In vivo ESR measurement of free radical reaction in living mice

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Abstract

Recently, free radicals such as active oxygen species, nitric oxide, etc are believed to be one of the key substances in physiological and pathological, toxicological phenomena, and oxidative damages, and all organism have defencing system against such as free radicals. Formation and extinction of free radicals may be regulated through bio-redox system, in which various enzymes and compounds should be involved in very complicated manner. Thus, direct and non-invasive measuement of in vivo free radical reactions with living animals must be essential to understand the role of free radicals in pathophysiological phenomena.

Electron spin resonance spectroscopy (ESR) is very selective and sensitive technique to detect free radicals, but a conventional ESR spectrometer has large detect in application to living animals, since high frequent microwave is absorbed with water, resulting in generation of high fever in living body. In order to estimate in vivo free radical reactions in living whole animals, we develop in vivo ESR-CT technique using nitroxide radicals as spin probes. Nitroxide radicals and their reduced forms, hydroxylamines, are known to interact with various redox systems. We found that! ! the signal decay due to reduction of nitroxyl radicals is influenced by aging, inspired oxygen concentration, ischemia-referfusion injury, radiation, etc. In the present paper, I will introduce in vivo ESR technique and my laboratory recent results concerning non-invasive evaluation of free radical reactions in living mice.

Nitroxyl radicals were dissolved in isotonic buffer. Female ddy mice (10¡-25g) or SD rats(250g) were anesthetized with pentobarbital or urethan. The solution containing nitroxyl radical was intravenously(i.v.), intratracheally(i.t.), or intraarticularly(i.a.) administered to living mice or rats, and then ESR spectrum was oberved with an in vivo ESR spectrometer. The imaging of nitroxyl radicals was obtained by filtered back-projection method with hand-made in vivo ESR-CT system. Antioxidant activity was also estimated with in vivo ESR measurement.

Nitroxyl radicals gave triplet sharp lines, and the ESR-CT imaging revealed that nitroxyl radicals localized in lung and articular cavity after i.t and i.a. injection, respectively. The radicals having hydrophobic property also distributed in fat and brain. The signal decresed, obeying pseudo first order kinetics. The decay constants differed among nitroxyl probes depending on their structures and physico-chemical properties. The decay arose from not only the reduction to the corresponding hydroxylamine but also penetration into tissue, metabolism, excretion, etc. The reduction rates were enhanced by oxidative stress, iron-overload, inflammation, treatment of xenobiotics, etc. The presence of antioxidants suppressed the enhancement of the reduction, indicating that the enhancement of nitroxyl reduction was due to active oxygen species generated ! ! in vivo during oxidative stress.

The time-resolved imaging was reconstructed with the signal decay rates, demonstrating the location and enhancement of free radical reactions, indicating radical as a probe has capability to visualize in vivo free radical reactions and in vivo activity of antioxidants in living body.

References

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