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Review Article

Klotho, Hypertension and Arterial Stiffness: A Review

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Abstract

Klotho is an important anti-aging protein expressed in kidney and in arterial, epithelial, endocrine, reproductive and neuronal tissue. Klotho exists in three forms including a full-length transmembrane α-Klotho, a truncated soluble α-Klotho, and a secreted α-Klotho. Tissue expression and serum levels of Klotho decrease concomitantly with aging. Hypertension and arterial stiffness are both aging related diseases, and previous studies have indicated that Klotho deficiency was closely related to hypertension and arterial stiffness in animal models and in several population studies. Several genetic mutations of the klotho gene (*KL*) have also been reported to be associated with hypertension. However, a causal relationship between Klotho deficiency, hypertension, and arterial stiffness, has yet to be established in large prospective population studies. This article reviews the evidence regarding the role of Klotho in the pathogenesis of hypertension and arterial stiffness and the most important underlying pathways involved, such as the FGF23/Klotho axis and the SIRT1 pathway.

Keywords: Klotho; Hypertension; Arterial stiffness; FGF23; SIRT1

Introduction

Hypertension is a major health problem worldwide and as the population ages the number of patients with hypertension is increasing. According to the Seventh Report of the Joint National Committee, more than 2/3 of individuals who are at least 65 years old have experienced hypertension, and individuals who are normotensive at 55 years of age have a 90% lifetime risk for developing hypertension [1]. Arterial stiffness can cause hypertension and reflects a gradual loss of elastin fiber and the accumulation of collagen fibers in the media of large arteries. Arterial stiffness is a powerful independent predictor of cardiovascular events and all-cause mortality [2]. Arterial stiffness has been reported to be associated with decreased compliance of the central vasculature, which alters arterial pressure and flow dynamics and impacts cardiac performance and coronary perfusion. Additionally, arterial stiffness is associated with a decreased estimated glomerular filtration rate (eGFR), hypertension, and diabetes mellitus [3] Arterial stiffness is an independent risk factor for hypertension, stroke, and myocardial infarction [4]. However, the etiology and pathogenesis of arterial stiffness remains largely unknown. A recent study revealed that Klotho deficiency led to aortic stiffness that preceded blood pressure elevation [4]. Furthermore, a growing body of evidence indicates that decreased Klotho levels are associated with arterial stiffness and hypertension. Thus, Klotho has become a topic of great interest in cardiovascular research over the last decade.

Previous studies have indicated that Klotho could be an important etiologic factor and a potential interventional target for arterial remodeling and stiffness [5]. Arterial stiffness occurs not only as a consequence of vascular biological aging, but also as a result of arteriosclerosis. Inflammation plays a major role in the development of arteriosclerosis, and thus inflammation is a major contributor to the stiffening of large arteries. Deleterious changes in carotid Intima-Media Thickness (IMT) and arterial stiffness parameters, including aortic augmentation index (AIx) and Pulse Wave Velocity (PWV), pose significant risks for stroke, myocardial infarction, heart failure, and overall mortality in older adults [6,7]. PWV was significantly higher in *KL* heterozygous (Klotho$^{+/-}$) mice compared to their age matched wild-type littermates, suggesting that deficiency of Klotho is sufficient for the development of arterial stiffening [8]. After discovery of *KL* in mice, its various functional aspects have been studied extensively leading to a greater understanding of its role in hypertension and arterial stiffness. The present article reviews updates regarding recent advances in the study of Klotho-related arterial stiffness and hypertension.

The Klotho Gene and Protein

KL was discovered by Kuro-o et al. in 1997 and the protein it encodes was named α-Klotho [9]. Subsequently, two Klotho-related genes were identified based on sequence similarity and designated as β-Klotho and γ-Klotho (also known as KLPH or LCTL) [10]. The *KL* gene is located on chromosome 13q12 spanning over 50kb in length and consists of 5 exons and 4 introns in humans [11]. Klotho was originally identified as putative aging-suppressor gene and its study generated tremendous interest and advanced the understanding of the aging process [10]. Several functional aspects of *KL* have been studied in context of aging [12]. Genetic mutation of *KL* causes multiple premature aging-like phenotypes and significantly shortens lifespan in mice [13]. In mice, *KL* mutation caused premature aging, arteriosclerosis, arterial calcification, hyperphosphatemia, and shortened lifespan while overexpression of *KL* extended lifespan by 20% [9]. Several mutations in the human *KL* gene have been identified. Among these, the functional *KL*-VS variant is composed of six SNPs in linkage disequilibrium, two of which result in amino acid

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substitutions F352V and C370S altering *in vitro* secretion and activity of the protein and its functionality [14,15]. The *KL*-VS variant has been associated with latent coronary artery disease [14], hypertension [16], high-density lipoprotein cholesterol, blood pressure, stroke, and longevity [15]. A systematic review and meta-analysis suggested that the G-395A (rs1207568) and F352V (rs9536314) SNPs might be a risk factor for hypertension [17].

Klotho is a 130kDa protein existing in two forms, membrane-bound Klotho and secreted Klotho, with distinct functions. In humans, α-Klotho is expressed in various tissues including arterial, epithelial, endocrine, reproductive and neuronal tissue. Klotho secretion can be regulated by many factors. For example, the calcitonin gene-related peptide and fibroblast growth factor 23 (FGF23) may up-regulate Klotho expression, while the renin-angiotensin system, inflammation, and oxidative stress may reduce Klotho expression [18]. α-Klotho also may function as a hormone [12,19] and as a cofactor/co-receptor regulating FGF23. Recent insights about the interplay between FGF23 and Klotho have led to a marked advancement in the interpretation of data on vascular aging [20].

α-Klotho

α-Klotho is a multifunctional protein that regulates the metabolism of calcium, phosphate, and vitamin D. Three α-Klotho protein types with different function have been identified: a full length transmembrane α-Klotho, a truncated soluble α-Klotho, and a secreted α-Klotho [12]. The α-Klotho protein was initially thought to be expressed in a limited number of tissues, most importantly the kidney, parathyroid gland, and choroid plexus [19]. Recent data have now demonstrated that Klotho is more widely expressed and that kidney is likely to be the principal source of circulating Klotho [21]. The wide distribution of α-Klotho in human tissues was first identified by immunohistochemistry [22], and confirmed by western blotting of both whole tissue and primary cells.

β-Klotho and γ-Klotho

β-Klotho and γ-Klotho are type 1 single-pass transmembrane proteins [23]. β-Klotho is composed of a β-glycosidase-like domain and shares 42% amino acid sequence homology with α-Klotho [24]. β-Klotho is highly localized to cell membranes in the liver, gastrointestinal tract, spleen, kidney, white adipose tissue, and the brain [22, 24]. β-Klotho is functionally active in both enterocytes and cholangiocytes. γ-Klotho is made of a family 1 glycoside-like extracellular domain and a short intracellular domain [10] and is expressed in kidney and skin [10]. The function of γ-Klotho is yet to be defined.

FGF23/Klotho axis

FGF23 is secreted into the plasma by bone and is known to suppress phosphate reabsorption and vitamin D production in the kidney [25,26]. FGF23 was originally identified as factor causing phosphate wasting, including autosomal dominant hypophosphamatic rickets and tumor-induced ostomalacia [27,28]. Klotho forms complexes with several fibroblast growth factor receptors (FGFRs) including FGFR1c, FGFR3c, and FGFR4 and increases their selectively for FGF23. Recently, αKlotho was confirmed as a non-enzymatic molecular scaffold for FGF23 hormone signaling, and it was incompatible with its purported glycosidase activity [29]. Klotho serves as a mediator for the actions of FGF23, namely urinary phosphate excretion, inhibition of calcitriol ($1,25(OH)_2D_3$) secretion, and inhibition of parathyroid hormone (PTH) synthesis and secretion. The soluble form of Klotho functions as a humoral factor and is involved in the regulation of nitric oxide production in the endothelium, the preservation of endothelial permeability, calcium homeostasis in the kidneys, and the inhibition of insulin and insulin like growth factor-1 signaling [30]. Recent studies have indicated that FGF23 levels increase before serum PTH when calcium and phosphate metabolism is impaired [31]. Reduced phosphate reabsorption from urine can occur through downregulation of sodium phosphate co-transporters in the renal proximal tubular epithelial cell [26]. FGF23 controls phosphate and calcitriol plasma concentration [25], and excessive amounts of circulating intact FGF23 leads to phosphate wasting as long as kidney function is normal [32]. Very high levels of FGF23 in renal failure are an independent risk factor for cardiovascular disease [33]. FGF23 is also associated with incident coronary artery diseases, heart failure and cardiovascular mortality even at normal kidney function [34]. FGF23 is an independent risk factor for all-cause and cardiovascular mortality in patients with normal renal function undergoing coronary angiography [35]. Increased circulating levels of FGF23 indirectly promote cardiovascular disease progression by contributing to sodium and water retention [36,37]. Unfortunately, it remains difficult to establish whether or not FGF23 acts directly on blood vessels [38]. However, the presence of Klotho in human arteries and vascular smooth muscle cells [39] indicate that the FGF23/Klotho axis could be involved in arterial stiffness and subsequent hypertension and subsequent cardiovascular events.

SIRT1 and its relationship with α-Klotho in pathogenesis of arterial stiffness and hypertension

It has been shown that the serum level of Klotho is decreased by approximately 45% in patients with arterial stiffness and hypertension compared to those without [5]. Klotho deficiency increased NADPH oxidase activity, and superoxide production, collagen expression, and elastin fragmentation in the aortic media [5]. SIRT1, a NAD-dependent deacetylase, and which has anti-inflammatory, anti-oxidant, and anti-apoptotic effects in endothelium, is involved in endothelial senescence and dysfunction [40,41]. Klotho deficiency led to arterial stiffness and hypertension in $Klotho^{+/-}$ mice [5], and the expression and activity of SIRT1 was significantly decreased in the aortic endothelium and smooth muscle cells in these animals suggesting that Klotho deficiency downregulates SIRT1 [5]. Treatment with SRT1720 (15mg/kg/d), a specific SIRT1 activator, abolished Klotho deficiency-induced arterial stiffness and hypertension in $Klotho^{+/-}$ mice. Additionally, Klotho deficiency was associated with significant decreases in AMP-activated protein kinase alpha (AMPKα) and endothelial NO synthase (eNOS) activity in the aorta [5]. Activation of SIRT1 by SRT1720 treatment abolished the downregulation of AMPKα and eNOS activity [5].

NADPH oxidase was originally found in the plasma membrane of phagocytes and generates superoxide to participate in host defense by killing or destroying invading microbes. Recent studies indicate that NADPH oxidase is a primary source of Reactive Oxygen Species (ROS) in vasculature [42]. The NADPH oxidases expressed in the cardiovascular system are membrane-associated enzymes that catalyze the one-electron reduction of oxygen using NADH or NADPH as an electron donor. It has been known for years

that vascular tissue is a rich source of ROS, including superoxide, hydrogen peroxide, and nitric oxide. Virtually every cell type in the vascular wall has been shown to produce and regulate ROS [43], with smooth muscle cells and fibroblasts accounting for the majority of superoxide production in normal vessel walls. Klotho not only downregulates NOX_2 protein expression and intracellular superoxide production, but also attenuates Angiotensin II-induced superoxide production, oxidative damage, and apoptosis [44]. However, Klotho did not affect NOX_2 mRNA expression suggesting inhibition may occur at the posttranscriptional level, perhaps via the cAMP-PKA-dependent pathway [44].

Physiological and pathophysiological role of Klotho

The three forms of Klotho have distinct functions, which are diverse and have been implicated in multiple biological processes including the regulation of energy metabolism, the anti-inflammatory process, the anti-oxidative process, modulation of ion transport, and regulation of mineral metabolism. The vascular phenotype of Klotho deficiency includes medial calcification, intimal hyperplasia, endothelial dysfunction, arterial stiffening, hypertension, and impaired angiogenesis [45]. High phosphate levels seem to be key to the pathogenic effect of Klotho deficiency with regard to the process of medial calcification [45]. Overexpression of Klotho seems to play a protective role against medial calcification, endothelial dysfunction, and hypertension. Thus, Klotho has many protective effects in the vasculature and constitutes a therapeutic target.

Klotho and Hypertension

Multiple epidemiological studies have indicated that the incidence of arterial stiffness, hypertension, and related cardiovascular disease increases with age [46]. Hypertension doubles the risk of cardiovascular diseases including coronary heart disease, congestive heart failure, ischemic and hemorrhagic stroke, renal failure, and peripheral arterial disease. In humans, serum Klotho levels decrease with age, while high levels of Klotho were independently associated with a decreased risk of hypertension and related cardiovascular disease [4]. Furthermore, Klotho deficiency caused salt-sensitive hypertension and renal damage in mice by CC chemokine receptor 2-mediated inflammation [47]. Systolic blood pressure in $KL^{+/-}$ mice began to increase at the age of 15 weeks, reached a peak level at the age of 17 weeks, and remained elevated thereafter. High salt intake further increased blood pressure only in $KL^{+/-}$ mice but not affect BP in WT mice. Blockade of CC chemokine receptor 2 (CCR2) by a specific CCR2 antagonist (INCB3284) abolished the high salt-induced increase in blood pressure in $KL^{+/-}$ mice [47]. *In vivo,* expression of Klotho abolished the downregulation of anti-inflammatory cytokines and the upregulation of Nox2 expression, NADPH oxidase activity, and superoxide production in aorta [48], suggesting that Klotho may exert its protective role against cardiovascular aging via inhibition of inflammation and oxidative stress. In addition, polymorphisms of Klotho are associated with changes in lifespan [14], the development of coronary artery disease, atherosclerosis [49], and salt-sensitive hypertension.

Klotho and Vascular Stiffness

Decreased compliance of the central vasculature alters arterial pressure and flow dynamics and impacts cardiac performance and coronary perfusion. There is good evidence to suggest the association of Klotho with vascular pathologies including vascular calcification, and with cytoprotecting of both the endothelium and vascular smooth muscle cells and the prevention of mineralization [50]. In patients with arterial stiffness (defined as branchial-ankle PWV ≥ 1400cm/sec), there were significant decreases in serum Klotho levels [30]. It is critical to understand the pathogenic role of Klotho in the endothelium and in the vascular smooth muscle. The entire cardiovascular and lymphatic system is lined with endothelium, which contributes to its physiological function, and intact endothelial structure and function is important for vascular integrity. Klotho may increase NO production in endothelial cells and consequently modulate vascular smooth muscle. It can also protect endothelial cells from high phosphate and attenuate oxidative stress, pro-inflammatory cytokines, induced cell senescence, and apoptosis in vascular smooth muscle cells. High phosphate levels are likely to be directly pathogenic and responsible for medial calcification, but more important determinants are pathways that regulate cellular senescence, suggesting that Klotho deficiency can make cells susceptible to phosphate toxicity. Overexpression of Klotho is shown to ameliorate medial calcification, endothelial dysfunction, and hypertension. Thus, Klotho can be a potential biomarker and a biologic therapeutic agent for arterial stiffness.

Conclusion

Accumulating evidence indicates that the anti-aging protein Klotho has a functional role in aging and age-related diseases including hypertension and arterial stiffness. Klotho deficiency is strongly associated with hypertension and arterial stiffness. However, the pathological role and underlying mechanisms are still poorly defined. Further studies examining organ protection and the association of Klotho with the development of hypertension and arterial stiffness are needed.

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