Klotho Expression and Regulation in the Early Pathogenic Development of Vascular Calcification in Uremic Rats

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SUMMARY

The purpose of the study was to analyze calcification and Klotho expression and regulation in the rat abdominal aorta. The basic procedures were subtotal (5/6) nephrectomy (Nx) and the division of Wistar rats into three groups (group at week 20 after a sham-operation – control, group at week 10 after subtotal Nx, and group at week 20 after subtotal Nx), von Kossa calcification staining and analyses of Klotho expression in freshly extracted or *in vitro*-stimulated abdominal aortas with inorganic phosphate (Pi), Klotho protein and Pi + Klotho protein for 48 hours. Main findings: Aorta calcification was higher at week 20 than at week 10 after subtotal Nx ($P = 0.004$). Klotho protein and messenger (m)RNA decreased at week 10 and week 20 after subtotal Nx when compared with controls ($P = 0.01$). Pi reduced Klotho expression (protein and mRNA) in the aortic wall of sham-operated rats when compared with the aortas stimulated with Klotho protein ($P = 0.01$). The addition of exogenous Klotho abrogated the Pi-induced reduction of Klotho in the aortic wall. Principal conclusions: Calcification and Klotho expression in the rat abdominal aortic wall are inversely proportional. Pi reduces the expression of Klotho and possibly promotes calcification in the rat abdominal aorta.

KEYWORDS

Abdominal aorta; *Calcification*; *Klotho*; *Nephrectomy*; *Phosphate*

Introduction

Vascular calcifications have emerged as a significant complication of chronic kidney disease (CKD)[1] and are closely associated with the development of cardiovascular diseases[2]. The prevalence of vascular calcifications tends to increase as the glomerular filtration rate declines in patients with CKD[3]. Patients with CKD have accelerated calcification of the intima in the large arteries and tunica media of the medium-sized arteries, but also in the heart valves and myocardium, developing the uncommon state of calcific uremic arteriolopathy (calciphylaxis)[4,5]. Apart from classic cardiovascular risk factors (arterial hypertension, dyslipidemia, diabetes, tobacco use, family history, age, male sex), nontraditional risk factors (homocysteine, microalbuminuria, defective endothelial nitric oxide synthesis, shedding of glycocalyx components, low antioxidant levels and low-grade inflammation)[6] patients with CKD typically exhibit an abnormal mineral metabolism characterized by hormonal dysregulation[1], a condition known as CKD-mineral and bone disorder (CKD-MBD)[7]. Disturbances in mineral ion homeostasis reflect bone quality and turnover, as well as cardiovascular health and soft-tissue calcifications in rodents[8] and humans[3,9]. While the clinical relevance of vascular calcification assessment is widely recognized, the most reliable quantitative methods still rely on radiography[10]. Many studies have analyzed the pathophysiological mechanism and designed biomarkers for the early prevention of vascular calcifications to prevent adverse events of cardiovascular diseases in patients with CKD-MBD[11,12]. The role of α-Klotho (called Klotho here) and fibroblast growth factor-23 (FGF-23) in the context of vascular calcification in CKD-MBD[11-14] and low-grade inflammatory disease was the focus of these research studies[15-17].

The Klotho gene was initially identified by Kuro-o as an anti-aging gene in mice[18]. The human Klotho gene is located on chromosome 13q12 and consists of approximately 50 thousand base pairs, spanning five exons[19]. The Klotho family of proteins comprises α-, β- and γ-homologous isoforms created by the transcription of the Klotho gene[20,21]. Klotho protein expression is observed in various organs and tissues, with the highest level of expression found in the kidneys[22,23]. Klotho protein is found in the human smooth muscle cell layers of the aorta with staining of the intima, media and vasa vasorum, as well as in medium-sized arteries, and it was always more prominent in healthy subjects than in patients with CKD-BMD[13,23]. However, Klotho was not proved in the renal and iliac arteries of healthy donors and kidney recipients, nor in cultured smooth muscle cells from the human aorta by another research group[24], suggesting a variable expression of Klotho in different arteries[13,23] and possibly culture conditions. Klotho acts physiologically as a co-receptor for fibroblast growth factor receptors (FGFRs), facilitating the activation of the FGF-23 signaling pathway[25,26] in the kidney[23] and vascular tissue[13]. The ligation of FGF-23 to the FGF receptor/Klotho complex in proximal renal tubules decreases phosphate reabsorption and serum phosphate concentration, whereas it increases phosphaturia[27]. FGF-23 decreases the synthesis of 1α, 25-dihydroxycholecalciferol in proximal kidney tubules and the resorption of phosphate ions while facilitating calcium resorption in distal kidney tubules[27]. This explains the complex mineral ion regulation mediated by the FGF-23 receptor/Klotho signaling in animal models and humans[26].

Both endogenous tissue Klotho and soluble Klotho have demonstrated vascular anti-calcification effects[11,22,26,28]. However, the interaction of vascular Klotho and the calcification process in CKD-MBD is not completely understood. The aim of this research was to analyze calcification and Klotho expression in the abdominal aorta after subtotal nephrectomy (Nx) in rats depending on the duration of CKD-MBD and *in vitro* regulation of Klotho expression in rat abdominal

aorta tissue culture condition by soluble Klotho and inorganic phosphate (Pi).

Methods

Animals

The study was conducted on male Wistar rats (10–12 weeks old) purchased from the Institute for Medical Research and Occupational Health (Zagreb, Croatia). Additionally, all experimental procedures on animals, postoperative care and animal sacrifice were carried out at the Institute for Medical Research and Occupational Health. Animals were housed at a temperature of 22 °C with a 12 h light-dark cycle and *ad libitum* access to food and water. All procedures on animals were carried out in accordance with the legal regulations of the Republic of Croatia (OG 135/96, 37/13, 125/13, 55/13, 39/17) and the guidelines of the Council of Europe (806/69/EEC). During the experiments, bioethical standards were applied for conducting experiments on experimental animals, avoiding unnecessary experiments and animal suffering in accordance with the European Directive 2010/63/EU. The study was approved by the Ethics Committee of the Faculty of Medicine, University of Rijeka, Croatia for grant no. Uni-ri-biomed-18-213, from March 7, 2019.

Animal experimental model of nephrectomy

The animals were operated on under general anesthesia with ketamine (0.1 mg/g body weight, intraperitoneal, IP) and xylazine (0.02 mg/g body weight, IP), both from Intervet International B.V., Boxmeer, Netherlands. An incision was made in the middle of the soft abdominal wall. The right kidney was exposed and removed. After a week, the animal was reoperated, and the left upper and lower kidney poles were removed to achieve 5/6 or subtotal nephrectomy (Nx). The remaining kidney tissue was sutured with absorbable suture 6.0. Sham-operated animals were used as controls, with their abdominal cavity opened twice at weekly intervals but not subjected to Nx. The rats were divided into three groups: group at week 20 after sham-operation (control), group at week 10 after subtotal Nx, and group at week 20 after subtotal Nx.

The group of animals sacrificed 10 weeks after Nx was used to investigate the early changes (calcifications and Klotho expression) of the abdominal aorta, whereas the group of rats sacrificed 20 weeks after Nx was used to investigate late changes.

Ten and 20 weeks after subtotal Nx and 20 weeks after sham operation, the rats were sacrificed with the above-mentioned anesthetic. After the sacrifice, animals were transcardially perfused with 0.9% sodium chloride (Kemika, Zagreb, Croatia) and 4% paraformaldehyde (PFA, Sigma Aldrich, St. Luis, Mo, USA) in phosphate buffer saline (2 PBS tablets (Gibco, Gaithersburg, MD, USA) dissolved in 1 L of distilled water, pH 7.4). The abdominal aorta was extracted and either immediately fixed in 4% PFA for 24 hours and embedded in paraffin or placed in a nutrient medium with stimulation for 48 hours and then fixed in 4% PFA for 24 hours and embedded in paraffin. The Klotho messenger ribonucleic acid (mRNA) analysis was performed in freshly isolated or *in vitro* stimulated abdominal aortas.

Labeling of Klotho protein

Samples of paraffin-embedded aortic tissue specimens of nephrectomized and sham-operated control rat groups were labeled following the

principles of the immunohistological method described earlier and applied in our laboratory[29]. Aortic tissue specimens were cut into 3 mm thin sections, which were deparaffinized and rehydrated in the paraffin cleaning agent Tissue Clear (Sakura Finetek Europe, Zoeterwoude, Netherlands), hydrated in decreasing concentrations of ethanol (from 100% to 75%) and washed in PBS. After antigen retrieval in 10 mM sodium citrate, pH 6.6 and washing in PBS, nonspecific binding was minimized by 3% bovine serum albumin (Sigma-Aldrich Chemie, Steincheim, Germany) in PBS (60 minutes) and endogenous peroxidase was blocked with 3% H_2O_2 (Sigma Aldrich) for 20 minutes. Indirect immunoperoxidase staining was performed using the Dako REAL Detection System (Dako, Glosrup, Denmark, K5007) following the manufacturer's instructions. The sections were incubated overnight at +4 °C with anti-Klotho rabbit polyclonal IgG (MyBioSource, San Diego, CA, USA, Cat. No. MBS150338, concentration 1 mg/mL, diluted 1:100 in PBS 1% BSA). The sections were then labeled with secondary biotinylated antibodies for 1 hour at room temperature. The binding of streptavidin-peroxidase with 3.3-diaminobenzidine (DAB) visualized the reaction of specific binding. The nuclei were stained with hematoxylin solution Gill No. 3 (Sigma-Aldrich Chemie, Steincheim, Germany). After washing, the sections were dehydrated by immersion in increasing concentrations of ethanol from 75% to 100% and subsequently dipped in Tissue Clear. The specimens were mounted using Entallan (MerckKgaA, Darmstadt, Germany). All the washes were performed in PBS, except the washes after DAB or hematoxylin solution, when distilled or tap water was used.

Detection of vascular calcifications

The von Kossa staining method was performed in aortic paraffin-embedded sections from rats, which were sacrificed at weeks 10 or 20 after subtotal Nx or 20 weeks after sham operation to visualize vascular calcification following the abovementioned principle[30]. The sections were deparaffinized and hydrated in distilled water, then incubated in an aqueous solution of 5% silver nitrate in distilled water for 60 minutes with exposure to ultraviolet light. After incubation, the sections were rinsed in distilled water for five minutes, incubated in sodium thiosulfate solution (5%) for three minutes and rinsed for five minutes in distilled water. After washing, the slides were incubated in nuclear-fast red 0.1% solution for 10 minutes and washed as in the previous step. The sections were dehydrated in absolute alcohol, cleared and mounted in Entallan (MerckKgaA). The analyses were performed as mentioned above.

Estimation of histological staining

Slides were analyzed using the Olympus BX51 light microscope (Tokyo, Japan). Microphotographs were acquired using the Olympus DP71 camera and Cell^A imaging software, version 3.0 (both from Olympus, Tokyo, Japan). Magnification was achieved using the Olympus UPlan objective lens 10×/0.25 (100×). We used the Image J program (HIH, Bethesda, Md, USA) for the analysis of photomicrographs. Calcification was quantified as the percentage of positive von Kossa stained (calcified) area of the abdominal aorta.

Tissue culture of abdominal aorta

Freshly isolated abdominal aortas from rats 20 weeks after sham operation were maintained in a nutrient medium (DMEM supplemented with 0.9 mM Pi, 1% Glutamine, Penicillin/Streptomycin, and 15% FCS, all from Sigma Aldrich) for 48 hours. The tissue was divided into three groups based on the treatment: group A was treated with 1.6 nM Klotho (Klotho recombinant protein, Cat. No. MBS2010008, MyBioSource, San Diego, CA, USA), group B with 3 mM Pi (Kemika, Zagreb, Croatia), and group C with 1.6 nM Klotho and 3 mM Pi. After the 48 hour-culture, the abdominal aortas were frozen at −80 °C until used for quantitative real-time polymerase chain reaction (qRT-PCR) analysis (for no longer than 6 months) or embedded in paraffin for immunohistology.

Detection of Klotho mRNA

Klotho mRNA was isolated and quantified in freshly isolated abdominal aortas from uremic groups of rats (10 weeks and 20 weeks after subtotal Nx) and from rats 20 weeks after a sham operation. The aortic specimens from 20w sham-operated rats after the 48 hour-culture *in vitro* were analyzed as well according to the above-mentioned principle[31].

mRNA was isolated using an RNA isolation kit (Thermo Fisher Scientific, MA, USA) following the manufacturer's instructions. The RNA underwent DNAse I digestion. Selected primers and Taqman sets (Thermo Fisher Scientific, MA, USA) were used for cDNA synthesis and real-time quantitative polymerase chain reaction (RT-qPCR) analysis of Klotho mRNA expression. Primer/probe sets were used specifically for the membrane-bound Klotho mRNA (TaqMan assay Hs00935388_m1, Thermo Fisher Scientific) and for the alternative Klotho mRNA (forward: 5'-AACTACATTCAAGTAAGTCAGC-3', reverse: 5'-CAGAGTGGTATCTACTAGTG-3', probe: 5'-56-FAM/TCAGCAGTC/ZEN/TCACCAAGCCCT/31ABkFQ-3', IDT). GAPDH was used as an endogenous control (forward sequence: GTCTCCTCTGACTTCAACAGCG, reverse sequence: ACCACCCTGTTGCTGTAGCCAA). RT-qPCR was performed on a StepOne Plus instrument (Thermo Fisher Scientific, MA, USA).

Statistical analysis

TIBCO Statistica, version 13.4.0.14 (Palo Alto, CA, USA) was used to calculate the difference between two groups with the non-parametric Mann-Whitney U test, whereas the difference among three groups was calculated with the Kruskal-Wallis non-parametric test and post hoc Dunn's test, using MedCalc Statistical Software, version 20.011 (MedCalc Software Ltd, Ostend, Belgium). Statistical differences with $P < 0.05$ were considered significant.

Results

Vascular calcification in the abdominal aorta

The rat aortic intima displayed weak calcification 20 weeks after sham operation (Figure 1A) and 10 weeks after subtotal Nx (Figure 1B), whereas intense calcification was found in the aorta 20 weeks after subtotal Nx (Figure 1C). The calcification formed a black ring in von Kossa staining and increased gradually with the survival time after subtotal Nx (arrows). The lower portions of the abdominal aorta were mutually compared in this research. The percentage of the ring calcification in the abdominal aorta from rats at

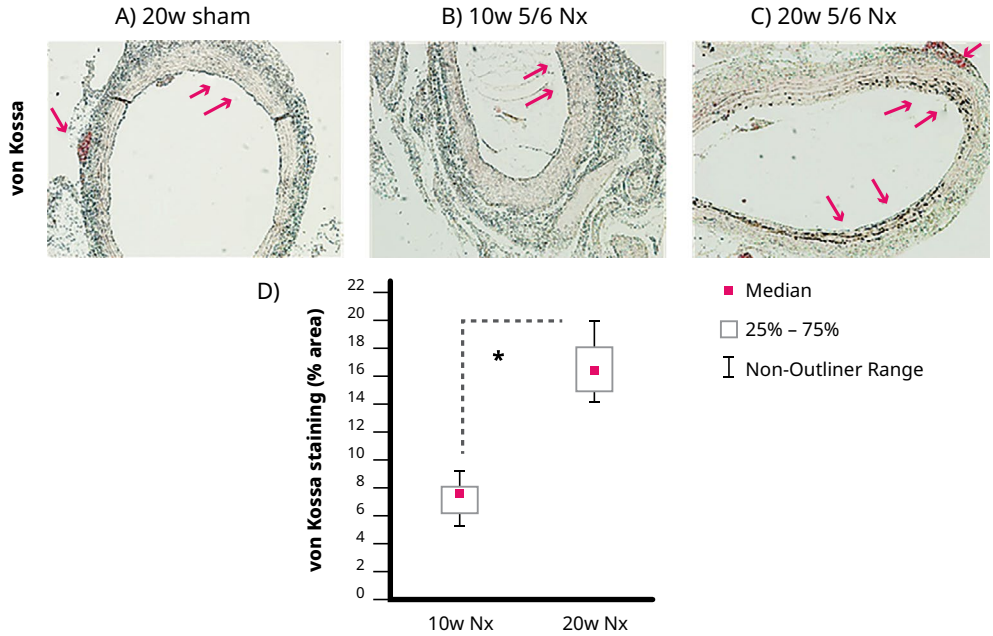

FIGURE 1. An evaluation of calcification in the abdominal aorta of rats. The representative microphotographs show paraffin-embedded lower rat abdominal aorta sections stained with the von Kossa method in rats 20 weeks after a sham operation — control (A), 10 weeks after 5/6 nephrectomy (B) and 20 weeks after 5/6 nephrectomy (C). Arrows denote intima calcification in black, and arrowheads point out the pink coloration of nuclei and cytoplasm. The chart shows the percentage of positive von Kossa stained areas in rings of the abdominal aortas from rats 10 and 20 weeks after 5/6 nephrectomy (D). Five independent experiments were performed in each group. Statistical significance was set at $*P = 0.004$. The original magnification was × 100.
Nx = nephrectomy; w = weeks.

week 20 after subtotal Nx (16.5 (14–20), median (range)) was statistically significantly higher than that of the rats that lived 10 weeks after subtotal Nx (7.5 (5–9), $P = 0.004$) (Figure 1D). Arrowheads show pink staining of the nucleus and cytoplasm, appearing ordinarily in von Kossa staining.

Klotho expression in the abdominal aorta

Immunohistochemical labeling of Klotho is visualized as brown precipitation at the antigen binding site (Figure 2). It revealed intense expression of Klotho protein in rats 20 weeks after sham operation and showed a cytoplasmic labeling pattern of the cells in the aortic intima and media, including vascular smooth muscle cells (VSMCs, Figure 2A). A less intense labeling of Klotho was found in aortic specimens from rats 10 weeks (Figure 2B) and 20 weeks (Figure 2C) after subtotal Nx. The expression of Klotho mRNA followed the dynamics of Klotho protein. Klotho mRNA was approximately three times higher in rats 20 weeks after sham operation (2.9 (2.68–3.3)) than in rats 10 weeks (0.86 (0.8–1)) or 20 weeks (0.76 (0.69–0.86)) after subtotal Nx ($P = 0.11$, Figure 2D).

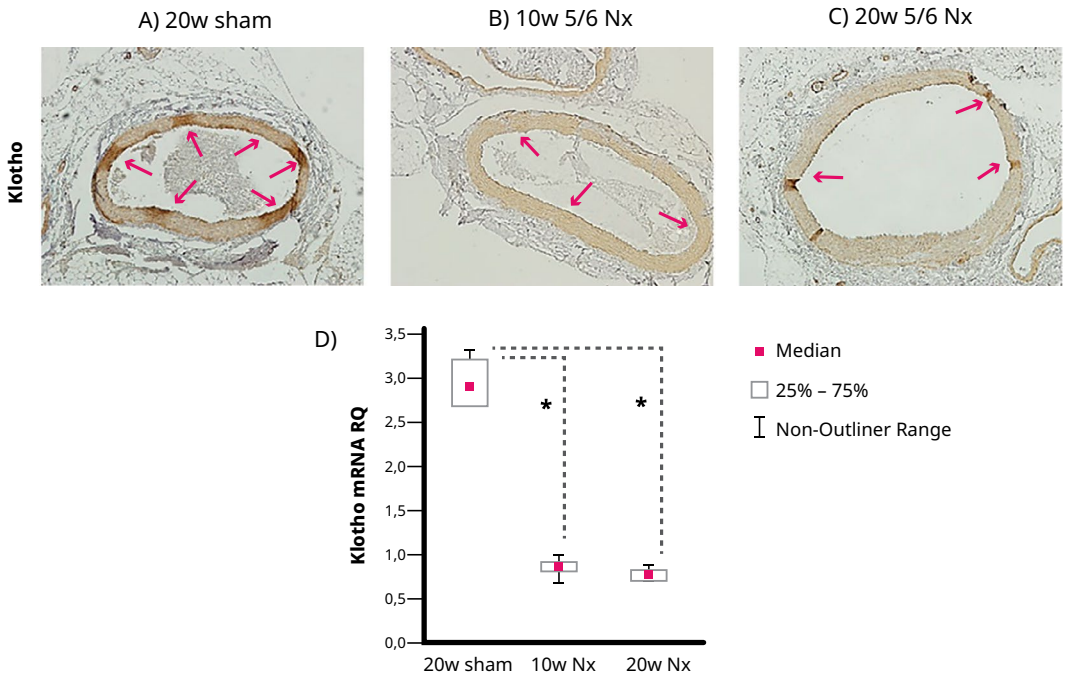

FIGURE 2. Klotho expression in the rat abdominal aorta. Representative paraffin-embedded aortic tissue sections from sham-operated rats — control (A), 10 weeks after 5/6 Nx (B) or 20 weeks after 5/6 Nx (C) groups, labeled by immunohistology using rabbit polyclonal anti-α-Klotho antibodies. The Klotho-positive cells appear as brown precipitation after incubation with 3.3-diaminobenzidine (indicated by arrows). The original magnification was × 100. The chart shows real-time quantitative PCR for Klotho mRNA expression in the above-mentioned groups (D). Five independent experiments were performed in each group. The level of statistical significance was set at $*P = 0.01$. Nx = nephrectomy; w = weeks; RQ = relative quantification.

In vitro regulation of Klotho expression in the abdominal aorta

We assessed the regulation of Klotho protein and mRNA expression in the abdominal aorta in rats 20 weeks after sham operation and after further 48-hour exposure to 1.6 nM Klotho, 3 mM Pi, or 1.6 nM Klotho and 3 mM Pi in tissue culture condition *in vitro* (Figure 3). Immunohistology revealed intense Klotho protein expression as positive staining in the intima and media of the aorta treated with 1.6 nM Klotho (Figure 3A). However, Klotho was labeled very weakly in the aortas stimulated with 3 mM Pi (Figure 3B). The stimulation of abdominal aortas with 1.6 nM Klotho and 3 mM Pi showed intense Klotho expression noticed in brown color (Figure 3C). The fluctuation of Klotho protein expression was followed by Klotho mRNA expression (Figure 3D). Klotho mRNA relative quantification was approximately four times lower in abdominal aortas treated with 3 mM Pi (0.6 (0.55–0.7), mean mRNA RQ (range)) than in abdominal aortas treated with 1.6 nM Klotho (2.1 (1.9–2.3)) or 1.6 nM Klotho and 3 mM Pi (1.9 (1.7–2.4)), ($P = 0.01$, Figure 3D).

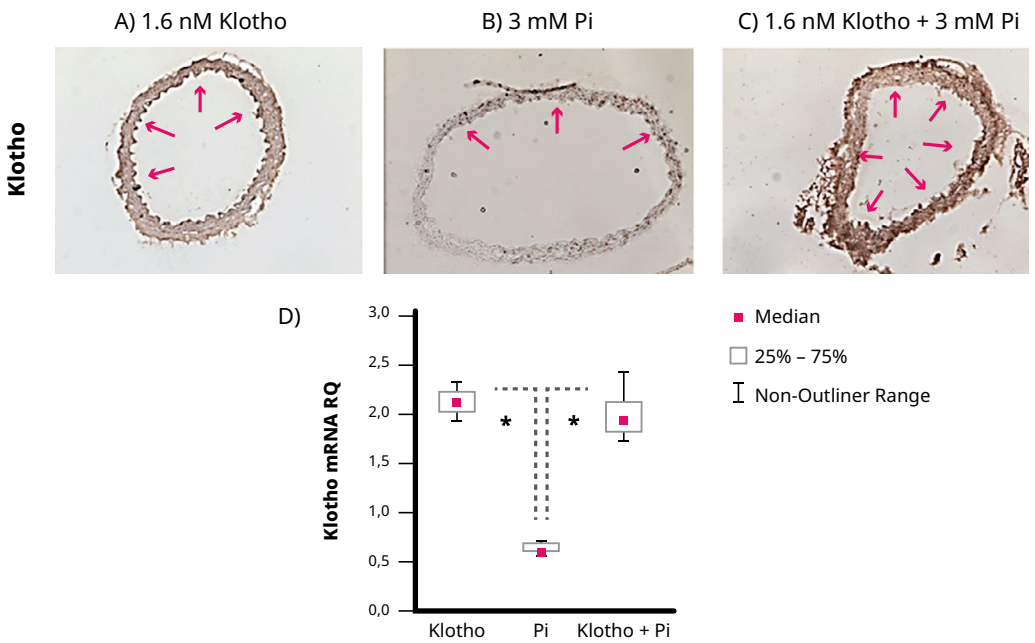

FIGURE 3. The regulation of Klotho protein and mRNA expression in the abdominal aorta *in vitro*. Samples of abdominal aortas show immunohistology labeling using rabbit polyclonal anti-α-Klotho antibodies in rats 20 weeks after sham operation and after further stimulation with 1.6 nM Klotho (A), 3 mM Pi (B) and 1.6 nM Klotho + 3 mM Pi (C) for 48 hours. Positive labeling appeared as brown color (arrows). The chart shows real-time quantitative PCR for Klotho mRNA expression in the abovementioned groups. Five independent experiments were performed in each group. The level of statistical significance was set at $*P = 0.01$.
Pi = inorganic phosphate; RQ = relative quantification.

Discussion

Vascular calcification in patients with CKD-MBD is still incompletely understood, and targeted therapies have been slow to emerge[1]. Here we showed a time-dependent increase in the calcification of the intima layer (atherosclerosis) in the abdominal aorta in subnephrectomized rats. In our experiments, the calcification of the rat aorta appeared as early as 10 weeks after subtotal nephrectomy and progressed rapidly and statistically significantly until 20 weeks, supporting the opinion that vascular calcification appears in the early stage of CKD-MBD, as proved in humans[32,33].

Soft tissue calcifications appeared concomitantly with hyperphosphatemia[11]. It is known that a deficiency of Klotho or FGF-23 in mice with CKD-MBD leads to hyperphosphatemia, hypervitaminosis D and hypercalcemia[26], contributing to an accelerated aging phenotype[34]. In humans, hyperphosphatemia due to chronic kidney dysfunction is one of the first ion disorders incapable of initiating a physiological response in terms of phosphate regulation/elimination[11,32] due to a lack of kidney tubule Klotho expression[23,35,36], which disables FGF-23-mediated phosphaturia and maintenance of calcium homeostasis. Low Klotho vascular expression is also implicated in

early vascular calcification in rodents and humans with CKD-MBD[1,8]. Vascular tissue is sensitive to FGF-23, and the knockdown of Klotho in vascular cells obstructs FGF-23-mediated intracellular signaling and accelerates calcification *in vitro*[13]. However, the regulation and particular function of Klotho in vascular tissue is not completely understood[33]. Lim et al.[13] initially identified endogenous Klotho expression in human arteries and human aortic smooth muscle cells. Other research did not prove Klotho in cultured smooth muscle cells from the human aorta[24], suggesting possible variable expression of Klotho in different culture conditions.

We showed herein a significant reduction of Klotho protein and mRNA concomitantly with the increase in calcification in the rat abdominal aorta after 10 and 20 weeks of subtotal nephrectomy, which is in accordance with intracellular Klotho decrease associated with endothelial senescence and the inverse correlation of plasma Klotho expression and vascular calcification[37]. Our results imply that Klotho protein might be used as a serum marker for monitoring vascular calcifications and subclinical atherosclerosis, as suggested by other studies[14,35,38]. Recently, it has been postulated that the initial decrease in Klotho appears in inflammatory conditions[16,17] at the beginning of CKD-MBD, even in arterial hypertension and unregulated metabolism of carbohydrates and lipoproteins — all of which could take part in CKD-MBD pathogenesis and have low-grade inflammation underlying the diseases[15,36]. Pro-inflammatory transcription factor NF-κB ligates the Klotho promoter and inhibits Klotho protein synthesis[37].

On the other hand, considerable Klotho expression in the abdominal aorta of sham-operated rats was not associated with intima calcification at 20 weeks. This is in accordance with the fact that transgenic mice with CKD-MBD overexpressing α-Klotho showed better kidney function with preserved glomerular function and enhanced phosphaturia, accompanied by diminished vascular calcification due to a direct restraint of phosphate intake in arterial smooth muscle cells when compared with wild-type mice with CKD-MBD[11]. Furthermore, a low-phosphate diet prevents soft tissue calcification despite a rise in vitamin D, encouraging the opinion that hyperphosphatemia is more substantial than vitamin D insufficiency in the process of aging[34]. Therefore, we stimulated abdominal aortas from sham-operated rats with Pi in tissue culture condition *in vitro*, which mimics hyperphosphatemia in CKD-MBD, and we found a significant reduction of Klotho (protein and mRNA) when compared with aortas stimulated with Klotho protein, mimicking a healthy condition. The results suggest that hyperphosphatemia might decrease vascular Klotho expression. Currently, we have no more data on the mechanism of action of Pi on Klotho expression in the aortic wall. In uremia, prominent pro-inflammatory and oxidative stress in endothelial cells decreased Klotho, which can be restrained by quenching inflammation or by the addition of soluble Klotho protein in our experiments and in experimental models of other research groups[37]. The addition of soluble Klotho in the medium with Pi abolishes the effect of Pi in rat abdominal aortas and increases endogenous Klotho protein and mRNA expression in intima and media. This is in accordance with the topical administration of Klotho, which reduced vascular calcification, as soluble Klotho directly suppresses the transport of phosphate-dependent sodium and calcification supported by phosphate in mice aortas[39]. The soluble form of Klotho, which circulates in the bloodstream, prevented endothelial damage induced by inflammation and oxidative stress in uremia and diminished the nuclear factor kappa B-DNA binding ability, Klotho expression, intima calcification and controlled cellular senescence[37].

Moreover, the substitution of Klotho in Klotho-deficient mice with severe vascular calcifications hampers calcification, and Klotho preserves

vascular smooth muscle cell differentiation *in vitro*[8]. It ameliorates vessel structure, kidney performance and serum phosphate levels, suggesting a significant influence of Klotho on vascular health and longevity[8,11,37], and possibly shedding light on atherosclerosis as a reversible process if the precipitating factor — hyperphosphatemia — is removed.

In conclusion, this research has brought forth a new understanding of Klotho regulation in the vascular wall of the rat aorta by Pi and inflammatory conditions within CKD-MBD, emphasizing the mutual relationship between Pi and Klotho in a rat animal model, which might be reminiscent of human disease.

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SAŽETAK

Ekspresija i regulacija Klotho proteina u ranom razvoju vaskularnih kalcifikacija u uremičnih štakora

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Svrha istraživanja bila je analizirati ulaganje kalcija te izražaj i reguliranje Klotha u trbušnoj aorti. Osnovni postupci bili su subtotalna (5/6) nefrektomija (Nx) i podjela Wistar štakora u tri skupine (skupina u 20. tjednu nakon lažne operacije – kontrola, skupina u 10. tjednu nakon subtotalne Nx i skupina u 20. tjednu nakon subtotalne Nx), von Kossa obilježavanje ulaganja kalcija i analiza izražaja Klotha u svježe izvađenoj ili *in vitro* stimuliranoj trbušnoj aorti s anorganskim fosfatom (Pi), Klotho bjelančevinom te Pi i Klotho bjelančevinom tijekom 48 sati. Glavni rezultati: Kalcifikacija aorte bila je veća u 20. tjednu nego u 10. tjednu nakon subtotalne Nx ($P = 0,004$). Klotho bjelančevina i glasnička (g)RNK smanjili su se u 10. tjednu i 20. tjednu nakon subtotalne Nx u usporedbi s kontrolom ($P = 0,01$). Pi je smanjio izražaj Klotho bjelančevine i gRNK u stijenci aorte lažno operiranih štakora u usporedbi s aortom stimuliranom Klotho bjelančevinom ($P = 0,01$). Dodatak Klotha u kulturu ukinuo je smanjenje Klotha potaknutu s Pi u stijenci aorte na razinama bjelančevine i gRNK. Glavni zaključci: Ulaganje kalcija i izražaj Klotha u stijenci trbušne aorte štakora obrnuto su proporcionalni. Pi smanjuje izražaj Klotha i vjerojatno potiče ulaganje kalcija u trbušnu aortu štakora.

KLJUČNE RIJEČI

Trbušna aorta; Kalcifikacija; Klotho; Nefrektomija; Fosfati