Microglia are activated and contribute to delayed neuronal death after cardiac arrest/cardiopulmonary resuscitation

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Background
Survivors of cardiac arrest and cardiopulmonary resuscitation (CA/CPR) frequently suffer from disabling memory problems. This is likely due to neuronal death in the ischemia-sensitive hippocampus. Hippocampal cell death is delayed after CA/CPR and accompanied by pronounced microglial activation. We hypothesized that activated microglia respond to danger-associated molecular patterns (DAMPs) released from injured neurons after CA/CPR, and release neurotoxic factors which cause the delayed death of injured neurons.

Methods
• male C57Bl/6 mice (20-25 g) are anesthetized with isoflurane and intubated, right internal jugular vein is cannulated
• CA is induced by KCl and verified by ECG tracing; during CA, tympanic temp. is maintained at 37.5°C, rectal temp. at 30°C
• resuscitation after 8 min CA (epinephrine/CPR); brains are harvested 1 day or 7 days after CA/CPR
• microglia are isolated after CA/CPR using magnetic bead-assisted cell sorting (MACS) with CD11b-beads (Miltenyi)
• isolated microglia are used immediately for RNA isolation or plated on Transwell inserts and incubated overnight
• neonatal microglia are harvested from mixed-glial cultures from postnatal C57BL/6 and plated on Transwell inserts
• microglia for ELISA analysis are plated in 96-well plates
• primary neurons from E16 C57Bl/6 mice are cultured in 24-well plates in Neurobasal medium with 2% B27 and exposed to 90 min oxygen-glucose deprivation (OGD) on day in vitro 9
• microglia are moved to serum-free medium 1 day after plating; additional groups are moved to medium conditioned by primary neurons after OGD (neuron-conditioned medium, NCM)
• microglia are added to neuronal cultures immediately before OGD
• neuronal survival is assessed 24 hours later by MTT assay
• TNF-α release measured by ELISA 24 hrs after stimulation
• TNF-α mRNA in microglia measured by quantitative RT-PCR
• group comparison by t-test or ANOVA; mean+/– SEM

Results

- isolated adult microglia (AMG) can be cultured after CA/CPR
- neuron-conditioned medium (NCM) increases microglial toxicity
  • neonatal microglia exacerbate neuronal death after OGD (left panel)
  • toxicity increases when microglia are treated with medium conditioned by injured neurons (NCM)
  • microglia also cause death in non-injured, baseline neurons (right panel)
  • pre-treating microglia with NCM exacerbates this microglia-induced neuronal death

CA/CPR increases AMG toxicity
- AMG isolated 1 or 7 days after CA/CPR exacerbate death of cultured neurons after OGD
- isolated AMG express elevated mRNA levels of pro-inflammatory cytokine TNF-α (left panel)
- TNF-α protein levels in mouse hippocampus are elevated 1 day after CA/CPR (right panel)

NCM induces TNF-α in neonatal microglia
- • neonatal microglia also cause death in non-injured, baseline neurons (right panel)
- • pre-treating microglia with NCM exacerbates this microglia-induced neuronal death

Conclusions
Adult microglia isolated from mouse brain after CA/CPR could be sustained in culture for several days. Addition of adult microglia to neuronal cultures exacerbated neuronal death after OGD. This was significantly more pronounced when microglia were isolated 7 days, rather than 1 day, after CA/CPR, suggesting that microglial toxicity increased in response to mediators released after CA/CPR. Microglial toxicity is similarly increased when microglia are pre-treated with medium conditioned by OGD-injured neurons. Protein expression and release of TNF-α increased as microglia became more toxic after CA/CPR. TNF-α released from microglia was sufficient to exacerbate neuronal death. We conclude that our model is useful to dissect the mechanisms of microglial activation and microglia-mediated neuronal death after CA/CPR. Microglia produce and release neurotoxic TNF-α after CA/CPR in response to DAMPs released from injured neurons.

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