

P015 Fluorescent probes for measurement of oxidative and nitrosative species in cells and tissues: what do they really measure?

**Peter Wardman, Marta Wrona, Kantilal Patel,
Lisa K Folkes and Mark Burkitt**

University of Oxford, Gray Cancer Institute

Chemical probes for free radicals in biology are important tools offering high detection sensitivity. The commonest probes for 'reactive oxygen and nitrogen species' are reduced fluorescein and rhodamine dyes that fluoresce when oxidized. Oxidation by radicals produces an intermediate which is normally oxidized further by oxygen to yield a fluorescent, stable product. These probes should be used with caution however. Generally there is a lack of selectivity for the common reactive species; hydrogen peroxide, hydroxyl, superoxide and thiyl radical; carbonate radical-anion; and nitric oxide, nitrogen dioxide, and peroxyxynitrite. Also oxidation may require a catalyst e.g. a peroxidase. Probes that detect peroxyxynitrite are probably actually measuring its free radical breakdown products, hydroxyl, carbonate, and nitrogen dioxide. Fluorescent products being measured can react further with radicals, and intermediate probe radicals are generally reactive towards antioxidants and oxygen, generating superoxide. Changes in fluorescence can be attributed to changes in the levels of cellular antioxidants which react with and 'repair' the probe radical intermediates rather than reflecting differences in the free-radical generation rate. Rational use of probes requires understanding and quantitation of the mechanistic pathways involved, and of environmental factors such as oxygen and pH.