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Cholesterol lowering treatment restores blood global DNA methylation in chronic kidney disease (CKD) patients

A. Zinellu [a,*], S. Sotgia [a], E. Sotgiu [a], S. Assaretti [a], A. Baralla [a], A.A. Mangoni [b], A.E. Satta [c], C. Carru [a,d]

[a] Department of Biomedical Sciences, University of Sassari, Sassari, Italy
[b] Department of Clinical Pharmacology, School of Medicine, Flinders University, Adelaide, Australia
[c] Department of Surgical, Microsurgical and Medical Sciences, University of Sassari, Sassari, Italy
[d] Quality Control Unit, University Hospital Sassari (AOU), Sassari, Italy

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Abstract *Background and aims:* Chronic kidney disease (CKD) is characterized by increased oxidative stress (OS). In consideration of the well-known link between OS and DNA methylation we assessed DNA methylcytosine (mCyt) concentrations in CKD patients at baseline and during cholesterol lowering treatment.

Methods and results: DNA methylation and OS indices (malonyldialdehyde, MDA; allantoin/uric acid ratio, All/UA) were measured in 30 CKD patients randomized to three cholesterol lowering regimens for 12 months (simvastatin 40 mg/day, ezetimibe/simvastatin 10/20 mg/day, or ezetimibe/simvastatin 10/40 mg/day) and 30 age- and sex-matched healthy controls. DNA methylation was significantly lower in CKD patients vs. controls (4.06 ± 0.20% vs. 4.27 ± 0.17% mCyt, $p = 0.0001$). Treatment significantly increased mCyt DNA concentrations in all patients (4.06 ± 0.04% at baseline; 4.12 ± 0.03% at 4 months; 4.17 ± 0.03% at 8 months; and 4.20 ± 0.02% at 12 months, $p = 0.0001$ for trend). A trend for a greater effect on DNA methylation was observed with combined treatment ezetimibe/simvastatin 10/40 mg/day (+5.2% after one year treatment). The treatment-associated mCyt increase was significantly correlated with the concomitant reduction in MDA concentrations and All/AU ratios.

Conclusion: Our results demonstrate that CKD patients have a lower degree of DNA methylation and that cholesterol lowering treatment restores mCyt DNA concentrations to levels similar to healthy controls. The treatment-associated increase in DNA methylation is correlated with a concomitant reduction in OS markers.

The study was registered at clinicaltrials.gov (NCT00861731).

Abbreviations: All, allantoin; CKD, chronic kidney disease; EZE, ezetimibe; MDA, malondialdehyde; mCyt, methylcytosine; OS, oxidative stress; UA, uric acid.

* Corresponding author. Department of Biomedical Sciences, University of Sassari, Viale San Pietro 43/B, 07100 Sassari, Italy. Fax: +39 079228275.
E-mail address: azinellu@uniss.it (A. Zinellu).

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Introduction

There is unequivocal evidence that patients with chronic kidney disease (CKD) develop accelerated atherosclerosis, with a consequent increase in cardiovascular morbidity and mortality [1–3]. The main mechanisms underlying the increased cardiovascular disease (CVD) risk in this population are related to the relatively high prevalence of hypertension, diabetes and obesity [4,5]. Moreover, patients with CKD often have alterations in lipoprotein metabolism, which might result in severe dyslipidemia [6]. Therefore, in view of the cardiovascular risk reduction reached in hypercholesterolaemic patients, the pharmacological management of hypercholesterolaemia represents a promising target to reduce CVD risk in CKD patients [7]. Several clinical studies show that statins, aside from decreasing plasma cholesterol concentrations, may have specific renoprotective properties and, when combined with renin–angiotensin system (RAS) inhibitors, may have additional antiproteinuric effects [8]. The combination of statins with ezetimibe (EZE), a cholesterol absorption inhibitor, exerts additional lipid lowering effects vs. statins alone [9]. We have recently reported that combined simvastatin/ezetimibe therapy reduces inflammatory status and decreases plasma markers of endothelial dysfunction through a reduction in oxidative stress (OS) in stage III–IV CKD patients [10,11]. OS occurs when reactive oxygen species (ROS) production in the body overcomes the intrinsic antioxidant capacity, leading to oxidative attack of cellular structures such as proteins, lipids and DNA [12]. Furthermore, OS may be associated with aberrant DNA methylation in some diseases such as cancer and cardiovascular disease [13,14]. OS is associated with DNA hypomethylation through several mechanism: i) generation of DNA base adducts, such as 8-hydroxyl-2′-deoxyguanosine (8-OH-dG) and O6-methylguanine, strongly inhibit methylation of adjacent cytosine residues yielding global DNA hypomethylation [15]; ii) redox regulation of S-adenosylmethionine (SAM)-dependent methyltransferases, that have been reported as potentially redox-sensitive enzymes [16]; iii) downregulation of methionine adenosyltransferase, which catalyzes the enzymatic addition of methionine to adenosine for the synthesis of SAM, in an oxidized environment [17]; iv) glutathione (GSH) depletion during chronic oxidative stress, leading to decreased global DNA methylation through the depletion of SAM in the folate/homocysteine pathway [18]. Under oxidizing conditions, cystathionine-β-synthase principally direct homocysteine (Hcy) metabolism through the transsulfuration pathway for the generation of GSH. This, in turn, reduces the amount of Hcy directed toward the regeneration of methionine, which may result in decreased SAM concentrations [19].

Despite the well-known presence of OS in CKD and its metabolic link with DNA methylation, little information is currently available on mCyt concentrations in DNA extracted from stage III–IV CKD patients [20,21]. Previous studies have either assessed global DNA methylation patterns in end-stage renal disease (ESRD) [22,23] or focused on site specific DNA methylation [24,25].

Therefore, the aim of our study was to assess a) the concentrations of mCyt in DNA of CKD patients, and b) whether OS improvement during cholesterol-lowering treatment is associated with a modification of DNA methylation pattern. Moreover, since hyperhomocysteinaemia, a raised concentration of the sulphur aminoacid homocysteine in plasma that often occurs in CKD, has been found to be associated with DNA hypomethylation in ESRD [23], quantification of Hcy was also performed.

Methods

Study population

Patients' recruitment was conducted as previously described [10,11]. In brief, 30 CKD patients (age 60.2 ± 10.5 years, 19 males) were identified at the Istituto di Patologia Medica – Azienda Ospedaliero Universitaria, University of Sassari, with the following inclusion criteria: age > 18 years, plasma LDL-cholesterol concentrations > 100 mg/dL (without concomitant hypolipidemic drugs), presence of proteinuric CKD defined as creatinine clearance > 20 ml/min/1.73 m^2 combined with urinary protein excretion rate > 0.3 g/24 h, without evidence of urinary tract infection or overt heart failure (New York Heart Association class III–IV). Patients were stage III–IV CKD not receiving dialysis. Exclusion criteria were: previous or concomitant treatment with steroids, anti-inflammatory and/or immunosuppressive agents, vitamin B6, B12, folate or statins; evidence or clinical suspicion of obstructive uropathy, type 1 diabetes, vasculitis and renovascular disease. The latter was ruled out by renal artery echo-Doppler or by following the American College of Cardiology/American Heart Association guidelines on Peripheral Artery Disease, that propose that diagnostic testing for renal artery stenosis should be performed in the presence of one of the following: onset of severe hypertension (blood pressure ≥ 180 mmHg systolic and/or 120 mmHg diastolic) after the age of 55 years; unexplained deterioration of kidney function during antihypertensive therapy, especially an acute and sustained elevation (>50%) in serum creatinine concentrations within one week of treatment with an angiotensin-converting enzyme (ACE) inhibitor or angiotensin II receptor blocker (ARB); severe hypertension in patients with diffuse atherosclerosis, particularly those aged >50 years; severe hypertension in a patient with an unexplained atrophic kidney or asymmetry in renal sizes of >1.5 cm; a unilateral small kidney (≤9 cm); severe hypertension in patients with recurrent episodes of acute pulmonary oedema or refractory heart failure with impaired renal function; a systolic–diastolic abdominal bruit that lateralizes to one side [26].

All patients were on stable treatment with RAS inhibitor therapy (ACE inhibition by benazepril plus angiotensin II antagonism by valsartan) for at least six months. Enrolled patients were randomized to a 12-month treatment with either 40 mg/day simvastatin