TIME-COURSE PATTERN OF NEURONAL DEATH AND GLIOSIS IN GERBIL HIPPOCAMPI INDUCED BY MILD, SEVERE AND LETHAL TRANSIENT GLOBAL CEREBRAL ISCHEMIA

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Introduction

Transient global brain ischemia happens by particular ischemic conditions such as return of spontaneous circulation after cardiac arrest. It brings selective neuronal death/loss to specific vulnerable substructures (fields or areas) of the brain. Transient global brain ischemia happens by particular ischemic conditions such as return of spontaneous circulation after cardiac arrest. It brings selective neuronal death/loss to specific vulnerable substructures (fields or areas) of the brain. Among these structures in the hippocampus, pyramidal cells or neurons of the Cornu Ammonis (CA) 1 field will die several days after transient global brain ischemia. This is called “delayed neuronal death”.

Astrocytes perform many functions, including biochemical support of endothelial cells to constitute the blood-brain barrier (BBB), maintenance of extracellular ion balance, provision of nutrients to nervous tissues, and participation in repair of the central nervous system (CNS) following injuries. In the BBB, astrocytes form neurovascular coupling by provision of cellular link between neuronal circuitry and blood vessels.

Microglia are immune cells in the CNS that secrete various kinds of inflammatory cytokines in some conditions such as cerebral ischemia. In addition, microglia communicate with neurons in order to maintain healthy environment in the CNS. Both astrocytes and microglia are activated in pathological status of the CNS, such as ischemic insults and neurodegenerative diseases. They release various factors in order to contribute to neuronal death as well as neuronal survival when nervous tissues are under pathological status.

It is well known that just for 5 min of bilateral common carotid artery occlusion (BCCAO) in gerbils can cause death/loss of pyramidal neurons in CA1 field of the hippocampus 4-5 days later. Furthermore, spontaneous hyperactivity following 5 min of BCCAO is generally shown in gerbils.

Experimental purpose

Chronological alterations of neuronal loss and gliosis in the hippocampal formation, which consists of hippocampus proper (CA1-3 fields) and dentate gyrus, following mild, severe, or lethal transient ischemia have not been fully investigated yet. Therefore, we chose 5, 15 and 20 min of BCCAO as mild, severe and lethal ischemic injury, respectively, as previously reported and examined differences in degrees of behavioral alterations, neuronal death/loss, and gliosis in the hippocampus proper and dentate gyrus of gerbils subjected to 5, 15 and 20 min of BCCAO.

In this study, we modulated durations of transient ischemia to be 5, 15 and 20 min in order to figure out differences in impact of locomotor activity, neuronal death, and glial activation among the three groups by using CV staining, immunohistochemistry for NeuN, FJB histofluorescence and immunohistochemistry for GFAP and Iba1.

Materials and Methods

Experimental animals

- Mongolian gerbil (male, 6 months of age)

- 1) Sham group ($n=28$)
- 2) 5 min BCCAO operated group ($n=28$)
- 3) 15 min BCCAO operated group ($n=28$)
- 4) 20 min BCCAO operated group ($n=21$)

Immunohistochemistry

Mouse anti - NeuN, GFAP

Rabbit anti - Iba-1

Tissue staining

Fluoro-Jade B (F-J B)

Cresyl violet stining (CV)

BCCAO operation

Tissue processing for histology

Results

Fig. 1. CV staining in the hippocampal formation of the 5 min and 15 min BCCAO operated groups of gerbils at sham (A), 1 (B), 2 (C) and 5 (D) days after BCCAO. CV stained cells are not changed in all subfields at 1 day after BCCAO. At 2 days after BCCAO, CV stainability is significantly decreased in the pyramidal layer (PL) of the CA1 field of the 15 min BCCAO operated group of gerbils. Five days after BCCAO, CV stained cells are reduced in number in the PL of the CA1 field of the 5 min BCCAO operated group of gerbils, and CV stained cells of the 15 min BCCAO operated group of gerbils are reduced in the PL of the CA1-3 fields. DG, dentate gyrus; GCL, granule cell layer; ML, molecular layer; OL, oriens layer; PoL, polymorphic layer; RL, radiant layer. Scale bars = 400 μ m (Aa, Ba, Be, Ca, Ce, Da, De) and 100 μ m (Ab-Ad, Bb-Bd, Bf-Bh, Cb-Cd, Cf-Ch, Db-Dd, Df-Dh).

Fig. 2. (A-D) NeuN Immunohistochemistry in the hippocampal formation of the 5 and 15 min BCCAO operated groups at sham (A), 1 (B), 2 (C) and 5 (D) days after BCCAO. At 1 day after BCCAO, NeuN-ir neurons are not altered in all subfields. At 2 days after BCCAO, NeuN-ir neurons are decreased in the pyramidal layer (PL, asterisk) of the CA1 field alone. At 5 days after BCCAO, NeuN-ir neurons are rarely shown in the CA1 PL of both groups, and, in the 15 min BCCAO operated gerbils, they are significantly decreased in number in the CA2/3 PL, dentate polymorphic layer (PoL) and lower granule cell layer (GCL, asterisk). Scale bar = 100 μ m ($n = 7$ at each time after BCCAO).

Fig. 3. (A-D) FJB histofluorescence in the hippocampal formation of gerbils subjected to 5 and 15 min BCCAO at sham (A), 1 (B), 2 (C) and 5 (D) days after BCCAO. No FJB positive cells are shown in the sham. One day after BCCAO, FJB positive cells (arrows) are detected in the dentate polymorphic layer (PoL). Two days after BCCAO, FJB positive cells are additionally detected in the CA1 PL in the 15 min BCCAO operated gerbils. Five days after BCCAO, FJB positive cells in the 5 min BCCAO operated gerbils are significantly increased in the CA1 PL, and, FJB positive cells in the 15 min BCCAO operated gerbils are significantly increased in the CA1 PL, dentate PoL and granule cell layer (GCL). Scale bar = 100 μ m ($n = 7$ at each time after BCCAO).

Fig. 4. (A-D) Immunohistochemistry for GFAP in the gerbil hippocampal formation after 5 and 15 min BCCAO at sham (A), 1 (B), 2 (C) and 5 (D) days after BCCAO. One day after BCCAO, the distribution of GFAP-ir astrocytes is not significantly altered in all subfields. At 2 days after BCCAO, GFAP-ir cells are significantly hypertrophied in all subfields. Five days after BCCAO, GFAP-ir cells are significantly hypertrophied in all subfields of both groups. Scale bar = 100 μ m ($n = 7$ at each time after BCCAO).

Fig. 5. Immunohistochemistry for Iba1 in the gerbil hippocampal formation after 5 and 15 min BCCAO at sham (A), 1 (B), 2 (C) and 5 (D) days after BCCAO. At 1 day after BCCAO, Iba1-ir cells are hypertrophied in all subfields. At 2 and 5 days after BCCAO, Iba1-ir microglia are more hypertrophied in all subfields of both groups: in particular, they are much more hypertrophied in the 15 min BCCAO operated gerbils than the 5 min BCCAO operated group. Scale bar = 100 μ m ($n = 7$ at each time after BCCAO).

Conclusion

In summary, this research showed that 15 min of BCCAO evoked earlier neuronal death/loss earlier in hippocampal subfields than 5 min of BCCAO. Together with the neuronal death/loss, astrocytes and microglia were significantly proliferated and hypertrophied in hippocampal subfields after 15 min of BCCAO compared to those after 5 min of BCCAO. Furthermore, gerbils died in at least 2 days after 20 min BCCAO. This study implies that we must consider the degree of impact of ischemic duration when we treat transient brain ischemic injury.