Impact of oxygen concentration on yields of DNA damages caused by ionizing radiation

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Abstract. Local hypoxia-induced radiation resistance is one of the major problems in current radiation therapy of solid tumors. Concentration of cellular oxygen is not uniform within tumor volume and changes during the course of radiotherapy. The aim of this work is to evaluate the influence of oxygen concentration on the water radiolysis process and subsequently on the yields of primary DNA damages caused by ionizing radiation. Monte Carlo based computer modeling approach was used for this purpose. Detailed track structures provided by TRIOL code and molecular structure of 100 bp DNA oligomer generated using Amber 8 molecular dynamics package were used as input for stochastic model RADAMOL. Diffusion and chemical reactions of radicals produced in the water radiolysis process were followed in a step-by-step simulation. Both unscavengable and scavengable DNA damage was taken into account. It was shown that concentration of diluted oxygen in water significantly influences yields of \( e_{aq}^{-} \), H and O and consequently modifies yields of primary DNA damage. However, this itself cannot explain the oxygen radiosensitizing effect in living cells. The influence of oxygen on DNA damage chemical repair and fixation should be included in further research.

1. Introduction
When a solid tumor grows above a certain size, its inner parts get hypoxic because of insufficient blood supply. The concentration of oxygen is not uniform within the tumor volume and in addition it changes during the course of radiotherapy. Hypoxic cells are two to three times more resistant to a single fraction of ionizing radiation than those with normal levels of oxygen. As this means higher doses necessary to attain tumor control, such locally induced radiation resistance is a serious problem in radiation therapy of solid tumors.

Although the radiosensitizing effect of oxygen has been first described in 1909, its exact mechanism is still not completely understood. It is suggested that at the cellular level the causes for this effect are the modification of the initial damage caused by ionizing radiation and the modification of the subsequent biological DNA repair processes. Two mechanisms have to be taken into account regarding the modification of the initial damage. First is the modification of water radiolysis process. The time evolution of yields of radical species is influenced by additional reactions of the radical.
species with oxygen. Therefore the number of free radicals interacting with the target DNA molecule should depend on the concentration of diluted oxygen in the cell environment.

The second mechanism is a possible chemical repair or fixation of the DNA damage. The DNA damage created by scavengeable or unscavengeable means may undergo a reaction with oxygen. Such reaction leads to state which cannot be chemically repaired by thiol compounds anymore. Biological repair mechanisms take place in later times.

In this study we focused entirely on the influence of cell oxygenation on the initial DNA damage yields at the end of chemical stage (about $10^{-6}$ s), without considering chemical damage repair or fixation. Theoretical model RADAMOL based on detailed description of time-space evolution of track structure and direct and indirect DNA damage has been used for this purpose.

2. Theoretical model

2.1. Structure of the theoretical model RADAMOL
Code package RADAMOL includes modules for detailed modeling of water radiolysis and direct and indirect damage to DNA [1]. Input track structures of ionizing particles in liquid water are provided by Monte Carlo code TRIOL [2]. Water radiolysis is described by the code STOCHECO. DIRADACK and RADACK codes are used for modeling of un- and scavengeable DNA damage respectively.

The calculations were performed for DNA oligomer represented at atomic level. Energy minimized molecular conformation of 100 bp DNA fragment was obtained using Amber 8 molecular dynamics package [3]. The oligomer is placed into a target volume, virtual sphere with radius 75 nm filled by non-structured water. During the simulation procedure the tracks of ionizing particles are superimposed over the target volume containing the DNA oligomer. Time-space evolution of the track is then followed and the resulting DNA damage is scored.

2.2. Track structures
Track structures of charged particles were generated by Monte Carlo code TRIOL [2]. This code enables calculating track structures of electrons in the energy range of 10 eV–2 MeV, photons of 1 keV–2 MeV and ions of $(0.3 \cdot Z^{4/3} - 200)$ MeV/a.m.u. in liquid water. Hundred tracks were generated for: 100 keV electrons, 2, 5, 10 MeV protons and 2, 5 MeV alpha particles. First 100 keV fragment of each track was used in the performed simulations.

2.3. Water radiolysis simulation
In this work, the time-space evolution of charged particle tracks has been followed from $10^{-12}$ to $10^{-6}$ s using the stochastic code STOCHECO [4]. The radiolytic species issued from ionized and excited water molecules diffuse and undergo mutual chemical reactions. The random flight method has been applied to simulate 21 most relevant chemical reactions and radical diffusion motion within charged particle tracks. Yields and spatial distributions of radical species and molecular products, $e_{aq}^-$, OH, H, O, H$_2$, H$_2$O$,^\cdot$, H$_2$O$_2$, OH$^-$, O$^\cdot$, O$_2$, HO$_2$, HO$_2^-$ have been scored.

Probabilities of $e_{aq}^-$, H and O$^\cdot$ reaction with oxygen homogenously dissolved in water are calculated as $p = 1 - \exp(-k \cdot c_{O_2} \cdot \Delta t)$ where $k$ is the reaction rate constant, $c_{O_2}$ is oxygen concentration and $\Delta t$ is the diffusion time step.

2.4. DNA damage model
Both unscavengeable and scavengeable DNA damage was followed. Energy deposition within particular DNA atoms has been assumed to lead to direct damage [5]. Radicals produced in bound water shell around DNA have been assumed to react with DNA, possibly causing unscavengeable damage. Diffusion and chemical reactions of radicals produced farther were followed in a step-by-step simulation [4] and reactions of OH$^-$, $e_{aq}^-$ and H$^\cdot$ radicals with DNA were scored for scavengeable radiation damage according to an improved version of RADACK code [6].
Following types of DNA damage were scored: modified base (MB), single strand break (SSB), double strand break (DSB), complex double strand break and non-DSB clustered damage. DSB was defined as cluster consisting of two SSB on the complementary strands, complex DSB as DSB plus another lesion and non-DSB cluster as a cluster of two or more lesions where no two SSB can form a DSB. It was assumed that to form a cluster the lesions have to be at most ten base pairs apart and independent clusters are separated by at least ten undamaged base pairs.

3. Results

The results of calculations confirm that oxygen diluted in water alters the yields of radical species present during the chemical phase and consequently the number of species reacting with DNA. Figure 1 shows the time evolution of yields of $e_{aq}^-$ and OH-, which are responsible for most of the unscavengeable DNA damage. Presented values are for simulation in water without target structure.

The effect of oxygen presence is most evident in case of $e_{aq}^-$ yields for 5.01 MeV protons. For higher LET the effect diminishes. OH- does not directly react with oxygen, but OH- radical yields are influenced indirectly by modified numbers of available reactants.

![Figure 1](image-url)  
Figure 1 – Kinetics of $e_{aq}^-$ and OH yields calculated for 5.01 MeV proton and 3.39 MeV α particles in presence and in absence of oxygen.

Changes in water radiolysis process influence the numbers of radical species reacting with DNA as well. Table 1 presents the probability of DNA damage by attack of $e_{aq}^-$, OH and H and its relative change in presence of oxygen. The probabilities in all the cases increase with increasing LET of the incident charged particle. The most influenced is DNA damage by $e_{aq}^-$ and H, the influence of oxygen on OH radical attack to DNA is lower.

| $O_2$ [mmHg] | $e_{aq}^-$ | OH  | H    |
|------------|-----------|-----|------|
| 100 keV e  | 0.004     | -57.2% | 0.01 | -9.6% | 0.0002 | -63.0% |
| 10 MeV p   | 0.021     | -57.9% | 0.02 | 7.4%  | 0.0012 | -62.7% |
| 5 MeV p    | 0.040     | -58.7% | 0.04 | 9.1%  | 0.0025 | -66.8% |
| 2 MeV p    | 0.087     | -62.8% | 0.09 | 4.7%  | 0.0069 | -65.0% |
| 5 MeV α    | 0.258     | -52.8% | 0.18 | 13.4% | 0.0445 | -67.5% |
| 2 MeV α    | 0.405     | -51.2% | 0.19 | 9.5%  | 0.0844 | -68.3% |

Table 1 – Probability of DNA damage by a particular radical type in anoxic and fully oxic conditions.
Above described classification of simple and clustered DNA damage has been used to score the damage for different levels of oxygen. The yields of selected types of DNA damage calculated for oxic and fully anoxic conditions are shown in Figure 2. The yields of simple modified bases are lower in oxygenated conditions. For single strand breaks the relation depends on LET – for protons and α particles the yields are higher in presence of oxygen and the difference grows with LET. For complex DNA damages the trends are given by the properties of the contributing simple damages – results for non-DSB clusters behave similarly to modified bases and simple DSB similarly to SSB.

4. Summary

Presented theoretical calculations confirm that the concentration of diluted oxygen in water influences the yields of radical species created by ionizing radiation, namely $e_{aq}^-$, H and O·. Yields of OH are modified to less extent, only indirectly due to changes in occurrence of particular chemical reactions. Changes to water radiolysis process result in modification of primary DNA damage yields. However, this itself does not explain the oxygen radiosensitizing effect in living cells. The effect of oxygen on chemical fixation and repair of primary DNA damage should be studied as the possible main source of this effect in the chemical stage of biological radiation action.

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