Role of Soluble Epoxide Hydrolase in Post-Ischemic Angiogenesis

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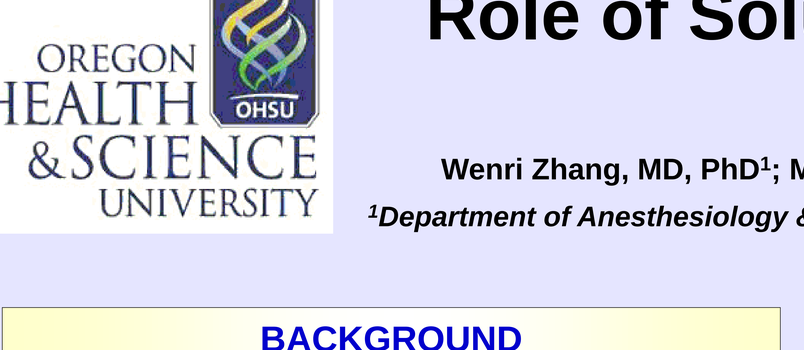
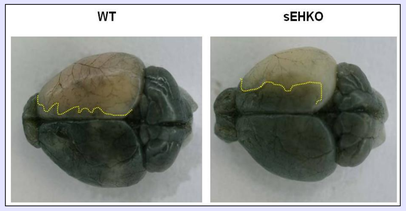
BACKGROUND

- Angiogenesis is a natural defense mechanism helping to restore oxygen and nutrient supply to damaged brain tissue.
- Cerebral ischemia induces cerebral angiogenesis, part of a vascular remodeling response that is necessary for regeneration and functional recovery. To take advantage of angiogenesis as a therapeutic target in stroke, it is important to understand the underlying molecular mechanisms.
- Epoxyeicosatrienoic acids (EETs), P450 eicosanoids produced in brain by astrocytes, play an important role in blood flow regulation and protection after cerebral ischemia. EETs' actions are terminated by soluble epoxide hydrolase (sEH).
- In a previous study, we found EETs to be increased, brain perfusion improved and infarct size reduced after middle cerebral artery occlusion (MCAO) in sEH knockout (sEHKO) compared to WT mice.



Cerebral blood flow images using optical microangiography (OMAG) in ipsilateral (I) and contralateral (C) cerebral cortex of WT and sEHKO mice at baseline and 30 min after MCAO. The white bar indicates 1 mm distance on X-Y projection.

- sEH gene deletion enhances post-ischemic perfusion, and sEHKO mice have better collateral blood flow.
- In addition to their vasodilator effect, EETs exhibit angiogenic properties in multiple tissue and experimental models. Thus, we tested the hypothesis that sEH gene deletion enhances angiogenesis and functional recovery after ischemia.



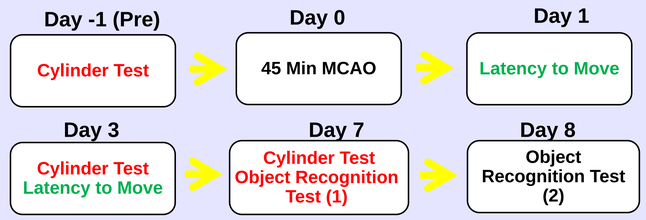
Post-ischemic collateralization in sEHKO vs WT mice visualized by carbon dye perfusion 30 min after MCAO.

HYPOTHESIS

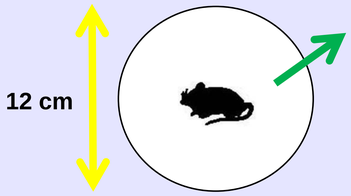
- sEH gene deletion promotes post-ischemic angiogenesis and functional recovery after focal cerebral ischemia in mice.

METHODS

- Animals: male sEH knockout (sEHKO) and Wild type (WT) mice
- Mice underwent 45 min of right-sided middle cerebral artery occlusion (MCAO) via the intraluminal filament technique followed by 8 days of recovery.
- A battery of somatosensory and cognitive neurobehavioral tests were performed during recovery.
- Brains were processed (perfusion-fixation, 4% PFA, free-floating 6 µm cryosections) and stained for CD34, which identifies endothelial cells, as well as endothelial progenitor cells. Unbiased, stereology-based estimates of vascular density were obtained from the density of CD34-positive vascular profiles in these brain sections using computer-assisted optical dissector probe.



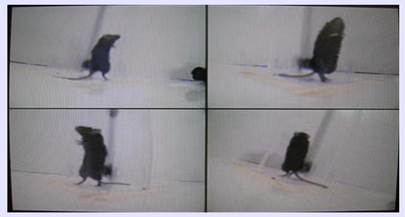
Latency to Move



- Each mouse was placed in the center of a 12-cm diameter circle on a flat surface and the time required for the mouse to move outside the circle was recorded.

Cylinder Test

- Cylinder was 9cm in diameter and 15cm in height.
- Four cameras placed around the cylinder.
- Maximum of two paw touches for one rearing were recorded.
- A total of 20 touches was recorded during 10 min.
- Left / total paw touches was calculated.

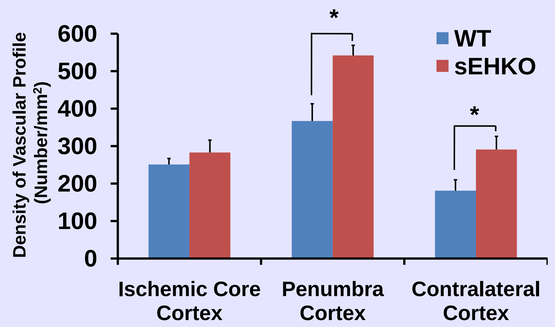


Object Recognition Test

- Identical objects were placed in two opposite corners of a white plastic box. The mouse was placed in the box for 5 minutes to explore the objects. Time of exploration of each object was recorded.
- 24 hours later, the object inspected for the least amount of time on day 1 got replaced with a novel object. The mouse was then placed into the box for 5 minutes, and the time spent investigating each object was again recorded.

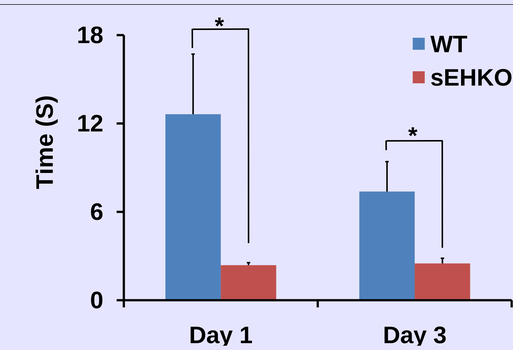
RESULTS

1. Higher density of new blood vessels in sEHKO mice brain



Density of new blood vessels in penumbra and contralateral cortex, but not in ischemic core, was significantly increased in sEHKO compared to WT mice (* p<0.05, n=8 per group).

2. Improved recovery of motor activity after stroke in sEHKO mice



sEHKO mice are significantly faster to move out of circle in day 1 and day 3 after 45 min MCAO compared to WT mice (* p<0.05, n=8, each).

3. No differences were found in Cylinder Test and Object Recognition Test between sEHKO and WT mice.

CONCLUSIONS

- sEHKO mice have higher post-ischemic angiogenic capacity.
- sEHKO mice perform better in the latency to move, but not the cylinder test or the object recognition test after stroke.

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