Microglia depletion is neuroprotective after cardiac arrest

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Background

Improved cardiopulmonary resuscitation (CPR) techniques led to improved survival rate after cardiac arrest (CA) over the last decades. Unfortunately, most survivors are left with brain injury, which leads to significant disability. Treatment options to reduce brain injury remain limited to hypothermia/targeted temperature management. New interventions are needed that selectively minimize neuronal death and reduce neurological deficit resulting from global cerebral ischemia during CA.

One potential target is the immune response. Microglia (MG), the brain’s resident immune cells, are rapidly activated after CA/CPR and mount an inflammatory that is believed to contribute to neuronal death. We previously observed strong activation of MG coinciding with neuronal death in our mouse model of CA/CPR (Wang 2013).

Methods

1) Selective depletion of microglia

Mouse model:
- CXCR1<sup>iDTR</sup> mice were crossed with mice carrying an inducible diphtheria toxin receptor (ROSA26iDTR; ‘DTR’), or with C57BL/6 mice (all strains obtained from The Jackson Laboratory) to obtain heterozygous CXCR1<sup>iDTR</sup>DTR experimental or CXCR1<sup>iDTR</sup>DTR control animals.
- Cre-recombination and subsequent DTR expression in CXCR1<sup>+</sup> positive cells (microglia, macrophages, and monocytes) was induced by tamoxifen injection.
- Short-lived peripheral DTR expressing monocytes and macrophages were replaced by non-DTR expressing cells from the bone marrow within 30 days after tamoxifen.
- Low-turnover brain microglia maintain DTR expression can be specifically ablated with diphtheria toxin (DT) injection 30 days after tamoxifen.
- Microglia ablation was confirmed by flow cytometry. Single cell suspensions of brain and spleen were prepared by mechanical dissociation. For brain tissue, Perls' gradients preceded and enzymatic digestion were further applied (Neural Tissue Dissociation Kit; Millenyi Biotec, Auburn, CA).
- Antibodies used for flow cytometry included:
  - CD11b: Anti-Microglia and Macrophage
  - CD45-APC-eFlour780: Microglia (CD45 intermediate), Macrophage (CD45 high)
  - CD19-PE: B lymphocyte
  - CD3-PE-Cy7: T lymphocyte
  - CXCR1(YFP+): CD11b+ populations gated on CD45int, CD19- and CD3-

2) The effect of microglial depletion after CA/CPR

CXCR1<sup>iDTR</sup>DTR (experimental) and CXCR1<sup>iDTR</sup>DTR (control) mice treated with tamoxifen and DT were subjected to CA/CPR (see Fig. 1). 3 days after CA, brains were harvested for histological assessment of neuronal death using Fluorojade B staining.

CA/CPR model:
- Under isoflurane (2%) anesthesia, a jugular vein was exposed, and endotracheal tube were placed.
- Under isoflurane anesthesia, a jugular catheter and endotracheal tube were placed.
- CA was induced by injecting 0.5 M potassium chloride into the internal jugular vein.
- 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.

Results

Diphtheria toxin injection selectively depleted microglia, leaving peripheral macrophages intact

The number of CD11b+ and YFP+ MG in brain was markedly reduced by 84% in experimental mice.
In contrast, CD11b+ and YFP+ macrophages in the spleen were not affected by DT injection.

Microglial depletion significantly reduced neuronal death

Neuronal death in the ischemia sensitive hippocampal CA1 region was dramatically reduced in experimental (MG depleted) mice, compared to controls.
Mortality was not different between experimental and control mice after CA/CPR (50% vs 55%, P = 0.69).

Conclusion

Using a new mouse line that allowed us to separate the effects of microglia from those of peripheral macrophages, we found that selective depletion of brain resident microglia while leaving the macrophage pool intact reduces neuronal death after CA. Based on our results, we propose that activated microglia after CA become neurotoxic and contribute to brain injury and resulting functional deficit. Blocking microglial toxicity by targeting the molecular switches underlying the toxic microglial activation will open new avenues for therapeutic intervention after CA/CPR.