IMMUNOSPIN-TRAPPING BASED PROTEIN FREE RADICAL IMAGING IN SUB-CELLULAR STRUCTURES

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Formerly believed to be exclusively deleterious, free radicals have been demonstrated to act in much more subtle ways as pivotal redox signaling agents. This new paradigm gained strength since the characterization of ($^{\bullet}NO$) as the endothelial derived relaxing factor. Since then interesting parallels were unveiled connecting oxidant production with human disease.Albeit the essential roles that biological oxidants play in physiology, there is no established technique that directly allows the probing of specific locations where oxidative stress occurs in cells. Because of that we decided to apply the newly developed immunospin-trapping technology, which combines the sensitivity of immunological techniques with the selectivity of the spin trapping reaction, to image protein free radical production in cells. So far, we have demonstrated that catalase radicals are produced upon hepatocyte treatment with hypochorite. Moreover, such radicals were unequivocally localized to the hepatocyte's peroxisomes what was confirmed by the use of catalase knockout mice. Such studies have been extended to other cell systems such as HL-60 cells treated with t-butyl hydroperoxide which showed radical production localized around the myeloperoxidase granulocytes. In addition, RAW 264.7 macrophages activated for $^{\bullet}NO$ and $O_2^{\bullet -}$ production, revealed increased levels of the spin trap 5,5-dimethyl-pirroline-1-oxide, (DMPO) bound to proteins distributed throughout the cytosol which is consistent with the diffusion properties of both $^{\bullet}NO$, $O_2^{\bullet -}$ and peroxynitrite. The attractive possibilities of a reliable method to localize free radical production in cells structures (a goal pursued for more than 30 years) warrant further investigation.
Key words: Immunospin-trapping, protein radical, free radical, imaging