In vivo assessment of diabetes-induced renal oxidative stress and response to therapy using hyperpolarized 13C dehydroascorbate magnetic resonance imaging

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Target Audience: Scientists and clinicians with interests in studies of renal metabolism and oxidative stress-related diseases.

Purpose: Diabetic nephropathy (DN) is the leading cause of end stage renal disease in the United States [1]. Non-invasive methods to evaluate DN are currently limited. Increased reactive oxygen species (ROS) and oxidative stress have been proposed to be a unifying cause for the onset and progression of DN, and a target for novel therapies [2]. A key source of renal ROS is NADPH oxidase 4 (Nox4), an enzyme activated by chronic hyperglycemia to produce superoxide, and has been shown to mediate several key histological features in diabetic nephropathy, including mesangial matrix expansion [3]. Hyperpolarized (HP) 13C-dehydroascorbic acid (DHA) is an endogenous redox sensor, which is reduced to the antioxidant vitamin C (VitC) in a glutathione (GSH, a key antioxidant) dependent manner [4]. Here, we apply HP 13C-DHA to interrogate the redox capacity associated with DN, and following treatment with an angiotensin-converting enzyme inhibitor, Ramipril, which has targeted effects on oxidative stress.

Methods: A mouse model of type 2 diabetes (db/db mice) was used in this study. Db/db mice at 8, 12, and 16 weeks of age, and their age-matched control (db/m) mice (n=4-5 in each group) underwent HP 13C-DHA MR spectroscopic imaging (MRSI). Additionally, a separate group of db/db mice (n=5) were treated with Ramipril for 4 weeks (8-12 weeks of age) followed by HP 13C-DHA MRSI. All mice underwent MRSI, which was acquired 25 seconds post injection of 15M of HP 13C-DHA, at 5mm isotropic resolution, using a 3T scanner equipped with multinuclear package and dual-tuned 1H-13C imaging coil as previously described [4,5]. Ratios of HP DHA and VitC were calculated from the peak integrals in 3D MRSI data. Mice were sacrificed following imaging and renal tissues 1) sectioned for staining with periodic acid-Schiff (PAS, for evaluation of % glomerular mesangial area), 2) tissue imaging, 3) oxidase (Nox4) expression level.

Results and Discussion: In the db/db mice, there was early decrease in renal redox capacity. The VitC/(VitC+DHA) ratios were 13%, 35%, and 33% lower compared to the age-matched db/m mice at 8, 12, and 16 weeks, respectively (p<0.05 for all). The observed in vivo alteration in the 13C-DHA reduction to VitC correlated to renal reduced glutathione (GSH) concentration, reflecting the redox coupling between VitC and GSH. The mean renal GSH concentration were 15%, 26%, and 29% lower in the db/db mice compared to age-matched db/m mice at 8, 12, and 16 weeks, respectively (p<0.05 for all), consistent with lower redox capacity in the diabetic kidneys. The renal mRNA expression of Nox4, a major source of renal ROS, was significantly increased in the db/db mice at all 3 time points (p<0.05 for all), consistent with lower redox capacity in the diabetic kidneys. The renal mRNA expression of Nox4, a major source of renal ROS, was significantly increased in the db/db mice at all 3 time points (p<0.05 for all). The metabolic phenotype resulting from increased Nox4 with decreased redox capacity was reflected by in vivo HP DHA reduction to VitC. The PAS stains of the renal slices demonstrated the % mesangial matrix area increased over time in the db/db mice at 13.9% for the 8 week old mice, and 20.4% for the 16 week old mice, confirming histologic features characteristic for diabetic nephropathy. Hyperglycemia has been shown to increase cellular angiotensin II production, which in turn activates Nox4 resulting in the generation of super oxide and increased oxidative stress [6]. Following 4 weeks of treatment with the ACE inhibitor Ramipril, the 12-week-old db/db mouse demonstrated normalization of the 13C-DHA reduction to VitC, with concomitant restoration of tissue GSH concentration, Nox4 mRNA expression, and renal histology measured by % mesangial matrix area (Fig. 1). Ramipril protects the kidneys from oxidative stress-induced diabetic nephropathy, and HP VitC levels in turn reflect the in vivo normalization of redox capacity following successful treatment.

Conclusions: Our study suggests that HP 13C DHA reduction to VitC strongly correlates to renal reduced glutathione concentration, and likely the impact of changes in superoxide generation. HP 13C DHA can noninvasively evaluate redox network alterations associated with diabetic renal injury and following successful treatment. This technique can be extended to the evaluation of other complications from diabetes or other oxidative stress-related diseases, and to develop biomarkers of early injury and response to targeted therapies.


Acknowledgement: NIH K99/R00 EB014328, NIH R01CA166766, NIH P41EB013598, Society of Abdominal Radiology Morton Bosniak Grant.