The HO• radical: friend or foe?

Abstract Free radicals levels are increased drastically in the human body by environmental stress, disturbing the delicate balance between free radical production and inbuilt antioxidant capabilities, resulting in widespread biological damage. Hydroxyl (HO•) radicals are the most reactive species formed, modifying the cell redox environment resulting in damage to DNA, proteins and lipids. Known for their damaging effects on living organisms, the HO• reactivity can be exploited in the fight that represents cancer treatment for example. This paper reviews the benefits *vs* damages of this species.

Keywords **Free radicals, HO• radicals, oxidation stress, radiotherapy, ionizing radiation.**

Résumé **Les radicaux HO• : ami ou ennemi ?**
Lors d'une exposition à un stress oxydant, la quantité de radicaux dans le corps humain croît considérablement et perturbe ainsi l'équilibre entre production et régulation, engendrant alors divers dommages oxydatifs. Parmi les espèces formées, les radicaux HO• sont les plus réactifs, susceptibles de dégrader autant l'ADN que des protéines et des lipides. La diversité des dommages créés est le reflet de la pluralité des pathologies qui en découlent. Pourtant, bien que souvent connu pour ses propriétés néfastes, HO• peut s'avérer être un allié de taille dans la lutte que représente le traitement du cancer par exemple. Cet article vise à examiner les avantages et les inconvénients que présente ce radical.

Mots-clés **Radicaux libres, radicaux •OH, stress oxydant, radiothérapie, rayonnement ionisant.**

From their controversial proposal of involvement in biological processes, to date, an increasing body of literature exists on the role of free radicals in pathology inducing reactions [1-4]. Free radicals – reactive oxygen species (ROS) and reactive nitrogen species (RNS) – are produced *in vivo* as metabolic by-products and are essential in many biochemical reactions. They can be involved in normal cell regulation in which oxidants and redox status are important in signal transduction, in cellular responses to noxia, in defense against infectious agents and in the induction of mitogenic response [5-6]. However, ROS/RNS are highly reactive and can act as mediators to oxidative damage to biomolecules and cell organelles, affecting their biological activity. ROS that are involved in oxidative damage include superoxide anion ($O_2^{\bullet-}$), hydroperoxyl radical ($HO_2^{\bullet}$), hydroperoxide (H_2O_2) and hydroxyl radical ($HO^{\bullet}$). Although H_2O_2 is not a free radical, it can act as a reservoir for $O_2^{\bullet-}$ and $HO^{\bullet}$. Among these, the $HO^{\bullet}$ is by far the most damaging ROS as it can react with biomolecules at a diffusion-controlled rate, having a half-life of about 10^{-10} s, causing indiscriminant and widespread cellular damages, that can lead to tissue injury and cell death. This latter property is exploited in radiation therapy for cancer treatments, as it can prevent cancerous cell proliferation and tumor development. This article reviews the sources of $HO^{\bullet}$ and their chemistry, exploring the potential benefits *vs* deleterious effects they have in living organisms.

Sources of HO•

$HO^{\bullet}$ are continuously produced in all mammalian systems as by-products of normal cellular metabolism after several monovalent reductions of O_2 (endogenous sources) and as a result of exposure to a wide range of external stimuli (exogenous sources).

Endogenous sources

Even though oxygen is necessary for living aerobic organisms, high concentrations in tissues is detrimental because of formation of oxy radicals *in vivo*. Biomolecules' reactions with O_2 are generally slow, but once initiated, they can form $O_2^{\bullet-}$, involved in several chain reactions catalyzed by the addition of transition metals such as manganese, iron and copper ions (*insert 1*). Many cellular sources produce $O_2^{\bullet-}$, and ultimately $HO^{\bullet}$, NADPH oxidase and mitochondria being the major ones.

Exogenous sources

In addition to the above processes, a variety of environmental agents – ultraviolet light, ionizing radiation, pesticides, carcinogenic metals (chromium, nickel, cobalt, arsenic), industrial solvents, fibrotic mineral dusts, atmospheric oxidants (ozone), tobacco smoke – can stimulate $HO^{\bullet}$ production.
Exposure to ionizing radiation leads to the production of a range of free radicals and non-radical species from ionization of intracellular water. Excited water molecules formed after passage of ionizing radiation will rapidly undergo hemolytic fission to produce $HO^{\bullet}$, while H_2O^+ will react, forming at its turn $HO^{\bullet}$. Hydrated electrons are powerful reductants that in the presence of O_2 can form $O_2^{\bullet-}$, which at their turn form H_2O_2 and $HO^{\bullet}$:

$$H_2O \rightsquigarrow H_2O^+, e^-_{sol}, H_2O^* \quad \text{Eq. 1}$$

$$H_2O^* \rightarrow H^\bullet + HO^\bullet \quad \text{Eq. 2}$$

$$H_2O^+ + H_2O \rightarrow H_3O^+ + HO^\bullet \quad \text{Eq. 3}$$

Upon exposure to non-ionizing radiation such as ultraviolet light (100-400 nm), certain molecules, called photosensitizers[(1)], can be brought into an excited state (*figure 1*). They rapidly convert into an excited triplet state and transfer energy to an adjacent O_2 molecule, while the photosensitizer returns to its ground state. O_2 can either convert to a singlet state, generating type I reactions in the living organisms, or it can produce $HO^{\bullet}$ and $O_2^{\bullet-}$, generating type II reactions that are responsible for damage in photochemical systems. H_2O_2 is for example photoreactive, its exposition to light or laser radiation generating $HO^{\bullet}$; this radical production being proportional to H_2O_2 concentration and irradiation time.

Insert 1:

HO• production via Haber-Weiss and Fenton reaction

In immunological responses and cell signaling, a key role is played by the activation of NADPH (nicotinamide adenine dinucleotide phosphate) oxidase, generating $O_2^{\bullet-}$, which through dismutation leads to hydrogen peroxide formation:

$$NADPH + H^+ + 2O_2 \rightarrow NADP^+ + 2H^+ + 2O_2^{\bullet-} \quad \text{Eq. 9}$$

$$2O_2^{\bullet-} + 2H^+ \rightarrow H_2O_2 + O_2 \quad \text{Eq. 10}$$

This radical is rather unreactive directly, but through the Haber-Weiss reaction forms HO• (Eq. 3) [27-29].

$$O_2^{\bullet-} + H_2O_2 \rightarrow HO^\bullet + OH^- + O_2 \quad \text{Eq. 11}$$

Redox-active iron ions found in various proteins, can act as catalyst for HO• production via Fenton chemistry [30]:

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + HO^\bullet + OH^- \quad \text{Eq. 12}$$

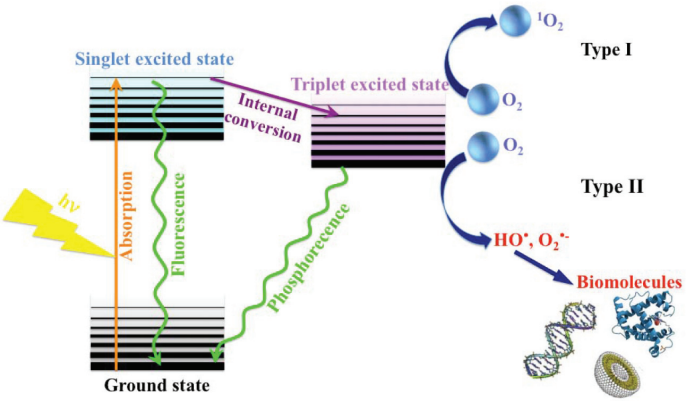

Figure 1 - HO• production by photosensitization.

Chemical behavior of HO•

HO• are the most reactive oxygen radicals known, with a standard reduction potential of $E_{HO^\bullet/H2O} = 2.31$ V *vs* NHE [7], therefore capable of oxidizing nearly all targets with reaction rate constants near the diffusion limit (10^9-10^{10} M^{-1} s^{-1}). Some second order reaction rate constants for HO• reactions are listed in *table I*.

In vivo, the diffusion distance of HO• was estimated to be ~ 3 nm [10], about the average diameter of a protein. Therefore, HO• radicals react only at their generation site, making most HO• damage local environment-specific [2, 11].

HO• ionizes at very alkaline, biologically irrelevant pH values:

$$HO^\bullet \rightarrow O^- + H^+ \qquad pKa \simeq 12 \quad [12] \qquad \text{Eq. 4}$$

Recombination of HO• is very rapid and can occur in ionization clusters (spurs) during pure water radiolysis, but *in vivo,* it is in competition with reactions with cellular targets (*table I*):

$$HO^\bullet + HO^\bullet \rightarrow H_2O_2 \qquad k = 5 \times 10^9\ M^{-1} \cdot s^{-1} \qquad \text{Eq. 5}$$

There are three main types of reactions, that HO• can undergo: hydrogen abstraction, addition and electron transfer. These reactions are usually initiators of oxidations in proteins and lipids or DNA bases, leading to degradation of these compounds.

Hydrogen atom abstraction from a C-H bond by HO•:

$$HO^\bullet + R - CH \rightarrow R - C^\bullet + H_2O \quad \text{Eq. 6}$$

Alpha-H abstraction reactions by HO• from any amino acids to form an alkyl radical derivative are one mechanisms of protein oxidation. H abstraction reactions can also occur on N-H and S-H bonds leading to reactive N-or S-centered free radicals.

HO• addition onto another molecule:

$$HO^\bullet + X \rightarrow (HO - X)^\bullet \quad \text{Eq. 7}$$

Addition reactions are faster than H abstraction reactions due to more favorable transition state energies, making addition reactions predominant over C-H or S-H abstraction reactions [9]. HO• reacts rapidly with C-C and C-N double bonds or aromatic cycles, but these reactions are regioselective due to the electrophilic nature of HO• [13].

Electron transfer (ET) reactions with HO•:

$$HO^\bullet + X^- \rightarrow X^\bullet + OH^- \quad \text{Eq. 8}$$

Generally, addition and ET reactions are in competition, and ET mechanism dominates if the favored positions for addition are blocked by bulky substituents. ET reactions often proceed by addition-elimination mechanisms and are observed mostly on heavier atoms like sulfur or phosphorus and proceed usually via complex two atom-three electron bonded intermediate formation.

HO•-induced damage to DNA

Considering its redox potential and its reaction rates with biological substrates, HO• is thermodynamically and kinetically the strongest oxidizer produced *in vivo*, inducing non-selective molecular oxidations. Given the plurality of a cell composition, it is not difficult to imagine the diversity of oxidized products induced by HO• [1] (*figure 2*). For many years, the scientific community was convinced that DNA was the hydroxyl radical target to investigate because it carries the genetic material and the consequences of its oxidation might be dramatic. Indeed, all DNA components can be damaged: the pyrimidine base, the deoxyribose or the phosphodiester [14]. As a consequence, it induces various degrees of degradations: single or double strand breaks, formation of abasic sites, inter-linking between strands… [15]. For example, hydrogen abstraction on deoxyribose leads to single strand break and formation of aldehydes [14]. Oxidation of the four bases – guanine, thymine, adenine, cytosine – is mainly initiated by HO• addition on double bonds. One of the most known DNA damage is guanine oxidation (*insert 2*) since its redox potential is the lowest of the four DNA bases. The consequences of double strand breaks, even if their occurrence probability is lower than other oxidations, are significantly more important as they are seldom repaired.

HO•-induced damage to membranes

Membrane oxidation also appeared as a crucial point considering their abundance as they separate all organelles

Table I - Reaction rate values of HO• with various biomolecules.
*Reaction rates values taken from [8-9].

Compound	Rate constant ($M^{-1}.s^{-1}$)*	Compound	Rate constant ($M^{-1}.s^{-1}$)*
Catalase	1.4×10^{11}	Casein	3.6×10^9
Albumin	8.0×10^{10}	Phenylalanine	3.5×10^9
Haemoglobin	3.6×10^{10}	Deoxyribose	3.1×10^9
Ribonuclease	1.9×10^{10}	Thymine	3.1×10^9
Guanine	1.0×10^{10}	Uracyl	3.1×10^9
Gluthatione	8.8×10^9	Adenine	3.0×10^9
Glutamic acid	7.9×10^9	Adenosine	2.5×10^9
Cysteine	7.9×10^9	DNA	8.0×10^8
Methionine	5.1×10^9	RNA	8.0×10^8

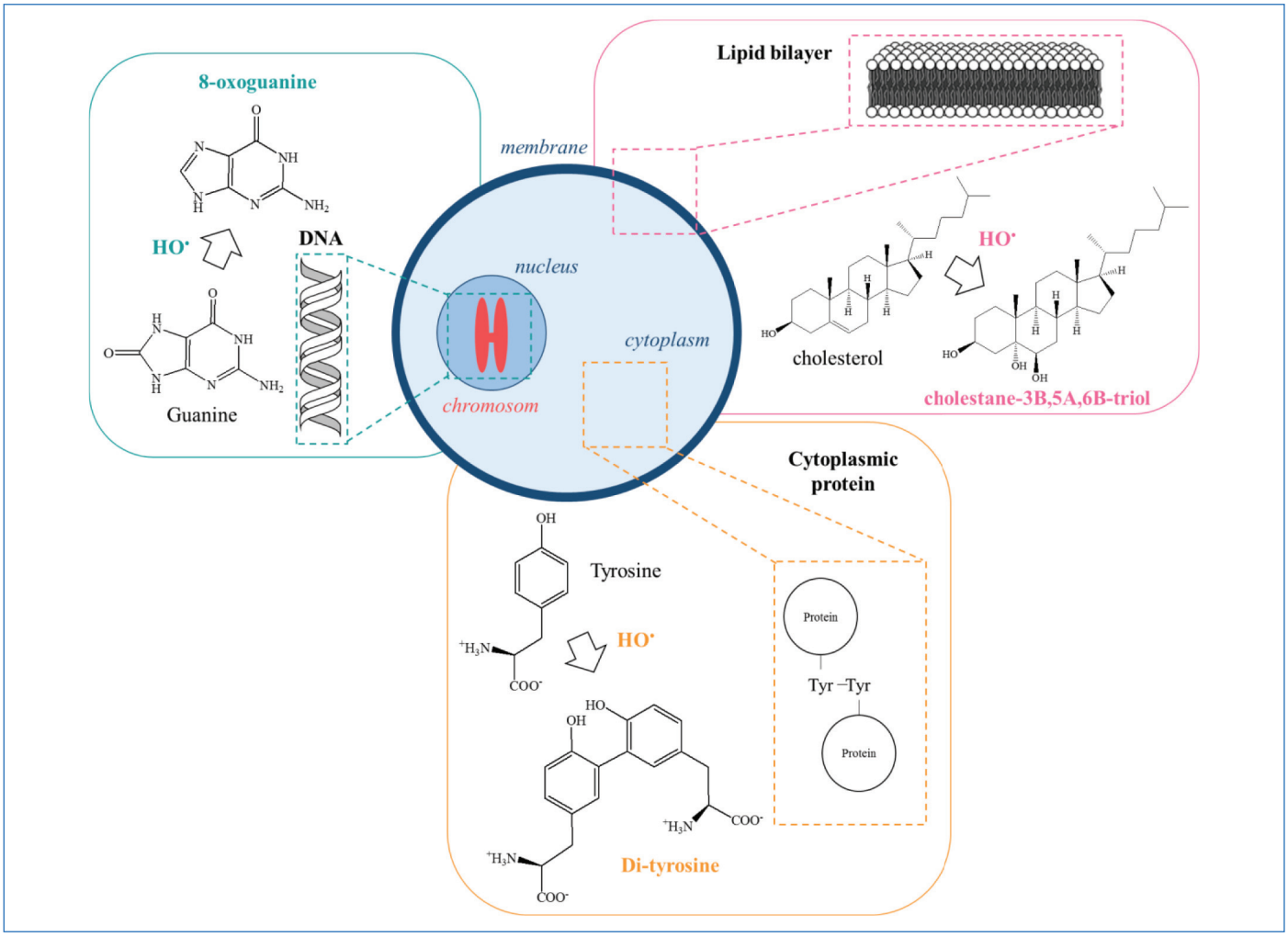

Figure 2 - Schematic drawing of some possible HO•-induced cellular damages.

Insert 2: 8-oxoguanine

After guanine oxidation by HO•, 8-oxoguanine is among the major products formed [31]. Normally, to form DNA double helix, guanine pairs with cytosine. Their binding is mediated by three hydrogen bonds. When guanine is oxidized, one of these bonds is no longer feasible. Then, instead of pairing with cytosine, 8-oxoguanine will pair with adenine leading to DNA mutations G-C → T-A which are the most common one found in human cancers. 8-oxoguanine is widely used as a biomarker of DNA oxidation and oxidative stress [32] (*figure 2*).

Insert 3: Oxysterols

Cholesterol is part of the cellular lipids: responsible for plasma membranes rigidity, it is important to consider its HO• oxidation pattern mainly composed of oxysterols. It was proved that oxysterols formation drastically modifies lipid bilayers permeability [17] and that patients suffering from atherosclerosis have higher oxysterols plasma level than healthy ones [33]. More specifically, cholestane-3A,5B,6A-triol was identified as a biomarker of Niemann-Pick C type 2 disease in which non-esterified cholesterol accumulates [33] (*figure 2*).

from the surrounding medium and as they also ensure information's transport. Membranes are mainly constituted of phospholipids, cholesterol and fatty acids made of non-conjugated double bonds making them sensitive to HO•. Their oxidation process, which is a three-step reaction with one being catalytic, is called lipid peroxidation: it starts with a hydrogen abstraction on the methylene group (initiation) which leads to peroxyl and hydroperoxyl radicals after oxygen addition.

These radicals are known to be very reactive species and can themselves react on lipids; the initial lipid radical formations are thus amplified (propagation). Most of the time, the final step (termination) is stable carbonyl formation [1, 3]. Isoprostans [16], oxysterols [17] (*insert 3*) and lipid peroxides may have dramatic consequences as the latter are involved in atherosclerosis expansion, hence they can also be used as biomarkers.

HO•-induced damage to proteins

Among cell components, proteins are the most abundant ones as they represent 70% of cellular dry mass [18]. HO• reaction rates with proteins are about ten times higher than those with DNA, making them the preferred targets of these radicals. HO• are able to attack each of the twenty amino acids both on peptidic and side chains [19]. Nevertheless, aromatic and sulfur amino acids are among the most sensitive residues. Like in DNA and lipids, HO• is capable of abstracting an H atom, leading to a C-centered protein radical, or adding on double bonds. The fastest reactions occur on sulfur residues leading to sulfur-centered radicals (*table 1*). In turn these radicals can propagate oxidation chain reactions. Therefore, proteins can be fragmented, oxidized at a local level (oxidation of a single residue) or at a larger scale. Then, protein structure can potentially be disrupted leading to a dysfunction of the protein or even to a total inactivation (*insert 4*) [18].

Insert 4: Di-tyrosine

Phenylalanine can be converted in tyrosine by addition of $HO^{\bullet}$ on the aromatic cycle and the latter can be oxidized again to form di-tyrosine [34] and oligomers of higher order. Formation of di-tyrosine in proteins can lead to protein dysfunction [35] and/or aggregation, as it is the case for amyloid peptide responsible for Alzheimer disease. Here also, di-tyrosine became one of the most used biomarkers of proteins oxidation and so oxidative stress and aging [36] (*figure 2*).

As proteins are not only cytoplasmic and can be found everywhere in the cell including at the membrane or close to DNA, some lipid-protein or DNA-protein crosslinks are generated, inducing the possible blockage of DNA replication for example [20].

All these oxidative damages are induced in every cell at any time and many repair systems exist *in vivo*, especially for DNA (six different pathways) and proteins (methionine sulfoxide reductase) degradations. However, for proteins, the most efficient repair is elimination followed by synthesis *de novo*. When the repair is not effective, cells commit "suicide" such as apoptosis or autophagy. Otherwise, damages can persist inducing long-term pathologies. Even though $HO^{\bullet}$ is not expected to be selective considering its very high redox potential, as mentioned, $HO^{\bullet}$-attack is local environment-specific, and as a consequence low accessibility could preserve target oxidation. Depending on the protein 3D structure, most accessible amino acids can be oxidized preferentially [21], except if intramolecular electron transfer modifies the location of the damage. In DNA-protein complexes for example, it is not absurd to imagine that some proteins "sacrifice" themselves to preserve DNA integrity.

$HO^{\bullet}$ reactivity's benefits

Hydroxyl radicals being responsible for cell death, different applications were developed taking advantage of their cytotoxicity: disinfection and therapy which goals are respectively to kill invading microorganisms or cancer cells. Considering the importance of clean water in the world, disinfection with radical formation in order to induce cell or pathogen death, was applied to water and wastewater treatments. The first method to kill pathogens used hydroxyl radical production through the Fenton reaction. Nevertheless, in order to limit chemicals in water, treatments now include the photochemical reaction of hydrogen peroxide with UV light and eventually a combination with ozone. H_2O_2 is photoreactive in the range of 185 to 400 nm [22]. This type of treatment is also well-adapted to water transportation where the risk is to introduce non-native species which could, at long term, disturb local environment and biodiversity [23]. This type of disinfection necessitates specific equipment, as hundreds of m^3 of water need to be treated in an hour. To a much lower extend, LED light can be used to decompose H_2O_2 in order to prevent oral infectious diseases by forming $HO^{\bullet}$ to kill pathogenic oral bacteria. The Food and Drug Administration guarantied the safety of a concentration of 3% of H_2O_2 for medical treatment of dental plaque [24]. Though disinfection exploiting the oxidizing properties of hydroxyl radical is quite recent, radiotherapy, which is based on the same capacity of $HO^{\bullet}$ to kill cells is much older. It was even developed though radio-induced radical species were not yet known.

In 1896, Emil Grubbe in Chicago, Victor Despeignes in Lyon and Leopold Freund in Vienna experienced the treatment of cancer with X-ray radiation. Few years later, Thor Stenbeck and Antoine Beclère confirmed that ionizing radiation could cure some cancers. Though some side effects were evidenced both on patients and medical staff, radiotherapy became one of the main treatments for cancer in 1920. Radiotherapy is based upon the interaction of ionizing radiations with water, the main constituent of the body, leading to the formation of $HO^{\bullet}$ radicals, among others.

Different types of therapies exist: internal therapy named Curie-therapy or brachytherapy and external therapy. For brachytherapy, sealed sources are placed inside the body near or in the tumor. This type of implant can be temporary or permanent and is made of radionuclides as ^{131}Cs, ^{60}Co, ^{125}I or ^{192}Ir emitting various types of radiation. The emitted radiations have different energies and different penetration powers in the tissues. Therefore, $HO^{\bullet}$ are produced at shorter or longer distances from their source. For external sources, many different types of equipment exist, as cancer therapy policy is country-dependent. X-rays used for decades are photon beam radiation therapy but have a very low penetration (few mm for energy of hundreds of keV), so they are mainly dedicated to skin tumors. For more deeply embedded tumors, more penetrating beams are required, as cobalt sources for example. But one should keep in mind that these types of radiations generate $HO^{\bullet}$ all along their track, so both cancer cells and normal tissues are destroyed inducing radiotherapy side-effects (*figure 3*). To get around that problem, new technological developments were designed. First, the type of beam can be changed. For example, Japan therapy is only performed with protons or heavy ions as carbon beams. The advantage of such beams if that their energy deposition is not all along the track but much more localized which means that $HO^{\bullet}$ will be generated in a more localized area (at the end of track) than with other beams (*figure 3*). Mathematical calculations can allow definition of parameters to focus the energy deposition in the tumor and therefore more precisely generate radicals. Another approach developed to spare normal tissues is three-dimensional conformal radiation therapy. To focus the irradiation dose only to the tumor, photon beams are delivered from different directions in order to match the tumor shape. Here also, high quantities of $HO^{\bullet}$ are produced in the tumor and only a few in normal tissues.

$HO^{\bullet}$, phobia or love affair?

$HO^{\bullet}$ are constantly being produced in our bodies and play a significant role in the regulation, induction and maintenance of a number of biological functions. However, if excessive amounts of $HO^{\bullet}$ are produced, they can induce a wide range of cellular damages, leading to cell death via apoptosis, necrosis and autophagy. Part of the damage can be repaired, and the first line of defense against $HO^{\bullet}$ damage is antioxidants. Antioxidants generally operate at different levels: prevention of $HO^{\bullet}$ formation or interception by scavenging reactive species and are classified as enzymatic antioxidants (superoxide dismutases, glutathion peroxidases), or dietary components (vitamin C, E, Mn^{2+}). Antioxidants are used to prevent damage to organs being prepared for transplantation, being under research as an adjuvant therapy (vitamin C, E) in the treatment of various conditions. However, when consumed

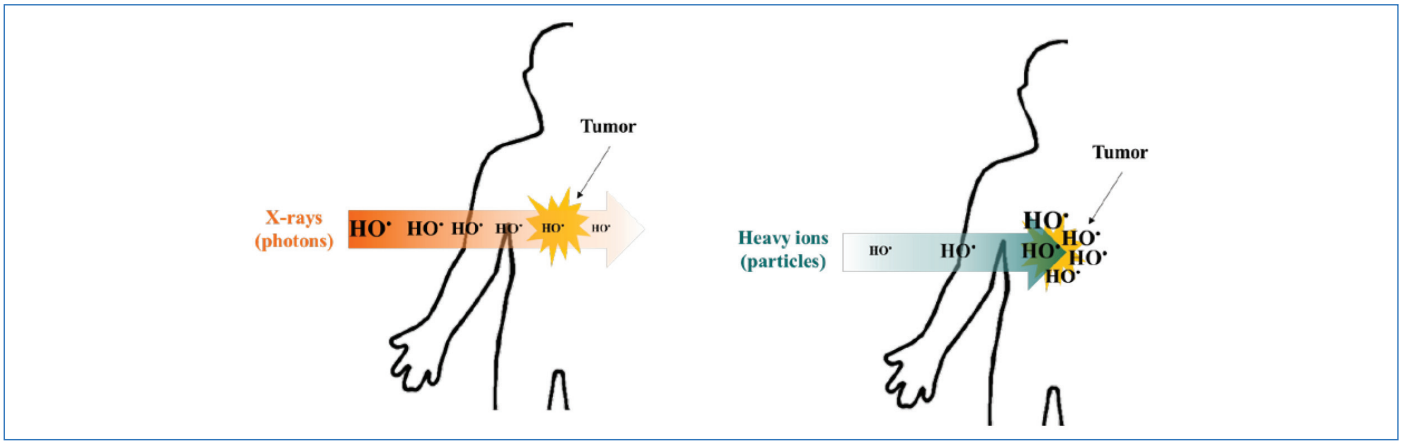

Figure 3 - Schematic representation of HO• localization in different beams used for therapy.

in large doses, these antioxidants can act as pro-oxidants [25-26]. Vitamin C for example can reduce transition metal ions, triggering the Fenton reaction, resulting in oxidative damage. Some polyphenols are believed to provide protection against cardiovascular diseases and cancer, but dietary polyphenolic supplements consumption during pregnancy was linked to infant leukemia. When HO• radicals *in vivo* are not scavenged, molecular oxidations occur and specific repair mechanisms of damaged molecules are triggered (sulfoxide reductase – DNA repairs). When these damages are too important, cellular death or pathologies are generated. Some benefits can be however derived from this highly reactive species in radiotherapy or industrial applications. Therefore, a more comprehensive and mechanistic understanding of the processes involving HO• is imposed.

Note and references

(1) *Photosensitizers*: agents that absorb visible-UV radiation, generating ROS, leading to inactivation of proteins or DNA damage or light-induces cell death.

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