40][41]. The current understanding of the mechanisms by which cystamine exerts its action in vivo [42][43] suggests that cysteamine is the key intermediate involved in the radiation-protective properties of this compound.

Below pH 8, cystamine is predominantly in the form of a doubly protonated molecule, represented by the symmetric formula NH_3^+–CH_2–CH_2–S–S–CH_2–CH_2–NH_3^+ (with pK_a values of 8.7–9 for both of the –NH_3^+ groups) [44][45]. The mutual Coulomb repulsion between the two positively charged groups at opposite ends of the molecule promotes an open conformation with a high accessibility of the –S–S– center to approaching radicals [44]. This conformational feature is a

significant determinant of this compound's ability as a water-based free-radical scavenger, which explains its strong antioxidant profile. In addition to its role as a radical scavenger in protecting against cellular oxidative stress, cystamine also exhibits antiapoptotic properties. These properties have the potential to delay, halt, or even reverse the progression of neuronal degeneration seen in central nervous system disorders, such as Huntington's disease, Alzheimer's disease, and Parkinson's disease, as demonstrated in animal models [40][41][42][43][46][47]. Cystamine has also demonstrated the ability to reduce brain swelling, cell death, and neurological deficits in rats following intracerebral hemorrhages [48]. Moreover, it has been shown to significantly suppress HIV replication in cultured lymphocytes and macrophages [49]. Nevertheless, the exact molecular mechanisms through which it operates remain unclear.

Several prior studies [13][50][51] have employed the well-known radiolytic oxidation of ferrous (Fe^{2+}) to ferric (Fe^{3+}) ions in the aqueous ferrous sulfate, or Fricke, chemical dosimeter [20][52][53] to evaluate the radical scavenging abilities of cystamine and, consequently, its potential as a radioprotective/antioxidant. Although the Fricke dosimeter was initially designed as a dose-measuring device, it also serves as a valuable tool at the molecular level for investigating the impact of adding any scavenger of the primary chemical species of water radiolysis on the radiolytic ferric ion, or Fricke, yield $G(Fe^{3+})$ (e.g., see [54][55][56][57]). By inference, if a scavenger molecule, such as cystamine, is present in the Fricke solution during irradiation, it will competitively react with the products of the radiolysis of water before they can react with Fe^{2+}, leading to a reduced yield of Fe^{3+} (i.e., there will be protection of Fe^{2+}). The observed reduction in $G(Fe^{3+})$ with cystamine present was further corroborated by Monte Carlo simulations of the radiolysis of Fricke–cystamine solutions, both with and without oxygen [13][14][15].

In earlier research, scientists employed the IONLYS-IRT Monte Carlo track chemistry computer code [58][59][60] to simulate the radiolysis of Fricke–cystamine solutions by using 300 MeV incident protons, mimicking the low LET of cobalt-60 γ-rays or fast electrons [13]. These simulations covered a wide range of cystamine concentrations (10^{-6}–1 M). Subsequently, the research delved into examining the influence of radiation's LET on cystamine's radioprotective ability by adjusting the energy of the irradiating protons from 150 keV to 500 MeV. This adjustment corresponds to LET values that range from approximately 72.3 keV/µm down to 0.23 keV/µm [14]. Finally, researchers explored the effect of dose rate on $G(Fe^{3+})$ by employing a multitrack irradiation model in conjunction with an enhanced version of the IONLYS-RT code [61]. This enabled them to simulate the radiolysis of Fricke–cystamine solutions with single and instantaneous (Dirac) pulses of 300 MeV incident protons [15]. This scenario is pertinent to the "FLASH effect" in radiobiology [62][63][64] or, for example, a nuclear power plant accident [65].

In the field of cancer treatment, especially for deep-seated and traditionally radioresistant local tumors, carbon ion hadrontherapy (currently, there are 10 centers actively treating patients with carbon ions and more under development worldwide) is recognized for its superior tumor control capabilities compared to conventional photon radiotherapy or even proton therapy [66][67][68][69][70]. This superiority is due to its enhanced sparing effects on healthy tissues and greater biological effectiveness, meaning that at a given dose of radiation, it kills tumor cells more efficiently than conventional radiation modalities [4]. These advantageous properties are attributed to the densely ionizing nature of carbon ions in the so-called "Bragg peak" region (e.g., see [71][72][73]), where they deposit most of the therapeutic dose at a specific depth within the tumor volume. Such a characteristic depth–dose distribution of ions makes carbon ion therapy highly effective in treating hypoxic (i.e., radioresistant) tumors [74].

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