Physiological Effects of Peroxynitrite
Potential Products of the Environment

Jakob Vinten-Johansen

Peroxynitrite is a potent oxidant formed from the reaction between superoxide radicals and NO in a one-to-one stoichiometry. This reaction occurs at $6.7 \times 10^9$ mol/L$^{-1}$ s$^{-1}$, and it is essentially irreversible because of its highly exothermic nature. Therefore, the reaction is diffusion-limited, and it out-competes the reaction of superoxide dismutase for superoxide radicals, which proceeds at a rate of $2 \times 10^9$ mol/L$^{-1}$ s$^{-1}$. A key regulator of peroxynitrite production is the concentration of NO, and the reaction proceeds when the concentration of NO increases and can overcome dismutation by superoxide dismutase. This situation occurs when NO achieves micromolar concentrations as when there is a burst of NO production during ischemia/reperfusion and when NO is produced by cytokine-stimulated inducible NO synthase (iNOS) activity. The toxic effect of ONOO$^-$ and its protonated form, peroxynitrous acid, may stem from its oxidation of zinc fingers, protein thios, membrane lipids, and iron and sulfur clusters of biological molecules. In addition, hydroxyl-like and nitrogen dioxide radicals are produced by homolytic cleavage, increasing the potential for oxidant-mediated tissue injury. Intermediates are also formed from the heterolytic cleavage of ONOO$^-$ to hydroxyl anion and nitronium ion (NO$_2^+$), catalyzed by the transition metal centers of superoxide dismutase and myeloperoxidase. The nitration of protein tyrosine residues gives rise to 3-nitrotyrosine, which is frequently used as an assay for ONOO$^-$ in tissues, blood, and perfusates.

Like NO, peroxynitrite has been associated with both deleterious and beneficial effects. An advantage of the oxidant-mediated deleterious effects of peroxynitrite is that it has been suggested to contribute to the host-defense response to bacterial invasion. Both neutrophils and macrophages produce peroxynitrite by the rapid biradical reaction between NO and superoxide anions generated simultaneously from those cells. However, oxidant injury is also a primary mechanism of myocardial dysfunction and infarction, and several studies have implicated peroxynitrite as a major cause of injury in the heart subjected to ischemia/reperfusion or cytokines. Liu et al demonstrated that NO and superoxide anions and the production of peroxynitrite were elevated in the ischemic-reperfused heart in vivo, which at least places peroxynitrite at the scene of the injury and is a prerequisite for suggesting that it participates in deleterious actions on tissue.

The study by Ferdinandy et al in this issue of Circulation Research demonstrates that the production of peroxynitrite (and the substrates NO and superoxide anion) is contributory to the cardiodepressant effects of the proinflammatory cytokines interleukin-1β, interferon-γ, and tumor necrosis factor-α. This study correlated the temporal appearance of peroxynitrite with cytokine-induced contractile dysfunction, the latter of which was reversed by attenuating the production of either of peroxynitrite’s precursors, NO (by l-nitroarginine) or superoxide anion (by titron). In addition, the degradation of peroxynitrite using 5,10,15,20-tetrakis-(4-sulfonatophenyl)-porphyrinato-iron(III), a catalyst of peroxynitrite decomposition to nitrate, attenuated cytokine-induced cardiodepression and decreased perfusate nitrotyrosine levels, used as a surrogate measure of peroxynitrite.

The depressed cardiac function induced by peroxynitrite may be related to a decrease in cardiac efficiency. The authors concluded that peroxynitrite promotes myocardial contractile dysfunction ostensibly secondary to its oxidant effects or alternatively to nitration of important contractile proteins.

The central thesis of the study by Ferdinandy et al relies on the endogenous production of peroxynitrite from superoxide anion and NO stimulated by the mixture of cytokines. The authors verified that the substrates for peroxynitrite were, indeed, generated by measuring the presence of superoxide anions and NO in ventricular tissue. They also removed the substrates of peroxynitrite using an appropriate inhibitor of NOS and scavenger of superoxide anion. They additionally showed that xanthine oxidoreductase and NAD(P)H oxidoreductase were sources of superoxide anions, and increased iNOS activity was associated with the increased generation of NO. Measuring the end product, peroxynitrite, is more problematic in that it cannot be measured directly, and the surrogate measures of nitrotyrosine footprints and dityrosine used by Ferdinandy et al are not uniquely specific for peroxynitrite.

Nitrotyrosine and dityrosine were measured in perfusate rather than in tissue. Alternative methods (ie, oxidation of dihydrorhodamine-123) also have limitations in that there may be significant interference with biological molecules. Tyrosine residues can be nitrated by other nitrogen-centered oxidants, but this requires relatively high concentrations. Myeloperoxidase activity can form nitrotyrosine possibly by oxidation of nitrite to NO$_2^-$. However, myeloperoxidase activity likely is not important in the isolated buffer-perfused heart devoid of neutrophils. Hence, the most likely source of nitrotyrosine residues is peroxynitrite.

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The concomitant measurement of nitrotyrosine and dityrosine (the latter formed from biradical reaction between two tyrosyl radicals) may increase the confidence that the indirect measures of peroxynitrite generation represent the physiological appearance and relative concentrations in perfusate.

In contrast to the reports of the cardiotoxic effects of peroxynitrite by Ferdinandy et al and others, numerous studies report apparent cardioprotective effects of peroxynitrite. Nossuli et al showed in a feline model of coronary occlusion and reperfusion that intraventricular infusion of authentic peroxynitrite (1 μmol/L) 10 minutes before reperfusion was associated with a reduction in infarct size and attenuation of endothelial function in ischemic-reperfused coronary arteries assayed by vasorelaxation responses to acetylcholine and by reduced ex vivo adherence of unstimulated neutrophils to the endothelial surface of postischemic coronary arteries. In addition, authentic peroxynitrite attenuated adherence of neutrophils to normal coronary artery endothelium stimulated by thrombin. In addition, Lefer et al reported that peroxynitrite attenuated neutrophils rolling alone in vivo mesenteric venules. In a subsequent study, Nossuli et al demonstrated that the physiological effects of peroxynitrite are dependent on its concentration; at either 0.2 or 20 μmol/L concentrations of intraventricular infusion of peroxynitrite, the cardioprotection observed at 2 μmol/L was not apparent. This concentration-dependent effect is in agreement with the study by Schulz et al, in which cardiodepression was observed with infusion of 40 but not 4 μmol/L peroxynitrite in isolated rat hearts.

The sensitivity of cardiac tissue to concentration-dependent actions of peroxynitrite may explain, in part, the presence or lack of physiological effects, but it cannot explain the dichotomous effects reported in the literature, that is, cardiotoxicity as opposed to cardioprotection. However, insight into this issue was provided in a study by Ma et al, which indicated that the physiological effects of peroxynitrite on myocardium depended on the type of environment adopted for the experiment, for example, the presence of a crystalloid or biological (ie, blood) environment. In the study by Ma et al, the peroxynitrite generator Sin-1 exerted cardiodepression in isolated rat hearts perfused with crystalloid buffer, whereas, in sharp contrast, it was cardioprotective in a blood-perfused environment. Similar results were reported by Ronson et al in a model of cardiac surgery in which a canine heart was subjected to 30 minutes of global ischemia followed by 1 hour of myocardial protection with either crystalloid cardioplegia or blood cardioplegia in the absence or presence of 5 μmol/L authentic peroxynitrite. After blood reperfusion of the hearts off bypass, the hearts receiving crystalloid cardioplegia containing peroxynitrite showed significantly depressed contractile function and elevated creatine kinase activity compared with crystalloid cardioplegia solution without peroxynitrite (but otherwise identical in composition). In contrast, peroxynitrite in the blood cardioplegia solution was associated with better recovery of postischemic ventricular systolic function and lower creatine kinase activity than its blood cardioplegia counterpart not containing peroxynitrite. Postischemic coronary artery endothelial function (not reported) was significantly worse in the crystalloid solution containing peroxynitrite, whereas it was better in the peroxynitrite-containing blood solution. Hence, these data support the notion put forth by Ma et al that the physiological effects of peroxynitrite can vary dramatically depending on the environment of the exposure.

A review of the literature suggests that the effects of peroxynitrite on the myocardium do show a dependency on the environment in which the anion is present. Studies demonstrating a deleterious effect were largely conducted in crystallloid media, including the study by Ferdinandy et al. In contrast, the studies reporting cardioprotective effects of peroxynitrite were conducted largely in vivo systems (ie, blood-perfused environments). One interpretation is that there is some component of blood that attenuates the toxic effect of peroxynitrite. Glutathione is a major component of blood, being in plasma and highly concentrated in red blood cells. Glutathione reacts with peroxynitrite to form NO, nitrosoglutathione, or a similar nitrosothiol that demonstrates vasorelaxant effects, which are inhibitable by hemoglobin and methylene blue, a conventional scavenger of NO and inhibitor of guanylly cyclase activity, respectively. Nossuli et al observed an increase in S-nitrosoglutathione when peroxynitrite was coincubated with reduced glutathione. Moro et al demonstrated that glutathione could reverse the proaggregatory effects of peroxynitrite on washed platelets in buffer and that the buffer containing glutathione showed measurable NO and S-nitrosoglutathione in concentrations sufficient to account for attenuation of platelet aggregation. Furthermore, Nakamura et al noted that the deleterious effects of peroxynitrite in crystalloid cardioplegia solution were largely reversed with the addition of 500 μmol/L glutathione. Hence, the presence of glutathione may prevent the accumulation of peroxynitrite to toxic levels and may convert peroxynitrite to secondary products with cardioprotective properties.

In the study by Ferdinandy et al, the lack of glutathione or other thiol-containing molecules in the crystalloid perfusate may have allowed peroxynitrite to accumulate to toxic levels in the myocardium and, in turn, exert oxidant or nitration effects. The cytokine-stimulated generation of peroxynitrite by the myocardium and associated cardiac depression were clearly shown and supported under the ex vivo conditions used in the experiment. However, these results should be considered within the context of the preparation in which they were obtained.

References


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