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Anatomical and Metabolic Changes in the Visual Cortex of Streptozotocin-Treated Type 1 Diabetic Rats

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Abstract

Diabetic retinopathy (DR) is one of a major complication of type 1 diabetes mellitus (T1DM) and a leading cause of blindness. People with DM often have vision impairments. Evidence of animal study showed that it is not only a microvasucular lesion of the eye, but also a neurodegeneration disease of the visual system. There were many reports about the brain atrophy and perturbation of cerebral metabolism induced by DM, and the visual cortex maybe involved. In this study, type 1 diabetes was induced in rats by a single-dose intraperitoneal injection of streptozotocin (STZ). High resolution anatomical images were scanned with a RARE sequence and in vivo 1H spectra of the visual cortex were acquired by a PRESS sequence at 12 weeks after induction. The STZ-treated rats showed significantly increased myo-inositol (mI)/creatine (Cre) ratio, and significantly reduced N-acetyl aspartate(NAA)/Cre ratio and thickness in the visual cortex. These results indicated that chronic diabetic complications involve anatomical and metabolic changes in the visual cortex.

Keywords

T1DM, MRS, STZ, Visual Cortex Thickness, Metabolites.

1. Introduction

Diabetes mellitus (DM) is a life-long metabolic disturbance. Diabetic retinopathy (DR) is one of a major complication of chronic diabetes and a leading cause of blindness [1]. Chronic diabetic patients often show impaired contrast sensitivity, deficits in perceptive resolution, color vision and night adaptation, and even visual loss [1]. Clinical neuroimaging studies have revealed that chronic diabetic complications can cause reduced grey matter density in the right occipital lobeand reduced fractional anisotropy in the optic radiation in type 1 DM patients [2,3]. Evidence of diabetic animal studies also reported that müller cell, astrocyte, microglial cell dysfunction and neuron loss [4]. Region-specific cerebral metabolic changes have been reported in early study [5].

As aforementioned, the progressive visual field defects caused by DR might prevent normal stimulation of visual cortex. A growing body of evidence suggested that progressive visual field defects would cause plasticity and adaptive changes at the brain level, especially to the visual cortex. Previous MRI studies of blindness also reported decreased gray matter density [6-8] and reduced anisotropy in the occipital lobe [9,10]. Besides, visual field defects such as glaucoma, can result in the visual cortex metabolites changes, in the glaucoma model study, the Choline(Cho)/Cre ratio in the visual cortex are decreased [11]. In the clinical research, the occipital cortex of the blind person

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have increased mI/ Cre ratio without cortex thickness change [12]. So it comes to speculate that brain plasticity and adaptive changes will happened when receive abnormal visual stimulations caused by DM, behaving as anatomical changes and/or metabolite disturbance in the visual cortex.

A RARE sequence based on MRI method can supply high resolution images of the brain, so we can use this method to discover the anatomical changes in visual cortex caused by diabetes. Besides, 1H magnetic resonance spectroscopy (MRS) is a potential noninvasive method that enables the in vivo investigation of metabolic alterations associated with brain pathology during the long term of the hyperglycemia. In the present study, we will apply RARE imaging method to investigate the anatomical changes, and in vivo 1H-MRS to find the metabolic changes in the visual cortex under chronic hyperglycemia induced by STZ diabetic rats. These anatomical changes and metabolites biomarker abnormity may supply diagnostic and prognosis utility of MRI tech in DM visual impairment.

2. Materials and Methods

2.1 Animal Preparation

Eight-week old male Sprague-Dawley rats, weighing 220-250 g were used. Type 1 diabetes was induced by a single dose intraperitoneal (i.p.) injection of streptozotocin dissolved in citric acid PH 4.5 (STZ, 65 mg/kg, Sigma s0130). Control animals received i.p. injection of the same amount of solvent (0.01 mol/L citric acid). At each time point, body weight and fasting blood glucose level were measured in both groups. The STZ-treated rats with fasting blood glucose < 12.4 mmol/L on day 3 after STZ injection were excluded.

2.2 MRI Protocol

At 2 weeks (2 w) and 12 weeks (12 w) after diabetic induction, all rats were scanned on a 7 T/20 cm Bruker Biospec scanner under isoflurane anesthesia (1.8-2.5%, in pure O2). A volume coil was used for RF transmission, and a quadrature surface coil for signal detection. High resolution anatomical images were acquired with a RARE sequence with FOV 3.5 cm × 3.5 cm, matrix size 512 × 384, slice thickness 0.58 mm, TR 5800 ms, TEeff 40 ms, RARE factor 4 and a total of 8 averages. A PRESS sequence was used for in vivo 1H-MRS, with a 4 mm × 1 mm × 4 mm voxel placed on the visual cortex (Fig. 2a, white rectangle), TR/TE 2000/20 ms, spectral bandwidth 4 kHz, 2048 data points and 128 averages. Due to high level blood glucose level will increase the brain glucose level, and the limitation of overlap in 1H-MRS, diabetic rats were treated by an intravenous injection of insulin (2 U/mL, 2.5 mL) at least 35 minutes before 1H-MRS acquisition. Peak heights were used to calculate metabolite level ratios.

2.3 Data Processing Methods

The in vivo MR spectra were processed using Topspin 2.0 beta, before manually baseline and phase were corrected. Spectral peaks were assigned in the references of the singlet peak of NAA (CH3-group) at 2.02 ppm. Metabolities relative concentration were measured by the peak of the interest metabolites to the Cretine peak ratio, referred to as X/Cre, such as Glucose(Glc)/Cre, my-inositol (mI)/Cre, Taurine(Tau)/Cre, Glutamate(Glu)/Cre, Choline(Cho)/Cre and NAA/Cre.

Fasting blood glucose level, body weight and visual cortex thickness were measured in both groups. Statistically, all data were presented as mean ± standard deviation (SD). Two-tailed paired Student's t-tests were performed between control and STZ groups of all measurements. Results were considered significantly when $p < 0.05$.

3. Results and Analysis

At 12 w, compared to the control rats (n = 8), the STZ-treated rats (n = 7) had significantly decreased body weight (Fig. 1a) and increased fasting blood glucose level (Fig. 1b). Significantly reduced thickness of visual cortex was shown in STZ-treated rats at 12 w (Fig. 2b, 1.55 ± 0.04 mm vs. 1.65 ± 0.01 mm) ,while there was no difference between that of two groups at 2 w. Figure 3 shows 1H spectra

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acquired from the visual cortex of a control rat and a STZ-treated rat at 12 w. The STZ-treated rats showed significantly increased mI/Cre ratio (0.62 ± 0.09 vs. 0.52 ± 0.09) and significantly decreased NAA/Cre ratio (1.04 ± 0.06 vs. 1.13 ± 0.07) in the visual cortex at 12 w relative to the controls (Fig. 4).

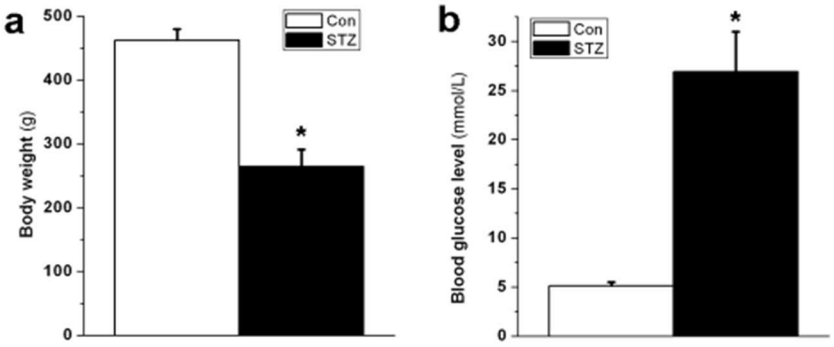

Fig. 1. Compared to control (Con), the STZ-treated rats had significantly decreased body weight (a) and increased fasting blood glucose level (b) at 12 w (*p<0.05).

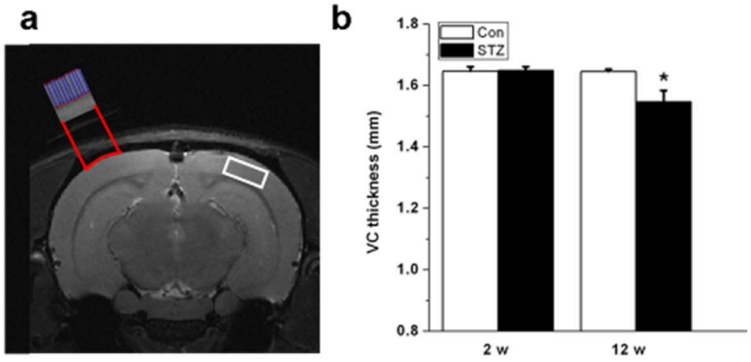

Fig. 2. Compared to the control rats (Con), the STZ-treated rats had thinner visual cortex (VC) thickness at 12w after induction (*p<0.05) (b). The regions for cortex thickness measurement and 1H-MRS are shown on the left and right sides of VC respectively (a).

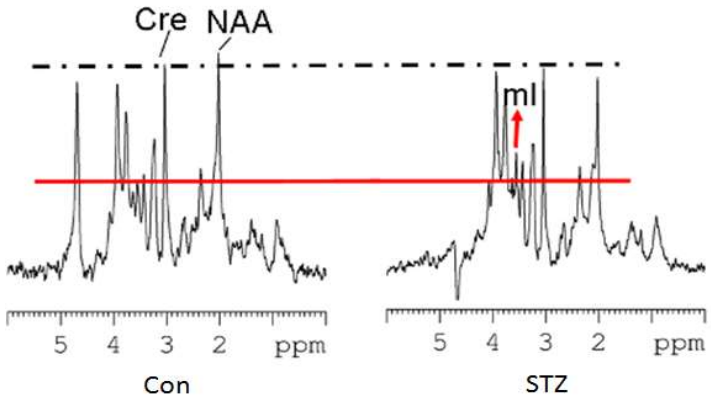

Fig. 3. In vivo 1H spectra from the visual cortex of a control rat (Con) and a STZ-treated rat at 12 w after induction. mI: myo-inositol; Cre: creatine.

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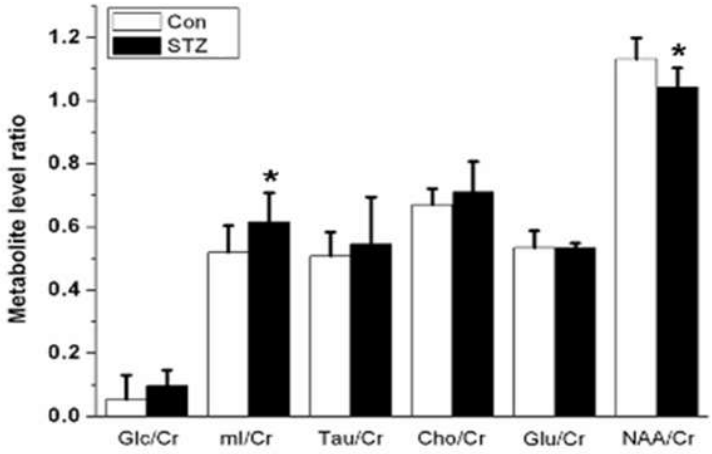

Fig. 4. Compared to the control rats (Con), the STZ-treated rats showed significantly (*p<0.05) increased mI/Cre ratio and decreased NAA/Cre ratio in the visual cortex at 12 w after induction.

4. Conclusion

In this study, we found that visual cortex thickness was thinner in the STZ-treated group compared to the control rats at 12 w after STZ induction, and this anatomical changes accompanied with a significantly increase of mI/Cre ratio and decrease of the NAA/Cre ratio. A previous study has showed that STZ-induced diabetic mice had significantly reduced cortex volume after 8 month of diabetes induction. Our results suggest that atrophy of the visual cortex may occur at earlier time. In addition to the anatomical changes, the visual cortex of diabetic rats also showed significant changes in metabolic profiles, such as increased mI/Cre ratio and decreased NAA/Cre ratio. This result is consistent with the previous study measuring metabolites in visual cortex of diabetic rats 12 weeks after diabetes induction. Zhang et al. showed that compared to control, the STZ-treated rats had significantly increased mI and reduced NAA concentrations in the hippocampus, but without any changes in metabolites ratios in visual cortex at 4 w after treatment. Increased mI level has also been reported in the occipital cortex of blind subjects and in the white matter of DM patients. We think that abnormal mI level in the visual cortex of the diabetic rats may have reflected glucose metabolic disorder and/or glia dysfunction. Since NAA is a biomarker of neuronal density and/or function, reduced NAA/Cre in the visual cortex of the STZ-treated rats might indicate that the neurons in this region are affected by long-term hyperglycemia.

In accordance with our result, poorly controlled DM patients had found low grey matter density (GMD) in the right occipital lobe, so it suggests that chronic hyperglycemia is harmful to this region. In other animal studies, under hyperglycemia for 2 months, dendritic atrophy and spine loss in the layer II–III pyramids of the parietal cortex have been found, and alteration of dendritic morphology in occipital cortex of diabetic rats has also been detected. As aforementioned, visual cortex may be a latent region that affected by hyperglycemia. Under the long-term hyperglycemia, due to glucose neurotoxicity, the neuron loss and axon degeneration may occur. The shrinkage of the visual cortex may be the result of brain plasticity and adaptive changes with the abnormal visual stimulations. Besides the visual cortex, the whole brain volume can also be found smaller in the young subjects who onset diabetes before 7 years old. Brain atrophy may be a common complication of DM, and the visual cortex is one of the regions that were involved in.

Usually, in vivo 1H-MRS can apply the metabolic information about neurons. Because the detectable NAA is an indicator of neuron integrity and function, the significantly decreased NAA/Cre ratio in STZ-treated group suggest neuronal loss and/or dysfunction in visual cortex. In agreement with our observation, Li et al. also found a decline of NAA concentration as a marker of neuronal loss in visual cortex for DM rats, which would be a result of transsynaptic injury and anterograde degeneration of

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RGCs and optic nerve. High glucose content will increase the blood viscosity and plasma osmolality, which would result in hypoperfusion of the brain and increased incidence of lacunar infarcts and stroke. Lower CBF in the cortex of diabetic animals and decreased regional CBF in the occipital lobe of DM have been found. The significantly decreased NAA/Cr ratio of the visual cortex at 12 w in diabetic rats in our study is likely to be a result of energy metabolism imbalance caused by long-term hyperglycemia.

MI is an important metabolite in the neuron cell, according to the precious study, the concentration is 10 times more than in the serum, it is not only control the osmolarity of the cell, but also involved in the cellular membrane-based second messenger system, as well as in direct action on membranes. MI, usually as a glia marker, the increase of which always indicates glia proliferation and/or glia dysfunction. In this study, the mI/Cr ratio is significantly increased in STZ-treated group compared to the control rats. This result is consistent with the clinic study that blind people have higher mI/Cr ratio in the occipital cortex, so it comes to make conclusion that maybe the increase of mI implies the glia plasticity to the abnormal vision stimulation caused by diabetes. The increase of mI concentration has been also found in visual cortex of diabetic rats accompanied by glial activation at 12 w after diabetes induction.

In conclusion, the STZ-treated rats showed significantly increased mI/Cre ratio, significantly reduced NAA/Cre ratio and thickness in the visual cortex compared to the controls. These MRI/MRS results indicated that chronic diabetic complications involve anatomical and metabolic changes in the visual cortex. It may provide useful neuroimaging information in the early diagnosis of DM visual impairment prior to neuropathological findings and even before any clinical manifestation of symptoms.

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