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Peroxiredoxin sets the brain on fire after stroke

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Abstract

How blood-borne inflammatory cells cause tissue damage in the brain after ischemic stroke remains elusive. Peroxiredoxins, cytosolic antioxidant proteins vital for redox balance, are released extracellularly from ischemic cells, acting as potent ‘danger signals’ that activate macrophages and lead to a harmful cytokine response, a new study shows. The findings unveil a new culprit in the delayed phase of ischemic injury and suggest new therapeutic approaches (pages 911–917).

The World Health Organization estimates that 15 million people suffer a stroke every year. Of these, one-third dies and another third becomes permanently disabled. Despite its enormous public health burden, second only to ischemic heart diseases in high-income countries, interventions to treat stroke remain extremely limited¹. Most strokes are caused by a sudden occlusion of a major cerebral artery (ischemic stroke) resulting in brain damage and permanent neurological impairment. Whereas energy deficit and glutamate excitotoxicity cause neuronal death immediately after ischemia, activation of brain inflammatory cells causes lingering damage that evolves over many hours². In this issue of *Nature Medicine*, Shichita *et al.*³ show that peroxiredoxins (Prxs), neuroprotective intracellular antioxidant enzymes essential for redox homeostasis, are released extracellularly after ischemia and, paradoxically, become potent proinflammatory signals that initiate a destructive immune response. The findings identify Prxs as a crucial link in the chain of events underlying post-ischemic inflammation and unveil a new powerful therapeutic target for the late stage of cerebral ischemia.

The factors responsible for the harmful inflammatory responses accompanying ischemic stroke have not been clearly defined, but activation of receptors of the innate immune system, including Toll-like receptors (TLRs) and scavenger receptors, has emerged as a key step in the signaling cascade^{4,5}. These receptors are the first line of defense against infectious agents, but they also contribute to the destructive effects of ‘sterile’ inflammation initiated by cell damage⁶. Located predominantly on inflammatory cells, TLRs are activated by danger signals, also termed danger-associated molecular pattern molecules (DAMPs), produced during tissue injury. Tissue injury generates a multitude of DAMPs, including proteins, complex lipids and nucleic acids derived from dying cells, as well as peptides produced by matrix proteolysis⁶. But the specific DAMPs responsible for TLRs activation in stroke have not been identified, an obstacle to developing much-needed therapies for the delayed phase of the injury.

The authors set out to shed light on the DAMPs, receptors and downstream factors triggering post-ischemic inflammation. In a previous study, they showed that macrophages invading the ischemic brain release the cytokine interleukin-23 (IL-23) and promote IL-17 production by $\gamma\delta$ T cells³, a T cell subset that reacts to tissue damage, which, in turn, contributes to the late phase of the injury⁷. In the present study, they found that mouse brain lysates were able to potently induce expression of IL-23 in bone marrow-derived dendritic cells (BMDCs)³. They found later that Prxs in the brain lysates are responsible for IL-23 induction in these cells³.

Six mammalian Prx isoenzymes (Prxs 1–6) exist in different subcellular compartments, wherein they detoxify hydrogen peroxide and a wide range of organic hydroperoxides⁷. However, oxidation of cysteine residues on Prxs inactivates their antioxidant properties⁸. Given that Prxs activate TLR4 in peripheral macrophages⁹, the authors hypothesized that after stroke Prxs may escape from damaged cells, losing their anti-oxidant property and becoming potent danger signals acting on TLRs. Indeed, Prx proteins were unable to induce production of IL-23 and other cytokines in TLR2- and TLR4-deficient BMDCs. After ischemic stroke in mice, Prx-positive debris-like granules were found in damaged areas, often associated with F4/80-positive phagocytic cells³, confirming a pathogenic role during stroke *in vivo*. Furthermore, infiltrating immune cells failed to produce IL-23, and IL-17-expressing $\gamma\delta$ T cells were suppressed in TLR2- and TLR4-deficient mice after stroke.

Finally, bone marrow chimera experiments using TLR2- and TLR4-deficient marrow showed that microglia, resident macrophages of the brain that express TLRs but are not replaced by bone marrow transplantation, are not the target of Prxs and are not a source of IL-23 (ref. 3). These findings, collectively, provide convincing evidence that extracellular Prxs released from injured cells in the ischemic brain activate TLR2 and TLR4 in infiltrating immune cells, leading to production of cytotoxic cytokines, including IL-23 and IL-17 (Fig. 1).

But do extracellular Prxs contribute to ischemic brain injury, and, if so, what is their therapeutic potential? To address these questions, the authors developed Prx-blocking antibodies that were systemically administered to mice after cerebral ischemia³. Remarkably, up to 12 h after ischemia, antibodies against Prxs ameliorated both infarct growth and motor deficits in mice, indicating that the treatment was effective even when administered in the late stages of the ischemic cascade. Such a wide therapeutic window has great translational relevance because most patients reach medical attention several hours after stroke onset, when thrombolysis with tissue plasminogen activator, the only treatment for acute stroke, is no longer safe or effective¹. The protective effect of Prx-specific antibodies was associated with a reduction in IL-23- and IL-17-producing cells in the ischemic brain; however, it was not observed in TLR2- and TLR4-null mice. Finally, by examining conserved regions among Prx proteins, they identified the domains responsible for the macrophage activation³. Administration of antibodies targeted against these domains was also strongly protective in the stroke mouse model. Although it remains to be determined whether these antibodies prevent the binding of Prxs to TLR2 and TLR4, the findings pinpoint a specific Prx domain that could be used for therapeutic development.

A large number of potential DAMPs, including modified lipids, heat shock proteins and β -amyloid peptides, are produced after stroke, but their role in ischemic brain injury and their therapeutic potential remain uncertain². One such DAMP, the nuclear protein high-mobility group protein 1 (HMGB1) is released from ischemic cells and is endowed with proinflammatory activity¹⁰. However, Shichita *et al.*³ found that HMGB1 acts predominantly in the early phase of the inflammatory cascade. In contrast, Prxs are produced later after ischemia and, as suggested by the efficacy of blocking antibodies up to 12 h after

ischemia, exert their pathogenic effect in the delayed phase of the damage. Blocking Prxs also enhanced the protective effect of blocking HMGB1 (ref. 3), attesting to the more powerful role of Prx in later stages of tissue damage. HMGB1 and Prx may therefore have temporally distinct roles in post-ischemic inflammation.

Several experimental therapies suppressing inflammation protect the ischemic brain with a wide therapeutic window, including, for example, those targeting complement, the cyclooxygenase-2 pathway or inducible nitric oxide synthase and those upregulating α B-crystallin^{11–14}. Therefore, the delayed protective effect of Prx-based approaches is not novel or unique. Nevertheless, counteracting Prxs is attractive because their sources, targets and pathogenic mechanisms have been clearly established^{3–5,9,15}. Furthermore, the domains responsible for their proinflammatory activity have been defined, opening the way to develop therapeutic approaches with small-molecule inhibitors. In combination with other treatment strategies, such as thrombolysis or targeting early events in the ischemic cascade, Prx-based therapies may increase our chances to forestall the relentless progression of ischemic damage.

There are several issues that need to be considered in evaluating the translational potential of the Prx pathway. First, the characteristics of the protection need to be better defined according to established criteria for preclinical stroke research¹⁶. For example, the effectiveness in the context of a more severe ischemic insult (permanent ischemia), in females or in other species and the efficacy in the presence of stroke risk factors, such as aging, diabetes and hypertension, needs to be established. Second, inflammation is crucial for tissue reorganization and repair, and suppressing inflammation may limit the potential of the brain to recover after stroke². It remains to be established whether Prx-TLR2/TLR4 signaling is involved in the recovery of the post-ischemic brain. Third, small-molecule-based approaches to neutralize the proinflammatory effect of Prx need to assure that the vital antioxidant function of the cytosolic enzymes is not impaired. These small molecules need to be cell permeable to cross the blood-brain barrier; if they penetrate into ischemic cells, they may inhibit the antioxidant activity of Prxs, which would prove devastating to the tissue at risk for infarction¹⁵. These considerations notwithstanding, the elegant work of Shichita *et al.*³ provides a cogent demonstration of the powerful destructive effects of sterile inflammation in ischemic brain injury and unveils a new therapeutic target with a remarkable translational potential.

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Figure 1.

Release of Prxs promotes post-ischemic inflammation after stroke. Prxs are intracellular enzymes crucial for antioxidant defense. Shichita *et al.*³ found that after stroke, Prxs are released from dying cells, lose their protective function and act as DAMPs by engaging TLR2 and TLR4 on infiltrating macrophages. The resulting nuclear factor-κB (NF-κB) activation in these immune cells leads to production of proinflammatory cytokines, which, in turn, trigger a destructive inflammatory response. One component of the inflammatory response involves IL-23, which contributes to the damage through IL-17-producing γδT cells that further enhance the activation of the inflammatory cascade.