Peroxiredoxin-1 is a novel danger signal involved in neurotoxic microglial activation after experimental cardiac arrest

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Methods

- adult male C57Bl/6 mice (20-25g)
- are anesthetized with isoflurane and orally intubated
- cerebral arrest (CA) is induced by iv injection of KCl and verified by EEG
- during CA, head temperature is maintained at 28°C, body temperature at 38°C
- mice are resuscitated after 10 minutes of CA (epinephrine injection and chest compression)
- brains are harvested 2 hours, 1 day, or 3 days after CA/CPR and protein extracted from hippocampi
- Prx1 is detected by immunoblotting (R&D Systems)
- cerebrospinal fluid (CSF) is harvested from the cisterna magna
- Prx1 is detected in CSF by ELISA (MyBiology)
- primary neurons are cultured from E16 mice and exposed to 90 minutes oxygen-glucose deprivation on DIV9 in the presence of absence of primary mouse microglia
- neuronal survival is measured 24 hours later using MTT assay
- primary microglia are harvested from confluent microglial cultures derived from postnatal (P4-2) mice
- TNF-α and IL-1β in microglial culture medium are measured by ELISA (eBioscience Ready-Set-Go)
- 0.5 L of 5 M recombinant human Prx-1 (ProSpec) or BSA control is injected stereotaxically into the hippocampal CA1 region
- brains are harvested 24 hours later and microglial activation assessed by immunohistochemistry for Iba1

Results

- Peroxiredoxin-1 is upregulated in hippocampus after CA/CPR
- While Prx1 was barely detectable in CSF from naive mice, it was rapidly released after CA/CPR and was easily detected in CSF within the first day after the insult.
- n=5, P<0.05

- Peroxiredoxin-1 is released into CSF after CA/CPR
- Neuron-conditioned medium (NCM) induced similar cytokine release from naive and Prx1-1-treated (A)
- Only NCM concentrate (NCM +) indicated significantly increased microglial neurotoxicity.
- Injection of Prx1 into the hippocampus (right panel) induced widespread activation of microglia (exposed processes, enlarged cell bodies, and increased expression of Iba1, yellow).
- Microglial activation was limited to the injection site (white star) after injection of BSA (left panel).

- Peroxiredoxin-1 induces pro-inflammatory cytokines
- Primary cultured microglia treated for 24 hours with NCM released TNF-α and IL-1β.
- Prx1 caused similar cytokine release, n=3, P<0.05 (A). Precipitation of Prx1 from NCM with Prx1 AB, but not control AB, reduced cytokine release (B).

- Peroxiredoxin-1 induces a neurotoxic microglial phenotype
- Addition of untreated microglia increased neuronal death after OGD.
- Death increased further when microglia where pre-treated for 24 hours with NCM (A).
- Pretreatment with Prx1 similarly increased microglial neurotoxicity (B), n=6, *P<0.05

- Peroxiredoxin-1 activates microglia in vivo
- Injection of Prx1-1 into the hippocampus (right panel) induced widespread activation of microglia (exposed processes, enlarged cell bodies, and increased expression of Iba1, yellow).
- Microglial activation was limited to the injection site (white star) after injection of BSA (left panel).

Abstract

Background: Cardiac arrest (CA) is a common manifestation of ischemic heart disease. While advances in cardiopulmonary resuscitation (CPR) and critical care have improved survival after cardiac arrest, survivors frequently suffer brain injury that leads to long-term cognitive dysfunction. Cardiac arrest causes inflammation and activation of microglia, the brain resident immune cells, which precipitates neuronal death in ischemia-sensitive brain regions. Prx1 is a novel danger signal that exacerbates neuronal death. We hypothesized that injured neurons release danger signals after CA, which activate microglia to a neurotoxic phenotype that exacerbates neuronal death. We tested whether the antioxidant Peroxiredoxin-1 (Prx-1) acts as a danger signal after CA/CPR.

Background:

While advances in cardiopulmonary resuscitation (CPR) and critical care have improved survival after cardiac arrest (CA) in recent years, survivors frequently suffer brain injury that leads to long-term cognitive dysfunction. CA causes inflammation and activation of microglia, the brain resident immune cells, which precipitates neuronal death in ischemia-sensitive brain regions. We hypothesized that injured neurons release danger signals after CA, which activate microglia to a neurotoxic phenotype that exacerbates neuronal death. We tested whether the antioxidant Peroxiredoxin-1 (Prx-1) acts as a danger signal after CA/CPR.

Conclusion:

We conclude that Prx1 is a novel danger molecule that is released from injured neurons after ischemic stress in vitro and after CA/CPR in vivo. We found that Prx1 is necessary and sufficient in our model to activate microglia to a pro-inflammatory and neurotoxic phenotype in response to neuronal injury. These novel observations improve our understanding of intracellular communication after ischemic brain injury. Future work will determine how Prx1 transforms microglia to the neurotoxic phenotype. Blocking the detrimental microglial signaling induced by neuronal Prx1 release after injury may provide a promising new target for neuroprotective intervention after CA/CPR. Support from AHA GRNT20380839.