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Original research article

Impact of soluble epoxide hydrolase inhibition on early kidney damage in hyperglycemic overweight mice

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ABSTRACT

This study addressed the hypothesis that inhibition of the EETs degrading enzyme soluble epoxide hydrolase affords renal protection in the early stage of diabetic nephropathy. The renal effects of the sEH inhibitor *t*-AUCB (10 mg/l in drinking water) were compared to those of the sulfonylurea glibenclamide (80 mg/l), both administered for 8 weeks in FVB mice subjected to a high-fat diet (HFD, 60% fat) for 16 weeks. Mice on control chow diet (10% fat) and non-treated HFD mice served as controls. Compared with non-treated HFD mice, HFD mice treated with *t*-AUCB had a decreased EET degradation, as shown by their higher plasma EETs-to-DHETs ratio, and an increased EET production, as shown by the increase in EETs + DHETs levels, which was associated with induction of CYP450 epoxygenase expression. Both agents similarly reduced fasting glycemia but only *t*-AUCB prevented the increase in the urinary albumine-to-creatinine ratio in HFD mice. Histopathological analysis showed that *t*-AUCB reduced renal inflammation, which was associated with an increased mRNA expression of the NFκB inhibitor Iκ≡ and related decrease in MCP-1, COX2 and VCAM-1 expressions. Finally, there was a marginally significant increase in reactive oxygen species production in HFD mice, together with an enhanced NOX2 expression. Both agents did not modify these parameters but *t*-AUCB increased the expression of the antioxidant enzyme superoxide dismutase 1. These results demonstrate that, independently from its glucose-lowering effect, sEH inhibition prevents microalbuminuria and renal inflammation in overweight hyperglycemic mice, suggesting that this pharmacological strategy could be useful in the management of diabetic nephropathy.

1. Introduction

Diabetic nephropathy is one of the most common microvascular complications of type 1 and type 2 diabetes, and is a leading cause of chronic kidney disease (CKD) and end-stage renal disease (ESRD) worldwide [1]. Diabetic nephropathy occurs early in the natural history of diabetes, with the progression from normal albuminuria to microalbuminuria and renal inflammation being considered as the initial steps [2,3]. Current treatment strategies for diabetic nephropathy include glycemic and blood pressure control, lipid-lowering drugs, and inhibitors of the renin-angiotensin system [4,5]. Although these therapeutic options slow the progression of diabetic nephropathy, the burden and mortality rate of the disease remains very high, and the majority of patients with diabetic nephropathy are at high risk of progression to ESRD [1].

In this context, new pharmacological targets are needed and soluble epoxide hydrolase (sEH) appears to be one of the most promising. The sEH metabolizes epoxyfatty acids, which are vasodilators with powerful anti-inflammatory properties, and notably converts cytochrome P450 epoxygenases-derived epoxyeicosatrienoic acids (EETs) to the less active dihydroxyeicosatrienoic acids (DHETs) [6]. Inhibitors of sEH have been developed and

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have shown promising results in animal models of cardiovascular diseases, lowering blood pressure and preventing vascular as well as cardiac remodeling and dysfunction [6–8]. Moreover, recent evidence indicates that inhibitors of sEH display interesting glucose lowering properties in animal models of insulin resistance and diabetes, mediated by the improvement in both insulin release and sensitivity [9,10].

However, the impact of sEH inhibition at the level of the kidney is more controversial. Data in experimental hypertension suggested beneficial renal effects that could be partly independent of the antihypertensive action of these agents [11–13]. However, although sEH inhibition is protective against acute renal failure [14,15], it may aggravate renal dysfunction in chronic kidney disease, by notably enhancing proteinuria [16]. Regarding diabetic nephropathy, one elegant study demonstrated that the genetic or pharmacological inhibition of sEH prevents renal inflammation and injury in streptozotocin-induced type 1 diabetes [17]. However, no data are available concerning the impact of such strategy on early kidney damage in insulin resistance and type 2 diabetes. This is particularly important to assess because the pathophysiology and natural evolution of diabetic nephropathy is somewhat different between T1D and T2D [2].

In this context, this study was designed to assess the effects of soluble epoxide hydrolase inhibition on renal function and structure in mice fed a high-fat diet (HFD), and to study the mechanisms involved.

2. Materials and methods

2.1. Animal treatments

The protocol was approved by a local institutional review committee and conducted in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals. Male FVB/N mice (Janvier), 6–8 weeks of age, weighing 26–30 g were fed with either a standard chow diet (control mice; D12450B, 10% energy by fat, Research Diets) or a HFD (D12492B, 60% energy by fat, Research Diets) for 16 weeks. Eight weeks after starting the HFD, mice were randomized to receive either the potent sEH inhibitor *trans*-4-(4-(3-adamantan-1-yl-ureido)-cyclohexyloxy)-benzoic acid (*t*-AUCB: 10 mg/l in drinking water) [9,17,18], the sulfonylurea glibenclamide (80 mg/l, Sigma-Aldrich) [19], or were not treated for the remaining 8 weeks. Glibenclamide is a hypoglycemic agent commonly used in the management of type 2 diabetic patients, devoid of direct effect on the kidney, allowing assessment of the hypoglycemic-independent renal impact of sEH inhibition.

2.2. Blood and urine analysis

The week before sacrifice, mice were fasted overnight and glycemia was measured in blood collected from the tail using a glucometer (StatStrip Xpress, Nova Biomedical). Urine samples were collected in the morning, allowing the determination of the urinary albumin-to-creatinine ratio. Finally, mice were anesthetized and blood samples were taken from the aorta at sacrifice. Plasma was prepared and stored at −80 °C until analysis. Urine albumin concentration was determined using a murine microalbuminuria ELISA kit (Albuwell M, Exocell). Plasma and urine creatinine was measured by an automatic biochemistry analyzer (COBAS c 502, Roche Diagnostics). Plasma levels of 5,6-, 8,9-, 11,12- and 14,15-EET and DHET regioisomers as well as whole blood level of *t*-AUCB were quantified by LC–MS/MS [18].

2.3. Renal histology and immunohistochemistry

Kidneys were harvested and decapsulated from anesthetized mice. Kidney weight was normalized to tibia length. Renal histological lesions were analyzed after Masson's staining. Briefly, the kidneys were fixed 24 h in 4% formalin and embedded in paraffin after conventional processing. Three μm slices were thereafter stained with Masson's trichrome solution. The slides were independently examined on a blinded basis for the level of interstitial inflammation, interstitial fibrosis, glomerulosclerosis, and tubular lesions, using a 0-to-4 injury scale as described previously [20]. In addition, 8-μm-thick sections of snap-frozen cryopreserved kidney were fixed with acetone for 10 min. After blockade of endogenous peroxidase with H_2O_2 0.3%, they were stained with anti-CD45 or F4/80 antibodies during 1 h at room temperature, used as markers of leukocytes and macrophages, respectively (Abd Serotec, MCA497GA and BD Biosciences, BD550539, respectively). Staining was revealed by 3-amino-9-ethylcarbazole (Sigma-Aldrich, A6926-50TAB), and hematoxyline (Labonord, 006-07125). Quantification of F4/80-positive and CD45 cells was performed using Image Pro Plus.

2.4. Real time quantitative PCR

Renal cortex was homogenized by grinding with Trizol (Invitrogen). RNA was extracted using chloroforme-isopropanol method and RNA quantity and purity were assessed with an ND1000 Spectrophotometer (NanoDropTechnologies). After digestion with DNase 1 (Invitrogen), RNA was reverse transcribed using 10 mM dNTP, 40 U of RNaseOUT and 200 U of M-MLV-Reverse Transcriptase (Invitrogen). The cDNA obtained was then amplified by PCR in a LightCycler 480 (Roche Molecular Biochemicals) with a commercial mix containing Taq DNA polymerase, SYBR Green I, and MgCl2 (FastStart DNA Master SYBR® Green I kit; Roche Molecular Biochemicals) under the following conditions: 95 °C for 10 min, 45 cycles at 95 °C for 10 s and 60 °C for 10 s. For each gene, the elongation time was calculated as (number of baspairs/25) + 3, at 72 °C. Specific primers for target mRNAs (Table 1) were designed using Primer 3 Plus (http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi/) and NCBI (http://www.ncbi.nlm.nih.gov). Results were normalized for 4 housekeeping genes: β-actin, GAPDH, 18*S* and β2-microglobulin and expressed as the percentage of gene variation in comparison to control mice.

2.5. Electron paramagnetic resonance (EPR) spectroscopy

The production of reactive oxygen species (ROS) in kidneys was evaluated using EPR spectroscopy. Briefly, the lower pole of the right kidney was frozen in liquid nitrogen and kept at −80 °C until analysis. Renal tissues were homogenized (Polytron) in Krebs/hepes buffer and incubated at 37 °C for 60 min in 50 mmol/l phosphate buffer containing 25 mmol/l deferrioxamine, 5 mmol/l diethyldithiocarbamate, and 500 mmol/l 1-hydroxy-3-methocarbonyl-2,2,5,5-tetramethyl pyrrolidine hydrochloride (CMH, Noxygen, Hamburg, Germany). The oxidation of CMH into the paramagnetic nitroxide CM•, driven mainly by superoxide, exactly 60 min after the beginning of incubation, tissues were introduced into an insulin syringe and frozen in liquid nitrogen and kept at −80 °C until EPR measurement. EPR Spectra were recorded at 77 K in a liquid nitrogen-cooled Dewar, using an MS400 spectrometer (Magnettech) with the following acquisition parameters: microwave power 20 mW; microwave frequency 9.5 GHz; modulation amplitude 5 G; modulation frequency 100 kHz; gain 200; sweep time 60 s; one scan. Intensity of the spectra was measured