Journal of Pharmaceutical and Biomedical Analysis 129 (2016) 383–388

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Effect of cholesterol lowering treatment on plasma markers of endothelial dysfunction in chronic kidney disease

Angelo Zinellu[a,*], Salvatore Sotgia[a], Arduino A. Mangoni[b], Elisabetta Sotgiu[a], Sara Ena[a], Andrea E. Satta[c], Ciriaco Carru[a,*]

[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

ARTICLE INFO

Article history:
Received 4 May 2016
Received in revised form 29 June 2016
Accepted 16 July 2016
Available online 18 July 2016

Keywords:
Asymmetric dimethylarginine
Chronic kidney disease
Ezetimibe
Intercellular adhesion molecule
Oxidative stress
Simvastatin
Vascular cell adhesion molecule

ABSTRACT

The elevated cardiovascular morbidity and mortality in chronic kidney disease (CKD) is linked with endothelial dysfunction secondary to the pro-inflammatory and pro-oxidative state typical of this pathology. In consideration of the well-known pleiotropic effect of statins, we investigated the effect of cholesterol lowering treatment on endothelial dysfunction markers (MED), asymmetric dimethylarginine (ADMA), vascular cell (VCAM) and intercellular (ICAM) adhesion molecule. Plasma MED concentrations, inflammation and oxidative stress indices [Kynurenine/Tryptophan (Kyn/Trp) ratio, malondialdehyde (MDA) and allantoin/uric acid (All/UA) ratio] 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). Treatment significantly reduced ADMA concentrations in all patients [0.694 μmol/L (0.606–0.761) at baseline vs. 0.622 μmol/L (0.563–0.681) after treatment, $p < 0.001$]. ADMA reduction was paralleled by a significant decrease of MDA, All/AU ratio and Kyn/Trp ratio, but not VCAM and ICAM plasma concentrations. Cholesterol lowering treatment was associated with a significant reduction in plasma ADMA concentrations in CKD patients. This might be mediated by reduced oxidative stress and inflammation.

1. Introduction

Chronic kidney disease (CKD) is a significant risk factor for coronary artery disease [1]. Even mild to moderate renal insufficiency is associated with adverse cardiovascular outcomes [2]. In stage III–IV CKD patients, the prevalence of cardiovascular disease (CVD) is 4- to 5-fold higher than that observed in the general population. This excessive risk could be explained, at least in part, to endothelial dysfunction (ED) and reduced availability of nitric oxide (NO). The latter plays a key role in the initiation and progression of atherosclerosis and might be a potential link between CVD and CKD [3]. The high prevalence of CVD risk factors in CKD, e.g. hypertension, diabetes and obesity, might contribute to ED in this setting [4,5]. Moreover, several observational studies have shown that patients with CKD exhibit significant alterations in lipoprotein metabolism, which might result in severe dyslipidemia [6]. Based on the CVD risk reduction achieved in hypercholesterolaemic patients prescribed cholesterol-lowering treatment with statins, management of hypercholesterolaemia represents a promising target to reduce cardiovascular risk also in CKD patients [7]. Experimental and clinical studies show that statins, in addition to lowering plasma cholesterol concentrations, may have specific renoprotective properties and, when combined with renin-angiotensin system (RAS) inhibitors, may have additive antiproteinuric effects [8]. The combination of statin with ezetimibe, a cholesterol absorption inhibitor, exerts additional lipid lowering effects vs. statin monotherapy [9]. We have recently reported that combined simvastatin/ezetimibe treatment improves oxidative stress, reducing the inflammatory status in stage III–IV CKD patients [10,11]. The activity of enzymes involved in the formation and degradation of asymmetric dimethylarginine (ADMA), an endogenous inhibitor of NO synthesis, such as protein arginine N-methyltransferases (PRMTs) and

Abbreviation: ADMA, asymmetric dimethylarginine; All, allantoine; Arg, arginine; CKD, chronic kidney disease; Cys, Cysteine; DDAH, dimethylarginine dimethylaminohydrolase; MED, markers of endothelial dysfunction; Hcy, homocysteine; ICAM, intercellular adhesion molecule; Kyn, Kynurenine; MDA, malondialdehyde; NO, nitric oxide; SDMA, symmetric dimethylarginine; Trp, Triptophan; UA, uric acid; VCAM, vascular cell adhesion molecule.

* Corresponding authors at: Dept. Biomedical Sciences – University of Sassari, Viale San Pietro 43/B – 07100 Sassari, Italy.
E-mail addresses: azinellu@uniss.it (A. Zinellu), carru@uniss.it (C. Carru).

http://dx.doi.org/10.1016/j.jpba.2016.07.025
0731-7085/

dimethylarginine dimethylaminohydrolase (DDAH) is regulated in a redox-sensitive fashion [12,13]. Therefore, ADMA concentrations in CKD patients (a disease commonly characterized by elevated levels of oxidative stress) might be sensitive to lipid lowering therapy. ADMA, an analogue of L-arginine, has been shown to impair endothelial function thus promoting atherosclerosis [14]. A number of studies have reported that ADMA concentrations predict CVD and are inversely related to endothelium-dependent vasodilation in subjects with hypercholesterolemia [15]. Plasma ADMA concentrations are increased in CKD as a consequence of both reduced renal excretion and reduced catabolism by DDAH [16]. Therefore, reducing ADMA concentrations in CKD might represent an effective strategy to curtail the excessive cardiovascular risk in this population. The aim of our study was to investigate whether oxidative stress improvement during cholesterol-lowering treatment is associated with a reduction in plasma ADMA concentrations. We enrolled 30 stage III–IV CKD patients randomized to three hypolipidemic regimens: simvastatin alone (40 mg/day) or ezetimibe/simvastatin combined therapy (10/20 or 10/40 mg/day). In addition to ADMA measurements, ED was also assessed by measuring the soluble forms of intercellular adhesion molecule (ICAM) and vascular cell adhesion molecule (VCAM) during therapy. VCAM and ICAM are endothelial adhesion molecules of the Ig gene superfamily that participate in atherogenesis by promoting monocyte accumulation in the arterial intima [17]. Considering the effect of oxidative stress on ADMA production/degradation enzymes, the evaluation of free plasma malondialdehyde (MDA) concentrations and allantoine/uric acid (All/UA) ratio was also performed to monitor oxidative stress during treatment. Inflammation status was assessed through quantification of the kynurenine/tryptophan (Kyn/Trp) plasma ratio, a recognized indicator of immune response activation [11,18]. Moreover, since homocysteine (Hcy) has been reported to inhibit DDAH activity in vitro, leading to increasing levels of intracellular ADMA, quantification of low molecular mass thiols Hcy and cysteine (Cys) was also performed [19].

2. Methods

2.1. Subjects

Patient 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, 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 3–4 CKD not receiving dialysis. Exclusion criteria were: previous or concomitant treatment with steroids, anti-inflammatory and immunosuppressive agents, vitamin B6, B12, folate or statin; evidence or clinical suspicion of renovascular disease, obstructive uropathy, type 1 diabetes and vasculitis. 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 (group 1, n = 10), ezetimibe/simvastatin 10/20 mg/day (group 2, n = 10) or ezetimibe/simvastatin 10/40 mg/day (group 3, n = 10). Patients were evaluated at baseline, 4, 8 and 12 months. Informed consent was obtained from each patient. The study was approved by the Ethics Committee of our Institution. The study complied with the principles of the Helsinki Declaration and was registered at clinicaltrials.gov (NCT00861731).

2.2. Biochemical analysis

Arg, ADMA, SDMA, MDA, All/UA ratio, Kyn/Trp ratio, Hcy and Cys were determined by capillary electrophoresis UV detection as previously described [20–25]. Inter-assay CV were 2.5% for Arg, 2.5% for ADMA, 2.9% for SDMA, 5.14% for MDA, 6.6% for All/UA ratio, 7.6% for Kyn/Trp ratio, and 4.9% for Hcy and Cys. Soluble forms of VCAM and ICAM were measured by ELISA kits (R&D System, Minneapolis, USA). Inter-assay CV were 6.4% for VCAM and 7.8% for ICAM.

Total plasma cholesterol, LDL, HDL and tryglicerides were measured by enzymatic methods using commercial kits (Boehringer-Mannheim, Mannheim, Germany).

2.3. Statistical analysis

All results are expressed as mean values (mean ± SD) or median values (median and interquartile range). The variables distribution was assessed by the Kolmogorov-Smirnov test. Differences between groups after randomization were tested by one-way ANOVA or Kruskal-Wallis test as appropriate. Correlation analysis between variables was performed by Pearson's correlation or Spearman's correlation. Multiple linear regression analysis was used to assess the contribution of different variables to plasma ADMA concentration at baseline. Only variables with p-value < 0.20 in the univariate analysis were entered into the multivariate model. The effect of drug treatment was evaluated by one-way repeated measures ANOVA or Friedman test as appropriate.

Statistical analyses were performed using MedCalc for Windows, version 12.5 64 bit (MedCalc Software, Ostend, Belgium) and SPSS for Windows, version 14.0 32 bit (IBM Corporation; Armonk, NY, USA).

3. Results

3.1. Clinical characteristics of CKD patients at baseline

Clinical characteristics of CKD patients at baseline are described in Table 1. Baseline ADMA concentrations were positively correlated with All/AU ratio (rho = 0.46, p = 0.011), plasma creatinine (rho = 0.51, p = 0.004), MDA (rho = 0.37, p = 0.042), Hcy (rho = 0.41, p = 0.025) and Kyn/Trp (rho = 0.48, p = 0.007), and negatively correlated with GFR (rho = −0.37, p = 0.043). A non-significant positive trend between ADMA and total cholesterol concentrations was also observed (rho = 0.33, p = 0.071). In multiple linear regression, with ADMA concentrations as dependent variable and All/AU, MDA, GFR, creatinine, Hcy, Kyn/Trp and total cholesterol as independent variables, only GFR (β=-0.42, P = 0.042) was independently associated with baseline ADMA (Table 2). A non-significant association trend was also observed with Hcy concentrations (β = 0.39, p = 0.06).

No significant correlations were observed between VCAM, ICAM, and other variables.

3.2. Effect of drug treatment on lipid profile and oxidative stress and inflammation indices

After randomization, no significant differences were found among the three treatment groups (Table 3). As previously reported [10,11], drug treatment significantly improved lipid profile in all groups already after 4 months. A decrease of 40% in total cholesterol, 62% in LDL, 21% in triglycerides, and 66% in LDL/HDL ratio was observed after 12 months. As previously described, a significant decrease in oxidative stress parameters, MDA and All/UA ratios, was observed in all patients (−19% for both MDA concentration and