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the massive increase in intracellular Ca^{2+} stimulates the activation of various enzymes (e.g., phospholipases, cyclooxygenases, nitric oxide synthase, and proteolytic enzymes) and stimulates the release of cytotoxic oxidant species and free radicals, which provoke lipid peroxidation, DNA injury, and mitochondrial damage. Tissue anoxia, calcium overload, and oxidant stress also stimulate the activation of several transcription factors, including NF- κ B, hypoxia-inducible factor 1, and STAT3. This, in turn, triggers the production of inflammatory cytokines (TNF- α , IL-1), enzymes

(inos, cox-2), adhesion molecules (icam-1, selectins), and the recruitment of activated phagocytes (refs. 1079, 587).

the diffusion-limited reaction of no with superoxide is similar to the reaction of molecular oxygen with other free radicals, as it is energetically favorable.

however, because no is a single electron biradical, the reaction can be more chemically and energetically facile, which allows the formation of a highly reactive nitrosonium ion (no⁺).

this biradical is highly reactive because it does not have a second unpaired electron. in aqueous solution, the 1:1 no:o₂

reaction produces a complex that has a very short lifetime and can be catalyzed by nitric oxide synthase (1). The reaction is: $2 \text{NO} + \text{O}_2 \rightarrow 2 \text{NO}_2$ (1) The reaction of NO with superoxide is important in host defense, as it allows NO to act as a potent oxidant to a variety of biological targets. Superoxide radicals can be formed in a variety of enzymatic processes, most notably through the actions of NADPH oxidase and monoamine oxidase. For example, in vascular smooth muscle cells, at least two different oxidases can produce superoxide radicals from the metabolism of

arachidonic acid: cytochrome b oxidase and xanthine oxidase. in polymorphonuclear leukocytes, nadph oxidase generates superoxide from the reduction of oxygen to form O_2^- .

peroxynitrite can readily react with superoxide to form nitrosonium (NO^+) and a short-lived peroxynitrous anion (ONO_2^-). the rapid reaction of NO with superoxide is similar to the oxidation of NO by molecular oxygen, because it involves a biradical. in particular, it is interesting that the reactivity of NO is similar to that of singlet oxygen.

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In addition to the direct effects of peroxynitrite on cells and tissues, NO may have indirect toxic effects through its effects on other free radicals, such as hydroxyl radicals. In chronic renal failure patients treated with PD-L1, peroxynitrite has been reported to accumulate at sites with increased inflammation (218). Prolonged accumulation of peroxynitrite could contribute to tissue damage in these patients as the free radical is believed to cause

endothelial dysfunction and disrupt the uptake of antioxidants and lipid-soluble vitamins, such as retinoids (218). Unilateral renal artery stenosis is a model of chronic renal failure and PD-L1 is frequently used as adjuvant therapy in this setting in animal models and human patients (315, 316). More recently, NO has been suggested to exert an indirect toxicity by the production of the hydroxyl radical. The role of NO in generating the hydroxyl radical is, however, still uncertain. Basigin in the basolateral membrane of the renal tubule has been reported

to be specifically nitrated in the presence of NO (216). In the final evaluation of an animal model, the normal level of expression of nitrotyrosine in the kidneys was significantly reduced in the presence of NO and the number of large cells with intracellular and perinuclear free radical staining appeared to be reduced. More importantly, at high doses of NO, which can only be achieved by combining a high dose of L-NAME and a high dose of arginase, these effects were abolished. These data are consistent with the suggestion that NO can generate a chain reaction by the formation of

peroxynitrite in which ONOO- can nitrate the vessel wall, reducing the arginine concentrations and then, paradoxically, leads to further production of NO. This hypothesis is consistent with the results that, in the non-NO-producing model, when the nitric oxide synthase inhibitor, L-NAME, is administered in conjunction with arginase, NO-dependent toxicity is ameliorated and even abolished, which implies that nitration of arterial wall proteins has a vasodilatory effect. In addition to the generation of NO and superoxide, most cell types are

capable of producing more nitric oxide (NO) than they can use as a source of energy. The overproduction of NO leads to the production of peroxynitrite in the cytosol, which is highly reactive and can damage proteins, lipids, and DNA. This possibility is supported by the fact that that release of NO per se in tissues can increase the uptake of free radicals (12, 59).

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