

Biological systems: from water radiolysis to carbon ion radiotherapy

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Abstract. Hadron therapy is an innovative cancer treatment method based on the acceleration of light ions at high energy. In addition to their interesting profile of dose deposition, which ensures accurate targeting of localized tumors, carbon ions offer biological properties that lead to an efficient treatment for radio- and chemo-resistant tumors and to provide a boost for tumors in hypoxia. This paper is a short review of the progress in theoretical, experimental, fundamental and applied research, aiming at understanding the origin of the biological benefits of light ions better. As a limit of such a vast and multidisciplinary domain, this review adopts the point of view of the physicists, leaning on results obtained in connection with CIMAP's IRRABAT platform.

1. Introduction

Interaction of fast ions with biological systems constitutes one aspect of the interdisciplinary researches performed with ion-beam facilities. This domain is as rich as it is complex since it encompasses several orders of magnitude in both space and time. The shortest space and time scale corresponds to atomic collisions, which may be as short as 10^{-18} s for the interaction of fast ions with individual atoms. At the opposite end of this domain, late effects – like cancer induction, chromosomal instability or organ dysfunctions – may appear or remain several years after irradiations. While irradiations may be limited to a very localized region, the whole behavior of an organ may be affected, possibly leading to human death, in particular when the irradiation dose and spatial extension are high. Between these two extreme scales, stands a great number of mechanisms, including for instance: the transport of the primary ejected electrons, the relaxation of the ionized and excited molecules, which may lead to direct damage in biological targets and to radical species and associated biochemical reactions. These early physical and chemical stages are followed by numerous and complex cell responses, such as the triggering of mechanisms to check DNA, to repair its damage, to manage the oxidative stress or to induce cell death. The numerous biological endpoints that have been studied reveal the complexity and the diversity of this biological response. These endpoints may involve particular structures of cells at the molecular scale (tracking of protein activities, damage in DNA, protein or lipid) or at the sub-cellular scale (chromosomes, nucleus, membranes, mitochondria...) and may concern cell organization (3D cell culture, tissues, organs, body). The domain of low doses



(\ll Gy) and low dose rates (e.g. lower than cGy/hour) is mainly explored in order to estimate the risks of ionizing radiations for health, while the domain of the so-called high doses ($>$ Gy) and high-dose rates (Gy/minute), is often addressed in the context of cancer treatment by external radiotherapy. The title of the present paper therefore refers to an extremely large topic, actually too vast to be summarized in one paper. Our contribution aims at providing, as illustrations of this promising research field, some key examples of scientific progress, mainly leaning on results obtained in connection with CIMAP's IRRABAT platform or made within an IPNL-GANIL collaboration, focusing in particular on the effects of high Linear Energy transfer (LET) in the context of research for hadrontherapy with light ions. Moreover, this contribution is presented with a physicist's point of view in mind.

2. Hadrontherapy with carbon ions

2.1. *A brief history of hadrontherapy*

The history of hadrontherapy goes back to the beginning of radiotherapy, and therefore, to the discovery of X-rays by Wilhem Conrad Röntgen in 1895. It may sound quite amazing today that, just one year after this discovery, a first clinical trial of treatment by X-rays was attempted in France (Lyon) by Victor Despeignes. At the opposite, many more years separated the invention of the cyclotron, by Ernest Lawrence in 1930, and the proposition in 1946 by Robert Wilson [1] to treat patients with fast ions. The idea was later applied in the 1950's with a proton beam at the Lawrence Berkeley Laboratory (LBL) and followed by other experiments e.g. in 1957 at the University of Uppsala, in 1961 at the MGH (Massachusetts General Hospital). These led to a first hospital-based proton facility at the Loma Linda University Medical Center. The first treatment trials with fast heavy ions were undertaken at Berkeley in the 1970's, but the first clinical center dedicated to cancer treatment with carbon ions was built at Chiba, Japan, (HIMAC Center [NIRS]) in 1994. Presently, eight centers for carbon therapy have been built in countries like Japan (1994 Chiba (HIMAC) - 2001 Hyogo (HIBMC) - 2010 Gunma University (GHMC)), Germany (1997 Pilot project in Darmstadt (GSI) - 2009 Heidelberg (HIT) - Marburg (CPT)), Italy (2013 Pavia (CNAO)), Austria (2013 MedAustron (first patient in 2015)), China (Langzhou (IMP)). Eleven new centers are in project.

2.2. *Physical properties of carbon beams*

As for protons, the so-called inverted dose-depth profile with the maximum dose deposition at the end of the ion path (Bragg peak), is a key feature of carbon ions. With respect to proton beam, the lateral straggling is even lower, offering more accuracy. While the nuclear fragmentation of carbon ions leads to a larger dose beyond the position of the Bragg peak in comparison to protons, this dose is drastically lower than that deposited by the high-energy photons used in radiotherapy. Moreover, fragmentation offers many opportunities to control the irradiation of patients, on-line or off-line: it is probable that the detection of prompt γ -rays [2, 3], secondary protons [4, 5] or the 511 keV γ -rays produced by positron emitters should increase the safety of treatments [6] in the future.

2.3. *Biological interest of carbon ions*

However, the major interest of carbon ions resides in the specific biological effect induced by carbon ions with respect to protons or any form of conventional radiotherapy. This effect is twofold and characterized by two indicators, the *Relative Biological Efficiency* (RBE) and the *Oxygen Enhancement Ratio* (OER). These coefficients will be described in the next sections, focusing in particular on the cell survival endpoint, since this quantity is strongly related to the local control of tumors. The dose dependency of radiation-induced biological effects is a complex phenomenon. At low dose, some effects, like hypersensitivity and enhanced radioresistance, have been observed and are still not clearly understood. When plotted in logarithmic scale, cell

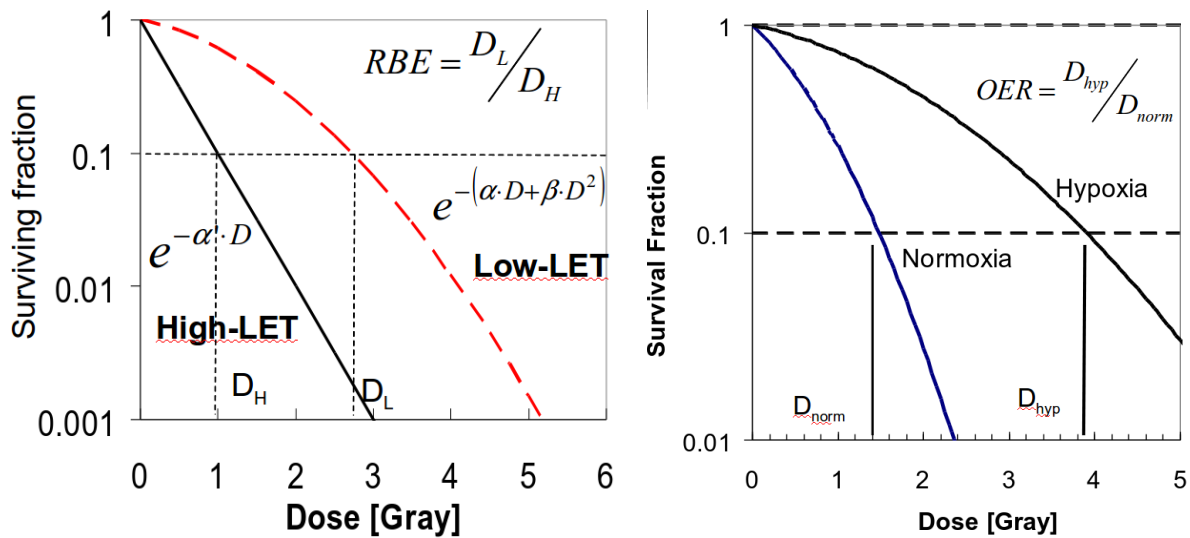


Figure 1. Illustration of cell survival curves. (Left) Effect of LET and definition of RBE; (Right) Effect of oxygenation and definition of OER.

survival at therapeutic doses shows linear dependency and a shoulder (figure 1). Practically, these curves for cell survival may be described by a linear quadratic model according to the following equation:

$$S(D) = \exp(-\alpha D + \beta D^2) \quad (1)$$

where α and β are parameters, which are fitted using experimental data.

Compared to high-energy photons, high-LET ions have a twofold impact: firstly, cell killing may be more efficient, leading to larger values for α ; secondly, β may vanish, leading to the disappearance of the shoulder on the curves. The *Relative Biological Efficiency* (RBE) is defined to help qualify the efficiency of a particular ionizing radiation in comparison to a reference one, typically high-energy photons: it corresponds to the ratio of the dose required with the reference radiation over the dose required with the studied irradiating particle, to induce a given endpoint. This coefficient is a quite complex function of many parameters, some biological (like the biological endpoint itself, the cell line and its environment), some physical. The latter are for instance the dose and the dose-rate, which, in the context of therapy, refer to the duration of each treatment session and the number of sessions (fraction) that are necessary to deliver the whole dose prescription. The dose-rate effects imply the dynamical response of cells to irradiations (e.g. DNA repair), and tissue recovery. The dependency of the RBE with respect to the type of ionizing particle cannot be reduced to the LET. A particle may induce less cell killing than another with lower LET (see for instance the results of our experiments at the GANIL/Irrabat facility [7]). Hence, the type of particle must be specified. For a fixed type of ions, the dependency of RBE with LET is typically the following: at low LET values, the RBE is close to unity, then it increases to a maximum and then decreases down to zero. This latter decrease is referred to as the “over-killing effect”. Considering all types of ions, the maximal value of RBE is the highest with protons. However, this value corresponds to low-energy protons (of the order of 1 MeV), i.e. ions for which the range is too low to kill more than one cell. In therapy, the number of ions is quite low compared to the number of cells to kill. Therefore, the RBE must be high for ions fast enough to reach many cells in the tumor volume, but low in the

healthy tissues. Carbon ions appear to be an interesting compromise. As an illustration, let us consider a volume of 120 cm^3 made of spherical cells each with radius $10 \text{ }\mu\text{m}$. The number of cells is therefore 6^{10} . An irradiation of this volume using carbon ions with 1 Gray requires an average of 7^8 incident carbon ions [8]. If this irradiation induces death in 50% of the cells present in this volume, this means that each carbon ion kills an average number of 50 cells. Even in the extreme scenario where one ion impact alone is sufficient to kill a cell, within this configuration, such “efficient” ions should range 1 *mm*, corresponding to the 18 MeV/n carbon-ion range in water. This rough estimation shows that the local control of tumors cannot be attributed to the highest-LET carbon ions, but relies on a compromise between a high value of RBE and the capacity for ions to cross many cells. It also explains why the average RBE in tumors irradiated with protons is close to 1, despite the large RBE value for MeV protons.

The Oxygen Enhancement Ratio (OER) refers to the oxygen effect. When irradiating cells at various levels of oxygenation, it has been observed that radiosensitivity increases with oxygenation up to a saturation for a partial pressure of oxygen lower than normoxia (normal oxygenation of tissue). In the same way as for RBE, the OER coefficient is defined for a given level of biological endpoint as the ratio of the dose required in hypoxia over the dose required with the tested level of oxygenation to induce this particular level of endpoint. The level of oxygenation in the cells of some tumors may be much lower than the one observed in normal conditions. The tumors in hypoxia become radioresistant thereafter. Such tumors are potentially indications for a boost with carbon ions, since the oxygen effect has been observed to decrease when the LET increases. In particular, close to the Bragg peak, the OER is as low as one.

3. Origin of RBE larger than 1

The origin of the biological efficiency of ions is still an open question. Somewhat arbitrarily, the basic scenarios have been divided into three classes.

3.1. Geometrical interpretation and microdosimetry

Historically, the first class refers to microdosimetry models and may be explained using the structure of ion-tracks. In the original framework of these models, the lethal lesions are induced by the interaction of sub-lethal lesions, with a typical distance of interaction in the order of one micrometer. As represented in figure 2a), the particularity of ion tracks leads to a spatial correlation in the distribution of sub-lethal events. In particular, when the ion LET increases, the distance between sub-lethal lesions decreases and therefore the probability for the occurrence of lethal lesions increases. In practice, the predictions are based on microdosimetry calculations. Presently, the most refined version of microdosimetry models, the Microdosimetry Kinetic Model (MKM) [9], is used for clinical treatment in Japan, and several research teams are extending the validity of MKM to carbon therapy [10, 11]. The targets that are considered in microdosimetry are not clearly defined. The creation of sub-lethal lesions by damaging DNA is nonetheless mentioned. Considering the typical distance of efficient sub-lethal interactions, those implied in such models could involve fragments of DNA or even chromosomes. Two arguments at least support microdosimetry models. First, it has been shown that high-LET ions could induce complex chromosomal rearrangements [12]. Second, calculations based on Monte Carlo simulations [13] have shown that high-LET ions generate smaller DNA fragments than low-LET ones do.

3.2. Local dose deposition and track-structure models

In this class of models, the high capacity of ions to deposit very high local dose is at the origin of their biological efficiency. Using the concept of radial dose to estimate the local dose is a common feature in models of this class. Historically, this idea was supported by Katz *et al.* in proposing the γ -kill and ion-kill model [14]. The Local Effect Model (LEM) was then proposed by Scholz *et*

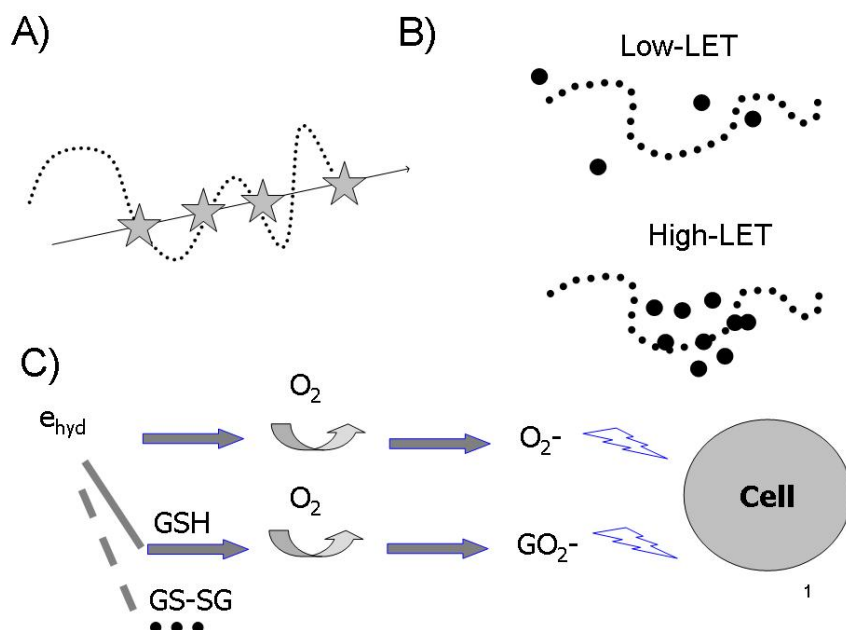


Figure 2. Illustration of the mechanisms based on a) the spatial correlation of sub-lethal lesions (stars) in DNA (dotted line); b) concentrations of radiation events (full circles) around DNA; c) direct or indirect interactions of hydrated electrons (or hydrogen radicals) with dioxygen molecules producing chemical species that are toxic for cells (illustrations of equation 3a and 3b).

al. [15]. In Katz's model, the target size is clearly defined to be of the order of the micrometer. In the LEM, the lethal events are considered as point-like, referring nevertheless to complex DNA damages (see figure 2b)). Both models are capable of predicting the main tendency of RBE with an acceptable accuracy. Both can also predict hook structures in the curves of inactivation cross section plotted as a function of ion LET. However, as pointed by Beuve *et al.* [16], the use of the radial dose concept in radiobiology has strong limitations regarding the stochastic nature of radiations, in particular for small targets. This raises questions in particular on the basic concepts of the LEM, as the target size is extremely small [17]. This conceptual problem led us to propose a new framework referred to as the Nanox model.

3.3. Core ionization

Alternatively, Chetioui *et al.* [18] pointed out a correlation between the cross-section for cell inactivation and the cross-section for core ionization when plotted as a function of LET (see figure 3). In particular, both show a section of increase with LET, followed by the hook structure. This idea was supported by an estimation of the efficiency for these core ionizations to induce lethal events, from a comparison of the cell killing induced by soft X-rays with photon energy below and above the binding energy of core electrons [19]. This efficiency, defined as the ratio of lethal-event number to the core ionization number, was estimated to 3-4 % [20]. Injecting this estimation into a Monte Carlo simulation led the authors to conclude that core ionization would represent 75 % of the lethal events produced by high-energy photons.

At this stage, this framework known as the model K, was not designed to allow cell survival prediction, but instead to allow discussions about the dominant mechanisms. Still, we propose

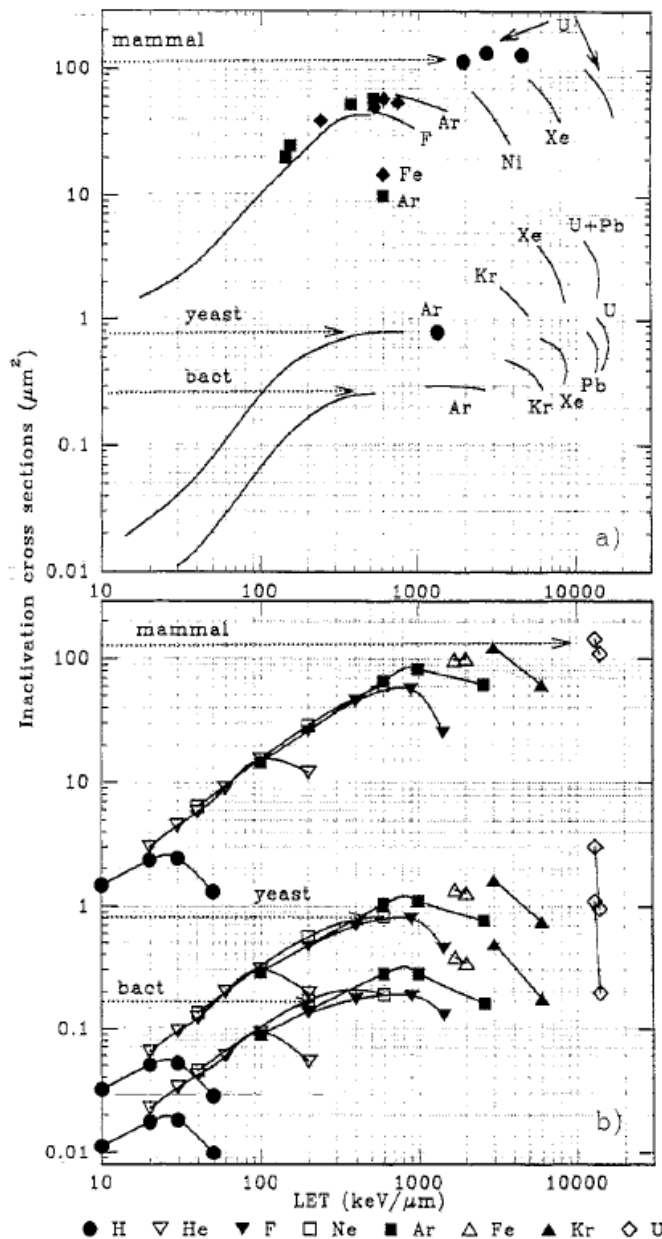


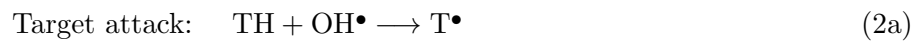
Figure 3. a) Experimental cross section for cell inactivation by heavy ions of various LET: mammal cells, yeast, bacillus subtilis; arrows indicate the geometrical cross section of the cell nucleus or of the DNA concentration. b) Calculated cross sections for the induction of at least one efficient K-vacancy in cell nucleus (from [21]).

to discuss about the application of the model K for hadrontherapy. Doing this raises at least two difficulties. The first one lies in the dose dependency of the lethal-event. Core ionization events are definitely localized events (atomic scale). These events, designated as lethal events in the model K, are therefore local lethal events. For such a class of events, it is possible to show that cell survival is a purely decreasing exponential (see for instance [17]). On the other hand, the measurements of cell survival show at least a linear quadratic dependency (as shown by the presence of a shoulder). This indicates that, in the framework of the model K, the efficiency ϵ to induce lethal events cannot be constant but should be a dose-dependent function $\epsilon(D)$. A second difficulty appears when comparing the efficiency of fast protons and carbon ions to induce lethal events and core ionizations. At a high but “fixed” ion velocity, both the LET and the core-ionization cross-section scale with the square of ion charge. Therefore, at a given fluence, the number of core ionizations on the one hand and the dose, on the other hand, also scale with

the ion charge square. Thus, the number of core ionizations per unit of dose is the same for protons and carbon ions. In the context of the model K, one expects the number of lethal events per unit of dose to be similar for protons and carbon ions (with equal velocities). However, the experimental value of this ratio, which corresponds to the α parameter in the linear quadratic model of cell survival, may differ by a factor as high as 5 or more when comparing these two ion species. This indicates either that core ionization is not the main source of lethal events alone, or that the efficiency defined in the model K is likely to be a complex function of the dose, the LET and the type of ions. Knowledge of this function would be necessary to apply the model K to hadrontherapy. When comparing the model K prediction to the effective cross-section based on the slope of cell survival at high doses (and not at low doses), the discrepancy is reduced to a factor 2. This remaining factor is interpreted by the authors of model K in two ways. Firstly, by the multi-ionisation implying several core levels, which increase with the charge of incident ions and would induce more severe damage in DNA. Secondly, by events, referred to *K events*, that combine the high density of ionizations generated by the ions and the core ionizations, including Auger electrons.

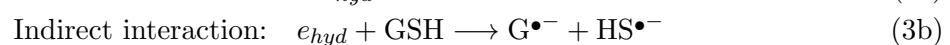
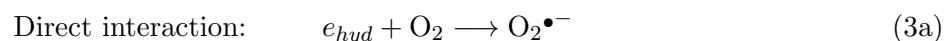
4. Origins of the OER reduction

The reduction of OER to 1 at high LET is all the more under question since the origin of the radiosensitive effect of oxygen is still unknown. One scenario is based on the capacity of oxygen molecules to fix damage before their restoration by an antioxidant. The following reaction scheme (T standing for “Target”) illustrates this scenario with hydroxyl radical attack ($\text{OH}\bullet$) and glutathione antioxidant (GSH):



In hypoxia, the fixation of damage would be inefficient and the damage capacity of radiation less important. As initially suggested by Neary [22], ions could produce oxygen molecules in their tracks, thus compensating the lack of oxygen in hypoxia. This production of oxygen has been observed and simulated [23, 24, 25, 26, 27, 28]. It is initiated by the multi-ionization of water molecules [28] and the reaction of the oxygen atoms issued with the chemical radicals of the track. However, as illustrated on figure 4, the production of oxygen in the track seems very low compared to the concentration of oxygen at which the oxygen effect saturates. This saturation appears at a partial oxygen pressure ranging from 3 up to 30 mmHg, while the normal partial oxygen pressure (normoxy) is 160 mmHg.

Alternatively, the production of superoxide anion ($\text{O}_2^{\bullet-}$ and its proton form $\text{HO}_2\bullet$) is suspected to be implied in the origin of the oxygen effect. This superoxide anion is produced by the following reactions:



This scenario is supported by chemical arguments based on the Fenton reaction and by biological evidence [30, 31, 32, 33, 34], in particular in relation with superoxide dismutase enzymes. Moreover, simulations of radical production by irradiation of water with various

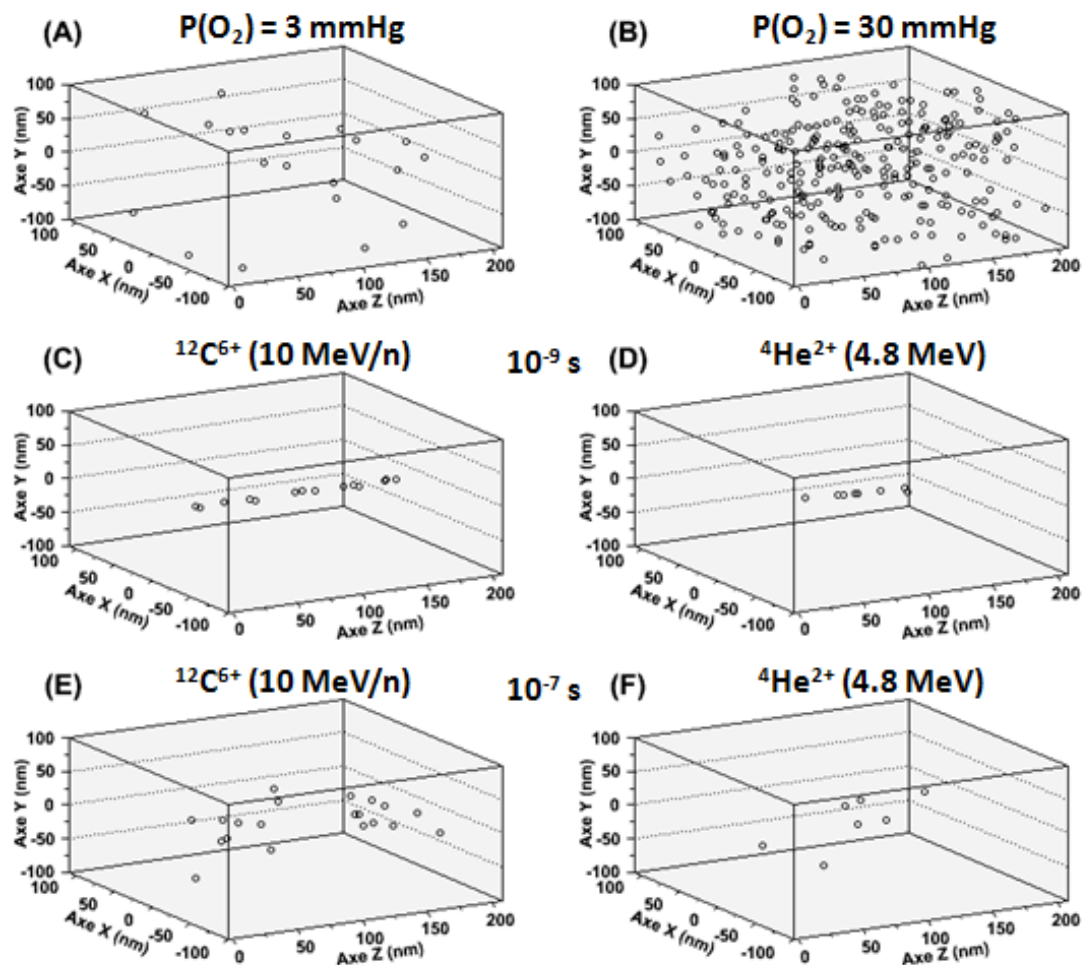


Figure 4. Spatial distributions of oxygen molecules dissolved in water at a partial pressure of 3 mmHg (A) and 30 mmHg (B) or produced in the track of C[10 MeV/n] (LET=168 keV/ μ m) and He[1.2 MeV/n] (LET=94 keV/ μ m) at 10^{-9} s (C and D) and 10^{-7} s (E and F) after ion impact [29]. 3 mmHg and 30 mmHg correspond to the range of partial pressures at which OER begins to saturate for various cell lines.

oxygen concentration have shown a correlation between the oxygen effect and the production of $\text{HO}_2^\bullet + \text{O}_2^{\bullet-}$ when varying LET, particle type and oxygen concentration [35]. However, considering the high probability for hydrated electrons (e_{hyd}) and hydrogen radicals (which are at the origin of the production of $\text{HO}_2^\bullet + \text{O}_2^{\bullet-}$) to be scavenged before reacting with oxygen, Colliaux *et al.* suggested a more general scenario: although hydrated electrons (e_{hyd}) and hydrogen radicals may have a low capacity of damage, by reacting either directly equation 3a) or through intermediate molecules, as for instance glutathione equation 3b), these two radicals may lead to the production of secondary chemical species that could be toxic for cells either by damaging some structures or by impacting some cell signaling (see figure 2c)).

5. DNA target

DNA is often considered as a crucial target in the mechanisms driving both the biological efficiency of light ions and the reduction of the oxygen effect. Although many other targets (and non-targeted events) should be considered, the DNA molecule is at least to be taken into account for such biological endpoints as mutation induction, chromosomal aberrations or instability and, to some extent, cell death. The detailed study of damage formation in DNA in cell appears quite complex both in *in vivo* and *in vitro* conditions. Alternatively, quantifying the damage generated by interactions of fast ions with biological elementary molecules, like sugar or DNA bases, in gas phase, appears quite simpler and more direct. However, such measurements may not provide enough information in the field of radiobiology. For instance, the effect of confinement induced by the molecule environment (mainly water in cells) may strongly modify the relaxation channels of the excited and ionized molecules: ionized molecules may fragment in the gas phase while they may find other modes of relaxation in liquid phase. Moreover, in a water environment, most of the damage in molecules is generated by the free radicals produced by water radiolysis. This mechanism, referred to as indirect action, cannot be ignored. Another limitation to the knowledge that these experimental systems may provide to the radiobiology domain is related to the geometry of these elementary molecules. Spothem-Maurizot *et al.* [36, 37] have shown that the geometrical structures of DNA molecules influence the signature of damage, due to the accessibility of free radicals to the sites of DNA. The sequence of nucleotides, but also the different levels of DNA compaction, should be considered when characterizing damage in DNA. Nevertheless, the quantification of the mechanisms induced by the interaction of ions with molecules in the gas phase may provide data to challenge complex models of the early interaction stage, like the quantum *ab initio* description of collision and molecule relaxations.

As an alternative target to elementary molecules in the gas phase, DNA plasmids in water constitute a step toward DNA in cells. With such systems, E. Sage *et al.* have demonstrated the capacity of high-LET ions to create complex damages. Known as clustered DNA lesions or locally multiply damaged sites (LMDS), these are defined as two or more damages in the DNA molecule within 1-2 helical turns [38]. Among them, the dirty double-strand break (DSB) [39], defined as a combination of a double-strand break close to a single base lesion, appears difficult to be handled by cells. All these complex damages increase the risk for mutation induction and cell death triggering.

Studies with plasmids have also shown that other elements played a role in the mechanisms of damage formation. The protection by antioxidants, that are able to scavenge the radiation-induced radicals before their interaction with DNA, reduces the number of damage [42]. The enzymatic activity modifies the yield and the signature of DNA damage, by repairing some lesions but also by inducing others. Urushiba *et al.* [43] have studied DNA damage induction by irradiating plasmids in solutions containing two types of enzymes (the endonuclease III (Nth), a base excision repair enzyme, and the formamidpyrimidine DNA glycosylase) at various concentrations. They showed that enzymatic activity could modify the yield of damage. They also confirmed the quite robust tendency that increasing LET decreases the yield of single-strand breaks (SSB) but increases the yield of DSB. These examples indicate that studies of DNA damage formation in cells are necessary to include all the mechanisms triggered in actual biological systems, a far from simple task in practice (see for instance [44]).

A question addressed in many papers concerns the relative contribution of direct and indirect actions to DNA damage formation. Often quoted, the pioneering work by Roots *et al.* [45] states that indirect actions dominate at low LET, while direct actions dominate at high LET. This work uses strong concentrations of hydroxyl radicals in scavenger (DMSO) and assumes that these concentrations are as high in the nucleus. Besides the fact that this methodology is questionable, we stress that Ito *et al.* have shown with the same protocol that indirect actions dominate for LET as high as $440\text{keV}/\mu\text{m}$ [46]. In studying the signature of base damage, J-L Ravanat *et*

al. [47] have shown that the indirect damage of DNA bases is also dominant at high LET. By modulating the concentration of glutathione in tumor cells, a major antioxidant in cell nucleus, Hanot *et al.* [48] have shown that a depletion of glutathione induce more cell killing and more residual DSB. Considering the high capacity of this antioxidant to scavenge free radicals, these observations are in favor of an important contribution of indirect actions for high-LET ions as well. Finally, the question on the relative contribution of direct and indirect actions still is opened and requires more experimental evidence.

6. Conclusion

Interaction of ions with biological systems is an active domain of research with an important impact on society, like the research for a better estimation of ionizing-radiation risks for health, or the improvement of cancer treatments by carbon ion therapy, an innovative therapy that benefits from the particular physical and biological properties of fast carbon ions. This latter opportunity was born from the results obtained through many years of research activities, combining modeling and experiments (performed with high-energy accelerator facilities, like GANIL) and many disciplines and actors, including physicists, chemists, biologists and physicians. Despite the numerous results, many fundamental or more clinically-oriented questions require clarification. As an illustration of this scientific and societal requirement, the national Infrastructure “France Hadron” aims at coordinating researches related to hadrontherapy and facilitating access to ion beams. This initiative will contribute to the increase of knowledge in such a complex but interesting field, which involves the interaction of high-energy ions with matter.

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