Peroxiredoxin-1 and Toll-like receptor 2 pathway contributes to neurotoxic microglial activation after cardiac arrest

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Background

Survivors of cardiac arrest (CA) and cardiopulmonary resuscitation (CPR) suffer cognitive decline related to delayed neuronal death in the hippocampus. We postulated that microglia are activated to a neurotoxic phenotype after CA/CPR, and that this contributes to delayed neuronal death. We tested whether danger signal peroxiredoxin-1 (Prx1), which is released from injured neurons, causes neurotoxic microglial activation after CA/CPR, and whether this requires microglial expression of toll-like receptor (TLR)2.

Methods

- Wild-type (WT) and TLR2-KO mice were subjected to CA/CPR.
- Mice were sacrificed 3 days after CA/CPR and hippocampal tissue was harvested.
- Neuronal death and microglial activation were evaluated histologically by H&E, Fluoro-Jade B (FjB) and anti-Iba1 antibody (Iba-1).
- Cerebrospinal fluid (CSF) was collected from the cisterna magna of additional WT mice 2hrs, 1day, or 3days after CA/CPR for quantification of Prx1 protein.
- Microglia were isolated from WT mice 2 or 7 days after CA/CPR for RT-PCR analysis of TLR2-expression.
- Neurotoxicity of microglia was assessed in vitro by measuring neuronal death in microglia-neuronal co-cultures after oxygen-glucose deprivation (OGD).
- Group differences were evaluated using ANOVA or Student's t-test, as appropriate. All data are presented as mean ±SEM.

CA/CPR model:

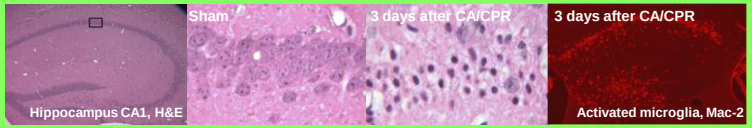
- Under isoflurane (2%) anesthesia, a jugular catheter and endotracheal tube were placed.
- CA was induced by injecting 0.5 M potassium chloride into the internal jugular vein and confirmed by ECG.
- CPR was initiated after 10 minutes of CA by injection of 11-16 µg of epinephrine and chest compressions at a rate of 300/minute.

Conclusion

Microglia are activated to a neurotoxic phenotype after CA/CPR, which is facilitated by danger signal Prx1. Microglial activation after CA/CPR and Prx1 stimulation requires TLR2, whereas neurotoxicity is abolished by TLR2-deletion in vitro only. Further studies are needed to understand the differential role of TLR2 in microglial activation and neurotoxicity.

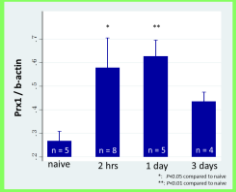
Results

Fig 1. CA/CPR causes neuronal death coinciding with microglial activation in hippocampus.



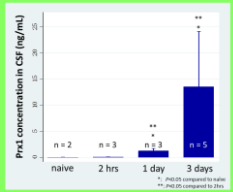
- No obvious neuronal death is apparent in the hippocampus of sham-operated animals.
- Eosinophilic (injured) and pyknotic (dead) neurons become apparent in the CA1 region 3 days after CA/CPR.
- Microglial activation coincide with the peak of delayed neuronal death.

Fig 2. Prx1 protein expression is upregulated in mouse hippocampus after CA/CPR



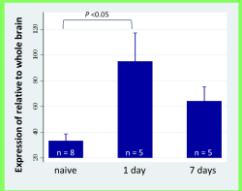
- Prx1 protein expression is upregulated in mouse hippocampus after CA/CPR.

Fig3. Prx1 is released and detectable in the CSF within the first day after CA/CPR



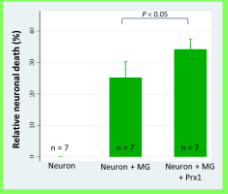
- CSF is collected from WT mice 2 hrs, 1 day, or 3 days after CA/CPR.
- Prx1 is released and detectable in the CSF within the first day after CA/CPR, followed by microglial activation and subsequent delayed neuronal death.

Fig 4. Microglial expression of TLR2 increases after CA/CPR



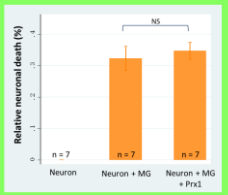
- Microglia are isolated from naïve WT mice or 1 or 7days after CACPR.
- Expression of TLR2 mRNA is analyzed by quantitative PCR.
- Microglial TLR2 expression is significantly higher 1 day after CACPR than naïve mice.

Fig 5. Prx1 exacerbates neuronal death after OGD in WT microglia-neuronal co-cultures



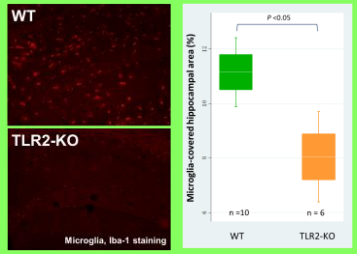
- Neuronal death in microglia-neuronal co-cultures after OGD increases when WT microglia are pretreated with Prx1.

Fig 6.TLR2 deletion abolishes exacerbation of neuronal death by Prx1



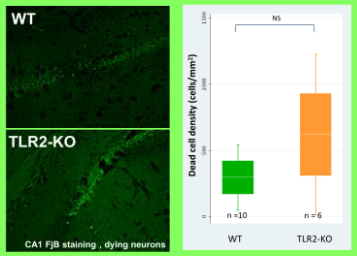
- There is no significant increase in neuronal death when TLR2-KO microglia are pretreated with Prx1.

Fig 7. Microglial activation is attenuated in TLR2-KO mice after CA/CPR



- Iba-1 staining is visualizing microglia.
- Microglial activation is attenuated in TLR2-KO mice 3 days after CA/CPR compared to WT mice.

Fig 8. Neuronal death is similar in TLR2-KO and WT mice after CACPR



- Fluoro-Jade B staining identifies dying neurons.
- Neuronal death is not different between the two groups.

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