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Iron and vascular calcification. Is there a link?

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Abstract

Iron deficiency is frequently seen in patients with end-stage renal disease, particularly in those treated by dialysis, this is because of an impairment in gastrointestinal absorption and ongoing blood losses or alternatively, due to an impaired capacity to mobilize iron from its stores, called functional

iron deficiency. Therefore, these patients may require intravenous iron to sustain adequate treatment with erythropoietin-stimulating agents. Aside from this, they are also prone to vascular calcification, which has been reported a major contributing factor in the development of cardiovascular disease and the increased mortality associated herewith. Several factors and mechanisms underlying the development of vascular calcification in chronic kidney diseased patients have been put forward during recent years. In view of the ability of iron to exert direct toxic effects and to induce oxidative stress on the one hand versus its essential role in various cellular processes on the other hand, the possible role of iron in the development of vascular calcification should be considered.

Keywords: anemia; iron; vascular calcification; osteoblast phenotype; oxidative stress; vascular smooth muscle cell

Introduction

Anemia and vascular calcification are common complications in patients with chronic kidney disease (CKD), particularly those treated by dialysis [1].

The anemia associated with end-stage renal disease is routinely corrected with recombinant human erythropoietin (r-HuEPO) and leads to substantial improvements in hemoglobin (Hgb) levels and reduced blood transfusion requirements which in turn are associated with general improvements in quality of life and decreased hospitalization rate [2, 3]. However, recent studies suggest that correction of anemia with erythropoiesis-stimulating agents (ESAs) might be associated with cardiovascular events and an increased risk of mortality [4–7]. The use of r-HuEPO leads to rapid utilization of iron and thus is associated with the progression of iron deficiency, therefore most dialysis patients require iron supplementation at some stage in order to maintain responsiveness to ESAs [8]. Literature indicates that excessive iron load may cause several toxic side effects. Rare acute adverse events caused by allergic reactions may occur [9]. Furthermore, iron overload may lead to ventricular arrhythmia and heart failure [10] and causes low density lipoprotein (LDL) oxidation, endothelial dysfunction and an increased infection rate thereby stimulating atherosclerosis [9, 11]. These findings suggest that iron administration may contribute to cardiovascular complications in CKD.

Cardiovascular disease accounts for 50% of all deaths in CKD. Vascular calcification is the major cause of vascular disease and is rapidly progressive in dialysis patients [12]. In CKD patients, compared with the general population, more severely calcified intimal atherosclerotic plaques and typical arterial medial calcifications are detected, both of which are independently associated with vascular mortality [13]. In addition, severe coronary artery calcification develops in young adult patients with end-stage renal disease [14]. Vascular calcification is a tightly cell-regulated pathological process that resembles osteogenesis [15–17] and results from an imbalance between inhibitors and inducers of mineralization [18, 19]. Although further evidence is needed, recent literature data point to a potential role of iron in the development of vascular calcification in CKD patients [20].

Clinical context and pathophysiological implications of vascular calcification

Coronary artery calcification is recognized as an independent predictor of cardiovascular disease [21] and mortality [22]. Aortic calcification promotes congestive heart failure by reducing arterial compliance. Ectopic vessel mineralization can be localized either in the tunica intima or the tunica media of the vessel. Although at present, there is no general consensus concerning the separation between intimal and medial calcification [23], a large body of evidence indicates that both types of calcification can appear independent of each other and have a distinct clinical presentation [24]. Intimal calcification is associated with atherosclerosis, mainly resulting from dyslipidemia and hypercholesterolemia and is characterized by lipid accumulation, foam cell formation, inflammation, oxidative stress and apoptosis. This type of vascular calcification results in focal calcification of atherosclerotic plaques, whereas medial calcification (arteriosclerosis or Mönckeberg's sclerosis) is more generalized and is found mainly in the elderly and in patients with CKD, osteoporosis, hypertension or diabetes mellitus. Mönckeberg's sclerosis leads to vessel stiffening, which is characterized by increased pulse pressure and elevated pulse wave velocity and is associated with increased cardiovascular risk [13, 25].

Vascular calcification is a cell-regulated pathological process showing striking similarities to osteogenesis. Structures similar to mineralized bone, cartilage and even bone marrow are detected in the arterial wall [26, 27]. Evidence has been presented that cell types responsible for normal bone metabolism, i.e. osteoblasts, chondrocytes and osteoclasts, are present in calcified vessels indicating that a similar regulation to bone formation and mineralization occurs during vascular calcification. In addition, various proteins involved in bone and mineral metabolism are induced in calcified arteries [16, 17, 28–30]. Transdifferentiation of vascular smooth muscle cells into osteochondrogenic cells is a well-known process underlying calcification in the vessel wall, triggered by a disturbed calcium, phosphorus and vitamin D metabolism together with a loss of calcification inhibitors such as pyrophosphate, matrix Gla protein and fetuin-A [31–34].

In general, intimal and medial calcification are not distinguished in epidemiological studies, a possible confounding factor that must be taken into account when interpreting literature data dealing with these pathological events.

Iron and vascular calcification in CKD

Oxidative stress and vascular calcification

In the context of various clinical settings, morphologic and functional characteristics at the cellular and molecular

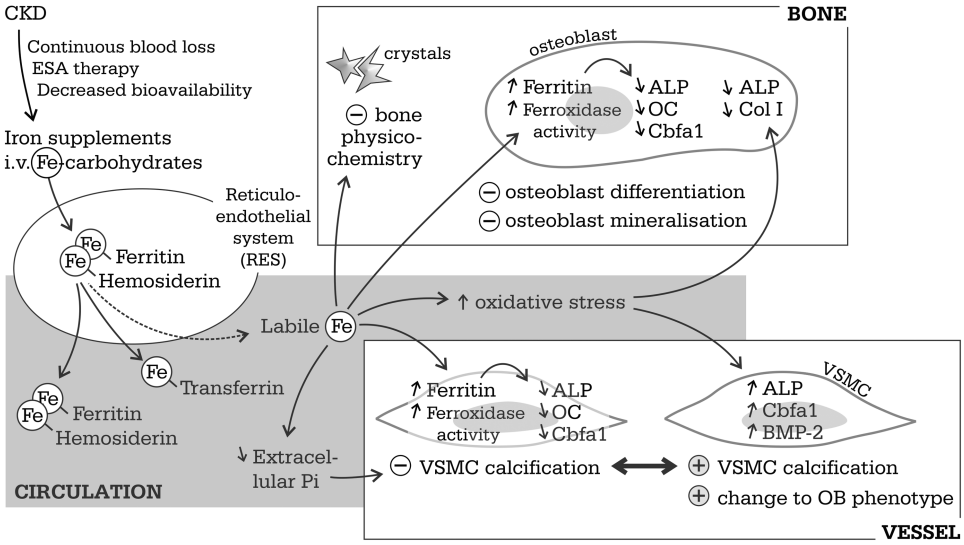

Fig. 1. *In vitro* effects of iron on osteoblasts and vascular smooth muscle cells (VSMC): opposite effects on smooth muscle cell calcification. Iron deficiency in patients with CKD results from reduced intake, blood loss during dialysis and depletion of the iron reserves for the production of red blood cells stimulated with ESA therapy and is treated with intravenous iron compounds. These compounds dissociate in the RES and are stored as ferritin or hemosiderin or finally transferred to transferrin. However, during these iron transfers, labile iron may be released in the circulation and may affect bone metabolism and target the vascular wall directly by inhibition of osteoblast differentiation and mineralization on the one hand and inhibition of VSMC calcification on the other hand, both of which occur at the cellular level through induction of ferritin and its ferroxidase activity. In addition, iron may also interact directly with the physicochemical formation of hydroxyapatite in bone. Alternatively, iron induces oxidative stress, which might be another pathway contributing to impaired osteoblast differentiation and mineralization. In contrast, iron-induced oxidative stress induces VSMC calcification and transdifferentiation toward osteoblast-like cells.

level determine the occurrence of aortic valve calcification, atherosclerotic calcification, diabetic medial artery calcification, vascular (both medial and intimal) calcification in CKD and calcific arteriolopathy also known as calciphylaxis [35]. Although vascular calcification has long been considered to take place via a passive process of dead and dying cells, work from laboratories worldwide has now highlighted that arterial biomineralization is an active tightly regulated form of calcified tissue metabolism. During recent years, an important role of oxidative stress signaling in vascular activation of osteogenic gene regulatory programs has been identified [35–37]. Various factors that have been linked to an increased prevalence of vascular calcification are associated with elevated oxidative stress, including hypercholesterolemia, hypertension, diabetes mellitus and (dialysis-dependent) end-stage renal disease. The uremic state is characterized by increased oxidative stress, which is the net balance between oxidant production and antioxidant activity. Pro-oxidants include reactive nitrogen species and reactive oxygen species (ROS). Although it has been known for many years that oxidative stress plays a critical role in the development of atherosclerosis and is involved in the formation of lipid laden macrophages and the development of inflammation, the contribution of oxidative stress-induced molecular signaling in the pathogenesis of vascular calcification has only been demonstrated very recently. In an elegant study, Byon *et al*. [37] found that exposure of primary cultures of vascular smooth muscle cells to hydrogen peroxide (H_2O_2), a well-known key player in oxidative stress in atherosclerotic lesions, promotes a shift of these cells from a contractile to an osteogenic phenotype (Figure 1) as indicated by an increased expression of Runx2, a key transcription factor for osteogenic differentiation, while inhibition of H_2O_2-activated AKT signaling blocked vascular smooth muscle cell calcification and Runx2 induction concurrently; without mediating apoptosis. Nakahara *et al*. [38] recently showed that H_2O_2 production and activation of s-Src and extracellular signal-regulated kinases 1 and 2 are required for fibroblast growth factor (FGF)-2 signaling which in turn was shown to activate osteoblast-marker gene expression by enhancing DNA-binding activity of Runx2 to its cognate binding site in vascular smooth muscle cells. In line herewith are findings demonstrating H_2O_2 and xanthine/xanthine oxidase to dose dependently enhance the osteoblastic phenotype marker alkaline phosphatase (ALP) in primary bovine aortic smooth muscle cells, while the authors observed the inverse effect in both the MC3T3-E1 mouse preosteoblast and M2-10B4 mouse marrow stromal cell lines [39] hereby supporting the opposite effects of oxidative stress on pathological vascular smooth muscle cell calcification in the vessel wall and physiological osteoblast mineralization in bone, the so-called 'calcification paradox' of the bone-vascular axis [40, 41]. Alternatively, oxidative stress has been shown to induce apoptosis in cultured vascular smooth muscle cells [42, 43]. Since apoptosis has been proposed to be one of the mechanisms initiating the calcification process by forming a nidus for the deposition of calcium–phosphate crystals [44–46], this may represent another pathway by which oxidative stress contributes to ectopic mineralization. Furthermore, accumulation of ROS has recently been demonstrated around calcifying foci in human vascular cells expressing osteoblast/osteoclast but not macrophage markers [47]. Using

8-oxo-7,8-dihydro-2′-deoxyguanosine (8-OHdG) as a marker of oxidative stress, Dalfino *et al*. [48] reported this parameter to be increased in CKD patients as compared to subjects with normal renal function. Moreover, serum 8-OHdG levels directly correlated with the serum level of the bone morphogenetic protein BMP-2, which belongs to the transforming growth factor-β superfamily involved in the pathogenesis of vascular calcification. Findings in humans could be confirmed *in vitro* after pre-incubating cultured vascular smooth muscle cells with H_2O_2, which enhanced the BMP-2-induced upregulation of ALP. Advanced oxidative protein products (AOPPs) directly increased the calcium deposition and expression of core-binding factor-alpha-1 (cbfa-1) and osteopontin and significantly decreased α-smooth muscle actin expression in human aortic smooth muscle cells *in vitro* [49].

Iron, oxidative stress and cardiovascular disease

There is little doubt that iron causes oxidative stress in cells and animals by its ability to induce ROS. Indeed, as iron has different oxidation states, it is redox-active by which electrons are transferred to or from organic compounds hereby inducing the process of radical formation. The initial reactive oxygen intermediate produced in almost all cases related to oxidative stress is $O_2^{\cdot-}$ (superoxide), which is rapidly converted to H_2O_2 (hydrogen peroxide) by the action of superoxide dismutases. Neither $O_2^{\cdot-}$ nor H_2O_2 are strong oxidizing agents and the only intracellular targets they can usually interact directly with are iron or iron containing molecules. However, when redox-active iron ions are available, the so-called 'Fenton-type' reaction (see below) takes place, producing the extremely reactive ·OH. Under inflammatory conditions, ferric iron (Fe^{3+}) is reduced by leukocyte-generated superoxide back to ferrous iron (Fe^{2+}), which in turn is involved in the generation of ·OH thus allowing iron to act as a catalyst of the reaction below [49–51].

$$Fe^{2+} + H_2O_2 \rightarrow \text{reactive intermediates} \rightarrow Fe^{3+} + {}^{\cdot}OH + OH^- \quad (1)$$

$$Fe^{3+} + O_2^{\cdot-} \rightarrow Fe^{2+} + O_2 \quad (2)$$

(adapted from Galaris and Pantopoulos [50]).

Although other metals, like copper, are able to catalyze Reaction 1 to an even higher extent than iron, the latter due to its availability in biological systems is believed to be the main catalyst in living cells [51, 52].

In view of (i) the involvement of iron in the induction of ROS, (ii) the notion that the therapeutic use of intravenous (i.v.) iron has been shown to increase biological makers of oxidative stress in cell cultures, animal models and CKD patients on hemodialysis [53] and (iii) the potential role of oxidative stress in the development of cardiovascular complications, the role of iron in cardiovascular disease/vascular calcification in CKD patients should be considered, especially since in CKD patients i.v. iron supplementation has been associated with an increase in bioactive iron [54].

In their excellent review, Kletzmayr and Hörl [11] put forward some hypotheses based on various studies suggesting iron overload to be associated with an increased cardiovascular risk in the general population. Although these hypotheses should be taken seriously, the authors stated that further research is needed to determine whether the association detected by some investigators between high iron stores and cardiovascular disease might have been due to non-controlled confounding factors such as serum lipid profile, dietary habits and cigarette smoking. With regard to the mechanism by which iron might underly cardiovascular disease within the general population, it was suggested that iron might either exert its role by promoting lipid peroxidation or by exacerbating the impairment of nitric oxide activity in the endothelium, a known component in atherosclerosis. Another pathway by which iron may contribute to cardiovascular complications is by favoring bacterial infection. Iron overload in hemodialysis patients is associated with an increased risk for infection [55, 56]. Decreased polymorphonuclear phagocytic function, reduced resistance to bacterial growth and an altered cytokine profile are proposed possible mechanisms by which iron may increase the infection rate [57]. Infection creates an inflammatory environment, which is a well-known key player in the atherogenesis and the development of vascular calcification.

Simple iron species originating from degradation of heme proteins and other sources have been reported to bind to negatively charged phospholipids under the formation of lipid peroxides [58]. Corroborative evidence for the association of iron status with lipid peroxidation and cardiovascular disease was provided in a series of patients from the Bruneck study in which serum ferritin and LDL cholesterol had a synergistic association with progression of carotid atherosclerosis which made the authors suggest that iron acted by promoting lipid peroxidation [11, 59]. In line herewith are data from another study in which smokers were randomized to either donate blood or not. In the blood donors, the concomitant decrease (44%) in serum ferritin was associated with a 20% decrease in maximal oxidation velocity, which was accompanied by a significant increase in the oxidation resistance of serum very low density lipoprotein/LDL [60].

Drüeke *et al*. [61] investigated the effect of i.v. iron therapy on plasma AOPP in 79 dialysis patients. These are considered reliable markers of oxidant-mediated protein damage and mediators of inflammation in uremic patients [61–63]. They found an association between these parameters and common carotid artery (CCA) intima-media thickness, serum ferritin and the annual i.v. iron dose administration which made them conclude that iron therapy with the doses recommended at that time (1.5–2 g of supplemental iron per year) could contribute to arterial wall damage in the early phase of atherosclerosis [62]. Data from Drüeke *et al*. later on were confirmed by findings of Reis *et al*. [64], showing a significant association between serum ferritin, i.v. iron dose and CCA intima-media thickness in 60 dialysis patients.

Iron, oxidative stress and vascular calcification

As discussed above, excess iron enhances oxidative stress, which in turn triggers molecular mechanisms that lead to vascular calcification. Patients with dialysis dependent CKD are frequently exposed to i.v. iron that comprises la-

bile, redox-active iron that can exacerbate the underlying state of oxidative stress and inflammation secondary to uremia *per se*, and oxidative stress can promote vascular calcification.

HO-1 (heat shock protein-32) one of the three known oxygenases is an important enzyme involved in vascular disease and inflammation. Using primary osteoblastic cells isolated from calvaria of 1-day-old Sprague-Dawley rats, Lin *et al*. [65] nicely showed that upregulation of HO-1 inhibits the maturation and mineralization of osteoblasts. Knowing that on the one hand, HO-1 can be upregulated by oxidative stress [66], while on the other hand, HO-1 catalyzes the degradation of heme into carbon monoxide, biliverdin but also ferrous iron, the decreased expression of ALP and Runx-2 after a 3-day exposure of the cells to ferrous histidinate is of particular interest and may indicate that iron is involved in the inhibitory effects of HO-1 [65]. Others also reported iron to inhibit osteoblast metabolism [67, 68]. With regard to the potential mechanism by which iron may suppress osteoblastic activity Zarjou *et al*. [69] recently demonstrated that the iron-induced inhibition of mineralization as well as the downregulation of a number of osteoblast-specific genes such as ALP, osteocalcin and cbfa-1 is provided by an iron-induced upregulation of ferritin and that the protein's ferroxidase activity plays a crucial role in this (Figure 1). Although with regard to vascular calcification, these findings at first glance are in contrast with iron's putative role as a promoter of differentiation of vascular smooth muscle cells toward an osteoblastic phenotype and subsequent calcification, they may also point to a dual role of iron in the cell biology and mineralization processes and be in line with the reported 'calcification paradox', i.e. the inverse correlation that may exist between physiological calcification of bone and pathological calcification in vessels [40]. In this respect, the opposite effects of lipid oxidation products on calcifying vascular cell and bone cell differentiation as reported by Parhami *et al*. [70] are worth mentioning. They indeed found that minimally oxidized low density protein as well as several other lipid oxidation products induced ALP acitivity and differentiation of vascular cells to a calcifying osteoblast-like cell type, while they inhibited the differentiation of MC3T3-E1 bone cells as evidenced by their stimulatory effects on proliferation and their inhibitory effect on the induction of ALP and calcium uptake. Another example in support of opposite actions at the bone and vessel level is the inhibitory effect of parathyroid hormone (PTH) on osteogenic vascular calcification, but the enhancing effect of PTH on bone mineralization in an low density lipoprotein receptor-deficient mouse model [71].

The role of iron in the development of vascular calcification is also not yet completely clear. Aside from the inductive effects of iron on differentiation/calcification of vascular smooth muscle cells, inhibitory effects have been reported also (Figure 1). In this context, recent *in vitro* findings by Zarjou *et al*. [20] should be mentioned as they may shed some new light on the role of iron and iron-containing molecules in these processes. The authors, following their above-mentioned work in osteoblasts [69], examined whether induction of HO-1 and ferritin alters the osteoblastic differentiation and mineralization of human aortic smooth muscle cells. These two molecules are highly inducible by heme and iron and of which ferritin also exhibits antioxidant properties. Exposing these cells to either heme or iron went along with a dramatic (i) upregulation of ferritin and HO-1 and (ii) inhibition of calcification and osteoblastic differentiation of the vascular smooth muscle cells. Strikingly, this latter effect was also seen when cells were exposed to either apoferritin, recombinant H-chain ferritin or even ceruloplasmin (three molecules with ferroxidase activity), while only a minor inhibitory effect was observed with the non-ferroxidase active L-ferritin chain and no effect at all with the H-mutant 222 ferritin lacking both ferroxidase activity and iron-storing capability. Overall, this made the authors put forward the hypothesis that inflammatory diseases such as CKD may favor binding of hepcidin to ferroportin leading to internalization and degradation of the latter transport protein, which ultimately will result in an increased sequestration of iron within the macrophages limiting its bioavailability to other cells. Aside from a possible role of the HO/ferritin pathway, the authors also found iron to decrease the level of intracellular phosphate which they attributed to the element's phosphate-binding capacity, a hypothesis which makes sense in view of the knowledge that phosphate is considered a major inducer of vascular calcification and made the authors conclude that derangements in iron metabolism in CKD may facilitate phosphate-induced vascular calcification [20].

Iron, FGF23, PTH and vascular calcification

Molecules or pathways influencing phosphate homeostasis such as FGF-23, and Klotho, have been shown to affect both vascular calcification and bone mineralization. FGF-23 is a phosphaturic hormone that inhibits renal phosphate reabsorption and negatively regulates renal 1α-hydroxylase activity. FGF-23-null mice exhibit hyperphosphatemia, in combination with skeletal abnormalities (decreased bone mineral density and osteoidosis) and vascular calcification. The antiaging protein Klotho acts as a cofactor for the binding of FGF-23 to the FGF receptor. Klotho-deficient mice show an accelerated aging syndrome, with osteopenia and vascular calcification, in combination with both hyperphosphatemia and hypercalcemia [72–74].

In view of previous observations that parenteral iron administration may be associated with hypophosphatemia and osteomalacia [75, 76], Schouten *et al*. [77] examined in a prospective hypothesis-driven study in iron-deficient outpatients what might be the effect of a single infusion of iron polymaltose on plasma phosphate and FGF-23. It was found that a single infusion of the compound caused a significant and prolonged FGF-23 elevation accompanied by a decrease in plasma phosphate, renal phosphate wasting and suppression of 1,25(OH)2vitD3, which, in contrast to a contemporarily published case report [78] was not due to a direct toxic effect of iron on renal tubular cells. Although the mechanism underlying these changes is still speculative, the authors put forward the interesting suggestion that iron probably may regulate the rate of enzymatic cleavage of intact FGF-23 with increased iron levels inhi-

biting and iron deficiency increasing protease activity which fits with the findings of elevated C-terminal FGF-23 fragments in patients with low serum ferritin [79]. While in subjects with intact renal function, increased FGF-23 levels lead to hypophosphatemia; in CKD, a further iron-triggered augmentation in the already increased FGF-23 levels (to compensate for persistent phosphate retention) will further reduce the production of 1,25-dihydroxyvitamin D which in turn leads to hypersecretion of PTH which can not be corrected for by FGF-23 due to the uremia-related reduced expression of the FGF-receptor1c complex at the level of the parathyroid gland [80]. This vicious circle will lead to an aggravation of the already existing hyperphosphatemia and further augment serum FGF-23, which both are shown to be important determinants of vascular calcification [81, 82] and mortality [83, 84] in CKD patients. In this context, it is worth mentioning that the phosphate-binding capacity of iron is well known and oral iron-based agents for the control of phosphate in CKD are under current investigation [85].

An alternative link between iron and vascular calcification might be the potentially harmful effect of iron overload on parathyroid gland function. In major β-Thalassemia patients, chronic iron overload due to repeated blood transfusions is associated with hypoparathyroidism. In this context, it is worth mentioning that extensive arterial calcification in hemodialysis patients has been associated with low serum PTH levels and low bone activity and adynamic bone [86].

Iron and calcific uremic arteriolopathy

Further support for a potential role of iron in ectopic calcification has been provided from studies dealing with calcific uremic arteriolopathy (CUA), previously known as calciphylaxis. As early as in the 1960s, experimental studies by Selye *et al*. [87] demonstrated that rats exposed to a 'sensitizing' medication, such as vitamin D or PTH, and then injected with a 'challenging' compound, such as ferric dextran, developed acute soft tissue calcification. Anghileri [88] later on found that local calcification of soft tissues in mice strongly depends upon the nature of the molecule and that ferric lactate is much more effective than iron dextran in inducing calcification. Based hereupon and in the knowledge that ferric lactate and iron dextran, respectively, are a strong and a weak calcium transport mediator, the author suggested that iron-mediated calcium transport is involved in iron-induced calcification [88]. Despite limited experimental evidence, only a few clinical case studies reported an association between iron exposure and CUA based on either retrospective iron dose assessment or (bio-)chemical markers of iron status such as tissue iron or serum ferritin [89–92]. It was only very recently that the contributive pathogenic role of iron in the development of CUA was further explored. Farah *et al*. [92] found iron deposition in areas of microvascular calcification in diagnostic specimens of 12 end-stage renal disease patients (9 of these with a record-based exposure to iron) diagnosed with CUA, while it was absent in unaffected microvasculature within the same biopsy specimens.

Iron therapy and oxidative stress in end-stage renal disease

As the correction of anemia in dialysis patients with r-HuEPO goes along with efficient utilization and rapid depletion of iron stores, most patients, particularly those on hemodialysis require iron supplementation at some stage in order to improve the response to erythropoiesis-stimulating agent (ESA) therapy to replace ongoing iron losses and keep hemoglobin within target range recommended by the National Kidney Foundation's Kidney Disease Outcomes Quality Inititiative (K/DOQI) [1]. Although safety concerns raised in literature against i.v. iron treatment need to be interpreted with caution as comorbid factors, such as inflammation and malnutrition may falsely elevate serum ferritin measures, the potential of free or labile iron (iron not bound to transferrin, incorporated as heme iron or stored as ferritin or hemosiderin) to act as a driver of the free radical reaction, which may lead to oxidative injury and cardiovascular disease should not be disregarded [93–95]. Today, different iron compounds are available for parenteral administration with slight differences in the pharmacological characteristics of each. Initially, free inorganic ferric iron was administered which, not to our surprise, was poorly tolerated by patients [96]. In order to combat the pro-oxidative effects of soluble iron salts, polynuclear iron complexes [molecular weight (MW): 50 000–300 000 Da] were created consisting of a core ferric-oxyhydroxide gel surrounded by a stabilizing carbohydrate shell [54, 97]. Although with these compounds, iron is introduced directly into the circulation, the body retains its ability to protect against free iron release and formation of labile iron (that is iron bound to serum albumin, citrate and other undefined negatively charged ligands) by taking up the iron-carbohydrate complex into the reticuloendothelial system (RES) in its bound form (Figure 1). In the RES, iron is dissociated from its carbohydrate ligand, stored as ferritin or hemosiderin and only then is transferred to transferrin, the body's primary buffer against free iron, for delivery to the erythroid marrow for Hgb generation [98, 99]. Nevertheless, with existing compounds, minute amounts of free and labile iron will be released into circulation having the potential to induce oxidative damage and non-specific uptake of iron involving secondary transport routes other than the tightly regulated transferrin receptor-mediated uptake. As a consequence, iron loading may occur in iron-sensitive tissues such as heart and vessels [99]. The degree at which ionic iron is released and labile iron is formed depends on the type of preparation and various studies have been performed studying their potential effect on oxidative stress [100]. As anaphylactic reactions limit the use of the so-called 'first-generation' iron compounds, the iron-dextrans, 'second-generation' agents such as iron sucrose and iron gluconate are now most widely used. While shown to have a low risk for allergic reactions, both these compounds have been reported to possess potent pro-oxidative effects as demonstrated *in vitro* [101, 102] and in clinical studies [103, 104]. Although further studies are needed 'next-generation' preparations such as ferric carboxymaltose and ferumoxytol (an iron oxide na-

noparticle coated with a polyglucose sorbitol carboxymethylether) have been reported to show less pro-oxidative activities [101, 105].

An alternative investigational approach for iron supplementation comprises infusion of soluble ferric pyrophosphate (SFP) via the dialysate during hemodialysis [106]. SFP is a small MW complex iron salt (~1000 Da) and unlike approved i.v. iron complexes is devoid of carbohydrate. Pyrophosphate is strongly complexed by ferric iron (stability constant K_{stab}: 22.2) and it has been shown that SFP derived iron is rapidly bioavailable and taken up directly by circulating apo-transferrin within minutes of exposure [107], similar to transfer of diet derived iron after absorption. A recent Phase II clinical trial of SFP in maintenance, hemodialysis patients demonstrated the safety of iron therapy using this approach to support erythropoiesis and maintain Hgb levels without any evidence for toxicity [108]. Pyrophosphate is known to be a potent antioxidant [109] and a potent inhibitor of vascular calcification [110, 111]. The delivery of iron, by administration of soluble ferric pyrophosphate, without aggravating the underlying inflammatory and pro-calcific diatheses in uremic patients is an intriguing potentially promising alternative for further research.

Conclusion

So far, the effects of iron, either directly or through induction of oxidative stress, on the development of vascular calcification has mainly been studied in *in vitro* experiments and further confirmation of the reported results is warranted. Therefore, experimental studies investigating the net *in vivo* effect of iron on arterial calcification are required, particularly in the setting of chronic renal failure as CKD patients are at high risk for cardiovascular complications and are frequently supplemented with iron compounds for optimal ESA treatment.

Conflict of interest statement. A.G. is an employee of Rockwell Industries Inc., Wixom, MI, USA.

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