Anti-inflammatory effect of DHA in mice submitted to acute ischemic stroke

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INTRODUCTION

Ischemic stroke is a major cause of death worldwide and responsible for serious long-time disability in adults. It was concluded that DHA has the potential for treating ischemic stroke, but the mechanism is unclear. The study aimed to investigate the anti-inflammatory effect of DHA in mice suffering from acute cerebral ischemia/reperfusion injury.

METHODS

- Cerebral ischemia in mice was induced by middle cerebral artery occlusion (MCAO) for 1 h.
- WT C57/BL6 mice submitted to tMCAO were treated with DHA (10mg/kg, every 24h, i.p. 3d) or Vehicle (Veh, saline).
- Stroke outcomes, systemic inflammatory status and microglia/macrophage phenotypic alterations were assessed 3 days after stroke.
- Macrophage depletions were induced by clodronate liposomes injection.
- Primary macrophage cultures were used to evaluate the direct effect of DHA on macrophages.

Figure 1

Fig.1 Post-stroke DHA treatment protects against acute cerebral ischemia. C57/BL6 mice were subjected to 60 min tMCAO followed by daily DHA (10mg/kg, i.p.) or saline (Veh, vehicle) treatment for 3 days. A) Infarct volume was quantified on NeuN (green)-stained coronal sections. B) Neurological deficit score was assessed at day 1-3 after tMCAO. C) Left: Representative images demonstrating TUNEL (red) co-labeling with NeuN (green) in infarct penumbra 3 days after tMCAO. Right: Quantification of the number of NeuN⁺TUNEL⁺ neurons. N=4. *P<0.05, ***P<0.001.

Figure 2

Fig.2 Systemic DHA therapy reduces immune cell infiltration into the ischemic brain. Immune cell infiltration in ipsilateral cerebral hemisphere was analyzed by flow cytometry 3 days after tMCAO. A) Gating strategy for macrophages, neutrophils, microglia, B lymphocytes and T lymphocytes. B-D) Representative FACS panels and quantification of the numbers of immune cells in the ipsilateral brain. N=5. *P<0.05, **P<0.01, ***P<0.001.

Figure 3

Fig.3 Post-stroke DHA therapy induces anti-inflammatory phenotype of macrophages in the ischemic brain. A) Representative FACS panels showing that DHA treatment induced an anti-inflammatory phenotype of macrophages. B) Representative FACS panels showing that microglia expressed low levels of CD206 and CD16. C) Immunofluorescent staining analysis of CD206 and Iba1. N=4. D-E) 3D reconstructed plot showing CD206-Alexa Fluor 488 and CD16-Alexa Fluor 488 fluorescence intensity. F) mRNA expression of pro-inflammatory and anti-inflammatory mediators was measured with RT-PCR in the ipsilateral brain 3 days after tMCAO. N=3. *P<0.05, **P<0.01, ***P<0.001. G-H) Representative FACS panels and quantification in blood (D) and spleen (C). N=5. ***P<0.001.

Figure 4

Fig.4 Systemic DHA injection shifts circulating macrophage polarity toward anti-inflammatory M2 phenotype after ischemic stroke without changing the immune cell numbers in blood and spleen. A-B) Immune cells in spleen (A) and blood (B) were assessed with flow cytometry 3 days after tMCAO. N=5.

Figure 6

Fig.6 Macrophages are essential for the neuroprotective effects of systemic DHA administration. A) Timeline of Clodronate liposomes application and stroke outcome analysis. Macrophages were depleted with Clodronate liposomes. B-C) Impact of Clodronate liposomes on blood macrophages (B) and brain microglia (C) in sham mice 36 hours after liposome application. N=3. D) Infarct volume was assessed with TTC staining 3 days after stroke. N=6. E) Neurological deficit score was assessed at 0-3 days after stroke. N=6. F) Representative images showing morphological alteration of macrophages after LPS activation with or without DHA treatment. (G) mRNA expression of inflammatory mediators in activated macrophages were measured by RT-PCR. *P<0.05, **P<0.01, ***P<0.001.

Figure 5

Fig.5 Systemic DHA injection reduces circulating inflammatory factors after ischemic stroke. Cytokine and chemokine production were measured in blood using protein array 3 days after tMCAO. Heat map (A), representative dot plots (B) and quantification (C) showed that post-stroke DHA treatment down-regulates chemokines, complement, cytokines and myeloid cell activators in blood 3 days after tMCAO. N=3. *P<0.05, **P<0.01.

CONCLUSION

- Post stroke DHA treatment decreased infarct volume significantly compared with control group
- Post stroke DHA treatment induces inflammatory resolving phenotype of macrophage after cerebral ischemia
- Post stroke DHA treatment down-regulates chemokines and other pro-inflammatory markers
- Post-stroke DHA treatment ameliorated acute ischemic brain injury in a macrophage-dependent manner

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