Magnetic Resonance Imaging 32 (2014) 1037–1042

Contents lists available at ScienceDirect

Magnetic Resonance Imaging

journal homepage: www.mrijournal.com

Brain metabolite alterations demonstrated by proton magnetic resonance spectroscopy in diabetic patients with retinopathy

Jia Tong [a,1], Houfa Geng [b,1], Zhengjun Zhang [b], Xuelei Zhu [b], Qiang Meng [b], Xinhai Sun [c], Min Zhang [b], Ruikun Qian [d], Lin Sun [b,*], Qiuhua Liang [b,*]

[a] Shandong University, Shandong, 250100, China
[b] Department of Endocrinology, Affiliated Hospital of Jining Medical University, Shandong, 272029, China
[c] Department of Magnetic Resonance Imaging, Affiliated Hospital of Jining Medical University, Shandong, 272029, China
[d] Shandong Academy of Medical Sciences, Shandong, 250001, China

ARTICLE INFO

Article history:
Received 19 December 2013
Revised 25 April 2014
Accepted 27 April 2014

Keywords:
Type 2 diabetes mellitus
Diabetic retinopathy
Brain damage
N-Acetylaspartate
Myo-inositol

ABSTRACT

Due to the homology between retinal and cerebral microvasculatures, retinopathy is a putative indicator of cerebrovascular dysfunction. This study aimed to detect metabolite changes of brain tissue in type 2 diabetes mellitus (T2DM) patients with diabetic retinopathy (DR) using proton magnetic resonance spectroscopy (^{1}H-MRS). Twenty-nine T2DM patients with DR (DR group), thirty T2DM patients without DR (DM group) and thirty normal controls (NC group) were involved in this study. Single-voxel ^{1}H-MRS (TR: 2000 ms, TE: 30 ms) was performed at 3.0 T MRI/MRS imager in cerebral left frontal white matter, left lenticular nucleus, and left optic radiation. Our data showed that NAA/Cr ratios of the DR group were significantly lower than those of the DM group in the frontal white matter and optic radiation. In the lenticular nucleus, MI/Cr ratios were significantly higher in the DM group than those in the NC group, while MI/Cr ratios were significantly lower in the DR group than those in the DM group. In the frontal white matter, NAA/Cho ratios were found to be decreased in the DR group as compared to the NC group. Additionally, our finding indicated that NAA/Cr ratios were negatively associated with DR severity in both the frontal white matter and optic radiation. A decrease in NAA indicated neuronal loss and the likely explanation for a decrease in MI was glial loss. In conclusion, we inferred that cerebral neurons and glia cells were damaged in patients with DR. Our data support that DR is associated with brain tissue damage.

1. Introduction

It is well known that diabetes mellitus is a risk factor for ischemic cerebrovascular disease. Diabetes is associated with an increased morbidity, recurrent frequency of stroke and worse functional outcome, higher mortality after stroke [1]. Owing to the homology between the retinal and cerebral microvasculatures, retinopathy is envisioned as a biomarker of cerebral vascular abnormalities [2]. Numerous researchers have paid attention to the association between retinal vascular lesions and cerebrovascular diseases recently. Emerging evidences that retinopathy is associated with stroke or white matter lesion as well as cognitive dysfunction or dementia have been showed by some large epidemiological surveys [3–10].

Proton magnetic resonance spectroscopy (^{1}H-MRS) is a non-invasive imaging method for detecting biochemical and macromolecular structures in brain, and both cerebral white matter and gray matter regions can be detected separately. ^{1}H-MRS technique has been increasingly utilized to investigate the metabolites in selected volumes of the brain. Nonetheless, ^{1}H-MRS findings related to changes in the brain are limited in patients with diabetic retinopathy (DR). We aimed to detect cerebral metabolite alterations in DR patients using ^{1}H-MRS technique and investigate the putative relationship between DR and brain damage.

2. Materials and methods

2.1. Study subjects and protocol

In the present study, 89 right-handed patients and controls were investigated, including 29 type 2 diabetes patients with DR (DR group), 30 type 2 diabetes patients without DR (DM group), and 30 age- and gender-matched control individuals who were free of

* Corresponding authors at: Department of Endocrinology, Affiliated Hospital of Jining Medical University, No. 79, Guhuai Road, Jining 272029, Shandong Province, China.
E-mail addresses: sunlinsddx@126.com (L. Sun), lqh_endocrinology@163.com (Q. Liang).
[1] These authors contributed equally to this article and should be considered to be co-first authors.

http://dx.doi.org/10.1016/j.mri.2014.04.020
0730-725X/

medically manifest disease especially pathoglycemia and ophthalmic diseases (NC group). Exclusion criteria for all the participants were as follows: (1) with signs or symptoms of cerebrovascular, cardiovascular, neurological or psychiatric disease, (2) head trauma, (3) experienced with ketoacidosis or severe hypoglycemia, (4) hypertension, (5) liver or kidney dysfunction, (6) previous or present alcohol abuse or drug abuse. Information of all the participants were obtained by a detailed questionnaire. The participants had no regular medication, except for insulin in the diabetic patients. The study was approved by the local research ethics committee. All subjects were fully informed and signed the informed consent form. All the procedures were performed in accordance with the guidelines of the Helsinki Declaration on human experimentation.

Diabetes was diagnosed according to the World Healthy Organization (WHO) criteria for diabetes. All the normal controls were screened through oral glucose tolerance test. Fasting plasma glucose (FPG) levels <6.1 mmol/L (110 mg/dl) and 2-hour plasma glucose values <7.8 mmol/L (140 mg/dl) after oral intake of 75 g of glucose were defined as normal glucose tolerance [11].

Resting blood pressure was repeated measure for three times in the right arm using a standard mercury manometer with all patients in the seated position. Subjects were classified hypertension if they had systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg or if they received antihypertensive treatment [12].

A fasting (>8 hours) blood sample was taken for analysis of FPG and hemoglobin A1C (HbA1C). FPG was tested by hexokinase method (enzymatic UV test) using Olympus AU 2700 (Olympus Diagnostic GmbH, Hamburg, Germany). HbA1C was measured using high-performance liquid chromatography method (HA-8140; Menarini Diagnostics, Florence, Italy).

2.2. Retinal examination

In retinal examination, a single ophthalmologist diagnosed retinopathy by fundoscopy after the best possible pharmacologic pupillary dilation obtained, using a clinical disease severity scale [13]. The minimum criterion for diagnosis of diabetic retinopathy was the presence of at least one define microaneurysm in any field of the eye. Most of the participants had mild to moderate non-proliferative diabetic retinopathy.

2.3. Proton magnetic resonance spectroscopy

The MRI examinations were examined on the 3.0 T System (Magneton Verio Tim, Simens, Germany). Routine imaging was carried out prior to spectroscopy to rule out any lesion or structural abnormality. The localization of voxels was confirmed by three orthogonal MR images in axial, sagittal, and coronal planes. Proton spectrum was acquired with the use of a single-voxel point-resolved spectroscopy sequence (TR/TE = 2000/30 ms, average = 80). Voxels were placed in the frontal white matter ($15 \times 15 \times 15$ mm^3), lenticular nucleus ($15 \times 15 \times 15$ mm^3), and optic radiation ($15 \times 15 \times 15$ mm^3), since the first two regions had been investigated by previous studies in diabetic population [14–16] and the optic radiation region was related to vision formation. Saturated zones were placed surrounding the voxels for avoiding influence of the skull and cerebrospinal fluid. Before spectra acquisition, shimming was performed using automated routines to optimize field homogeneity. The water signal was suppressed by a chemical shift selective saturation pulse (CHESS). Spectra post-processing steps were consisted by water reference processing, filter, zero-filling, fourier transformation, baseline correction, phase correction, curve fitting and automatically accomplished with software provided by the manufacturer. In the metabolite spectrum, the resonances were quantified as follows: *N*-acetylaspartate (NAA), 2.0 parts per million (ppm); creatine (Cr), 3.02 ppm; choline (Cho), 3.2 ppm; myo-inositol (MI), 3.6 ppm. The peak integrals, which represent the absolute concentration of compounds, were used to calculate metabolic ratios, such as NAA/Cr, Cho/Cr, MI/Cr and NAA/Cho.

2.4. Statistical analysis

All statistical analysis was performed with SPSS version 13.0 for Windows (SPSS, Chicago, IL). Normally distributed figures were expressed as means ± standard deviations (S.D.) and non-normally distributed data as the median and the interquartile range (25%, 75%). Age in different groups was compared with one-way ANOVA with least significant difference (LSD) post hoc correction. FPG and HbA1C in different groups were compared with independent-samples T test. Mann–Whitney U test was used to compare the diabetes duration of the DM and DR groups. X^2 test was used to compare the gender distribution of the three groups. The metabolic ratios (except for NAA/Cr and NAA/Cho values in the lenticular nucleus) among the three groups were compared with one-way ANOVA with LSD post hoc correction. NAA/Cr and NAA/Cho values in the lenticular nucleus (unequal variances among the three groups) were compared with Kruskal–Wallis test. Spearman's rank correlation were used to calculate the correlation between DR severity and metabolite ratios. A P value <0.05 was considered to be statistical significant.

3. Results

3.1. Clinical and laboratory characteristics

The age, gender distribution, disease duration, FPG and HbA1C of the participants were shown on Table 1. No statistically significant difference was found in age and gender distribution among the three groups. Disease duration, FPG, and HbA1C were also similar between the DM and DR groups ($P > 0.05$).

3.2. Metabolic parameters

Brain metabolic parameters of all the regions of interest (ROIs) were presented in Table 2. The representative spectra from each ROI in the DR group were showed in Figs. 1–3. In the DR group, NAA/Cr ratios were 11.7% lower ($P = 0.011$) in the frontal white matter and 6.5% lower ($P = 0.038$) in the optic radiation, than in the DM group. NAA/Cr ratios were also significantly decreased in DR group as compared to the NC group in both the frontal white matter ($P = 0.000$) and the optic radiation ($P = 0.037$). NAA/Cr ratios of the DR group were reduced in the lenticular nucleus as compared to the DM group, but the difference was not significant ($P > 0.05$). There was no significant difference in NAA/Cr ratios between the DM and NC groups in all the ROIs ($P > 0.05$).

In the lenticular nucleus, MI/Cr ratios were increased by 22% ($P = 0.009$) in the DM group as compared to the NC group, while MI/Cr ratios of the DR group were decreased by 24.6% ($P = 0.001$) as compared to the DM group. Although MI/Cr ratios of the DR group

Table 1
Clinical and biochemical characteristics of the study population.

	NC group	DM group	DR group
Age (years)	51.4 ± 5.3	51.2 ± 5.0	51.2 ± 5.7
Gender (M/F)	13/17	10/20	14/15
Diabetes duration (years)	–	8.5(7, 13)	9.5 ± 3.0
FPG (mmol/L)	4.8 ± 0.5[a]	8.7 ± 2.2	8.5 ± 2.1
HbA1C(%)	–	8.2 ± 1.7	8.2 ± 1.5

Data are expressed as means ± SD or median (interquartile range) or frequency.
[a] Statistically significant the NC group compared with the DM/DR group.