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White matter damage and glymphatic dysfunction in a model of vascular dementia in rats with no prior vascular pathologies

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ABSTRACT

We investigated cognitive function, axonal/white matter (WM) changes and glymphatic function of vascular dementia using a multiple microinfarction (MMI) model in retired breeder (RB) rats. The MMI model induces significant ($p < 0.05$) cognitive decline that worsens with age starting at 2 weeks, which persists until at least 6 weeks after MMI. RB rats subjected to MMI exhibit significant axonal/WM damage identified by decreased myelin thickness, oligodendrocyte progenitor cell numbers, axon density, synaptic protein expression in the cortex and striatum, cortical neuronal branching, and dendritic spine density in the cortex and hippocampus compared with age-matched controls. MMI evokes significant dilation of perivascular spaces as well as water channel dysfunction indicated by decreased Aquaporin-4 expression around blood vessels. MMI-induced glymphatic dysfunction with delayed cerebrospinal fluid penetration into the brain parenchyma via paravascular pathways as well as delayed waste clearance from the brain. The MMI model in RB rats decreases Aquaporin-4 and induces glymphatic dysfunction which may play an important role in MMI-induced axonal/WM damage and cognitive deficits.

1. Introduction

Vascular dementia (VaD) is a progressive disease that affects cognition and memory. VaD accounts for nearly 20% of all dementia patients and is the second leading form of dementia after Alzheimer's disease (Plassman et al., 2007). VaD, also known as multi-infarct dementia, is caused by a decrease in blood flow to the brain and is typically associated with a cerebrovascular accident such as a stroke or series of minor strokes. In multi-infarct dementia, emboli composed of cholesterol or of fragments of atheromatous plaques or thrombus, typically lodge in and block small arteries and induce multiple microinfarcts in the cortex and striatum (Venkat et al., 2015). Atheroemboli are the most common type of emboli encountered in VaD. The multiple microinfarction (MMI) model using cholesterol crystals closely mimics atheroembolization. MMI in rodents induces cognitive dysfunction and pathological features similar to human VaD, and induces delayed demyelination, hippocampal damage, blood brain barrier damage, and inflammation (Laloux and Brucher, 1991; Rapp et al., 2008b; Steiner et al., 1980; Venkat et al., 2015).

In this study, we employ a previously described and accepted MMI model using cholesterol crystals to induce VaD (Rapp et al., 2008a,b; Wang et al., 2012) and investigate for the first time selective pathophysiological events driving white matter (WM) damage and cognitive deficits.

Aquaporins are integral membrane pore proteins that transport and regulate water movement in the brain. Aquaporin-4 (AQP-4) is predominantly present in astrocytic endfeet near capillaries and in cells lining the ventricles which are key sites for water movement between the cellular, vascular, and ventricular compartments (Papadopoulos et al., 2002). The glymphatic system is an effective waste clearance pathway that removes metabolic wastes and neurotoxins from the brain along paravascular channels (Jessen et al., 2015). The paravascular space is filled with cerebrospinal fluid (CSF) which flows parallel to blood flow (Jessen et al., 2015). Around penetrating vessels, paravascular spaces take the form of Virchow–Robin spaces, also called perivascular space. While the perivascular spaces terminate within the brain parenchyma,

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paravascular CSF can continue traveling along the basement membranes surrounding arterial vascular smooth muscle, to reach the basal lamina surrounding brain capillaries. CSF movement along these paravascular pathways is rapid, and arterial pulsation been implicated as a major driving force for paravascular fluid movement (Rennels et al., 1985). AQP-4 mediated water channels facilitate extensive movement of CSF into the brain, CSF-ISF (interstitial fluid) exchange and interstitial solute clearance, and postinjury edema formation and resolution (Badaut et al., 2011; Iliff et al., 2014). Disruption of the AQP-4–mediated water channel and failure of the glymphatic system burdens the brain with accumulating waste and has been reported in aging as well as several neurological diseases such as Alzheimer's disease, stroke, and diabetes (Jessen et al., 2015; Jiang et al., 2016). However, whether glymphatic dysfunction and associated decreased AQP-4 expression influences vascular dementia has not been investigated.

In this study, we investigated cognitive deficits induced by an MMI-based VaD model in young and retired breeder (RB) male rats and its underlying disease mechanisms such as axonal/WM damage, synaptic disruption, as well as water channel and glymphatic system dysfunction.

2. Materials and methods

All experiments were conducted in accordance with the standards and procedures of the American Council on Animal Care and Institutional Animal Care and Use Committee of Henry Ford Health System.

2.1. Cholesterol crystals preparation and MMI model

In this study, we employ a previously described MMI model. Cholesterol crystals were freshly prepared (Rapp et al., 2008a,b; Wang et al., 2012) (summarized in Supplementary Fig. 1) and were filtered using 100-μm cell strainer with filtrate passed through a 70-μm cell strainer to collect residual crystals of size 70–100 μm. The crystals were counted on a hemocytometer and diluted to yield a final concentration of 500 ± 100 crystals/300 μL saline/per rat.

Male young adult (young, 3–4 months) and retired breeder (RB, 6–8 months) Wistar rats were subjected to the MMI model, as previously described (Rapp et al., 2008a,b; Wang et al., 2012). Briefly, rats were anesthetized with 2% isoflurane in a jar for preanesthetic, and spontaneously respired with 1.5% isoflurane in 2:1 N_2O:O_2 mixture using a facemask connected and regulated with a modified FLUOTEC 3 Vaporizer (Fraser Harlake, Orchard Park, NY, USA). Rectal temperature was maintained at 37 °C throughout the surgical procedure using a feedback regulated water heating system. A midline neck incision was made and the right common carotid artery (CCA), external carotid artery (ECA), and internal carotid artery (ICA) were exposed under an operating microscope. Carefully avoiding the vagus nerve, the CCA and ICA were temporarily clamped using microsurgical clips and a 5–0 silk suture was tied loosely at the origin of the ECA and ligated at the distal end of the ECA. A 1-mL syringe connected to a polyethylene-50 tube, with its tip tapered by heating near a flame was inserted into the ECA through a small incision made with micro scissors. The tube was gently advanced from the ECA into the lumen of the ICA and the microsurgical clip was repositioned to only block the CCA. The freshly prepared cholesterol crystals were slowly injected into the ICA over a minute. The tube was gently removed, ECA ligated, microsurgical clips removed, and the neck incision was closed. The animals were moved to their home cages to awaken. A battery of cognitive tests were performed, and rats were sacrificed at 4 weeks after MMI. Additional sets of RB rats were prepared and sacrificed at the second, fourth, and sixth week after MMI to assess VaD progression (N = 6/group). Naive age-matched controls were employed throughout this study (N = 6/group).

2.2. Function tests

An investigator blinded to the experimental groups performed a battery of neurological function and cognitive tests.

Neurological function tests: modified neurological severity score (mNSS) is a composite of motor, sensory, balance, and reflex tests with scores between 0 and 18 (normal score 0; maximal deficit score 18) (Chen et al., 2001). mNSS was performed at days 1, 7, 14, 21, and 28 after MMI.

Cognitive tests: the novel object recognition test (Stuart et al., 2013) with a retention delay of 4 hours was carried out to assess visual learning and short-term memory based on animal bias to explore new objects. The odor test (Spinetta et al., 2008) that evaluates olfactory learning and memory based on an animal's preference for new smells was conducted with a retention delay of 24 hours and used to test long-term memory. An open field evaluation (Brown et al., 1999) was performed for 5 minutes to assess locomotor activity and anxiety-like behavior. The Morris water maze test (Darwish et al., 2012) was used to evaluate spatial and visual learning and memory with averse motivation to assess hippocampal memory deficits.

2.3. Histological and immunohistochemical assessment

The animals were sacrificed and transcardially perfused with 0.9% saline. Brains were immediately removed and fixed in 4% paraformaldehyde. Seven coronal sections of tissue were processed and stained with hematoxylin and eosin for identification of infarctions. Brain coronal tissue sections were prepared and antibody against NG2 (oligodendrocyte progenitor cell [OPC] marker, Chemicon [EMD Millipore], Billerica, MA, USA; 1:400), synaptophysin (synaptic protein, Abcam, Cambridge, MA, USA; 1:400), fluorescein isothiocyanate (FITC)–labeled AQP-4 (EMD Millipore 1:1500) were used. BS (Bielschowsky silver, axon marker), LFB (Luxol fast blue, myelin marker), and Golgi staining (FD NeuroTechnologies, Columbia, MD, USA; Rapid Golgi stain kit, and manufacturer's protocol) were used. Control experiments consisted of staining brain coronal tissue sections as outlined above, but nonimmune serum was substituted for the primary antibody. In addition, internal positive controls were also employed.

2.4. Quantification analysis

Slides from each brain containing 4 fields of view of ipsilateral striatum, cortex, or corpus callosum were digitized under a 20× objective (Olympus BX40) using a 3-charge-coupled devices color video camera with an MCID image analysis system as indicated in Fig. 1A close to regions of expected damage. In the WM bundles of the striatum and/or corpus callosum, using MCID image analysis the numbers of immunoreactive cells were counted or positive stained areas were measured (densitometry function) with a density threshold above unstained set uniformly for all groups (Chen et al., 2016; Yan et al., 2014, 2015). All striatal vessels with diameter >10 μm were selected without bias and perivascular space was quantified as previously described (Ampawong et al., 2011). To evaluate water channel dysfunction, AQP-4 positive areas were measured around these blood vessels under 40× magnification. Neurite branching was counted under a 40× objective and 10 intact neurons from the layer III of cortex were chosen and primary and secondary branching counted. For evaluation of spine density, 10