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Effects of Uremic Toxins on Vascular and Bone Remodeling

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ABSTRACT

Chronic kidney disease (CKD) is associated with both extensive vascular calcification and abnormal bone remodeling, namely renal osteodystrophy. Moreover, there is increasing evidence for a close relationship between bone and vessel function. Pathological vascular calcification has been recently recognized as an active, cell-mediated process with similarities to physiological skeletal mineralization. Accordingly, we described the concept of vascular remodeling, in analogy to bone remodeling. In this review, we discuss the role of uremic toxins in the cross-talk between bone and vessel, and emphasize their potential contribution to the development of both vascular and bone remodeling disorders in patients with CKD.

Vascular calcification (VC) is a process that affects the arterial wall. It may occur in both intima layer (associated with atherosclerotic plaques) and media layer (Mönckeberg's sclerosis). Initially viewed as a passive phenomenon, VC has been recently recognized as an active, cell-mediated process. This process shares many similarities with that of skeletal mineralization, including the presence of a calcifying osteo/chondrocyte-like cells and an osteogenic extracellular matrix, as well as the expression of bone-related proteins (such as collagen type 1, osteopontin, and alkaline phosphatase) in calcified, atherosclerotic areas (1). More recently, it has also been shown that monocyte/macrophage cell types are able to differentiate directly into osteoclasts in calcified vessels (2). The coexistence of these two bone-like cell types suggests that VC may mimic the remodeling process which is normally observed only in bone tissue. Furthermore, according to the vascular remodeling hypothesis, VC occurs as the result of an imbalance between calcification formation and resorption processes, in favor of the former (Fig. 1) (2).

Vascular calcification in CKD patients has been linked to both traditional and uremia-related risk factors. One of these is renal osteodystrophy (ROD), a common complication of uremia that appears early in the course of CKD. ROD is characterized by abnormal

bone remodeling, that is a disturbance of the delicate balance between bone formation and resorption, which may range from a state of high bone turnover to that of low bone turnover (3). It is noteworthy that recent studies have generated an increasing body of evidence linking vascular and bone health in CKD. Indeed, low-turnover bone disease has been associated both with VC and arterial stiffness in chronic hemodialysis patients (4). Furthermore, improved bone remodeling has been linked to slower progression of coronary calcification (5). In view of these perspectives, "Kidney Disease: Improving Global Outcomes" (KDIGO) has recommended integrating the anomalies of mineral and bone metabolism and extra-skeletal calcification observed in uremic patients into a unique clinical entity, named CKD-mineral bone disorder (CKD-MDB) (3).

In this review, we will focus on several uremic toxins that may participate in the cross-talk between the skeletal and the vascular system, and interfere with remodeling at both sites.

Parathyroid Hormone

Chronic kidney disease is associated with a disorder of mineral homeostasis that generally manifests itself as hypocalcemia, hyperphosphatemia, and low serum levels of vitamin D sterols. It leads to the development of secondary hyperparathyroidism, characterized by an increase in parathyroid hormone (PTH) secretion, and parathyroid gland hyperplasia. Secondary hyperparathyroidism is a common finding in CKD patients. It generally occurs early in the course of the condition. It is associated with a number of negative outcomes in a variety of tissues and systems, including the bone and the
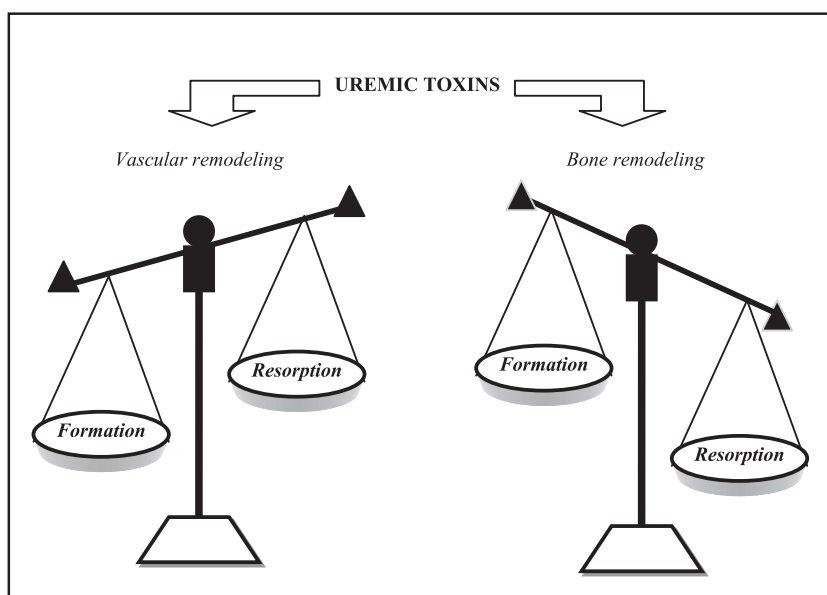
Fɪɢ. 1. Schematic representation of the effect of uremic toxins on vascular and bone remodeling. In bone, uremic toxins promote an uncoupling of the physiological remodeling process and the predominance of bone resorption. Conversely, in the vessels, the effects of uremic toxins result in a preponderance of heterotopic bone formation and inhibition of its resorption.

cardiovascular system. Given this variety of adverse effects, PTH has been considered as a major uremic toxin.

High PTH levels induce the development of a high-turnover bone disease called osteitis fibrosa (3). It is characterized by increased activity of osteoblasts and osteoclasts responsible for bone formation and resorption, respectively. However, the bone resorption rate often exceeds bone formation rate, leading to the bone loss that may progress to osteoporosis. Ultimately, this results in an increased efflux of phosphate and calcium from bone into the blood.

The direct effects of PTH on the vasculature remain subject to debate. In a rat model of CKD in which the animals underwent parathyroidectomy, continuous infusion of supraphysiological rates of synthetic PTH was associated with the development of extensive aortic calcification—independently of phosphate levels or the presence or the absence of uremia (6). Conversely, it has been reported that intermittent doses of synthetic PTH given by the I.P. route have a beneficial effect on vascular calcification and aortic osteogenic differentiation in a murine model of diabetic dyslipidaemia (7). Differences in methodology (i.e., continuous vs. intermittent PTH replacement) may explain these conflicting results. Importantly, a large epidemiological study recently reported an association between high PTH levels (intact-PTH > 600 pg/ml) on the one hand, and both cardiovascular hospitalization and poor survival in CKD patients on the other (8). Whether these clinical effects are mediated by a direct effect of PTH on VC development needs to be determined in further studies.

Phosphate

Phosphate is critical for many physiological functions, including skeletal development, mineral metabolism,

energy transfer via mitochondrial metabolism, and cell membrane phospholipid content and function. Eighty-five percent of the body's phosphorus stores are contained in bone as hydroxyapatite. Freely circulating phosphorus corresponds only to 0.15% of total body stores.

Urinary excretion of phosphate is regulated by several factors, including calcitriol, PTH, fibroblast growth factor-23 (FGF23) together with klotho, and, to a lesser extent, metabolic acidosis, volume expansion, glucocorticoids, calcitonin, growth hormone, and thyroid hormone. As CKD progresses, the kidneys are less able to adequately regulate phosphate levels, resulting in phosphate retention. Hyperphosphatemia has been recognized as a potent stimulus of secondary hyperparathyroidism. High phosphate levels may directly stimulate PTH secretion, PTH gene expression and parathyroid cell proliferation. Moreover, due to its inhibitory effect on 1-α-hydroxylase activity, phosphate may also indirectly enhance PTH secretion by reducing circulating calcitriol levels. High phosphate levels have also been linked to uremic skeletal resistance to the calcemic action of PTH (9). This view is supported by the demonstration that high inorganic phosphate concentrations in culture media directly inhibit (i) the generation of new osteoclasts and (ii) bone resorption by mature osteoclasts via an up-regulation of osteoprotegerin (OPG) expression (10).

Phosphate has also been implicated in the pathogenesis of VC. In vitro studies have confirmed that cultured vascular smooth muscle cells (VSMCs) calcify when exposed to elevated phosphate levels in the culture medium. Concomitantly, the VSMCs undergo a profound phenotypic transition characterized by (i) the loss of expression of markers of the VSMCs' contractile phenotype and (ii) simultaneous expression of osteochondrogenic markers [such as osteopontin, core binding factor

1 (Cbfa-1), osteocalcin] (1). In line with these in vitro observations, it has been recently demonstrated that the administration of oral phosphate binders in an animal model of CKD is able to prevent phosphate-induced vascular calcification (11). In chronic hemodialysis patients, it has been demonstrated that controlling phosphate levels with the noncalcium and nonaluminum containing phosphate binder sevelamer slows the progression of coronary artery calcification, as compared to calcium-containing binders (12).

Recently, our group has provided additional evidence of the involvement of phosphate in VC. We demonstrated that phosphate down-regulates the differentiation of monocyte/macrophage precursors into osteoclast-like cells via a negative feedback mechanism that interferes with the receptor activator of nuclear factor kappa B ligand (RANKL) and its receptor (RANK) (13). This finding may explain, at least partially, the difficulty in identifying osteoclast-like cells in calcified vessel areas. Thus, in addition to inducing the differentiation of VSMCs into osteoblast-like cells, phosphate may inhibit osteoclast-like cell differentiation in the vessel wall. Our postulate is that phosphate plays an important role in the proposed vascular remodeling imbalance not only by stimulating extra-osseous bone formation but also by blunting its resorption (Fig. 1).

Indoxyl Sulfate

Indoxyl sulfate (IS) is produced by the metabolism of dietary tryptophan. Briefly, tryptophan is metabolized to indole by intestinal bacteria. After intestinal absorption, indole is metabolized in the liver to indoxyl and then IS. As excretion of this compound depends on proximal tubular secretion, IS accumulates in the blood of CKD patients. It is noteworthy that the serum IS concentration is 30–80 times higher in nondialyzed CKD patients than in healthy subjects (14). It has been suggested that IS plays a role in the response of blood vessels and bone to the uremic environment (15,16).

In a rat model of chronic renal failure with low-turnover bone disease, osteoblast function worsens with the progression of kidney disease. This observation is compatible with the hypothesis that uremic toxins (including IS) accumulating in blood may be involved in low-turnover bone disease, as proposed by Iwasaki-Ishizuka et al. (16). In an additional study, this same group demonstrated that the oral charcoal adsorbent AST-120, which prevents IS accumulation in the blood, protected the animals against the development of low-turnover bone disease (17). Taken together, these findings indicate that IS may be at least one of the factors responsible for the skeletal resistance to PTH observed in the uremic state. Studies in CKD patients are required to test this hypothesis further.

Indoxyl sulfate may also act as a vascular toxin. It directly stimulates rat VSMC proliferation in a concentration-dependent manner (18). Furthermore, an increase in aortic wall thickness and severe aortic calcification, with colocalization of osteoblast-specific proteins such as Cbfa-1, osteopontin, and alkaline phosphatase,

were observed in salt-sensitive hypertensive Dahl rats to which IS was administered together with a high-salt diet (15). However, the effect of IS on human cells in vitro and in vivo remains to be proven.

Leptin

Leptin is a 16 kDa peptide hormone that regulates food intake and stimulates thermogenesis by acting on the hypothalamus. It is mainly produced by white fat cells. Therefore, serum leptin levels usually reflect the amount of adipose tissue. They are elevated both in progressive obesity and chronic renal failure, the latter as a consequence of reduced leptin ultrafiltration by the diseased kidney (19). Leptin exerts its effects by interacting with six different receptors (ObRa, ObRb, ObRc, ObRd, ObRe, and ObRf). In the circulation, leptin is carried by the ObRa receptor, released from vascular endothelial cells, and transported across the blood–brain barrier, where it activates ObRb receptors in the hypothalamus. This signal then stimulates expression of a hypothalamic osteoblast inhibitory factor (HOBIF), which lowers the osteoblasts' matrix-making ability (20). It has also been demonstrated that leptin has a direct osteotropic effect by (i) promoting the differentiation of bone marrow stromal cells into osteoblasts (21) and (ii) inhibiting osteoclast generation (22). The postulated relation between serum leptin levels and bone mass in humans is not yet well established. Concerning CKD, an inverse correlation between serum leptin levels and histomorphometric findings of bone resorption was reported in a cohort of 46 chronic hemodialysis patients (23). These apparent discrepancies may reflect a subtle balance between leptin's direct stimulatory action on osteoblast precursors and its indirect suppressive action on bone activity via the hypothalamus.

It seems plausible that leptin also plays a role on heterotopic bone formation, i.e., vascular calcification. It has been shown that mice express the leptin receptor in the artery wall and that in vitro, leptin induces osteoblast differentiation and VSMC calcification (24). Although it has been suggested that leptin may act selectively on certain VSMC subpopulations, which are more prone to calcification, additional evidence is required to define the precise role of leptin in vascular calcification unambiguously.

Conclusion

In recent decades, many steps involved in the pathogenesis of vascular calcification have been elucidated. It has been clearly demonstrated that active mechanisms, very much resembling those of skeletal ossification, are also involved in heterotopic bone formation. Recent studies have reinforced the link between abnormal bone remodeling and vascular calcification in CKD. Several uremic toxins such as PTH, phosphate, leptin, and IS may affect both skeletal and vascular remodeling by interfering with osteoblast function, osteoclast function, or both and thus contribute to renal osteodystrophy and

TABLE 1. The effects of uremic toxins on bone and vessels

Uremic toxin	Action on bone	Action on vessels
PTH	Increased osteoblast activity Increased osteoclast activity	Promotion of VC?
Phosphate	Induction of skeletal resistance to PTH	Induction of VSMC osteoblastic differentiation Inhibition of monocyte/macrophage osteoclastic differentiation Raising phosphate–calcium products
Indoxyl sulfate	Osteoblast dysfunction	Induction of VSMC proliferation
Leptin	Osteoblast dysfunction (indirect effect, via the hypothalamus) Promotion of differentiation of bone marrow precursors into osteoblasts Inhibition of osteoclasteogenesis	Induction of VSMC osteoblastic differentiation

PTH, parathyroid hormone; VC, vascular calcification; VSMC, vascular smooth muscle cell.

vascular calcification (Table 1). In particular, phosphate may play an important role in this context, as it has been demonstrated that phosphate may restrict osteoclast differentiation and activation and promote VSMC osteoblast differentiation. Other uremic toxins such as proinflammatory cytokines, advanced protein oxidation products, and advanced glycation products may also play a role. An integrative approach is essential in evaluating the respective roles of each of these factors.

Therapeutic interventions aimed at reducing or preventing vascular calcification should be seen in the context of each treatment's potential effects on bone and vice versa. Approaches that reduce levels of uremic toxins, e.g., via the use of phosphate binders and AST-120, have already been shown to exert beneficial effects, albeit in relatively small patient cohorts. Randomized clinical trials addressing the impact of such therapeutic interventions on vascular calcification, bone remodeling, and morbidity/mortality in CKD patients are clearly required. Stimulating osteoclast activity in calcified vascular areas appears to be a promising field as well, given that active osteoclasts are capable of demineralizing calcified elastin thereby limiting calcification (25). However, whether focal stimulation of osteoclast activity in calcifying blood vessels is possible in the absence of effects on osteoclast activity in bone, and if so, whether it is beneficial or not remains to be determined.

References

1. Jono S, McKee MD, Murry CE, Shioi A, Nishizawa Y, Mori K, Morii H, Giachelli CM: Phosphate regulation of vascular smooth muscle cell calcification. *Circ Res* 87:E10–E17, 2000
2. Massy ZA, Mentaverri R, Mozar A, Brazier M, Kamel S: The pathophysiology of vascular calcification: are osteoclast-like cells the missing link? *Diabetes Metab* 34(Suppl. 1):S16–S20, 2008
3. Moe S, Drüeke T, Cunningham J, Goodman W, Martin K, Olgaard K, Ott S, Sprague S, Lameire N, Eknoyan G, Kidney Disease: Improving Global Outcomes (KDIGO): definition, evaluation, and classification of renal osteodystrophy: a position statement from Kidney Disease: Improving Global Outcomes (KDIGO). *Kidney Int* 69:1945–1953, 2006
4. London GM, Marchais SJ, Guérin AP, Boutouyrie P, Métivier F, de Vernejoul MC: Association of bone activity, calcium load, aortic stiffness, and calcifications in ESRD. *J Am Soc Nephrol* 19:1827–1835, 2008
5. Barreto DV, de Carvalho Barreto F, de Carvalho AB, Cuppari L, Draibe SA, Dalboni MA, Moyses RM, Neves KR, Jorgetti V, Miname M, Santos RD, Canziani ME: Association of changes in bone remodeling and coronary calcification in hemodialysis patients: a prospective study. *Am J Kidney Dis* 52: 1139–1150, 2008
6. Neves KR, Graciolli FG, dos Reis LM, Graciolli RG, Neves CL, Magalhães AO, Custódio MR, Batista DG, Jorgetti V, Moysés RM: Vascular calcification: contribution of parathyroid hormone in renal failure. *Kidney Int* 71:1262–1270, 2007
7. Shao JS, Cheng SL, Charlton-Kachigian N, Loewy AP, Towler DA: Teriparatide (human parathyroid hormone (1-34)) inhibits osteogenic vascular calcification in diabetic low density lipoprotein receptor-deficient mice. *J Biol Chem* 278:50195–50202, 2003
8. Tentori F, Blayney MJ, Albert JM, Gillespie BW, Kerr PG, Bommer J, Young EW, Akizawa T, Pisoni RL, Robinson BM, Port FK: Mortality risk for dialysis patients with different levels of serum calcium, phosphorus, and PTH: the Dialysis Outcomes and Practice Patterns Study (DOPPS). *Am J Kidney Dis* 52:519–530, 2008
9. Bover J, Jara A, Trinidad P, Rodriguez M, Felsenfeld AJ: Dynamics of skeletal resistance to parathyroid hormone in the rat: effect of renal failure and dietary phosphorus. *Bone* 25:279–285, 1999
10. Kanatani M, Sugimoto T, Kano J, Kanzawa M, Chihara K: Effect of high phosphate concentration on osteoclast differentiation as well as bone-resorbing activity. *J Cell Physiol* 196:180–189, 2003
11. Phan O, Ivanovski O, Nguyen-Khoa T, Mothu N, Angulo J, Westenfeld R, Ketteler M, Meert N, Maizel J, Nikolov IG, Vanholder R, Lacour B, Drüeke TB: Sevelamer prevents uremia-enhanced atherosclerosis progression in apolipoprotein E-deficient mice. *Circulation* 12:2875–2882, 2005
12. Chertow GM, Burke SK, Raggi P: Sevelamer attenuates the progression of coronary and aortic calcification in hemodialysis patients. *Kidney Int* 62:245–252, 2002
13. Mozar A, Haren N, Chasseraud M, Louvet L, Mazière C, Wattel A, Mentaverri R, Morlière P, Kamel S, Brazier M, Mazière JC, Massy ZA: High extracellular inorganic phosphate concentration inhibits RANK-RANKL signaling in osteoclast-like cells. *J Cell Physiol* 215:47–54, 2008
14. Vanholder R, De Smet R, Glorieux G, Argilés A, Baurmeister U, Brunet P, Clark W, Cohen G, De Deyn PP, Deppisch R, Descamps-Latscha B, Henle T, Jörres A, Lemke HD, Massy ZA, Passlick-Deetjen J, Rodriguez M, Stegmayr B, Stenvinkel P, Tetta C, Wanner C, Zidek W, European Uremic Toxin Work Group (EUTox): Review on uremic toxins: classification, concentration, and interindividual variability. *Kidney Int* 63:1934–1943, 2003
15. Adijiang A, Goto S, Uramoto S, Nishijima F, Niwa T: Indoxyl sulphate promotes aortic calcification with expression of osteoblast-specific proteins in hypertensive rats. *Nephrol Dial Transplant* 23:1892–1901, 2008
16. Iwasaki-Ishizuka Y, Yamato H, Nii-Kono T, Kurokawa K, Fukagawa M: Downregulation of parathyroid hormone receptor gene expression and osteoblastic dysfunction associated with skeletal resistance to parathyroid hormone in a rat model of renal failure with low turnover bone. *Nephrol Dial Transplant* 20:1904–1911, 2005
17. Iwasaki Y, Yamato H, Nii-Kono T, Fujieda A, Uchida M, Hosokawa A, Motojima M, Fukagawa M: Administration of oral charcoal adsorbent (AST-120) suppresses low-turnover bone progression in uraemic rats. *Nephrol Dial Transplant* 21:2768–2774, 2006

18. Yamamoto H, Tsuruoka S, Ioka T, Ando H, Ito C, Akimoto T, Fujimura A, Asano Y, Kusano E: Indoxyl sulfate stimulates proliferation of rat vascular smooth muscle cells. *Kidney Int* 69:1780–1785, 2006

19. Whitfield JF: Leptin: brains and bones. *Expert Opin Investig Drugs* 10:1617–1622, 2001

20. Ducy P, Amling M, Takeda S, Priemel M, Schilling AF, Beil FT, Shen J, Vinson C, Rueger JM, Karsenty G: Leptin inhibits bone formation through a hypothalamic relay: a central control of bone mass. *Cell* 100:197–207, 2000

21. Thomas T, Gori F, Khosla S, Jensen MD, Burguera B, Riggs BL: Leptin acts on human marrow stromal cells to enhance differentiation to osteoblasts and to inhibit differentiation to adipocytes. *Endocrinology* 140:1630–1638, 1999

22. Holloway WR, Collier FM, Aitken CJ, Myers DE, Hodge JM, Malakellis M, Gough TJ, Collier GR, Nicholson GC: Leptin inhibits osteoclast generation. *J Bone Miner Res* 17:200–209, 2002

23. Coen G, Ballanti P, Fischer MS, Balducci A, Calabria S, Colamarco L, Di Zazzo G, Lifrieri F, Manni M, Sardella D, Nofroni I, Bonucci E: Serum leptin in dialysis renal osteodystrophy. *Am J Kidney Dis* 42:1036–1042, 2003

24. Parhami F, Tintut Y, Ballard A, Fogelman AM, Demer LL: Leptin enhances the calcification of vascular cells: artery wall as a target of leptin. *Circ Res* 88:954–960, 2001

25. Simpson CL, Lindley S, Eisenberg C, Basalyga DM, Starcher BC, Simionescu DT, Vyavahare NR: Toward cell therapy for vascular calcification: osteoclast-mediated demineralization of calcified elastin. *Cardiovasc Pathol* 16:29–37, 2007