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Original Article

Determinants of thromboxane biosynthesis in patients with moderate to severe chronic kidney disease

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ABSTRACT

Background: Mechanisms of accelerated atherothrombosis in patients with chronic kidney disease (CKD) are only partly characterized. The aims of this study were to evaluate the extent of thromboxane (TX)-dependent platelet activation in patients with CKD, and to characterize the determinants of altered TX biosynthesis in this setting, with particular reference to enhanced lipid peroxidation, low grade inflammation and CKD-related anemia.
Patients and methods: A cross sectional comparison between urinary 8-iso-$PGF_{2\alpha}$ and 11-dehydro-TXB_2, *in vivo* markers of oxidative stress and platelet activation, respectively, was performed in 115 patients with stage 1–4 CKD.
Results: Levels of both urinary 11-dehydro-TXB_2 and 8-iso-$PGF_{2\alpha}$ increased sequentially across the four CKD stages ($P < 0.0001$, Kruskal–Wallis test). Both urinary prostanoids were inversely associated with either estimated glomerular filtration rate (eGFR, $P < 0.0001$) or hemoglobin levels ($P < 0.0001$). A significant direct correlation was also observed between urinary 11-dehydro-TXB_2 and 8-iso-$PGF_{2\alpha}$ (Rho = 0.620, $P < 0.0001$). On multivariate analysis, urinary 8-iso-$PGF_{2\alpha}$ ($\beta = 0.459$, $P < 0.0001$), hemoglobin levels ($\beta = -0.261$, $P = 0.002$) and eGFR ($\beta = -0.172$, $P = 0.032$) were independent predictors of urinary 11-dehydro-TXB_2 (adjusted $R^2 = 0.488$).
Conclusions: This study provides biochemical evidence of persistent platelet activation in patients with CKD. This condition occurs early in the natural history of the disease and is related to kidney function and oxidative stress. Moreover, we found an independent inverse relationship between hemoglobin levels and TX-dependent platelet activation. This finding may provide a mechanistic link between CKD-related anemia and increased cardiovascular risk.

1. Introduction

Cardiovascular disease (CVD) has been shown to be a critical factor influencing quality of life and mortality in patients with chronic kidney disease (CKD). There is a well-established association of CKD with the development of atherosclerosis and its thromboembolic complications [1–5]. In particular, an independent, graded, inverse relationship between estimated glomerular filtration rate (eGFR) and cardiovascular event rates has emerged from large-scale observational studies [1,6] and CVD is increased in early-stage CKD [7].

Despite these findings, the mechanisms responsible for accelerated atherogenesis remain elusive. Clustering of cardiovascular risk factors such as sustained arterial hypertension, altered lipoprotein levels, diabetes mellitus, or obesity is often observed in CKD [8]. Nonetheless, classical risk factors poorly perform in the prediction of cardiovascular outcomes in this population, thus fostering interest into emerging risk factors.

Oxidative stress has been postulated to be a relevant risk factor for CVD in CKD patients [9–11], developing from an imbalance between increased free radical production due to dysfunctional mitochondria and reduced antioxidant defenses.

Abbreviations: CVD, cardiovascular disease; CKD, chronic renal disease; eGFR, estimated glomerular filtration rate; ROS, reactive oxidant species; PG, prostaglandin; Hb, hemoglobin; Scr, serum creatinine; MDRD, modification diet of renal disease study; EPO, erythropoietin; hs-CRP, high-sensitivity C reactive protein; TX, thromboxane; Clp, clopidogrel.

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Increased reactive oxidant species (ROS) formation leads to increased lipid peroxidation. A series of bioactive prostaglandin (PG) F2-like compounds (isoprostanes) has been discovered, which represent specific end-products from free radical damage [12,13]. Among these products, the most meaningful is 8-iso-$PGF_{2\alpha}$, which induces vasoconstriction and modulates the function of human platelets [12] and whose levels are increased in association with CVD [12,14]. F2-isoprostanes can be reliably measured both in plasma and in urine [12]. However, since they are rapidly metabolized, any increase in plasma isoprostane concentration may be due not only to their increased formation from lipid peroxidation, but also to decreased metabolism [15]. Thus, only measurement of F2-isoprostanes in the urine provides a reliable measure of persistent whole-body oxidative stress [12,15]. However, most of the available studies on isoprostane formation and loss of kidney function have reported increased plasma levels of these iso-eicosanoids, early in the progression of CKD [16–18].

Enhanced generation of 8-iso-$PGF_{2\alpha}$ and other biologically active iso-eicosanoids could contribute to persistent platelet activation, as reflected by enhanced urinary 11-dehydro-thromboxane (TX)B_2, an enzymatic metabolite of TXA_2 [12,19]. Therefore, the present study was specifically designed to investigate whether urinary 8-iso-$PGF_{2\alpha}$ excretion rate is altered in CKD patients and whether it correlates with the rate of TXA_2 biosynthesis. We also assessed the relative contribution of oxidative stress and TX-dependent platelet activation to CKD stage, impairment of eGFR, proteinuria, CVD factors including diabetes mellitus, plasma uric acid, and hemoglobin (Hb) levels, which have been inversely associated with mortality [20].

2. Methods

2.1. Study design and population

This is a cross-sectional observational study. A total of 115 adult Caucasians [median age (IQR) 63 (57–68) years, 81 (70.4%) males] were consecutively recruited from four nephrology outpatient clinics. All patients included in the study were diagnosed as having CKD according to National Kidney Foundation K/DOQI Guidelines [21].

We used a modified National Kidney Foundation classification of CKD, which classifies estimated GFR in the following ranges: at least 60 mL per minute per 1.73 m^2 (stages 1–2), 45 to 59 mL per minute per 1.73 m^2 (stage 3a), 30 to 44 mL per minute per 1.73 m^2 (stage 3b), 15 to 29 mL per minute per 1.73 m^2 (stage 4) [1]. The most common underlying causes of CKD were hypertension (87.8%) and/or diabetes (23.5%).

We excluded patients with end-stage kidney failure (K/DOQI stage 5: eGFR < 15 mL per minute per 1.73 m^2 or renal replacement therapy), evidence of clinically significant hepatic, cardiac or pulmonary insufficiency, uncontrolled hypertension, history of malignant neoplasms (diagnosed and treated within the last 5 years) other than in-situ carcinoma of the cervix or non-melanoma skin cancer, use of vitamin dietary supplements. Patients requiring chronic non-steroidal anti-inflammatory drug therapy or low-dose aspirin (ASA) were also excluded.

The study protocol was approved by the local ethics committee and all participants provided written informed consent. The study was performed in accordance with the principles embodied in the Declaration of Helsinki.

2.2. Risk factor assessment

Each examination included CVD clinical assessment and blood testing. Previous cardiovascular disease was defined by history of acute coronary syndrome, transient ischemic attack, stroke or a revascularization procedure. Diabetes was defined as having a fasting glucose level ≥ 126 mg/dL (7.0 mmol/L) or being treated with insulin and/or oral hypoglycaemic medications. Hypertension was defined as having systolic blood pressure ≥ 140 mm Hg or diastolic blood pressure ≥ 90 mm Hg (average of two readings taken by the examining physician) or being treated with antihypertensive medications. Hypercholesterolemia was defined according to NCEP-ATP III [22]. Smoking status was defined as smoking one or more cigarettes per day in the year preceding the examination. Anemia was defined by the World Health Organization criteria as a hemoglobin concentration < 13 g/dL in men and <12 g/dL in women.

2.3. Biochemical measurements

Biochemical tests were performed in the laboratory of Thrombosis and Haemostasis unit within the Center for Excellence on Ageing (CeSI). Serum lipids, glucose, uric acid and hemoglobin levels were measured in fasting blood samples using standard procedures. Serum creatinine (Scr) was measured using a modified kinetic Jaffe reaction, and eGFR was estimated using the simplified Modification Diet of Renal Disease study (MDRD) equation [eGFR (mL/min/1.73 m^2) = 186 × (Scr) − 1.154 × (age) − 0.203 × (0.742 if female)] [23]. Proteinuria was defined by the presence of at least 30 mg/dL protein on urinalysis. For serum preparation for erythropoietin (EPO) and high-sensitivity C reactive Protein (hs-CRP) measurement, the blood was allowed to clot for 2 h at room temperature and then centrifuged at 2000 *g* for 10 min at 4 °C. The serum samples were aliquoted, coded and stored at −80 °C until analysis.

Serum EPO levels were determined using a commercially available two-site enzyme immunoassay (Biomerica EPO ELISA, Biomerica Inc. Irvine, CA, USA) on an ALISEI automated equipment (Radim Diagnostics, Pomezia, RM, Italy) according to the manufacturers' instructions. Intra- and inter-assay variation coefficients for EPO determination were both below 8%. The reference range for normal sample was 4–32 U/L.

hs-CRP determination was performed using a quantitative immunoturbidimetric ultrasensitive assay on an ARCHITECT c8000 System (Abbott Labs, Chicago, IL, USA). The limit of quantification for hs-CRP using this method was equal to 0.01 mg/dL with a linearity of the assay up to 16 mg/dL. The reference range for normal sample was 0.5 mg/dl.

2.4. Eicosanoid assessment

Urinary eicosanoids were quantified on morning urine specimens and indexed to urinary creatinine concentration (Enzo Life Sciences, Penn, USA; intra-assay CV = 2–4 (%). Urinary 8-iso-$PGF_{2\alpha}$ and 11-dehydro-TXB_2 were measured by previously described radioimmunoassay methods [24]. Measurements of urinary 8-iso-$PGF_{2\alpha}$ and 11-dehydro-TXB_2 by these radioimmunoassays have been validated using different antisera and by comparison with gas chromatography/mass spectrometry, as previously described [24].

Immunoreactive 11-dehydro-TXB_2 was extracted from 20-ml aliquots of each urine sample (the pH was adjusted to 4.0 to 4.5 with formic acid) on SEP-PAK C18 cartridges (Waters Associates, Milford, Mass.) and eluted with ethyl acetate. The eluate was subjected to silicic acid column chromatography and further eluted with benzene:ethyl acetate:methanol (60:40:30). The overall level of recovery, as determined by the addition of 11-dehydro-[3H]TXB_2, averaged 80 ± 6%. Immunoreactive 11-dehydro- TXB_2 eluted from silicic acid columns was assayed at a final dilution of 1:15 to 1:1000, as described elsewhere [25].

For 8-iso-$PGF_{2\alpha}$ measurement, 10-mL urine aliquots were extracted on Sep-Pak C18 cartridges (Waters Associates) after adjusting the pH to 4 with formic acid and eluted with 10 mL ethyl acetate. The eluates were subjected to silicic acid chromatography and further eluted with benzene/ethyl acetate/methanol (60:40:30, vol/vol/vol). These eluates were dried, recovered with 5 mL of buffer, and assayed in the radioimmunoassay system at a final dilution ranging between 1:30 and 1:60.