Emerging Roles of Klotho in Cardiovascular Diseases

Abstract

Klotho is a putative anti-aging gene, which in recent years has emerged as an important factor in the etiologic and cure/prevention of varied pathologies. The expression of this protein in the vascular wall and its role in maintenance of vascular homeostasis indicates its importance in cardiovascular diseases (CVD) such as diabetes, hypertension, vascular calcification, cardiac hypertrophy, atherosclerosis, etc. It seems to be a panacea and as such holds a major influence on future medicine. This review attempts to provide an overview of the part played by Klotho in the various pathologies included in CVD.

Keywords: Diabetes; Hypertension; Preeclampsia; Cardiac hypertrophy

Introduction

In 1997, mutation of a single gene in Chromosome 13q12 causing extensive aging phenotypes identified a new anti-aging factor. It was named after the Greek Goddess, Klotho, spinner of the thread of life. Genetic mutations in this gene were shown to cause multiple premature aging-like phenotypes and strikingly shorten lifespan. The aging phenotypes included arteriosclerosis, vascular calcifications, soft tissue calcifications, emphysema, hypo-activity, gonadal dysplasia, infertility, skin atrophy, ataxia, hypoglycaemia and severe hyperphosphatemia [1]. While, over expression of the Klotho gene in mice suppressed aging and was shown to extend lifespan via regulation of the insulin and insulin-like growth factor-1 (IGF-1) signalling, which is an evolutionarily conserved mechanism for extending life span [1,2]. The discovery of Klotho has a great impact on aging research.

The human Klotho gene, spanning over 50kb in length, encodes a single pass transmembrane protein (α-Klotho) and is expressed primarily in the kidney [3,4], the parathyroid gland [5,6], and the choroid plexus in brain [7]. The protein has a large extracellular amino-terminal domain and a small intracellular carboxy-terminal domain [1]. Circulating α-Klotho results either from direct secretion by the cell or from cleavage of the intracellular domain of the full length protein by secretases [1,8]. This secreted α-Klotho protein found in blood circulation is also referred to as a peptide hormone due to its action on target sites other than the site of production [9]. It inhibits the actions of IGF-1 through binding to a specific cell membrane receptor which results in the suppression of tyrosine phosphorylation of the IGF-1 receptor as well as insulin receptor substrates (IRS), leading to disruption of IGF signals. This signalling activity in tissues shows close association with the extended life span and is known to regulate various metabolic processes [2]. It also influences other growth factor signalling pathways including p53/p21, cAMP, protein kinase C (PKC) and Wnt, [10-13]. It acts as a sialidase that modifies glycans on the cell surface which may explain the ability of secreted Klotho protein to regulate activity of multiple ion channels [13]. It protects against endothelial dysfunction and regulates the production of nitric oxide. It inhibits the phosphorylation of transcription factors forkhead box O (FoxO) which then increase the expression of antioxidant enzymes, thereby protecting against oxidative stress [13,14]. The transmembrane form of α-Klotho protein, mainly expressed in kidney distal convoluted tubule, is widely studied. It functions as an obligatory co-receptor for a bone derived phosphaturic hormone, Fibroblast Growth Factor-23 (FGF-23). FGF-23 requires Klotho for its signalling. The Klotho protein binds to multiple FGF receptors and increases their affinity for FGF-23 and helps in secreting phosphorus from kidney [15]. Thus, α-Klotho regulates phosphate and vitamin D metabolism through FGF-23. The Klotho gene reportedly encodes two other Klotho proteins, β-Klotho and Klotho-related protein (Klrp). Klrp is a transmembrane protein that binds to FGF receptor (FGFR)-1b, FGFR-1c and FGFR-2c but its function is unknown [16-18]. β-Klotho is not found in secreted form and is predominantly expressed in liver and adipose tissue. Its main function involves metabolic regulation, glucose uptake, bile acid synthesis and fatty acid metabolism, independent of β-Klotho [9,17].

The α-Klotho gene is highly conserved in humans, mice, rats and Danio rerio and Caenorhabditis elegans [9]. Mouse Klotho cDNA shows 80% and Klotho protein shows 86% homology with that of human [19]. Klotho knock out mouse model (Klf-/-) represents human progeroid syndrome and exhibits various phenotypes of aging in a wide range of organs including arteriosclerosis, neural degeneration, skin and gonadal atrophy, pulmonary emphysema, calcification of soft tissues, and cognition impairment [1,2]. Studies in this mouse model are expected to provide new insights into human aging as well as in diseases associated with aging. α-Klotho is involved in the protection of vasculature through various mechanisms, including prevention of endothelial dysfunction, anti-inflammatory effects, and reduction of vascular calcification or attenuation of cardiac hypertrophy [20]. On the other hand, it has also been demonstrated that Klotho mRNA is down regulated under sustained cardiovascular or metabolic stress, such as hypertension, diabetes mellitus, and hyperlipidemia [3]. This review attempts to provide an overview of the part played by Klotho in cardiovascular diseases (CVD) (Table 1).
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Table 1: Klotho in CVD.

| No | Study                                      | Findings                                                                 |
|----|--------------------------------------------|--------------------------------------------------------------------------|
| 1  | Utsugi et al. [21]                         | Deceased insulin production in pancreas and increased insulin sensitivity in Klotho deficient mice |
| 2  | Zhao et al. [22], Kacso et al. [23]        | Decreased serum α-Klotho levels in diabetic nephropathy                   |
| 3  | Wu et al. [24]                             | Decreased serum α-Klotho levels T2DM                                     |
| 4  | Leahy [25]                                 | β-cell failure occurs from early phase in T2DM                            |
| 5  | Lin and Sun [26]                           | Klotho gene and protein expressed in pancreatic islets                   |
| 6  | Lin and Sun [27]                           | β-cell-specific expression preserved β-cell function                      |

Klotho in Hypertension

| No | Study                                      | Findings                                                                 |
|----|--------------------------------------------|--------------------------------------------------------------------------|
| 1  | Su and Yang [31]                           | Systolic or elderly hypertension due to reduction in serum Klotho levels  |
| 2  | Xiaoli et al. [32]                         | In mutant Klotho mice persistent elevation of SBP                       |
| 3  | Saito et al. [33]                          | Klotho gene delivery in animal model of atherosclerotic disease reduced elevated BP and prevented medial hypertrophy |
| 4  | Wang and Sun et al. [34]                   | Klotho gene delivery in spontaneous hypertensive rats stopped further increase in BP |

Klotho in Preeclampsia

| No | Study                                      | Findings                                                                 |
|----|--------------------------------------------|--------------------------------------------------------------------------|
| 1  | Cecati et al. [38] & Guannubilo et al. [39]| Significantly (80-83%) lower expression levels of placental Klotho        |
| 2  | Loichinger et al. [40]                     | AVM with reduced levels of α-Klotho                                       |

Klotho in Other CVD related Cardiac Pathologies:

| No | Study                                      | Findings                                                                 |
|----|--------------------------------------------|--------------------------------------------------------------------------|
| 1  | Semba et al. [41]                          | Higher plasma Klotho with lower likelihood of developing CVD             |
| 2  | Navarro-González et al. [42]               | Lower mRNA and soluble Klotho in vascular wall with CAD                  |
| 3  | Xie et al. [43]                            | Klotho deficient mice with atherosclerotic pathologies                   |

Discussion

Klotho in Diabetes

Physiologically, pancreatic β-cells constantly synthesize insulin, which is stored within vacuoles and released once triggered by an elevation in blood glucose level. Utsugi et al. [21] have demonstrated that Klotho mutant mice have deceased insulin production in the pancreas and increased insulin sensitivity. However they could not elucidate the precise mechanism involving Klotho. Subsequent studies by Zhao et al. [22], Kacso et al. [23] and Wu et al. [24] have reported decreased serum α-Klotho levels in diabetic nephropathy and Type 2 Diabetes Mellitus (T2DM) respectively. T2DM is now recognized not only owing to insulin resistance, but also due to β-cell failure from early phase [25]. Thus, apart from the normal glucose control, one of the goals in the treatment of T2DM is to preserve functional β-cells in pancreatic islets. Lin and Sun in their study [26] have demonstrated that Klotho gene and protein are expressed in pancreatic islets. In their subsequent study [27], they further established that β-cell-specific expression of Klotho decreased hyperglycemia, enhanced glucose tolerance, preserved β-cell function and protected against the development of T2DM in murine model of T2DM (db/db). In addition, β-cell-specific expression of Klotho decreased intracellular superoxide levels, oxidative damage, apoptosis, and DNAJC3 (a marker for endoplasmic reticulum stress) in pancreatic islets, increased expression levels of Pdx-1 (insulin transcription factor), PCNA (a marker of cell proliferation), and LC3 (a marker of autophagy) in pancreatic islets in db/db mice. Thus, these results reveal that β-cell-specific expression of Klotho improves β-cell function and attenuates the development of T2DM by suppressing oxidative stress, endoplasmic reticulum stress, apoptosis; increasing cell proliferation and normalizing autophagy in pancreatic islets. Therefore, in vivo expression of Klotho in pancreatic β-cells may offer a new and effective therapeutic strategy for overcoming β-cell dysfunction in T2DM and warrants further mechanistic investigation into the therapeutic role of Klotho in protecting β-cell function. However, Lin and Sun further documented that β-cell-specific expression of Klotho attenuates but does not...
prevent the development of T2DM [27]. Therefore, simultaneous management of hyperglycemia and insulin resistance is also important for the protection of β-cells in T2DM.

**Klotho Hypertension**

Blood pressure usually refers to the arterial pressure in systemic circulation which when elevated leads to Hypertension. The pathophysiology associated with hypertension involves mechanical stretch on the vascular wall, increased systemic vascular resistance, increased vascular stiffness, activation of the renin-angiotensin system (RAS) responsible for increased Angiotensin II, which further activates NADPH/NADH oxidase of the vascular smooth muscle cells, resulting in release of Reactive Oxygen Species (ROS), which in turn trap Nitric Oxide (NO), an endothelium-derived relaxing factor [28, 29].

It is well known that with increase in age, systolic blood pressure (SBP) increases [30] and recent findings demonstrate decrease in α-Klotho protein with age [13]. Su & Yang [31] have demonstrated that Systolic or elderly hypertension may be partially attributed to reduction in serum Klotho levels. In mutant Klotho mice, Xiaoli et al. [32] showed that, Klotho is essential for the maintenance of normal BP and its deficiency caused significant and persistent elevation of SBP. Saito et al. [33] observed that in Otsuka Long-Evans Tokushima Fatty (OLETF) rat, animal model of atherosclerotic disease, adenovirus-mediated Klotho gene delivery ameliorated vascular endothelial dysfunction, increased nitric oxide production, reduced elevated blood pressure, prevented medial hypertrophy and perivascular fibrosis. In a similar study by Wang & Sun [34], Klotho gene delivery via an adeno-associated virus (AAV) in spontaneous hypertensive rats stopped further increase in BP but did not decrease the BP levels to that of controls. Additionally, Klotho gene delivery resulted in prolonged up-regulation of Klotho gene expression. Thus, AAV delivery of Klotho may be a new approach for long term control of hypertension.

**Klotho in Preeclampsia**

Preeclampsia is the most common pregnancy hypertensive disorder characterized by a peripheral vasoconstriction and decreased arterial compliance that induce various clinical manifestations including failure of several maternal organs as liver, kidney and brain which complicates about 5–7% of pregnancies, depending on the populations studied [35]. Preeclampsia and cardiovascular disease share many risk factors, including endothelial dysfunction, obesity, hyperglycemia, insulin resistance, diabetes mellitus, hypertension, and dyslipidemia. Women who develop preeclampsia are exposed to an increased risk of coronary heart disease, stroke, and cardiovascular disease in general [36]. The relative risk of future cardiovascular events after a pregnancy complicated by preeclampsia has been reported varying from 1.3 to 3.3, with a range of 2.7–8.1 in more severe preeclampsia states [37].

To date, for preeclampsia, there are no reliable predictors, specific preventive measures, or treatments other than delivery. Klotho may be involved in the pathogenesis of preeclampsia. Cecetti et al. [39] & Guannubulo et al. [39] found that the expression levels of placental Klotho were significantly (80-83%) lower in preeclampsia group as compared with controls. Prolonged oxidative damage in the placenta gives rise to characteristic placental histology, defined as accelerated villous maturation (AVM) which within the preeclampsia group, were associated with reduced levels of α-Klotho [40]. Thus, α-Klotho is a potentially important protein in preeclampsia.

**Klotho in Other CVD related Cardiac Pathologies**

Coronary artery disease (CAD) and its clinical manifestations including vascular stiffening, peripheral arterial disease, left ventricular hypertrophy and myocardial infarction are further complications of CVD [20]. The disruption in the homeostasis of Klotho seems to be a key element in the development of these diseases. Sembra et al. [41] found that individuals with higher plasma Klotho concentrations were independently associated with lower likelihood of developing CVD. Correspondingly, lower concentrations of soluble Klotho as well as reduced mRNA expression of Klotho in vascular wall were associated independently of established CVD risk factors in CAD patients as reported by Navarro-González et al. [42]. Xie et al. [43] showed that Klotho deficient mice developed an exaggerated pathological cardiac hypertrophy and remodelling in response to stress. They further demonstrated that, Klotho over expression ameliorated pathological cardiac pathologies in these mice and improved their long-term survival. The cardiac protection bestowed by Klotho was via down-regulation of TRPC6 channels which are Ca²⁺-permeable cations channels expressed in the cardiac membrane, which function as an important modulator of cardiac hypertrophy and are responsible for aberrant cardiac development and premature death. The central role of Klotho in the pathogenesis of CVD makes its use possible as a diagnostic biomarker or as a therapeutic factor for treatment of vascular diseases. However, further studies are needed to clarify the relationship between this factor and promotion of vascular health [20].

**Conclusion**

Since its discovery, Klotho has been hailed as the novel anti-aging gene. There are strong evidences suggesting an important role for Klotho in the etiology of CVD and its clinical manifestations such as diabetes, hypertension, cardiac hypertrophy etc. Various *in-vitro* and animal studies have demonstrated that Klotho over expression ameliorates cardiac pathologies and ensures vascular health. Though its mechanism of action remains elusive, research conducted worldwide postulates that Klotho may be the elixir that mankind has been in search of. Identifying the specific pathways linking to its known actions or novel functions will enhance our understanding of the exact role of this anti-aging protein.

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**References**

1. Kuro-o M, Matsunami Y, Akawa H, Kawaguchi H, Suga T, et al. (1997) Mutation of the mouse klotho gene leads to a syndrome resembling ageing. Nature 390(6655): 45-51.

2. Kurosu H, Yamanoto M, Clark JD, Pastor JV, Nandi A, et al. (2005) Suppression of aging in mice by the hormone Klotho. Science 309(5742): 1829-1833.
3. Aizawa H, Saito Y, Nakamura T, Inoue M, Imanari T, et al. (1998) Downregulation of the Klotho gene in the kidney under sustained circulatory stress in rats. Biochem Biophys Res Commun 249(3): 865-871.

4. Kato Y, Anawaka E, Kinosita S, Shirai A, Furuya A, et al. (2000) Establishment of the anti-Klotho monoclonal antibodies and detection of Klotho protein in kidneys. Biochem Biophys Res Commun 267(2): 597-602.

5. Hofman-Bang J, Martuseviciene G, Santini MA, Olgaard K, Lewin E (2010) Increased parathyroid expression of klotho in uremic rats. Kidney Int 78(11): 1119-1127.

6. Krasnij T, Olausson H, Mirza MA, Hellman P, Akerström G, et al. (2010) Parathyroid Klotho and FGPR receptor 1 expression decline with renal function in hyper parathyroid patients with chronic kidney disease and kidney transplant recipients. Kidney Int 78(10): 1024-1032.

7. Li SA, Watanabe M, Yamada H, Nagai A, Kinuta M, et al. (2004) Immunohistochemical localization of Klotho protein in brain, and reproductive organs of mice. Cell Struct Funct 29(4): 91-99.

8. Chen CD, Podvin S, Gillespie E, Leeman SE, Abraham CR (2007) Insulin stimulates the cleavage and release of the extracellular domain of Klotho by ADAM10 and ADAM17. Proc Natl Acad Sci USA 104(50): 19796-19801.

9. Xu Y, Sun Z (2015) Molecular basis of Klotho: from gene to function in aging. Endocrinology 156(2): 174-193.

10. Tatar M, Bartke A, Antebi A (2003) The endocrine regulation of aging by insulin-like signals. Science 299(5611): 1346-1351.

11. Unger RH (2006) Klotho-induced insulin resistance: a blessing in disguise? Nat Med 12(1): 56-57.

12. Liu H, Fergusson MM, Castilho RM, Liu J, Cao L, et al. (2007) Augmented Wnt signaling in a mammalian model of accelerated aging. Science 317(5839): 803-806.

13. Kuro-o M (2009) Klotho and aging. Biochim Biophys Acta 1790(10): 1049-1058.

14. Yamamoto M, Clark JD, Pastor IV, Gurnani P, Nandi A, et al. (2005) Regulation of oxidative stress by the anti-aging hormone klotho. J Biol Chem 280(45): 38029-38034.

15. Kuroso H, Ogawa Y, Miyoshi M, Yamamoto M, Nandi A, et al. (2006) Regulation of fibroblast growth factor-23 signaling by klotho. J Biol Chem 281(10): 6120-6123.

16. Yahata K, Morii K, Arai H, Koida S, Ogawa Y, et al. (2000) Molecular cloning and expression of a novel klotho-related protein. J Mol Med (Berl) 78(7): 399-394.

17. Ito S, Fujimori T, Hayashizaki Y, Nasahisa Y (2002) Identification of a novel mouse membrane-bound family 1 glycosidase-like protein, which carries an atypical active site structure. Biochem Biophys Acta 1576(3): 341-345.

18. Yayağolu MB, Titmus A, Visel A, Alvarez-Bolado G, Thaller C, et al. (2005) Comprehensive expression atlas of fibroblast growth factors and their receptors generated by a novel robotic in situ hybridization platform. Dev Dyn 234(2): 371-3786.

19. Wang Y, Sun Z (2009) Current understanding of klotho. Aging Res Rev 8(1): 43-51.

20. Martin-Núñez E, Donate-Corra J, Muros-de-Fuentes M, Mora-Fernández C, Navarro-González JF (2014) Implications of Klotho in vascular health and disease. World J Cardiol 6(12): 1262-1269.

21. Utsumi T, Ohno T, Ohyama Y, Uchiyama T, Saito Y, et al. (2000) Decreased insulin production and increased insulin sensitivity in the klotho mutant mouse, a novel animal model for human aging. Metabolism 49(9): 1118-1123.

22. Zhao Y, Banerjee S, Dey N, LeJeune WS, Sarkar PS, et al. (2011) Klotho depletion contributes to increased inflammation in kidney of the db/db mouse model of diabetes via RelA (serine) 536 phosphorylation. Diabetes 60(7): 1907-1916.

23. Kacso IM, Bondor CI, Kacso G (2012) Soluble serum Klotho in diabetic nephropathy: relationship to VEGF-A. Clin Biochem 45(16-17): 1415-1420.

24. Wu C, Wang Q, Lu C, Qin N, Lei S, et al. (2014) The changes of serum sKlotho and NGAL levels and their correlation in type 2 diabetes mellitus patients with different stages of urinary albumin. Diabetes Res Clin Pract 106(2): 343-350.

25. Leahy JL, Hirsch IB, Peterson KA, Schneider D (2010) Targeting beta-cell function early in the course of therapy for type 2 diabetes mellitus. J Clin Endocrinol Metab 95(9): 4206-4216.

26. Lin Y, Sun Z (2012) Antiglomerular klotho enhances glucose-induced insulin secretion by up-regulating plasma membrane levels of TRPV2 in MIN6 β-cells. Endocrinology 153(7): 3029-3039.

27. Lin Y, Sun Z (2015) In vivo pancreatic β-cell-specific expression of antiaging gene Klotho: a novel approach for preserving β-cells in type 2 diabetes. Diabetes 64(4): 1444-1458.

28. Arakawa T, Ogawa T, Ueda M, Nishikawa T, et al. (2002) Angiotensin II stimulates NADH and NADPH oxidase activity in cultured vascular smooth muscle cells. Circ Res 74(6): 1141-1148.

29. Nguyen Dinh Cat A, Montezano AC, Burger D, Touyz RM (2013) Angiotensin II, NADPH oxidase, and redox signaling in the vasculature. Antioxid Redox Signal. 19(10): 1110-1120.

30. Pinto E (2007) Blood pressure and ageing. Postgrad Med J 83(976): 109-114.

31. Su XM, Yang W (2014) Klotho proteinlowered in elderly hypertension. Int J Clin Exp Med 7(8): 2347-2350.

32. Xie B, Wen L, Zhaojie S (2014) Klotho Deficiency Causes Hypertension and Renal Damage and Its Mechanism. J Am Coll Cardiol 64(16_S): (GW25-e0435).

33. Saito Y, Nakamura T, Ohyama Y, Suzuki T, Iida A, et al. (2000) In vivo klotho gene delivery protects against endothelial dysfunction in multiple risk factor syndrome. Biochem Biophys Res Commun 276(2): 767-772.

34. Wang Y, Sun Z (2009) Klotho gene delivery prevents the progression of spontaneous hypertension and renal damage. Hypertension 54(4): 810-817.

35. Redman GW, Sargent IL (2005) Latest advances in understanding preeclampsia. Science 308(5728): 1592-1594.

36. Bellamy L, Casas JP, Hingorani AD, Williams DJ (2007) Pre-eclampsia and risk of cardiovascular disease and cancer in later life: systematic review and meta-analysis. BMJ 335(7627): 974.

37. Neustedt J, van Damr T, Magee LA (2007) Preeclampsia and future cardiovascular risk. Expert Rev Cardiovasc Ther 5(2): 283-294.

38. Cecati M, Giannubilo SR, Saccucci F, Sartini D, Ciavattini A, et al. (2016) Potential Role of Placental Klotho in the Pathogenesis of Preeclampsia. Cell Biochem Biophys 74(1): 49-57.
39. Giannubilo SR, Cecati M, Saccucci F, Corradetti A, Emanuelli, M et al, (2012) PP035. Placental klotho protein in preeclampsia: A possible link to long term outcomes. Pregnancy Hypertens 2(3): 260-261.

40. Loichinger MH, Towner D, Thompson KS, Ahn HJ, Bryant-Greenwood GD (2016) Systemic and placental α-klotho: Effects of preeclampsia in the last trimester of gestation. Placenta 41: 53-61.

41. Semba RD, Cappola AR, Sun K, Bandinelli S, Dalal M, et al. (2011) Plasma klotho and cardiovascular disease in adults. J Am Geriatr Soc 59(9): 1596-1601.

42. Navarro-González JF, Donate-Correa J, Muros de Fuentes M, Pérez-Hernández H, Martínez-Sanz, R et al. (2014) Reduced Klotho is associated with the presence and severity of coronary artery disease. Heart 100(1): 34-40.

43. Xie J, Cha SK, An SW, Kuro-O M, Birnbaumer L, et al. (2012) Cardioprotection by Klotho through downregulation of TRPC6 channels in the mouse heart. Nat Commun 3: 1238.