Triethylenetetramine Treatment in Diabetic Heart Failure: An Animal Trial

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Introduction: During the last 20 years the number of people with diabetes worldwide has risen from 30 million to 230 million, according to the International Diabetes Federation. The disease is expected to affect 380 million people worldwide by 2025. End-stage diabetes is often associated with cardiac complications that are the leading cause of death in diabetic patients. The usual characteristics of diabetic heart failure are left ventricular dysfunction, myocellular hypertrophy, interstitial fibrosis, and altered myocardial fat metabolism (1). Streptozotocin (STZ)-induced diabetic rats have been used as a model for diabetic cardiac dysfunction (2, 3), and triethylenetetramine (TETA), a copper (II) selective chelator used for the treatment of Wilson's disease, appears to be effective in treating heart failure in this model (4) and in clinical trials (4, 5). Here, we investigated effects of TETA treatment on cardiac function in STZ-rats over an extended treatment period using High Field MRI. We used MRI cine images to calculate cardiac function. We hypothesized that TETA treatment would improve diabetes-mediated cardiac dysfunction over time and that longitudinal MRI study would reveal that trend.

Methods: Forty age matched Wistar rats underwent cardiac examination by MRI, of which 11 were controls (SHAM) and 29 were rendered diabetic by a single intravenous tail vein injection of STZ (Sigma; 55 mg/kg bodyweight) in isotonic saline. Eight weeks later, 16 out of 29 STZ-diabetic rats were treated with TETA.2HCl at 20 mg/day/rat via their drinking water for a further 8-week period, and the other 13 rats remain untreated. Blood glucose was monitored weekly via tail-glucose values. Cardiac MR images were acquired before STZ injection, and at 8 weeks and 16 weeks after STZ injection for each rat, using a Varian (Palo Alto, CA, USA) 4.7-T horizontal-bore magnet controlled by a Unity Inova spectrometer. Animals were prepared for MRI study by induction of general anaesthesia with 4% isoflurane in air, placement of ECG, respiration and temperature monitoring electrodes (SAII, Stony Brook, NY) and mounting in a 72-mm ID circularly polarized bird-cage coil (M2M Imaging, Cleveland, OH, USA). Core body temperature was maintained at 35-38°C throughout the study by directing a regulated warm air source over the animal. Following acquisition of scout images, a cardiac- and respiration-gated T_1 weighted gradient-echo cine study was acquired in the cardiac short axis orientation spanning from the apex to the base of the left ventricle (TR = 2* R-R interval, ~ 280-360 msec, TE = 2.2 msec, cardiac phases = 20, flip angle = 20°, slices = 6, thickness = 2 mm, averages = 2, FOV = 60x60 mm, matrix = 128x128, gap between slices = 0.6-1 mm). A second cine study was then performed in the cardiac long-axis view using the same acquisition parameters (slices = 3, and oriented to give 2-, 3- and 4-chamber views). End-diastolic volume (EDV), end-systolic volume (ESV), ejection fraction (EF) and left ventricular mass (LVM) were determined from regions of interest that were manually drawn in the cine images using ImageJ.

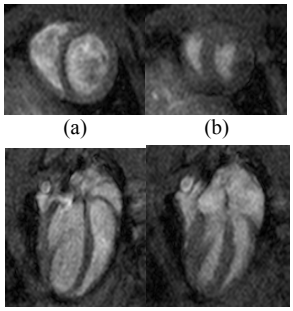

Figure 1. Cardiac cine images of a rat heart at diastole (a. short axis and c. long axis) and systole (b. short axis and d. long axis).

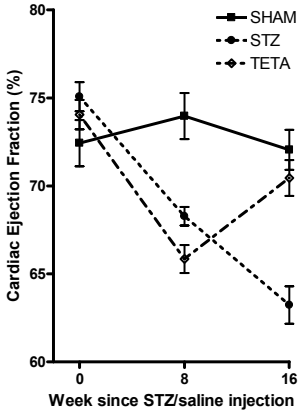

Figure 2. Cardiac EF of rats at Week 0, 8 and 16 of saline or STZ injection. SHAM: rats injected with saline (n = 11); STZ, rats injected with STZ (n = 13); TETA: rats injected with STZ and treated with oral TETA from Week 8 for the next 8 weeks (n = 16).

Results: Blood glucose levels in STZ-injected rats rose from < 8 mmol/L to > 25 mmol/L two days after STZ injection and remained at that level thereafter. SHAM rats had significantly higher body weight than STZ rats with or without TETA treatment. There was no significant difference in body weight between treated and untreated STZ rats. No significant change in LVM/body-weight ratios was observed in any treatment group at any time point. Eight weeks' TETA treatment did not modify blood glucose levels in STZ rats. Left ventricular images with high blood/myocardium contrast were generated using the cine protocol (Figure 1). Cardiac EF in SHAM rats did not show any significant change between Weeks 0, 8 and 16 (Figure 2, $p > 0.50$, one-way ANOVA). However, there was a significant decrease in EF values in STZ-injected animals after 8 weeks ($p < 0.001$, one-way ANOVA) which continued to decline after 16 weeks for STZ-rats without treatment ($p < 0.001$, one-way ANOVA). However, STZ rats with 8 weeks of TETA treatment show significant improvement in EF values ($p < 0.01$, one-way ANOVA). Comparison among the three groups of rats over treatment-time showed that all rats started with similar EF values. After 8 weeks, STZ-diabetic rats had significantly lower EF values than SHAM rats. After another 8 weeks, at Week 16, the EF values of the group of STZ rats without treatment had fallen further. By contrast, the EF values of the group of STZ rats treated with oral TETA for 8 weeks were significantly higher than those of STZ rats at Week 16 (Figure 2, $p < 0.001$, two-way ANOVA). There was no significant difference between EF values of TETA-treated and SHAM rats at Week 16 ($p > 0.05$, two-way ANOVA).

Discussion: This is the first comprehensive study to look at longitudinal cardiac functional change in STZ-diabetic rats with and without TETA treatment. The non-invasive MRI technology enabled us to track cardiac function of each individual rat over the 16-week experimental period. A significant decrease in EF was detected in rats 8 weeks after diabetes induction by cine MRI. Without treatment, this deterioration continued to Week 16 (Figure 2). Introduction of oral TETA treatment at Week 8 for an 8-week period restored EF values towards normal. This finding is consistent with our previous end-point studies on isolated perfused hearts of STZ rats, which showed that cardiac output was significantly attenuated by diabetes, and that TETA treatment partially restored that output (4, 6). Histological studies in the same report also showed significant structural damage at cellular level in LV of STZ rats and that TETA treatment repaired damaged LV structure. Diabetes in humans is often accompanied by LV hypertrophy and LV dysfunction, and diabetic heart failure is a leading cause of death in diabetes. Our results show that STZ-diabetic rats can mimic the cardiac impairment observed in human disease, and that this impairment is reversible by oral TETA treatment. A recent clinical trial, also using MRI to determine cardiac function, showed that 12-month's oral administration of TETA.2HCl was effective in improving LV hypertrophy in type 2 diabetic patients (5). The results of our longitudinal study here provide further concrete evidence that TETA can improve diabetes-impaired cardiac function, and future clinical study of this drug in the treatment of diabetic heart failure is warranted.

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