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TYPE 2 DIABETES REDUCES THE PROLIFERATION AND SURVIVAL OF OLIGODENDROCYTE PROGENITOR CELLS IN ISHCHEMIC WHITE MATTER LESIONS

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Abstract—Diabetes mellitus (DM) is a major risk factor for stroke and it exacerbates tissue damage after ischemic insult. Diabetes is one of the important causes of the progression of white matter lesion, however, the pathological mechanisms remain unclear. The present study evaluated the influences of type 2 DM on ischemic subcortical white matter injury and the recruitment of oligodendrocyte progenitor cells (OPCs) under chronic cerebral hypoperfusion using type 2 diabetic (*db*/*db*) mice. After bilateral common carotid artery stenosis (BCAS), the rarefaction in the white matter was more severe in *db*/*db* mice than in *db*/+ mice, and the number of glutathione S-transferase-pi (GST-pi)-positive mature oligodendrocytes (OLG) was lower in *db*/*db* mice than in *db*/+ mice at 4 and 8 weeks after ischemia. There were no significant differences in the number of single-stranded DNA (ssDNA)-positive apoptotic cells in the deep white matter between the *db*/*db* and *db*/+ mice. We found a transient increase in the platelet-derived growth factor receptor-α (PDGFRα)-positive OPCs in white matter lesions after ischemia. However, significantly fewer PDGFRα-positive OPCs were detected in *db*/*db* than *db*/+ mice from 4 weeks after BCAS. The number of Ki67-positive proliferating cells in the deep white matter was significantly lower in *db*/*db* mice than in *db*/+ mice from 4 to 8 weeks after BCAS. Most of the Ki67-positive cells were PDGFRα-positive OPCs. Finally, we assessed the survival of 5-bromo-2′-deoxyuridine (BrdU)-positive proliferating cells in ischemic white matter, and found significantly poorer survival of BrdU/PDGFRα-positive OPCs or BrdU/GST-pi-positive OLGs in the *db*/*db* mice compared to the *db*/+ mice in the white matter after BCAS. Our findings suggest that the type 2 DM mice exhibited more severe white matter injury 8 weeks after chronic ischemia. Decreased proliferation and survival of OPCs may play an important role in the progression of white matter lesions after ischemia in diabetics.

Key words: diabetes, white matter lesion, oligodendrocyte progenitors, chronic ischemia, dementia.

INTRODUCTION

Diabetes mellitus (DM) is one of the most important risk factors for cardiovascular disease and ischemic stroke (Emerging Risk Factors Collaboration et al., 2010). The incidence of not only diabetes, but also stroke patients with comorbid diabetes, continues to increase worldwide (Towfighi et al., 2012; Holman, 2013). Patients with diabetes are known to have a poor short-term prognosis and worse functional recovery after stroke (Megherbi et al., 2003; Ergul et al., 2009; Tanaka et al., 2013). Moreover, the diabetic patients with cerebral ischemia are younger than their non-diabetic counterparts, with greater mortality and slower recovery (Jorgensen et al., 1994). Furthermore, several epidemiological studies have demonstrated that diabetes and insulin resistance are related to dementia, including vascular dementia and Alzheimer's disease (Ott et al., 1999; Ohara et al., 2011).

It has been reported that diabetes correlates with a larger white matter lesion volume than that found in non-diabetic controls in an imaging study (Jongen et al., 2007). Diabetes and high blood glucose concentrations are important risk factors for the progression of white matter lesions (Gouw et al., 2008). Such white matter lesions are considered to be important risk factors for cognitive decline and the future occurrence of dementia (Debette and Markus, 2010). Several experimental studies of acute ischemic stroke have confirmed the presence of more extensive tissue damage and worse functional impairment in diabetic animals compared with non-diabetic animals (Kumari et al., 2007; Tureyen et al., 2011). However, the impact of diabetes on the extent of white matter lesions caused by chronic cerebral ischemia remains unknown.

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Abbreviations: BCAS, bilateral common carotid artery stenosis; BGA, blood gas analysis; BrdU, 5-bromo-2′-deoxyuridine; CBF, cerebral blood flow; CC, corpus callosum; DCX, doublecortin; DM, diabetes mellitus; GFAP, glial fibrillary acidic protein; GST-pi, glutathione S-transferase-pi; Iba-1, ionized calcium binding adapter molecule-1; OLG, oligodendrocyte; OPC, oligodendrocyte progenitor cell; PBS, phosphate-buffered saline; PDGFRα, platelet-derived growth factor receptor-α; ssDNA, single standard DNA; SVZ, subventricular zone.

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The genetically altered *db*/*db* mouse (Hummel et al., 1966) manifests adult-onset type 2 DM and hence is a useful animal model for studying the pathophysiological mechanisms related to diabetes. The *db*/*db* mice have a point mutation in the ''diabetic gene'' (*db*), which encodes a G-to-T mutation of the leptin receptor, with a resultant truncated, nonfunctional protein (Chen et al., 1996; Lee et al., 1996) that produces a phenotype of diabetic hyperinsulinemia and hyperglycemia (Coleman, 1982). The mice are characterized by significant hyperglycemia with hyperphagia, hyperinsulinemia and obesity by 6 weeks of age.

In this study, the main hypothesis was that diabetes accelerates the white matter injury caused by chronic brain ischemia. To confirm this hypothesis, we analyzed white matter lesions at different time intervals after chronic cerebral hypoperfusion induced by bilateral common carotid artery stenosis (BCAS) in *db*/*db* mice. We also investigated the cell death, proliferation and survival of oligodendrocyte progenitor cells (OPCs) in this area, because these cells are thought to contribute to the tissue damage after BCAS.

EXPERIMENTAL PROCEDURES

Animals and experimental design

Adult male *db*/*db* mice (*C57BLKS*/*Jm* + / + *Leprdb*/*db*; type 2 diabetic) and *db*/ + (normoglycemic genetic controls of *db*/*db*) mice (both eight-weeks-old) were purchased from Charles River Institute (Kanagawa, Japan) and were maintained on a 12-h light/dark cycle with free access to food and water. To produce constriction of both common carotid arteries, micro coils were prepared from piano strings with an inner diameter of 0.18 mm, pitch of 0.50 mm and total length of 2.5 mm (Samini Co., Shizuoka, Japan), as described previously (Shibata et al., 2004). The mice were anesthetized with 1–2% isoflurane in 30% oxygen, and then anesthesia was maintained with 70% nitrous oxide. During surgery, a temperature probe was inserted into the rectum, and a heat lamp was applied to maintain the body temperature at 37.0–37.5 °C using a heating pad. The blood pressure was monitored using a noninvasive tail-cuff system (Softron BP-98A NIBP, Softron Inc., Tokyo, Japan). Through a midline incision, each common carotid artery was carefully separated from the cervical sympathetic and vagal nerves, and wound with the microcoils just below the level of the carotid bifurcation to induce BCAS. The mice in the sham (pre-operation) group underwent the same surgery but no microcoils were used.

Twenty mice in each group were examined for histological changes and were maintained in cages and provided with food and water *ad libitum*. At weeks two, four and eight after BCAS, the mice were re-anesthetized with 1% isoflurane and 70% N_2O:30% O_2, and then were transcardially perfused with phosphate-buffered saline (PBS) and 4% paraformaldehyde. The brain was dissected out immediately, postfixed in 4% paraformaldehyde for 24 h, and stored in 30% sucrose in 0.1 M PBS. For immunohistochemistry, 20 μm-thick free-floating coronal sections of the corpus callosum (CC) were prepared for staining.

All animals were acquired and cared for according to the guidelines published by the National Institutes of Health Guide for Care and Use of Laboratory Animals. All experiments described in this study were conducted after obtaining the approval of the Animal Care Committee of Juntendo University.

Measurement of the cerebral blood flow (CBF)

Following BCAS, the CBF was measured in a left temporal window using laser Doppler flowmetry (Laser tissue Blood Flow Meter FLO-C1; Omega Wave, Inc., Portland, OR, USA). The probe, in the shape of a straight rectangular sheet (7.5 mm in length and 1.0 mm in-depth), was positioned between the temporal muscle and the lateral aspect of the skull, as described previously (Harada et al., 2005). In these experiments, there was no need for craniotomy. The CBF was monitored continuously for 3–5 min, before, immediately after, and at weeks two, four and eight after BCAS. Reproducible recorded CBF velocities were obtained.

Blood gas analysis (BGA)

As a preliminary analysis, we performed BGA before and after BCAS. The BGA was performed using the i-STAT system and EG7 + cartridges (Abbott Point of Care Inc., Princeton, NJ, USA). The external carotid artery was used for the collection of blood samples before and immediately after BCAS from anesthetized mice and the pH, pCO_2, pO_2 and bicarbonate levels were measured. Approximately 75 μl of whole blood were collected in a heparinized precision capillary tube (75 mm in length, inner diameter of 1.1–1.2 mm, Drummond Scientific Company, Broomall, PA, USA) to prevent clotting. Anticoagulated blood was expressed into the wells of the cartridges within 30 min of collection. The values were read on an i-STAT Portable Clinical Analyzer within 2 min, and the results were printed by the infrared transmission of information to the system printer.

A one-way analysis of variance (ANOVA) followed by Tukey's post hoc tests was used to examine the differences in various parameters before and immediately after BCAS. A p value < 0.05 denoted the presence of a statistically significant difference.

5-bromodeoxyuridine labeling

To determine the phenotype and survival rate of the proliferating and dividing cells, 5-bromo-2′-deoxyuridine (BrdU) (Sigma–Aldrich, St Louis, MO, USA) was dissolved in saline and then injected intraperitoneally (50 mg/kg) three times at 4-h intervals. The BrdU was injected at 2 weeks after BCAS, and then the mice were sacrificed 2 weeks later (4 weeks after BCAS, each group $n = 5$).

Immunohistochemistry

Immunohistochemistry was performed on 20 μm-thick free-floating coronal sections, which were prepared as described previously (Miyamoto et al., 2010). Briefly, after incubation in 3% H_2O_2 followed by 10% block ace in 0.1%