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Editorial

Peroxynitrite induced cytotoxicity and detection in cardiovascular, neurodegenerative and inflammatory disorders

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The biological endogenous oxidant peroxynitrite (ONOO-), which is the result of the diffusion-limited interaction between superoxide (O_2) and NO, was originally discovered in 1990.[1] Peroxynitrite, which disrupts mitochondrial activity and results in cell death through oxidation and nitration processes, is a major contributor to nitric oxide- and superoxide-dependent cytotoxicity. When peroxynitrite interacts with other biomolecules, it can further break down into other harmful reactive species. Peroxynitrite is difficult to accurately detect in biological systems due to its high reactivity and short lifespan. Massive efforts have been undertaken over the past decade to create accurate methods for determining the production of peroxynitrite in various cellular and animal investigations.[2]

A biological oxidant called peroxynitrite is created when NO and O_2 combine. It produces oxidizing and nitrating species due to its high degree of reactivity. Thiols, amines, lipids, and proteins are just a few of the biomolecules that can interact with peroxynitrite. Peroxynitrite exposure can cause a variety of harmful biological reactions, some of which might affect cell viability and function. Both peroxynitrite anion (ONOO-) and peroxynitrous acid (ONOOH) can directly take part in one- and two-electron oxidation reactions with biomolecules. Peroxynitrite reactivity is pH-dependent. The nitration and dimerization of tyrosine residues, the oxidation of cysteine thiol groups, and the oxidation of methionine sulfur-groups are the most common protein changes caused by peroxynitrite. In addition, it has been shown that the disruption of metal-sulfur clusters causes the oxidative deactivation, modulation, or inhibition of enzyme activity.[3] The majority of ONOO-mediated oxidations, however, cause interference with cellular redox-signaling, cell damage, or the death of the entire organism, because ONOO- has been shown to contribute to pathophysiological conditions in many cardiovascular, neurodegenerative, and inflammatory diseases.[3,4]

There is evidence that glyceraldehyde-3-phosphate dehydrogenase's active site thiol can be covalently modified by peroxynitrite (GAPDH). Heart contractile dysfunction could result from myofibrillar creatine kinase activity being nitrated by peroxynitrite. Numerous ion pumps, including calcium pumps, calcium-activated potassium channels, and membrane Na+/K+ATPase activity, have been found to be inhibited by peroxynitrite. The proteasome accelerates the destruction of the cellular proteins that have undergone peroxynitrite modification. Superoxide dismutase, glutaredoxin, and glutathione are all inhibited by peroxynitrite, which increases intracellular oxidant production and exacerbates oxidative cellular injury. Through the activation of matrix metalloproteinase precursors, oxidative stress can harm tissue (proMMPs). A major mechanism of tissue injury in inflammation and reperfusion, MMP activation, may be reduced by peroxynitrite. A terminal mediator of cell damage, as well as enhancing and inciting a number of pro-inflammatory processes.[3] Both the superoxide anion radical that is free and the superoxide anion radical that is attached to the iron of the heme group are involved in the creation of peroxynitrite. A peroxynitrite complex, which is composed of heme-associated peroxynitrite, is created as a result of this interaction.[5,6]

Low levels of peroxynitrite can cause apoptotic cell death, which depends on the release of cytochrome c from the mitochondria and the activation of caspases 3, 2, 8, and 9, whereas exposure to high levels of peroxynitrite causes cell death. As a result of the interaction between peroxynitrite and lipids, malondialdehyde and conjugated dienes are formed, as well as nitrito-, nitro-nitrosoperoxo-, and/or nitrated lipid oxidation adducts. Tetrahydrobiopterin (BH4) has been shown to oxidize *in vitro* to the quinonoid 5,6-dihydrobiopterin under the influence of peroxynitrite. Thus, pathophysiologically low levels of BH4 can encourage a cycle of its own demise in endothelial cells and other cell types through NO synthase

dependent peroxynitrite production. This pathway may cause an imbalance in cellular pyrimidine nucleotide levels, a positive feedback cycle of cytotoxic oxidant production, and vascular endothelial dysfunction brought on by oxidative stress in a variety of illnesses.[3]

Because ONOO- plays a variety of roles in biological systems, it is crucial to develop methods for detecting and tracing ONOO-. However, it is a laborious task, because ONOO- has a relatively limited lifetime (10 ms) and occasionally only traces of it are present. An obstacle that dampens interest in investigations of ONOO- bioactivities is the lack of precise and direct detection methods. Electrochemical sensors, nitrotyrosine production detection, and fluorescent probes are the three main methods for peroxynitrite detection.[2]

For the purpose of finding ONOO- in chemical and biological systems, several electrochemical ultramicrosensors have been created. Studies using illness models that typically exhibit changes in pH value may not be appropriate candidates for electrochemical peroxynitrite detection.[7]

Finding NT in proteins is regarded as a diagnostic parameter for endogenous peroxynitrite activity, because it indicates a particular peroxynitrite-mediated protein modification.[3] For understanding ONOO-induced cytotoxicity and nitrosative stress-associated pathological index in many diseases, such as diabetes and its complications, cerebral ischemia-reperfusion injury, myocardial ischemia-reperfusion injury, neurodegenerative diseases, and inflammatory lung diseases, nitrotyrosine formation is regarded as a representative biomarker. For instance, by detecting nitrotyrosine, enhanced ONOO production has been seen in both diabetes people and experimental diabetic animal models. The expression of 3NT in the samples is frequently too low to be seen. More crucially, additional RNS including NO, •NO2, and •N2 O3 can also trigger 3NT production. As a result, ONOO is not required for 3NT detection. Furthermore, this approach makes it impossible to track the production of ONOO- in real time.[2,8]

The advantages of fluorescent probes include the capacity to provide real-time spatial imaging and reasonably high sensitivity for monitoring ONOO-. Recent developments have made it possible to use fluorescent probes to monitor ONOO- synthesis in living cells. Numerous fluorescence probes have been created and tested in chemical and biological systems nowadays using various fluorophores. In more than 2000 experiments, the reduced fluorophores dichlorodihydrofluorescein (DCHF) and dihydrorhodamine (DHR) have both been utilized. These probes can be oxidized by peroxynitrite to produce luminous chemicals. Since arylboronates may quickly and directly react with ONOO- to produce strong luminous products, boronate-containing fluorescent probes have been developed for ONOO- detection. They seem to be superior to DHR and DCHF. Since they can also be used to detect H_2O_2, it is currently challenging to use boronate-based probes to discriminate ONOO- from H_2O_2. A brand-new genetically encoded probe called boronic acid-derived circularly permuted green fluorescent protein has been created to address these issues. In addition, a number of interesting fluorescent probes based on various chemical processes and principles have recently been created with relatively high sensitivity and selectivity for ONOO-.[2,9,10]

Peroxynitrite is known to disrupt the physiological balance in organisms when it is present in excess, which causes nitro-oxidative stress. Clinical correlations exist between its protracted effect over time and numerous chronic disorders, including neurological, autoimmune, and cardiovascular diseases.[2] Recent years have seen the development of new generation probes that are based on various chemical mechanisms and principles. These probes have the capacity to specifically and sensitively detect ONOO-. However, several tests must yet be conducted to further evaluate their values. Despite the tremendous progress that has been made in this field, there are still a lot of obstacles to overcome before a method can be developed for the noninvasive detection and real-time imaging of ONOO- *in vivo*. It can now be inferred that the discovery of peroxynitrite has opened up new research avenues and that the clinically validated probe will provide information on the diagnosis and treatment of disorders that are mediated by peroxynitrite.

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