Association of Circulating Sclerostin Levels with Carotid Artery Atherosclerosis and Endothelial Function in Prevalent Haemodialysis Patients

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Abstract

Background and Objectives: Sclerostin has emerged as a novel regulator of bone metabolism. This 22.5 kDa protein is synthesized in osteocytes and is a potent down-regulator of bone metabolism by reducing osteoblast differentiation and function via canonical Wnt-signalling inhibition. Recent studies suggested that serum sclerostin may have a potential role in atherosclerosis. High serum sclerostin was described in patients with CKD so it may have a role in accelerated atherosclerosis in those patients. The aim of this study is to investigate the relationship of circulating sclerostin levels with carotid artery atherosclerosis and endothelial function in prevalent haemodialysis patients.

Methods: In this cross-sectional study, serum sclerostin concentrations were measured using a commercially available ELIZA kit. CIMT was measured and carotid plaques were identified by B-mode ultrasound imaging. Endothelial function was assessed by measuring brachial artery flow mediated dilation by B-mode ultrasound imaging.

Results: Ninty prevalent haemodialysis patients were involved in the study. Serum sclerostin levels were higher in patients with plaques in CCA than patients free of plaques (2.43 ± 0.83 vs 2.08 ± 0.71 ng/ml, $p = 0.04$). A significant positive correlation was recorded between serum sclerostin levels and CIMT ($r = 0.469$, $P < 0.0001$). A significant negative correlation was recorded between serum sclerostin levels and brachial artery FMD ($r = -0.507$, $p < 0.001$).

Conclusions: Circulating Sclerostin is positively associated with carotid atherosclerosis and negatively associated with endothelial function in prevalent hemodialysis patients.

Keywords: sclerostin, canonical Wnt signaling, flow mediated dilation.

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ADMA	Asymmetric dimethylarginine
APC	Adenomatous polyposis coli
AT1	Angiotensin receptor 1
BMD	Bone mineral density
CKD	Chronic kidney disease
CVD	Cardiovascular disease
DKK1	Dickkopf-related protein 1
Dsh	Dishevelled
EGF	Epidermal growth factor
ESRD	End-stage renal disease
FGF23	Fibroblast growth factor 23
FMD	Flow mediated dilation
FrzA	Frizzled A
FZD	Frizzled
GFR	Glomerular filtration rate
GSK3	Glycogen synthase kinase 3
ICAM-1	Intercellular adhesion molecule 1
KRM	Kremen
LDLA	Low density lipoprotein type A
LRP5/6	Low-density lipoprotein receptor-related protein 5 or 6
MBD	Mineral and bone disorder
MIA	Malnutrition– inflammation–atherosclerosis
NO	Nitric oxide
OPG	Osteoprotegerin
PAI-1	Plasminogen activator inhibitor-1
RANKL	Receptor activator of nuclear factor-κB ligand
ROS	Reactive oxygen species
SMC	Smooth muscle cell
sFRP-1	Secreted frizzled related protein-1
VCAM-1	Vascular cell adhesion molecule 1
Wnt	Wingless integration

Introduction

A growing body of evidence indicates that abnormalities of bone and mineral metabolism in chronic kidney disease (CKD) may contribute to the development of cardiovascular disease (CVD) and increased cardiovascular mortality, with the most likely link being the development of vascular calcification **(Raggi and Kleerekoper, 2008)**. The signalling pathways involved in these processes remain incompletely understood **(Thompson and Towler, 2012)** and accumulating data have raised interest in understanding bone and mineral metabolism regulation and its consequences in patients with CKD.

Sclerostin is a soluble inhibitor of the Wnt/b-catenin (canonical) signalling pathway. The main action of sclerostin is a decrease in bone formation via inhibiting osteoblast proliferation and differentiation, and promoting osteoblast apoptosis **(Drüeke and Lafage-Proust, 2011)**. Recently, increased levels of sclerostin were shown to be associated with decreased bone turnover and osteoblast number in dialysis patients (**Cejka et al., 2011)**.

On the other hand, sclerostin has been demonstrated to be upregulated during vascular smooth muscle cell (SMC)

calcification in vitro **(Zhu et al., 2011)**. It also was shown that high serum sclerostin was associated with the extent of aortic valve calcification and that in aortic valve tissue, sclerostin strongly co-localized with areas of calcification in dialysis patients **(Brandenburg et al., 2013)**.

Wnts have a role in SMC proliferation, migration, and survival. Consequently, Wnt proteins are likely to play an important role in atherosclerosis and intimal thickening. Studies have quantified the expression of Wnt proteins and b-catenin as well as Wnt receptors and endogenous soluble inhibitors in atherosclerotic lesions and provide a powerful argument to this notion **(Mill and George, 2012)**.

Endothelial dysfunction has been proposed to be an early event of pathophysiologic importance in the atherosclerotic process and provides an important link between diseases such as hypertension, chronic renal failure, or diabetes and the high risk for cardiovascular events that patients with these conditions exhibit. Because endothelial dysfunction is an early event, it may be of prognostic value. In patients with and without coronary artery disease, endothelial dysfunction in coronary arteries was associated with cardiovascular events **(Endemann and Schiffrin, 2004)**.

Flow-mediated vasodilatation of the arm arteries (FMD) has become the most widely used technique to measure endothelial function due to its noninvasivness. The technique measures the ability of the arteries to respond with endothelial NO release during reactive hyperemia (flow mediated) after a 5-minute occlusion of the brachial artery with a blood pressure cuff. Celermajer and colleagues were the first to measure this response in vivo by measuring the respective diameter changes of the brachial or radial artery by ultrasound, a response later demonstrated to be mainly nitric oxide (NO) dependent **(Flammer et al., 2012)**.

Since CKD mineral and bone disorder influences cardiovascular event rate and mortality in CKD populations, the relationship between serum sclerostin levels and the future outcome was analysed in CKD cohorts. High levels of serum sclerostin was associated with higher mortality in non-dialysed CKD patients **(Kanbay et al., 2014)**. In contrast, low serum sclerostin levels were shown to be associated with increased mortality risk in haemodialysis patients **(Viaene et al., 2013; Drechsler et al., 2015)**. These studies were performed in subjects with different clinical characteristics and with

different sclerostin assays, yielding more questions as to whether sclerostin will be used as a biomarker for both cardiovascular and bone health status in CKD.

Wnt signaling activity in general and sclerostin activity in particular are associated with ectopic and vascular calcification processes beyond bone mineralization **(Brandenburg et al., 2016)**. Further evidence is needed to answer the question regarding the effects of sclerostin on arteriosclerosis (pro- or anti-calcific).

Aim of study

To investigate the relationship of circulating sclerostin levels with carotid artery atherosclerosis and endothelial function in prevalent haemodialysis patients.

Chapter 1

Sclerostin: new player in renal bone and vascular disease

Molecular structure of sclerostin and its interaction with LRP5/6

Sclerostin is a 190-residue secreted glycoprotein that contains a cystine-knot motif, which comprises six cysteine residues forming a knot from three disulfide bonds. Three loops emanate from the cystine-knot core. The first and the third loops, which are running in parallel from the central cystine-knot, are structured forming two stranded β-sheets, termed fingers 1 and 2 and the second loop, which runs in the opposite direction, is highly flexible **(Veverka et al., 2009; Weidauer et al., 2009)** (Figure 1). Several studies indicate that flexible loop is important for sclerostin's ability to neutralize Wnt signaling. First, Veverka et al showed that effective sclerostin monoclonal antibody binds to this region to interfere with the sclerostin-LRP interaction (**Veverka et al., 2009)**. Second, structure–function studies showed that mutations in the tip of that loop impair binding of sclerostin to LRP6 as well as its Wnt inhibitory capacity **(Boschert et al., 2013)**.

The LRP5/6 is a transmembrane receptor. The extracellular domain of LRP5/6 consists of four β-propeller domains separated by four EGF domains, followed by LDLA repeats,

transmembrane domains and a cytoplasmic domain **(Holdsworth et al., 2012)**. The cytoplasmic domain is involved in recruiting the GSK3/ Dsh/Axin/APC complex, whereas the extracellular domain is responsible for Wnt ligand binding and subjected to antagonist inhibition. Structural analysis reveals that the first two propeller domain of LRP6 are necessary for both sclerostin and Wnt ligand binding, suggesting that sclerostin and Wnt1 compete for the same binding site of LRP6 **(Holdsworth et al., 2012)**. Conformational changes in the LRP6 propeller domains and/or steric hindrance effects of sclerostin may also be responsible for sclerostin inhibition of other Wnt ligands binding to LRP6 **(Holdsworth et al., 2012)**.