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INTERACTION BETWEEN HYPERTENSION AND CEREBRAL HYPOPERFUSION IN THE DEVELOPMENT OF COGNITIVE DYSFUNCTION AND WHITE MATTER PATHOLOGY IN RATS

J. Y. CHOI, [a,b,c†] **Y. CUI** [a,c†] **AND B. G. KIM** [a,b,c*]

[a] *Department of Brain Science, Ajou University School of Medicine, South Korea*

[b] *Department of Neurology, Ajou University School of Medicine, South Korea*

[c] *Department of Biomedical Sciences, Ajou University Graduate School of Medicine, South Korea*

Abstract—Hypertension is the most significant modifiable risk factor for vascular cognitive impairment. However, influence of hypertension on the development of ischemic white matter injury and cognitive dysfunction is not fully understood. We compared cognitive functions and neuropathological outcomes of chronic cerebral hypoperfusion induced by bilateral common carotid artery occlusion (BCCAO) between normotensive rats (NRs) and spontaneously hypertensive rats (SHRs). SHRs developed earlier and more severe deficits in spatial memory performance than NRs following BCCAO. Although no significant changes in the gross structure of myelinated white matter or oligodendrocyte number were noted, BCCAO resulted in subtle myelin degeneration and paranodal structural alterations at the nodes of Ranvier, regardless of hypertension. Disruption of the blood–brain barrier (BBB) was predominantly observed in the white matter of SHRs following BCCAO, implying a role of hypertension in BBB dysfunction in chronic cerebral hypoperfusion. In chronic cerebral ischemia, long-standing hypertension may aggravate impairment of BBB integrity, and the leaky BBB may in turn exacerbate dysfunction in the white matter leading to worsening of spatial cognitive performance.

Key words: blood–brain barrier, hypertension, myelination, node of ranvier, white matter stroke.

INTRODUCTION

Axonal fibers in the white matter convey neural information between various cortical and subcortical structures. Therefore, injuries to the white matter impede efficient communications between groups of neurons engaged in diverse cognitive domains. It has been shown that ischemic injuries in the subcortical white matter are a frequent cause of cognitive decline in elderly individuals (de Groot et al., 2000; Schmidt et al., 2007). Moreover, vascular contribution to Alzheimer's dementia has been established (Sonnen et al., 2007; Gorelick et al., 2011; Toledo et al., 2013), warranting researches into the detailed pathomechanism underlying ischemic white matter injury in order to prevent age-related cognitive dysfunction. An animal model that can closely replicate the pathology and cognitive dysfunction observed in human white matter ischemia would be invaluable for such a mechanistic study. Yet there is no consensus on an optimal animal model for vascular cognitive impairment (Jiwa et al., 2010; Gorelick et al., 2011).

The vascular cognitive impairment due to subcortical white matter injury is frequently caused by modest but chronic reduction of cerebral blood flow (Pantoni, 2010). Chronic cerebral ischemia induced by bilateral common carotid artery occlusion (BCCAO) in rodents has been employed to mimic subcortical ischemic vascular dementia (Farkas et al., 2007). One potential pitfall of this model would be that vascular risk factors found in a majority of human patients with vascular cognitive impairment are not taken into consideration. Chronic cerebral hypoperfusion in human patients is not an isolated pathophysiologic entity, rather is mixed with various vascular risk factors including hypertension. Therefore, integrating vascular risk factors in animal models of chronic cerebral hypoperfusion should be justified to resemble human pathophysiology more closely.

The most significant modifiable risk factor for cerebral ischemia is hypertension. Many epidemiologic studies and clinical drug trials have emphasized the importance of hypertension on the development of the vascular cognitive impairment (Lindsay et al., 1997; Peila et al., 2006). Although several studies have combined a white matter ischemia model with hypertension (Masumura et al., 2001; Jalal et al., 2012), potential interactions between hypertension and chronic cerebral ischemia in the development of cognitive dysfunction and white matter pathology have not been systemically studied. The

*Correspondence to: B. G. Kim, Departments of Brain Science and Neurology, Neuroscience Graduate Program, Ajou University Graduate School of Medicine, San 5, Woncheon-dong, Yongtong-gu, Suwon, Gyeonggi Province 442-749, Suwon, South Korea. Tel: +82-31-219-4495; fax: +82-31-219-4530.

E-mail address: kimbg@ajou.ac.kr (B. G. Kim).

† These authors contributed equally to this work.

Abbreviations: BBB, blood–brain barrier; BCCAO, bilateral common carotid artery occlusion; CC, corpus callosum; d-MBP, degraded MBP; EB, Evans Blue; MWM, Morris Water Maze; NOR, novel object recognition; NRs, normotensive rats; ROIs, regions of interests; SHRs, spontaneously hypertensive rats.

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current study therefore compared cognitive and neuropathological outcomes after BCCAO in normotensive and spontaneously hypertensive rats (SHRs).

EXPERIMENTAL PROCEDURES

Animals and surgical procedures

Adult male 12-week-old Wistar rats and SHRs on the Wistar background were used in the present study. All animals were housed in the Animal Experiment Center at Ajou University School of Medicine throughout the entire experimental procedures. All animal protocols were approved by the Ajou University Institutional Animal Care and Use Committee. Animals were classified into four groups: (1) normotensive Wistar rats (NRs) with sham operation, (2) NR with BCCAO, (3) SHR with sham operation, and (4) SHR with BCCAO. A total of 34 rats were used for the assessment of both behavioral and pathological outcomes (NR sham = 8, NR BCCAO = 7, SHR sham = 9, and SHR BCCAO = 10). To induce BCCAO, rats were anesthetized with chloral hydrate (400 mg/kg, intraperitoneal injection), and a midline neck incision was performed. Careful dissection was carried out to separate the vagus nerve from the common carotid artery (CCA) on each side. Dissected CCAs on both sides were occluded with 4-0 black silk with double ties. Sham operation rats were subjected to the same procedure but without CCA occlusion. After surgery, animals were assigned new identification codes by an experimenter (YC) to ensure blinded evaluation of behavioral performance by a different experimenter (JYC). A separate group of animals was used to assess leakage of the blood–brain barrier (BBB) using Evans Blue (EB) dye (N = 20, five animals per group). EB dye (3%, 1 ml) was injected 30 min before perfusion fixation through the tail vein 4 weeks after surgery.

Behavioral assessment

All behavioral tests were performed by an experimenter (JYC) who was blinded to the experimental grouping. Before sham or BCCAO operation, animals were pretrained for the Morris Water Maze (MWM) for 5 days and exposed to the Y-maze and the apparatus for the novel object recognition (NOR) test for 5 min each for 5 days.

Y-maze. The Y-maze test was performed 3 weeks after surgery. In order to have animals familiarized to the maze, animals were placed onto the Y-maze for 5 min per day for five consecutive days before the test day. The Y-maze consisted of three arms (each 50 cm long, 10 cm wide, and 15 cm deep) diverging at a 120° angle from the central point. Spontaneous alternation behavior was measured with some modification of a previously described procedure (Hughes, 2004). Briefly, rats were placed randomly in one of the three arms and then allowed to move freely into any of the three arms for 8 min. The sequences of entries into the arms were recorded and analyzed in overlapping triplet sets. Spontaneous alternation behavior was defined as entry into all three arms in a consecutive sequence. The percent alternation was calculated as the proportion of alternations out of the number of alternation opportunities. For example, if a sequence of entries into arms labeled A, B, and C is ABCBCABC, for example, the number of alternation opportunities would be six (total entries minus two, because the first entry is determined by an experimenter and alternation behavior cannot be determined until the third entry), the number of alternations is four (ABC, BCA, CAB, ABC), and the percent alternation is 66.7% ($100 \times 4/6$).

NOR test. The NOR test was performed 3 weeks after surgery using a box with 65 cm length, 45 cm width, and 45 cm height. After habituation to the apparatus, rats were familiarized to two identical objects in the box for 5 min per day for a total of 5 days. On the test day, rats were placed in the test box in which the two familiar objects were placed with one at each corner for 5 min. One and a half hours after familiarization, one of the objects was replaced with a novel object of a similar size but with different morphology. Rats were allowed to explore the apparatus again with the novel object. Exploration of the novel object was defined as sniffing or touching with snout or forepaw within 2 cm of the novel object with snout oriented toward the novel object. All explorations during the test period were recorded and analyzed using the SMART video tracking system (Panlab, Barcelona, Spain). The discrimination index was calculated using the following formula: $100 \times$ (time for the novel object − time for the familiar object)/(time for the novel object + time for the familiar object).

MWM test. The MWM test was performed 2 and 4 weeks after surgery. The MWM apparatus was comprised of a large pool (200 cm in diameter and 50 cm high), a platform (10 cm in diameter) and several extramaze cues in the test room visible to animals. The platform was submerged to 1.5 cm below the opaque water surface at a fixed location in the pool. In the pretraining and acquisition period, rats were trained in six trials each day for five consecutive days and the final data were obtained on the 6th day. In each trial, rats were placed in the pool at one of three starting positions and allowed to swim until finding the platform for up to 1 min. Once rats located and escaped to the platform, they were allowed to remain on it for 20 s. If animals failed to find the platform within 60 s, they were guided to the platform and allowed to remain on it for 20 s. The swimming movement in the pool was recorded and analyzed using with the SMART video tracking system (Panlab, Barcelona, Spain). The time taken for a rat to escape to the platform was recorded as the escape latency. For the probe trial, the platform was removed from the pool and rats were allowed to swim for 60 s. The time spent in the quadrant that previously harbored the platform (time in quadrant) was recorded for each rat.

Tissue processing and immunohistochemistry

All rats were perfused 1 day after the 4-week MWM test had been completed. Rats were anesthetized deeply