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Estimation of free radical formation by β-ray irradiation in rat liver

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Abstract

In vivo free radical reactions in rat liver as a result of exposure to low-dose β-radiation was evaluated with electron paramagnetic resonance (EPR) spectroscopy by monitoring the reduction of the nitroxyl spin probe after intravenous administration. The EPR signal intensity of a nitroxyl probe as a function of time in bile flow was monitored by cannulating the bile duct through the cavity of an X-band EPR spectrometer. The results show that the rate of nitroxyl signal loss was higher in rats whose livers were exposed to β-rays compared to unexposed rats. However, the rate of signal loss was lower in animals whose organs were exposed to air by opening the abdominal cavity. In vitro experiments also showed that the nitroxyl EPR signal loss was greater in an atmosphere of nitrogen than in air. Results suggest that under low levels of tissue oxygen, exposure to β-rays results in nitroxyl signal loss, which may be mediated by free radical dependent pathways. When tissue oxygen were higher, hydrogen peroxide mediated oxidation of hydroxylamine may predominate resulting in a signal loss of smaller magnitudes. This study

Abbreviations: EPR, electron paramagnetic resonance; ROS, reactive oxygen species; $^{\cdot}OH$, hydroxyl radical; $O_2^{\cdot -}$, superoxide; $^{\cdot}H$, hydrogen atom; e_{aq}^-, hydrated electron; GSH, glutathione; NADH, reduced-nicotinamide adenine dinucleotide; NADPH, reduced-nicotinamide adenine dinucleotide phosphate; 3CP, 3-carbamoyl-2,2,5,5-tetramethylpyrrolidine-1-yloxy; GM counter, Geiger–Müller counter.

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shows possible evidence of reactive oxygen species formation by low-dose β-ray irradiation in a living animal.
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Keywords: Electron paramagnetic resonance; Nitroxyl radical; Spin probe; Spin clearance; Reactive oxygen species; Redox

1. Introduction

Exposure of a living organism to ionization radiation is known to produce various reactive oxygen species (ROS) such as hydroxyl radical ($^{\cdot}OH$), superoxide ($O_2^{\cdot -}$), and hydrogen peroxide (H_2O_2). The ROS are mainly initiated in a living organism by the radiolysis products of water (H_2O), which contributes to 80% of the cell weight. Radiolysis of water gives rise to such species as the hydrogen atom ($^{\cdot}H$), $^{\cdot}OH$, H_2O^+, and the hydrated electron (e_{aq}^-). Hydroxyl radical and hydrogen atom attack cell components such as proteins, lipids, and nucleic acids by oxidation and reduction reactions, respectively. The e_{aq}^- is a strong reducing agent and reactive species. For ^3H β-ray (mean energy ~5.6 keV) radiolysis of deaerated pure water, the yields of three main products, $^{\cdot}OH$, e_{aq}^- and $^{\cdot}H$ are calculated as 1.40, 1.23 and 0.59 molecules/100 eV, respectively [1]. The formation of H_2O_2 by recombination reaction of $^{\cdot}OH$ in turn increases with time, but not all of the $^{\cdot}OH$ recombines to produce H_2O_2. In a living cell, these active species react rapidly with oxygen to give $O_2^{\cdot -}$, H_2O_2 and other alkoxy radical species, which can damage biologically important molecules in the cell.

Several types of ionizing radiation are used in the treatment of cancer. In systemic radiotherapies, molecules having a β-ray emitting nuclide are administered to the patient. In conventional radiotherapy, the patient is irradiated with external γ- or X-ray beams. Reactive species generated by the ionization of water molecules by the high-energy radiation, destroy the cancer cell by damaging DNA. Approximately 70% of the damage is caused by indirect reactions induced by radiation, and 70–80% of these indirect effects of radiation are caused by $^{\cdot}OH$, which is one of the most active species of ROS.

A recent electron paramagnetic resonance (EPR) spin trapping study showed the formation of $^{\cdot}OH$ in aqueous solution irradiated with 290 MeV/nucleon carbon ion beam [2]. However the direct detection of this unstable free radical species, having very short life time, is difficult, especially in vivo, and therefore no direct evidence is available to show in vivo $^{\cdot}OH$ formation by radiation unless very high doses are used [3].

EPR spectroscopy can measure exogenous stable free radicals such as nitroxyl radicals. The nitroxyl radicals have been used as spin probes in cells and animal models. When nitroxyl spin probes are administered to a living animal, they get reduced to the corresponding hydroxylamine by enzymatic and/or chemical one-electron reduction reactions depending on tissue redox status. The in vivo decay rate of nitroxyl spin probes increased in tissue by oxidative stress such as ischemia–reperfusion [4], iron overload [5], streptozotocin-induced diabetes [6], and radical generation in lung by diesel exhaust particles [7]. It is known that $O_2^{\cdot -}$ and $^{\cdot}OH$

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