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The quantification of blood-brain barrier disruption using dynamic contrast-enhanced magnetic resonance imaging in aging rhesus monkeys with spontaneous type 2 diabetes mellitus

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ABSTRACT

Microvascular lesions of the body are one of the most serious complications that can affect patients with type 2 diabetes mellitus. The blood-brain barrier (BBB) is a highly selective permeable barrier around the microvessels of the brain. This study investigated BBB disruption in diabetic rhesus monkeys using dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI). Multi-slice DCE-MRI was used to quantify BBB permeability. Five diabetic monkeys and six control monkeys underwent magnetic resonance brain imaging in 3 Tesla MRI system. Regions of the frontal cortex, the temporal cortex, the basal ganglia, the thalamus, and the hippocampus in the two groups were selected as regions of interest to calculate the value of the transport coefficient K^{trans} using the extended Tofts model. Permeability in the diabetic monkeys was significantly increased as compared with permeability in the normal control monkeys. Histopathologically, zonula occludens protein-1 decreased, immunoglobulin G leaked out of the blood, and nuclear factor E2–related factor translocated from the cytoplasm to the nuclei. It is likely that diabetes contributed to the increased BBB permeability.

1. Introduction

A stable supply of nutrients and oxygen from the capillaries to the brain tissue is essential to develop and sustain neurons, and it is critical to maintain homeostasis in the brain to protect neurons from circulatory toxins. Physiologically, the blood-brain barrier (BBB) keeps the balance between the nutrient supply and toxic invasion from the circulation. The BBB acts as a physical and metabolic barrier that restricts the penetration of molecules, unless that they are both lipid soluble and have molecular weights of <400 Da (Samiotaki et al., 2012). The BBB usually consists of cerebral endothelial cells, the basal lamina, pericytes, astrocyte endfeet, and axonal projections (Abbott et al., 2010). The BBB is characterized by tight junctions between endothelial cells, and these junctions significantly reduce the permeability of macromolecules and most polar solutes. Tight junctions are maintained by transmembrane protein occludins and claudins that are linked to a number of intracellular scaffolding proteins (e.g., zonula occludens protein-1 [ZO-1], ZO-2, ZO-3) and cingulins. These proteins constitute the initial barrier that prevents entry into the endothelial cells between the blood and the brain cells (Wolburg and Lippoldt., 2002). In addition, there are adherent junctions, which are composed mainly of vascular endothelium cadherin and which play a significant role in the maintenance of tight junctions (Obermeier et al., 2013). Perivascular astrocytes are also thought to be critical to the maintenance of BBB function, because astrocyte endfeet are abundant in water channel aquaporin 4. This factor may endow the BBB with the ability to regulate the volume of the brain (Haj-Yasein et al., 2011). The astrocytic expression of vascular endothelial growth factor-A is also a key driver of BBB permeability (Argaw et al., 2012). The endothelial cell phenotype in BBB is dependent on astrocyte-derived signals and transforming growth factor-β1 (Hawkins et al., 1608). Pericytes can constrict capillaries, change blood flow, and regulate angiogenesis (Kutcher and Herman, 2009).

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The BBB provides a stable environment for neural and synaptic signaling and thus protects the central nervous system against serum neurotoxins (Lim et al., 2007). However, this does not necessarily mean that the permeability of the BBB is constant. In fact, it is susceptible to regulation and damage caused by diseases such as cerebral ischemia or hemorrhage, stroke, hypertension, inflammation, tumors, hyperlipidemia, and diabetes (Yang and Rosenberg, 2011).

An increasing amount of evidence from recent basic and clinical studies has suggested that ongoing type 2 diabetes mellitus (T2DM) may give rise to a propulsive impairment of neuronal functions (Kerti et al., 2013). In response to oxidative stress, the nuclear factor E2-related factor (Nrf-2), which is the transcription factor of the nuclear factor, partially shifts from the cytoplasm into the nucleus and activates the expression of genes with antioxidant response. T2DM has been reported to be significantly involved in the development of Alzheimer's disease (Holscher, 2011) and deterioration after stroke as a result of the metabolic and cerebrovascular abnormalities of hyperglycemia and BBB disruption, respectively, with hyperglycemia being the leading cause of BBB disruption in diabetic patients (Starr et al., 2003; Hawkins et al., 2007). BBB leakage may also lead to inflammation, the imbalance of electrolytes, edema, and, eventually, neuronal dysfunction and degeneration (Chodobski et al., 2011).

Despite the large number of studies of T2DM-reduced microvascular complications of the kidney and the retina, the pathophysiologic relationship of T2DM with its cerebrovascular complications and the relevant encephalic neural diseases has not been fully elucidated. Because the BBB is the gatekeeper of the brain and the victim of diabetes-induced encephalic complications, it is extremely important to quantify the BBB's permeability in patients with T2DM.

The extravasation of Evans Blue is the traditional histopathological method that has been used to assess BBB disruption; however, its use involves the sacrifice of animals, and it does not allow for longitudinal quantification (Poittevin et al., 2015). In addition, it has been reported that the BBB transport coefficient that reflects its permeability, K^{trans}, can be measured with the use of dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI). During DCE-MRI, a contrast agent is injected into the blood flow before the acquisition of a series of T1-weighted images with fast imaging techniques. The signal intensity of the voxels in regions of interest (ROIs) changes over time as a result of the changed contrast agent concentration. The temporal and spatial distributions of the contrast agent depend largely on plasma flow, tissue perfusion, vessel permeability, and extracellular and extravascular volumes. Therefore, it can reflect detailed information about the anatomy, structure, and function of the ROIs (Larsson et al., 2009). DCE-MRI in combination with tracer kinetic modeling is widely used to assess perfusion and permeability in patients with conditions such as cancer (Singanamalli et al., 2015; Vos et al., 2013; Drisis et al., 2015), stroke (Heye et al., 2016; Aksoy et al., 2013), heart disease (Tarroni et al., 2012), aging (Montagne et al., 2015; Iadecola, 2015), demyelinating diseases (Cramer et al., 2014; Cramer et al., 2015) and dementia (van de Haar et al., 2015).

Unlike Evans Blue extravasation, DCE-MRI can be used to perform a non-invasive longitudinal assessment of an impaired BBB (Li et al., 2014), which may provide a promising diagnostic and therapeutic index for doctors. In the current study, we hypothesized that T2DM may cause BBB disruption, so we explored the influence of T2DM on the cerebral microvessels.

2. Materials and methods

2.1. Reagents and apparatus

The following supplies were used to complete this study:

- Isoflurane (United States Pharmacopeia grade 100%; RWD Life Science, San Diego, CA, USA)
- Injection gadopentetate dimeglumine (Magnevist, 469.01 mg/mL, 15 mL; Bayer Schering Pharma, Berlin, Germany)
- Pentobarbital sodium (P3761; Sigma-Aldrich, St. Louis, MO, USA)
- Anesthesia ventilator (Matrx Model 3000; Midmark Animal Health, Versailles, OH, USA)
- Anti–zonula occludens protein-1 antibody (1:100; Abcam, Cambridge, MA, USA)
- Anti–glial fibrillary acidic protein antibody (1:400; Abcam)
- Anti–the nuclear factor E2-related factor antibody (1:100; Abcam)
- Anti–Neuronal Nuclei antibody (1:100; Zhongshan Jinqiao Biological Technology, Beijing, China)
- Anti–immunoglobulin G antibody (1:100; Zhongshan Jinqiao Biological Technology, Beijing, China)
- Alexa Fluor 488 (goat anti-chicken, 1:100; Abcam)
- 647-labeled secondary antibodies (goat anti-chicken, 1:100; Abcam)

2.2. Ethics

All of the rhesus monkeys used in this study were provided by Sichuan Primed Bio-Tech Group Co., Ltd., of Chengdu, China. Our methods fully met the requirements of the National Institutes of Health Guide for the Care and Use of Laboratory Animals and of the Association for Assessment and Accreditation of Laboratory Animal Care. This experiment was also in accordance with the protocols of and was approved by the Experimental Animal Ethics Committee of the West China Hospital, Sichuan University, Chengdu 610041, China. This experiment was reported in compliance with the ARRIVE guidelines (Animal Research: Reporting in Vivo Experiments).

2.3. Animals

As in human beings, insulin resistance and hyperinsulinemia occur in certain middle-aged monkeys, and eventually these changes can lead to T2DM (Wagner et al., 2006; Okabayashi et al., 2015). We selected monkeys with spontaneous T2DM as our study objects, and they were held in stainless steel pair-housing monkey cages (size, 2 m × 1.7 m × 2 m; lighting cycle, 12 h of day/12 h of night; temperature: 18 °C to 26 °C; humidity, 40% to 70%; ventilation rate: 10 air exchanges per hour). The animals were all fed standard monkey fodder (calories provided from protein, 17%; from fat, 18%; and from carbohydrate, 65%), with approximately 300 g given to each monkey each morning. All of the monkeys had free access to both the fodder and the water. The inclusive criteria for the monkeys with T2DM included an age of 10 to 20 years, a fasting plasma glucose level of 5.5 mmol/L or more, and a glycated hemoglobin level of 4.5% or more (Ding et al., 2007; Gong et al., 2013; Hansen, 2012).

Five rhesus monkeys with T2DM (one female and four males; age, 13.20 ± 2.39 years; duration of T2DM, 3.40 ± 1.67 years; fasting plasma glucose level, 7.67 ± 1.77 mmol/L; glycated hemoglobin level, 6.12% ± 2.63%), three of which had a family history of T2DM, and six age-matched, healthy monkeys with well-controlled glucose levels (all male; age, 13.67 ± 3.14 years; fasting plasma glucose level, 4.60 ± 0.35 mmol/L; glycated hemoglobin level, 4.28% ± 0.16%) were selected. Because the monkeys had free access to the fodder, it was difficult to accurately measure the plasma glucose level 2 h postprandially. None of the monkeys had a history of medical treatment. The general condition and related blood biochemical indicators of all of the monkeys are shown in Table 1.

2.4. Magnetic resonance imaging acquisition and sequence parameters

All of the MRI examinations were performed on a 3 Tesla MRI system (Magnetom TrioTim; Siemens, Erlangen, Germany). The right antecubital vein passage was established in each animal before scanning began. The animals were anesthetized via the intravenous injection of 3% pentobarbital sodium, and this was followed by tracheal intubation with