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Original article

Uremic toxicity and sclerostin in chronic kidney disease patients

Toxicité urémique et sclérostine dans la maladie rénale chronique

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ABSTRACT

Background and aims. – Sclerostin is a circulating inhibitor of the Wnt/β-catenin pathway and may have a role in chronic kidney disease (CKD)-mineral and bone disorder. Blood sclerostin levels are known to be elevated in patients undergoing maintenance dialysis. The aims of the present study were to evaluate sclerostin levels in patients at different CKD stages and study potential associations between sclerostin levels and (i) biochemical parameters that are disturbed in CKD, (ii) markers of vascular disease and (iii) mortality.

Methods. – One hundred and forty patients at CKD stages 2-5D were included in the present study. Routine clinical biochemistry tests and assays for sclerostin, protein-bound uremic toxins (indoxylsulphate [IS] and p-cresyl sulphate [PCS]) and the toxin β2 microglobulin (β2M) were performed. Aortic and coronary calcification and arterial stiffness were assessed by multislice spiral computed tomography and pulse wave velocity measurements. The enrolled patients were prospectively monitored for mortality.

Results. – Sclerostin levels were found to be elevated in CKD patients (especially those on hemodialysis). Furthermore, sclerostin levels were positively correlated with inflammation markers, phosphate, fibroblast growth factor 23, IS, PCS, β2M and arterial stiffness. A multivariate linear regression analysis indicated that sclerostin levels were independently associated with IS, PCS and β2M levels. Elevated serum sclerostin appeared to be associated with mortality (independently of age and inflammation). However, this association disappeared after adjustment for a propensity score including age, phosphate, interleukin-6, CKD stage and PCS.

Conclusion. – Our results indicate that sclerostin levels are elevated in CKD patients and are associated with inflammation, vascular lesions, uremia and (potentially) mortality.

1. Introduction

Sclerostin was initially characterized as a bone morphogenic protein antagonist. It was subsequently demonstrated that sclerostin was also a circulating inhibitor of the Wnt-signalling pathway, which modulates processes such as inflammation, endothelial activation, vascular muscle cell proliferation and ostoblastogenesis. Sclerostin is produced by osteocytes and is a soluble inhibitor of osteoblast function [1–4]. Sclerostin deficiency results in sclerosteosis, which is characterized by elevated bone mineral density [5,6]. Knockout of the sclerostin gene in mice is associated with a high bone mass phenotype [7]. Similarly, functional inhibition of sclerostin with an anti-sclerostin antibody in humans results in a massive increase in bone formation [8].

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Only a few studies have evaluated the clinical importance of sclerostin in patients with chronic kidney disease (CKD) [9,10]. However, the repression of osteocyte Wnt-signalling (suggested by elevated serum levels of sclerostin and dickkopf-1 [Dkk1]) appears to be an early event in the course of CKD-mineral and bone disorder (CKD-MBD) [11,12]. It has recently been shown that sclerostin levels are positively correlated with the presence of bone disease in pre-dialysis and hemodialysis CKD patients [10,13]. Recently, Cejka et al. hypothesized that sclerostin could have a role in renal osteodystrophy [9]. Indeed, sclerostin was presented as a strong predictor of low bone turnover in 60 patients on haemodialysis. It is noteworthy that sclerostin levels in these CKD stage 5D patients were higher than in healthy control subjects. Furthermore, sclerostin levels are already abnormally high in CKD stage 3 patients [12].

To date, studies of sclerostin in CKD have focused on the protein's links with bone disease. However, it is well known that CKD-MBD (which is characterized by elevated phosphate and fibroblast growth factor 23 [FGF23] levels) and the accumulation of uremic toxins could have a preponderant impact only on cardiovascular problems as well as bone status [14–20].

Hence, the objectives of the present study were to:

- evaluate sclerostin levels in patients at different CKD stages;
- assess the link between sclerostin levels on one hand and biochemical parameters that are disturbed in renal failure and markers of vascular disease (including aortic calcification and arterial stiffness) on the other;
- and evaluate the putative association between sclerostin levels and mortality.

2. Materials and methods

2.1. Ethics statement

The study was performed in accordance with the principles of the Declaration of Helsinki and in compliance with the International Conference on Harmonization's guidelines on Good Clinical Practice. The study protocol was approved by the local independent ethics committee (*Comité de Protection des Personnes Nord-Ouest II*) prior to the initiation of any study-specific procedures. The study was registered with the French health authorities (project number: 06H3). All patients were provided with full information on the study's objectives and procedures and gave their written, informed consent to participation.

2.2. Patient selection

Over an 18-month period (from January 2006 to June 2007), a total of 140 Caucasian, prevalent CKD patients were recruited from the nephrology department's outpatient clinic at Amiens university hospital.

Included patients had to be over the age of 40, with available serum sclerostin assay results and a confirmed diagnosis of CKD. The latter was defined as being on hemodialysis or having two previous estimated creatinine clearances (calculated according to the Cockcroft and Gault formula < 90 mL/min/1.73 m^2, with an interval of 3 to 6 months). Stage 5D CKD patients had been on chronic hemodialysis three times a week for at least three months. The exclusion criteria consisted of the presence of chronic inflammatory disease, atrial fibrillation, complete heart block, abdominal aorta aneurysm, aortic and/or femoral artery prosthesis, primary hyperparathyroidism, kidney transplantation and any acute cardiovascular event in the three months prior to screening for inclusion. The 140 patients who met all the inclusion criteria and none of the exclusion criteria were included in the present analysis.

2.3. Study protocol

All patients were hospitalized for the day in order to perform laboratory blood tests, blood pressure measurements, a pulse wave velocity (PWV) determination, a lateral lumbar X-ray and a multislice spiral computed tomography (MSCT) scan. For a given patient, all examinations were performed between 9 am and 2 pm on the same day. Hemodialysis patients were seen on a dialysis-free day or, if this was not possible, the morning before the dialysis session. A patient interview focused on comorbidities, the personal disease history and (in particular) any previous vascular events. The patients' medical files were reviewed in order to identify and record any concomitant medications. For descriptive purposes, patients who reported current or past use of insulin and/or orally administered hypoglycemic drugs were considered to be diabetics. Previous cardiovascular disease was defined as a history of any of the following events: myocardial infarction, stroke, heart failure, angina pectoris, peripheral artery disease and any surgical procedure or percutaneous transluminal angioplasty because of vascular disease.

2.4. Laboratory tests

Blood samples were collected in the morning, before the other investigations were undertaken. Selected assays were performed after the samples had been frozen and stored at $-80\,^{\circ}$C. Serum calcium, phosphate, albumin, cholesterol, hemoglobin, creatinine (Scr) and C-reactive protein (CRP) levels were assayed in an on-site biochemistry laboratory using standard auto-analyzer techniques (the Modular IIP® system, Roche Diagnostics, Basel, Switzerland). Serum intact parathyroid hormone (iPTH 1-84) was determined in a chemiluminometric immunoassay (Liaison N-tact PTH CLIA®, Diasorin, Stillwater, USA). Plasma intact FGF23 was determined using a two-site (N-terminal and C-terminal) enzyme-linked immunosorbent assay (Immunotopics, San Clemente, CA). Sclerostin and dkk1 were measured using a commercially available enzyme-linked immunosorbent assay (ELISA) (Immunodiagnostik AG, Bensheim, Germany). Reference mean $\pm$ standard deviation (SD) values for sclerostin and Dkk1 in healthy subjects were 49.8 ± 38.8 pmol/L and 16.1 ± 9.6 pmol/L, respectively. Due to a lack of aliquots, some measurements of sclerostin were performed on plasma samples thawed twice.

To determine the concentration of free p-cresyl sulphate (PCS), serum samples were deproteinized by heat denaturation and then analyzed by reverse-phase high-performance liquid chromatography (RP-HPLC). The serum concentrations were then determined by fluorescence spectrophotometry (excitation 265 nm; emission 290 nm). The reference value for free PCS in healthy subjects was 0.008 ± 0.009 mg/dL. For the determination of serum indoxyl sulphate (IS) levels, samples were deproteinized by heat denaturation and analyzed with RP-HPLC [21]. The serum concentrations were then determined by fluorescence spectrophotometry (excitation 280 nm, emission 340 nm) using a reference value for IS in healthy controls of 0.113 ± 0.06 mg/100 mL. The plasma concentration of β2 microglobulin (B2M) was measured by immunonephelometry (BNProSpec®, Siemens Healthcare Diagnostics GmbH, Eschborn, Germany).

Serum cystatin C (CysC) levels were also determined by immunonephelometry (BNProSpec analyzer, N latex Cystatin C® assay, Siemens Healthcare Diagnostics GmbH, Eschborn, Germany). In order to assess the true GFR in non-dialyzed patients as accurately as possible, the estimated GFR combining Scr and CysC measurements was calculated according to the following, recently published "CKD-epi" equation [22]: