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Minireview

Fibroblast growth factor 23: a potential cause of cardiovascular diseases in chronic kidney disease patients

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ABSTRACT
Fibroblast growth factor 23 (FGF-23) has been identified as one of the risk factors for the development of cardiovascular diseases (CVDs) in chronic kidney disease (CKD) patients. Although FGF-23 is necessary for the maintenance of phosphate balance, it has been implicated in the pathogenesis of left ventricular hypertrophy, vascular dysfunction, and hypertension in CKD patients. FGF-23 induced alterations in intracellular calcium is hypothesized to be a key mechanism in the development of these CVDs. In addition, increased angiotensin II levels, upregulation of the renal Na^+Cl^- co-transporter and inhibition of endothelium-dependent vasorelaxation are among the potential mechanisms by which elevated FGF-23 levels cause hypertension in CKD. This review discusses these mechanisms and how these mechanistic pathways may be targeted in future experiments aimed at generating preventive and management therapies for CVDs in CKD patients.

INTRODUCTION

Systemic phosphate homeostasis is maintained through several hormonal mechanisms which involve fibroblast growth factor 23 (FGF-23), α-klotho, vitamin D and parathyroid hormone. FGF-23 is known to be the major regulator of phosphate balance (Mirams *et al*., 2004). FGF-23 is a phosphaturic hormone, which is synthesized by osteocytes in bone (Yoshiko *et al*., 2007). Increase in serum phosphate levels stimulates the release of FGF-23, which decreases phosphate levels independent of parathyroid hormone and vitamin D (Shimada *et al*., 2005).

In chronic kidney disease (CKD), the regulation of serum phosphate levels becomes significantly impaired resulting in rapidly increasing levels of FGF-23 in the circulation (Diniz and Frazao, 2013). With increasing evidence linking the incidence of cardiovascular diseases (CVDs) in CKD to elevated FGF-23 levels (Levin, 2003; Shuto *et al*., 2009; London *et al*., 2003), there is a dire need to review the potential mechanisms underlying this link. Understanding the mechanisms by which elevated FGF-23 levels leads to the occurrence of CVDs in CKD will significantly contribute to the generation of preventive measures for mitigating mortalities due to CVDs in CKD patients.

This review highlights the potential mechanisms linking elevated FGF-23 to CVDs in CKD. First, we will review the physiological role of FGF-23 in phosphate homeostasis, and the clinical and epidemiological studies linking FGF-23 to CVD in CKD patients.

FGF-23 and its role in phosphate homeostasis

FGF-23 is a 32kDa protein, which is made up of 251 amino acid residues, with an N-terminal region and a C-terminal fragment (Shimada *et al*., 2001). FGF-23 exists in two forms: biologically active intact form and the C-terminal fragments (Fukumoto and Martin, 2009). The biological significance of the C-terminal fragments is still unclear, and they are currently considered to be inactive (Shimada *et al*., 2002).

The hormone FGF-23 primarily acts in the proximal tubules of the kidneys where it increases the excretion of phosphate by downregulating the sodium-phosphate transporters (Andrukhova *et al*., 2012). It also inhibits the synthesis of vitamin D by downregulating the expression of the 1-α-hydroxylase enzymes, and

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increases the breakdown of vitamin D by upregulating the expression of 24-α-hydroxylase enzymes (Shimada *et al*., 2004). There are also reports that FGF-23 affects the synthesis of parathyroid hormone (Komaba and Fukagawa, 2010) (Fig. 1).

The action of FGF-23 in its targets is mediated by the interaction with the FGF-23 receptors (FGFRs), which are encoded by four genes, FGFR1 – FGFR4 (Yu *et al*., 2005; Li *et al*., 2011). There is evidence that the binding of FGF-23 to its co-receptor α–klotho is required for the FGF-23-FGFR complex to activate FGF-23 specific signaling pathways (Urakawa *et al*., 2006).

FGF-23 and CKD

In CKD, the progressive decline in renal functions is hypothesized to stimulate the increase in FGF-23 as a compensatory response for maintaining phosphate balance (Isakova *et al*., 2011). As a result, FGF-23 levels increases even before the occurrence of phosphate imbalance. The stages of CKD (stages 1 – 5) are characterized by progressively decreasing glomerular filtration rate (GFR). Pavik *et al*. (2013) showed that from early CKD (stage 2), the levels of FGF-23 begin to increase exponentially as the GFR decreases. Their findings also revealed that excess serum phosphate is only observed in late stages of CKD (stages 4 and 5).

Association of FGF-23 and CVDs in CKD

There is increasing rate of CVD mortality and morbidity in CKD patients compared with the general population. Approximately 40% of patients in early CKD stages, and up to 80% of patients in late CKD stages manifest left ventricular hypertrophy (Levin *et al*., 1996). Arterial calcification has also been reported in early CKD stages, which becomes very significant in over 60% of new dialysis patients (Block *et al*., 2007).

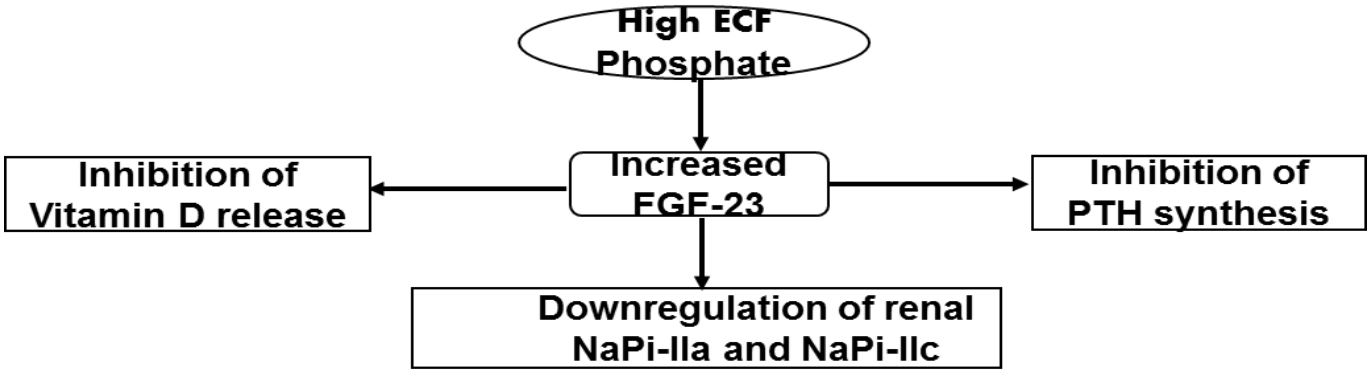

Fig. 1: Physiological functions of FGF-23. FGF-23 is released in response to high extracellular phosphate levels. Circulating FGF-23 downregulates the renal phosphate transporters, NaPi-IIa and NaPi-IIc; inhibits the release of active vitamin D and inhibits the synthesis of parathyroid hormones. These actions of FGF-23 restore extracellular phosphate levels back to normal.

The prevalence of hypertension has also been shown to be higher among patients with CKD (U.S Renal Data System, 2010). The U.S Renal Data System (USRDS) in 2010 reported that hypertension occurs in approximately 23.3% of individuals without CKD, 35.5% of stage 1 CKD- and 84.1% of stage 4-5 CKD patients.

Increased FGF-23 levels in CKD patients has been suggested as a novel risk factor for cardiovascular disease events (Seiler *et al*., 2010; Jean *et al*., 2009). Elevated FGF-23 levels, which is a consequence of disturbances in mineral metabolism has been shown to be associated with CKD progression (Noordzij *et al*., 2008). Kramer *et al*. (2005) reported a link between these disturbances in mineral metabolism and cardiovascular morbidity and mortality in CKD, possibly as a result of cardiovascular calcification.

A better understanding of the potential mechanisms underlying the occurrence of CVDs in CKD patients may prove invaluable for future investigation.

Potential mechanisms linking elevated FGF-23 and CVDs in CKD

FGF-23 and left ventricular hypertrophy (LVH)

Recent findings have shown that the pathological cardiac hypertrophic effect of FGF-23 is mediated by FGFR-dependent activation of the calcineurin-nuclear factor activated T cells (NFAT) signaling pathway (Faul *et al*., 2011). Calcineurin is a calcium-calmodulin activated protein, which is a serine/threonine-specific phosphatase that is activated by persistent elevation of intracellular calcium concentration (Klee *et al*., 1998; Dolmetsch *et al*., 1997). As a phosphatase, activated calcineurin dephosphorylate the members of the NFAT

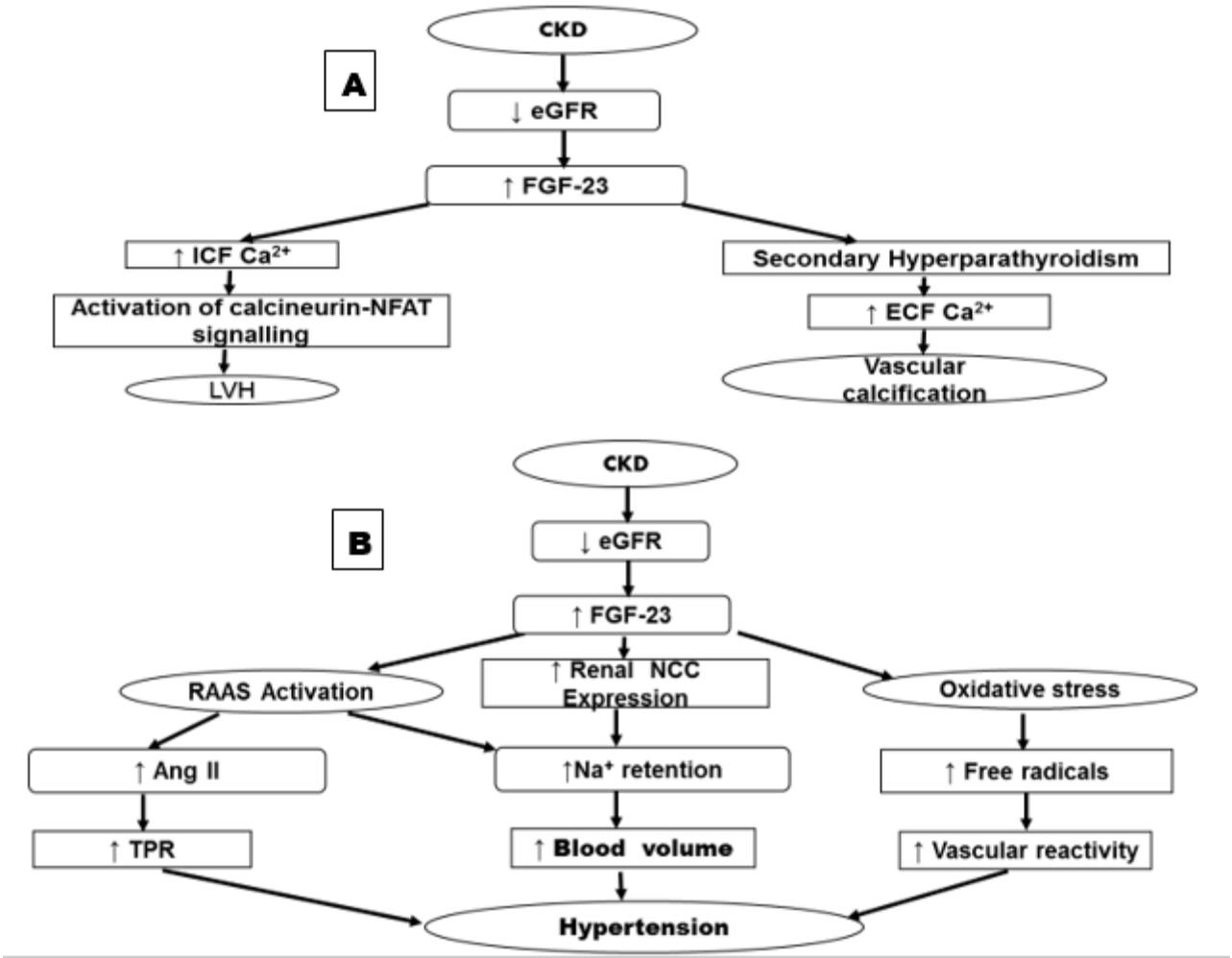

Fig. 2. Pathophysiology of cardiovascular diseases mediated by FGF-23 in CKD. A) High FGF-23 levels result in increased intracellular and extracellular Ca^{2+} levels. Excess intracellular Ca^{2+} in the left ventricle of the heart activates the calcineurin-NFAT signaling pathway, which results in left ventricular hypertrophy. Excess extracellular Ca^{2+} due to secondary hyperparathyroidism in CKD patients results in vascular calcification. B) High FGF-23 levels directly activate the $Na^{+}Cl^{-}$ co-transporter (NCC) expression in the renal distal tubule, stimulate the renin-angiotensin aldosterone system (RAAS), and also initiate oxidative stress. Increased sodium retention due to increased NCC and aldosterone activities as well as increased vascular resistance due to the vascular effects of angiotensin II and free radicals result in hypertension.

expressed in the myocardium, thus activating the hypertrophic pathway (Wilkins *et al*., 2002). This FGF-23-FGFR activation of the calcineurin-NFAT signaling pathway was shown to be independent of the FGF-23 co-receptor α-klotho (Faul *et al*., 2011). This rules out the involvement of α-klotho in the mechanisms underlying FGF-23 mediated cardiac hypertrophy.

A more recent report suggests that FGF-23 directly regulates intracellular calcium concentration in the myocardium (Touchberry *et al*., 2013), and this may play a role in activating the calcineurin-NFAT pathway; thus, implicating FGF-23 in the pathogenesis of LVH.

Furthermore, targeting the activation of calcineurin in CKD patients may prove invaluable in the prevention of LVH. Since calcineurin is physiologically activated by sustained elevations in intracellular calcium levels, it is plausible that hypercalcaemia in CKD may contribute to pathological LVH. The use of calcineurin inhibitors is not considered a therapeutic option in preventing LVH due to their nephrotoxic nature (Ojo *et al*., 2003). Further investigations on the potential role of FGFR blockers in the prevention of LVH in CKD may significantly advance knowledge about the pathogenicity of LVH in CKD.

FGF-23, vascular dysfunction and hypertension

Vascular calcification, a common form of vascular dysfunction, and hypertension are among the various CVDs associated with CKD. While hypertension has been strongly linked with elevated FGF-23 in normal

and CKD patients (Gutierrez *et al*., 2011), it is still unclear if elevated FGF-23 levels directly result in vascular calcification (Kendrick and Chonchol, 2011). The inhibition of vitamin D synthesis and the increased excretion of phosphate by FGF-23 suggest that FGF-23 may not directly play a role in vascular calcification. However, the extremely high levels of parathyroid hormone due to secondary hyperparathyroidism in CKD may be responsible for the development of vascular calcification in CKD patient. The role of FGF-23 in the activation of the renin-angiotensin aldosterone system (RAAS), renal sodium retention, and vascular reactivity may contribute to the development of hypertension in CKD.

Recent reports suggest that FGF-23 directly stimulates the RAAS by inhibiting the expression of angiotensin-converting enzyme 2 (ACE2)(Dai *et al*., 2012). ACE2 is an exopeptidase that catalyses the conversion of angiotensin I to angiotensin I-9 (Donoghue *et al*., 2000). It also catalyses the conversion of angiotensin II to angiotensin I-7 (Keidar *et al*., 2007). The net effect of ACE2 is to reduce circulating levels of angiotensin II. Hence, the suppression of ACE2 expression by FGF-23 will lead to increased levels of angiotensin II in the circulation. Angiotensin II acts on type 1 angiotensin II receptors to stimulate vasoconstriction, thus directly increasing the total peripheral resistance in blood vessels. Stimulation of the type 1 angiotensin II receptors by angiotensin II activates phospholipase C, which hydrolyses phosphatidylinositol-4, 5-biphosphate to generate inositol-1,4,5-triphosphate and diacylglycerol. Inositol-1,4,5-triphosphate triggers the release of calcium from intracellular stores resulting in an increase in intracellular calcium. Thus, the resultant effect of suppressing ACE2 expression by FGF-23 is increased levels of intracellular calcium, suggesting that FGF-23 induced increase in intracellular calcium levels may play a key role in the development of vascular dysfunction and hypertension in CKD. Angiotensin II is also a potent stimulus for aldosterone secretion. Taken together, these mechanisms support the role of FGF-23 in the development of hypertension in CKD.

Emerging evidence suggests that FGF-23 directly regulate the abundance of the Na^+Cl^- co-transporter (NCC) in the apical membrane of the renal distal tubules (Andrukhova *et al*., 2014). Loss-of-function mutations and gain-of-function studies by Andrukhova *et al*. (2014) showed that FGF-23 interacts with its co-receptor α-klotho to directly upregulate distal tubular NCC expression. Increased Na^+retention due to NCC upregulation results in a consequent increase in plasma volume and hypertension.

Another potential mechanism by which FGF-23 affect vascular function and blood pressure is its action on vascular smooth muscle activity. Studies on the localization of FGFRs and α-klotho in vascular tissue have shown that the expression of α-klotho is significantly lower than the expression levels of the FGFR1-4 (Donate-Correa *et al*., 2013; Lindberg *et al*., 2013). This suggests that the effects of FGF-23 on vascular function may be independent of α-klotho.

FGF-23 has been shown to directly impair endothelium-dependent vasorelaxation by reducing the bioavailability of nitric oxide (Silswal *et al*., 2014). To gain more insight into the potential mechanisms underlying this role of FGF-23 on nitric oxide bioavailability, reports by Six *et al*. (2014) indicate that endothelial nitric oxide synthase is unaffected by FGF-23 (Figs 1 and 2).

Furthermore, oxidative stress has been suggested to be a vital link between CVD and CKD (Andrukhova *et al*., 2014). With previous reports that oxidative stress induces endothelial dysfunction (Capellini *et al*., 2010), it is plausible that excess FGF-23 in CKD causes oxidative stress, which then affect the bioavailability of nitric oxide. Beckman and Koppenol (1996) previously hypothesized that oxidative stress increases the levels of superoxide, which rapidly combines with nitric oxide and converts it to peroxynitrite. It has recently been shown that an increased level of superoxide was observed following the preincubation of primary endothelial cells and aortic rings with FGF-23 (Andrukhova *et al*., 2014). Taken together, these findings suggest that FGF-23 reduces the bioavailability of nitric oxide by increasing the abundance of superoxide.

CONCLUSION

Increased angiotensin II levels, increased Na^+ retention and inhibition of endothelium-dependent vasorelaxation by FGF-23 are among the potential mechanisms by which elevated FGF-23 levels cause hypertension in CKD patients. Intracellular calcium plays a central role in FGF-23 induced LVH. Furthermore, investigating the role of FGF-23 in the regulation of intracellular calcium levels in vascular smooth muscle cells may reveal novel signaling pathways that may be targeted in the prevention of vascular dysfunction in CKD.

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