Mechanisms Of SAH-Induced Hippocampal Disfunction.


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Introduction.

Ninety five percent of subarachnoid hemorrhage (SAH) survivors experience various long-term disabilities including abnormalities in memory along with chronic fatigue, depression, and seizures. These disabilities are sufficiently significant that 44% of the SAH patients are unable to continue with their professional activities.

SAH is often followed by general brain atrophy including the temporomesial area which correlates with neurocognitive aberrations. These observations are suggestive of hippocampal abnormalities following SAH.

Earlier we observed neural activity-blood flow uncoupling in animals following the filament-induced SAH. Others demonstrated abnormalities in hippocampal synapses and long-term potentiation in brain slices using intra chiasmatic cistern blood injection model of SAH in rats.

We explored the indirect mechanisms, which may lead to hippocampal disfunction following the SAH induced by perforation of the circle of Willis mice.
Methods.

Studies were performed in 10-14 weeks old male C56BL/6J mice. Animals were anesthetized with Isoflurane, and SAH was induced using monofilament perforation of the Willis circle. Control animals underwent similar surgery but no perforation was performed. The hemorrhage was scored using modified grading scale developed by Sugawara et al., (2008) as it correlates with the neurological status. Animals were allowed to recover. Four days following the SAH animals were deeply anesthetized, euthanized, transcardially perfused with phosphate buffered saline followed by 4% formaldehyde solution. Brains were extracted, postfixed, cryoprotected, and slices 20 µm thick were prepared using freezing microtome. Slices were mounted on microscopic slides and histologically processed (immunohistochemistry, Golgi and Nissl staining) to explore histopathological consequences of SAH.
Results.

Upon histological examination we did not observe presence of blood in the ventricles of experimental animals. Prussian blue staining did not reveal presence of iron in the hippocampal area. Using Nissl stained images we counted number of cells in hippocampal CA1 area (4 fields in 3 slices/animal). Number of cells was comparable in naïve and SAH animals. We also did not observe fluoro-jade C positive cells in CA1 or dentate gyrus hippocampal areas. Immunohistochemical staining for activated caspase 3 was also negative.

However, immunohistochemistry revealed significant activation of astro- and microglial cells in CA1 and dentate gyrus hippocampal areas in which the circle of Willis was perforated 4 days before compared to naïve or animals with sham perforation. Immunoreactivity of glial fibrillary acidic protein (GFAP, specific astrocyte marker), increased two-fold (p<0.01) compared to sham control suggesting activation of astroglia. Immunoreactivity of ionized calcium-binding adapter molecule 1 (Iba1), marker of microglial activation, increased by 1.5 fold (p<0.04). Hippocampal neuroinflammation was accompanied by the loss of dendritic spines of hippocampal neurons (p<0.00002) of the dentate gyrus neurons.
Astroglia is activated at 4 days following the SAH in the hippocampal dentate gyrus.

Fluorescent immunohistochemical staining of astroglial cells using GFAP antibodies (green) in the hippocampal dentate gyrus in Naïve animals and animals in which circle of Willis was perforated 4 days before. (Blue – cell DAPI labeled nuclei).
Fluorescent immunohistochemical staining of microglial cells using Iba1 antibodies (green) in the hippocampal dentate gyrus in Naïve animals and animals in which circle of Willis was perforated 4 days before. (Blue – cell nuclei labeled with DAPI).

Microglia is activated at 4 days following the SAH in the hippocampal dentate gyrus.

A. Naïve

B. SAH
Density of dendritic spines of hippocampal neurons is decreased in the animals four days after SAH.

Golgi stained dendrites of the dentate gyrus hippocampal neurons in Naïve and animals, which received perforation of the circle of Willis 4 days before. Graph represents average density of dendritic spines/10 µm.

Scale bar 10 µm
Discussion.

Our data suggest that four days after SAH hippocampus undergoes significance changes. In agreement with data obtained by Han et al., 2014 we were not able to observe frank neuronal loss in the hippocampal area. However, at four days after SAH significant activation of astro- and microglia took place. Strong glial reaction was accompanied by significant loss of dendritic spines of hippocampal neurons. These changes may explain the hippocampal disfunction observed after SAH.

At four days no visible blood or iron were observed in the hippocampus in our experiments. Taking into account relatively small size of hemorrhage in mice after perforation of the circle of Willis it is conceivable that observed changes in hippocampus result from the indirect effect of the hemorrhage such as release of chemokines.

Our findings along with our earlier observation of CBF-oxygenation uncoupling in the hippocampus (Galeffi et al., 2015) along with the data from other laboratories (Han et al., 2014) strongly suggest that SAH-induced hippocampal abnormalities may underlie long-term SAH memory and cognitive consequences. Understanding the mechanisms of SAH-induced hippocampal damage will allow developing therapeutic approaches to improve patients recovery following the SAH.
Summary points.

Four days after SAH induced by perforation of the circle of Willis in mice:

- hippocampal astro- and microglia are severely activated;
- no neuronal loss is observed;
- density of dendritic spines of hippocampal neurons is significantly decreased;
- there is a possibility of involvement of other than direct blood effect factors in the post-SAHel hippocampal damage.