Effect of short- and long-term type 1 diabetes on the neurochemical profile in STZ-induced diabetic rats at 9.4 T

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INTRODUCTION

The neurological consequences of diabetes mellitus have been studied in both clinical and experimental settings. *In vivo* proton magnetic resonance spectroscopy (^{1}H MRS) at high magnetic fields provides an opportunity to noninvasively study neurochemical changes in diabetes, which may improve characterization of the disease progression as well as diagnosis and early identification of the disease. The aim of this work was to study the effect of short- and long-term diabetes on the neurochemical profiles in the brain of streptozotocin (STZ)-induced diabetic rats using ^{1}H MRS at 9.4 T.

METHODS

Eighteen Sprague-Dawley rats (mean ± SD = 304 ± 29 g) were injected with STZ (Sigma, 65mg/kg) to induce type-1diabetes. During MR measurements, the rats were anesthetized (air:oxygen = 1:1 with 1-2% isoflurane) and their core temperatures were maintained at 37˚C. The first MR measurement was performed before the injection (control, CTL) and the second measurement on the third day after injection (DM3). Subsequent measurements were performed every other week for up to 10 weeks after DM3. For each MR measurement, blood glucose levels were measured using a glucose oxidase method (Accu-Chek glucometer, Roche Diagnostics, Indianapolis, IN).

Ultra-short echo-time STEAM (TE=2ms, TR=5s, TM=20ms) [1] was used to acquired spectral data from a voxel (90 µl) in the neocrotex of the animal brain. The voxel was localized using T_2^*-weighted gradient echo images. All measurements were performed on a Varian 9.4 T MR scanner equipped with an 12-cm gradient coil (40 G/cm, 250 µs) and a shim coil (Magnex Sceintific, Abingdon, UK) with second-order shim strength up to 0.4 G/cm^2. The magnet was interfaced to a Varian INOVA console (Varian Inc., Palo Alto, CA). A quadrature surface RF coil consisting of two geometrically decoupled loops was placed on animal head for transmitting and receiving at 400 MHz proton frequency.

First- and second-order shims were adjusted using FASTMAP [2]. The water signal was efficiently suppressed using variable power RF pulses with optimized relaxation delays (VAPOR) technique. The acquired spectra were corrected for phase and frequency drift based on total creatine (Cr+PCr) signal at 3.03 ppm before being processed using LCModel [3] analysis.

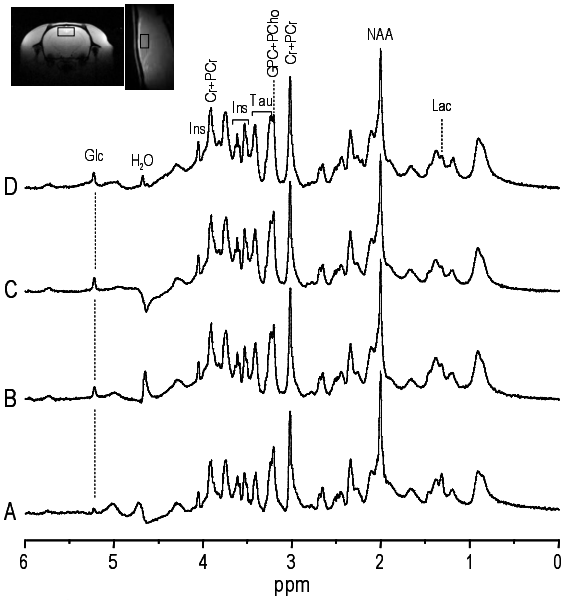

Fig. 1. ^{1}H MR spectra of the rat brain *in vivo* were measured before (A: control) and after STZ injection (B: DM3, C: DM17, D: DM59) at 9.4 T.

RESULTS AND DISCUSSION

In Figure 1, the spectra acquired from the rat brain (A) before (n = 18), (B) DM3 (n = 18) and (C) DM73 (n = 13) were shown. Excellent water suppression allowed visualizing the brain glucose signal at 5.23 ppm that increased after STZ-injection. The brain glucose levels matched well with the measured blood glucose levels, whose corresponding levels were 137, 490, 600+, and 556 mg/dL respectively. Figure 2 shows the changes in the concentrations of alanine (Ala), aspartate (Asp), β-hydroxybutyrate (bHB), glucose (Glc), glutamine (Gln), glutamate (Glu), glutathione (GSH), *myo*-inosital (Ins), lactate (Lac), N-acetylaspartate (NAA), taurine (Tau), creatine (Cr), phosphor-creatine (PCr), and the sum of glycerophosphocholine (GPC) and phosphocholine (PCho). The concentrations of these metabolites on DM3, DM17, and DM73 were compared to those on CTL. The brain Glc level was 3.27 µmol/g on CTL and was significantly increased by 90%, 151%, and 141% on DM3, DM17, and DM73 respectively with all significance levels $p<10^{-5}$, providing *in vivo* evidence of increased brain glucose in hyperglycemia [4,5]. Concurrent to the increase in Glc concentration, concentrations of several metabolites had significant changes as early as three days after STZ injection. They included GPC, Gln, Ins, Lac, Tau, Cr+PCr, and GPC+GPCho, whose concentrations were 0.27, 2.74, 6.23, 1.70, 6.76, 8.98, 1.08 µmol/g on CTL respectively, had significant changes of 37%, 8%, 7%, -9%, 7%, 3%, and 24% respectively on DM3.In addition, the level of bHB was 0.16 µmol/g on CTL and had increases of 116% on DM3, 137% on DM17, and 209% on DM73, indicating ketoacidosis in the hyperglycemic diabetic brain [6]. On DM17, the Ala level (0.53 µmol/g on CTL) changed significantly by -9.5%. These short-term changes persisted to DM73, except changes in Gln and Cr+PCr. The levels of Gln and Cr+PCr had initial increases ranging from DM3 to DM31 followed by marginal increases. In the chronic diabetes, concentrations of Asp, GSH, and NAA (2.11, 0.88, 9.19 µmol/g on CTL) had significant changes of -22%, -23%, -11% respectively.

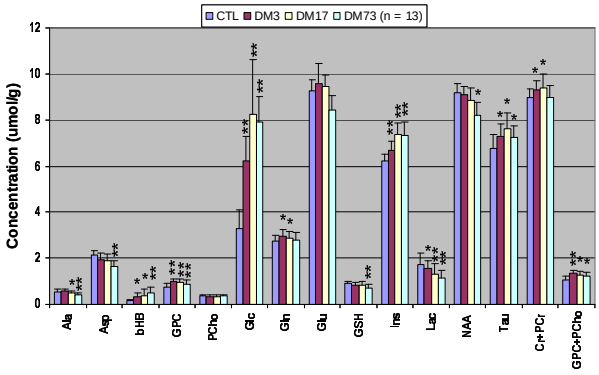

Fig. 2. Effect of shot- and long-term diabetes on the neurochemical profiles in the rat brain *in vivo*. Error bars denote standard deviations. Significance levels: *p<0.05, **p<0.005.

In summary, by employing ^{1}H MRS technique, we were able to detect subtle changes in the metabolite concentrations in the early and the chronic stages of the diabetes. The results showed that with short-term hyperglycemia there were significant changes in the concentration of several metabolites, including Ala, bHB, GPC, Gln, Ins, Lac, and Tau. These changes tend to persist to the chronic stage of the disease, when there were significant alterations in the concentrations of a number of additional metabolites, including Asp, GSH, and NAA. Delineation of these short- and long-term effects may provide better insight into understanding the disease progression.

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