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Losartan therapy decreases albuminuria with stable glomerular filtration and permselectivity in sickle cell anemia

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ABSTRACT

Sickle cell nephropathy begins with hyperfiltration and microalbuminuria and may progress to renal failure. The aim of this study was to determine the effects of losartan on glomerular function and albumin excretion in sickle cell anemia (SCA). Individuals with SCA on hydroxyurea with persistent albuminuria were enrolled in a 1-year study of losartan. Glomerular filtration rate (GFR) measured by iohexol clearance, albumin excretion rate (AER), and fractional clearance of dextran were assessed at baseline, short-term (1–2 month), and long-term (≥ 12 month) intervals. Twelve subjects (6 microalbuminuria, 6 macroalbuminuria) completed short-term studies; 8 completed long-term studies. Baseline GFR was 112 ml/min/1.73 m^2 (71–147 ml/min/1.73 m^2). AER decreased significantly at the short-term (median decrease −134 mcg/min, $p = 0.0063$). GFR was not significantly-different at short-term or long-term intervals. Dextran clearance improved for diameters smaller than albumin (< 36 Å) but not larger sizes. Losartan therapy for ≥ 1 year in sickle nephropathy results in lower albumin excretion with stable GFR. Filtration of neutral molecules ≥ 36 Å was not changed by losartan, suggesting that the effect of losartan is a mechanism other than alteration of glomerular filtration size-selectivity.

1. Introduction

Sickle cell nephropathy is a chronic complication of sickle cell anemia (SCA) that begins in childhood and progresses through adulthood. Up to 12% of adults with SCA progress to renal failure, which is an independent predictor of early death [1–3]. Early renal abnormalities are seen universally in the first decade of life with glomerular hyperfiltration and hypertrophy; progressive glomerular damage results in microalbuminuria and proteinuria, glomerulosclerosis, and gradual decline in glomerular filtration rate (GFR) during adolescence and adulthood [4]. Albuminuria is a sensitive marker of renal damage that may reflect both loss of glomerular permselectivity as well as impaired proximal tubular protein reabsorption [5]. Previously we reported the prevalence of albuminuria in SCA increases with age, occurring in < 10% of very young children, in 35% of adolescents, 61% of adults 18 to 30 years, and 79% of adults over age 40 years, demonstrating the progressive nature of sickle nephropathy. Multicenter trials in SCA demonstrate the associations of proteinuria with pulmonary hypertension and increased 3-year mortality, highlighting the importance of screening and treating sickle nephropathy [6].

Hydroxyurea (HU) is a mainstay of SCA treatment for the reduction of vaso-occlusive complications, chronic organ damage, and mortality in adults and children with SCA [7–10]. HU therapy has been shown to decrease albuminuria in adults and children with SCA; [11–13] however HU alone is not sufficient treatment to reverse or halt progression of albuminuria or nephropathy in many individuals, particularly those with more advanced nephropathy.

Angiotensin is a pathogenic mediator in several glomerulopathies; however there are few studies of renin-angiotensin system (RAS) blockade in sickle nephropathy. Angiotensin-II mediates glomerular damage via vasoconstriction of post-glomerular vessels, resulting in intraglomerular hypertension and increased glomerular permeability to macromolecules including albumin [14,15]. Angiotensin-II induces proximal tubular cell hypertrophy and mesangial proliferation, changes which are seen in sickle nephropathy [16,17], and inhibits proximal tubular expression of megalin, the receptor for tubular reabsorption of albumin [18,19].

A randomized controlled trial of the angiotensin converting enzyme

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(ACE) inhibitor captopril in 22 adults with SCA showed reduction in urinary albumin excretion without significantly lowering blood pressure [20]. Angiotensin Receptor Blockers (ARB) are selective antagonists of angiotensin-II receptor 1 (AT1R), allowing for inhibition of angiotensin-II generated by non-ACE pathways while preserving potential renoprotective effects through Angiotensin-II receptor 2 (AT2R) pathways. ACE-I and ARB medications are used in the long term management of nephropathies; however there is limited evidence for their use in SCA [21]. A recent phase 2 trial of losartan in 32 patients with SCA (18 with albuminuria) showed reduction in albuminuria, without change in estimated GFR [22].

As patients with SCA have unique renal hemodynamics, this study aimed to assess both the short-term and longer-term effects of losartan therapy on urinary albumin excretion, direct measurements of GFR, glomerular permselectivity, and urinary biomarkers of renal damage as well as potential secondary effects on blood pressure and serum potassium in adults and children with SCA with persistent albuminuria on chronic HU therapy.

2. Materials and methods

This was a single-arm, therapeutic trial of losartan therapy given for 1 year or longer to adults and children with SCA and persistent albuminuria. The trial was approved by the Institutional Review Board (IRB) of Emory University and is registered with ClinicalTrials.gov, identifier NCT01989078. Participants provided informed consent per the Declaration of Helsinki and were enrolled between January 2013 and December 2014 in the hematology clinics at Grady Hospital and Children's Healthcare of Atlanta. Eligible participants were age ≥ 10 years, had HbSS or Sβ^0-thalassemia, were on HU therapy for ≥ 6 months prior to enrollment, and had a spot urinary albumin/creatinine ratio (ACR) ≥ 30 mg/g on ≥ 2 occasions prior to enrollment. Subjects were excluded for chronic transfusion therapy, other conditions associated with proteinuria (e.g. diabetes, lupus, HIV), chronic daily use of non-steroidal anti-inflammatory drugs, estimated GFR < 60 ml/min/1.72 m^2, pre-existing hyperkalemia (serum K$^+ > 5.5$ mEq/l), or pregnancy. Participants who were previously receiving ACE-inhibitors underwent a wash-out period of at least 1 month off of the medication before entering the study and were included if albuminuria was persistent off ACE-inhibitors.

Renal function testing occurred at baseline (prior to losartan) and at 2 time points after starting losartan: a short-term interval defined as ≥ 1 month (range 1–3 months) and a long-term interval of ≥ 12 months (range 12–24 months). The variability in timing was allowed due to unforeseen shortages of PAH and dextran-40 used in the permselectivity measurements. Baseline ACR was defined as the mean of ≥ 2 ACR measurements done within 6 months of study entry. Chronic Kidney Disease (CKD) was categorized per KDIGO International Consensus guidelines [23]. Hyperfiltration was defined as GFR > 130 ml/min/1.73 m^2 [24]. Estimated GFR (eGFR) was calculated using the CKD-EPI Cystatin C equation if age ≥ 18 years and the CKiD Schwartz formula if age < 18 years [25–27].

2.1. Losartan dosing, adherence, and safety monitoring

At enrollment and at each subsequent study visit, losartan was dispensed directly to the subject. Safety and adherence visits occurred at 2 weeks, monthly for 3 months, then every 2 months until completion. The medication possession ratio (MPR) was calculated as the difference between total number of pills dispensed and the number of pills returned, divided by the total number of days on study.

Losartan starting dose and final dose were dependent on patient's weight and blood pressure (BP). For adults and children ≥ 50 kg with baseline systolic BP ≥ 100 mm Hg, the dose started at 50 mg daily and increased to 100 mg at the 2 week study visit if there was no hyperkalemia and if systolic BP was not ≥ 10 mm Hg below baseline. For children < 50 kg and adults with baseline systolic BP < 100 mm Hg, the dose started at 25 mg daily and increased to 50 mg at 2 weeks if no hyperkalemia or decrease in systolic.

At each study visit, blood pressure, serum potassium, CBC to assess HU adherence, and symptoms were assessed. Hyperkalemia was categorized as grade 1 (5.3–5.7 mEq/l), grade 2 (5.8–6.5 mEq/l), or grade 3 (> 6.5 mEq/l); safety monitoring called for losartan to be held for grade 2 hyperkalemia or higher.

2.2. Glomerular function testing

GFR, renal plasma flow (RPF), and glomerular permselectivity characteristics were determined by differential clearance of intravenous iohexol, para-aminohippurate (PAH), and dextran-40 respectively. Priming doses of iohexol 50 mg/kg, PAH 10 mg/kg, and dextran 130 mg/kg were given intravenously followed by continuous infusion to maintain constant plasma levels of each solute. After a 60 min equilibration period, timed urine and blood specimens were collected every 30 min until 240 min post infusion. Iohexol concentration was measured by high-performance liquid chromatography (HPLC), and GFR was calculated as the average of 4 urinary iohexol clearances. PAH concentration was measured by the Marshall's method (Bran & Luebbe Autoanalyzer II, Buffalo Grove, IL), and RPF was calculated by dividing the averaged PAH clearance by a constant extraction ratio [28]. The concentration of dextran was assayed by the anthrone method after separation of the timed urine and deproteinized plasma samples into narrow 2 Å fractions by means of gel permeation chromatography (GPC) with precalibrated Sephacryl HR-300 columns (Pharmacia) [29]. To characterize the size-selective properties of the glomerular filtration barrier, fractional clearance curves for uncharged dextrans in the 22–66 Å range were analyzed using a heteroporous model of the glomerular capillary wall, as previously described [30].

2.3. Measurement of biomarkers of renal and endothelial damage

At each of the 3 glomerular function test visits, albumin excretion rate (AER, mcg/min) and IgG excretion rate were calculated from comparisons of timed urine specimens obtained over a 4-h period. All urinary biomarkers were measured on the first urine sample obtained after oral water load and were expressed as a ratio to urine creatinine. Enzyme linked immunosorbent assay (ELISA) was used to measure urinary levels of albumin and immunoglobulin G (IgG) (Bethyl Laboratories, Montgomery, TX), α1-microglobulin (α1-MG) (MyBiosource, San Diego, CA), Kidney Injury Molecule-1 (KIM-1) (R & D Systems®, Minneapolis, MN), neutrophil gelatinase-associated lipocalin (NGAL) (R & D Systems®, Minneapolis, MN), nephrin (Exocell, Philadelphia, PA), transforming growth factor beta-1 (TGF-β1) (Promega Corporation, Madison, WI), and endothelin-1 (ET-1) (R & D Systems®, Minneapolis, MN). Urine creatinine was measured by the Jaffe method via the Beckman Creatinine Analyzer (Beckman Coulter, Brea, CA) [31].

2.4. Statistical analysis

Intra-patient change (Δ) in blood pressure, AER and IgG excretion, GFR, and RPF were calculated as the difference from baseline to each time point on losartan therapy. The Sign test for non-parametric comparison was used to determine if the change from baseline was significantly different. Baseline GFRs of subjects with macroalbuminuria vs. microalbuminuria were compared by Wilcoxon Rank Sum test. AER was compared to each urinary biomarker, including all evaluable time points using Pearson's correlation. The Benjamini-Hochberg procedure based on false discovery rates (FDR) was used to adjust for type I errors due to multiple comparisons [32,33]. Statistical analysis was done using SAS (version 9.4, SAS Institute, Cary, NC) and Microsoft Excel (Microsoft Corp., Redmond, WA). Fractional clearance of dextran was