International Journal of Geriatrics and Gerontology

Li X, et al. Int J Geriatr Gerontol 8: 189.

www.doi.org/10.29011/2577-0748.100089

www.gavinpublishers.com

OPEN ACCESS

GAVIN PUBLISHERS

Research Article

Aging Impairs the Capacity of Cardiac Functional Recovery Following Endotoxemia: Modification of Myocardial Klotho Level for Remedy

Xueting Li[1,2], Yufeng Zhai[1], Qingzhou Yao[1], Erlinda The[1], Lihua Ao[1], David A. Fullerton[1], Kai-Jiang Yu[2,3*], Xianzhong Meng[1*]

[1]Department of Surgery, University of Colorado Denver, Denver, USA

[2]Department of Critical Care Medicine, Harbin Medical University Cancer Hospital, Harbin, China

[3]Department of Critical Care Medicine, the First Affiliated Hospital of Harbin Medical University, Harbin, China

***Corresponding authors:** Xianzhong Meng, Department of Surgery, University of Colorado Denver, Denver, USA

Kai-Jiang Yu, Department of Critical Care Medicine, Harbin Medical University Cancer Hospital, Harbin, China.

Citation: Li X, Zhai Z, Yao Q, The E, Ao L, et al. (2024) Aging Impairs the Capacity of Cardiac Functional Recovery Following Endotoxemia: Modification of Myocardial Klotho Level for Remedy. Int J Geriatr Gerontol 8: 189. DOI: 10.29011/2577-0748.100089

Received Date: 28 March, 2024; **Accepted Date:** 30 April, 2024; **Published Date:** 06 June, 2024

Abstract

Background: Endotoxemic/septic, cardiac dysfunction occurs frequently in elderly patients undergoing major surgery and contributes to post-surgery morbidity and mortality. This study evaluated the effect of aging on cardiac functional recovery following endotoxemia and explored therapeutic approaches for promotion of the recovery. **Methods & Results:** A small dose of endotoxin (0.5 mg/kg, iv) was administered to young adult (3-4 months) and old (18-22 months) mice with or without subsequent treatment with recombinant interleukin-37 (IL-37, 50 μg/kg, iv) or recombinant Klotho (10 μg/kg, iv). Cardiac function was analyzed using a microcatheter at 24, 48 and 96 h following administration of endotoxin. Myocardial levels of Klotho, ICAM-1 and IL-6 were determined by immunoblotting and ELISA. Compared to young adult endotoxemic mice, old endotoxemic mice had worse cardiac dysfunction accompanied by greater myocardial levels of ICAM-1 and IL-6 at each time point and failed to fully recover cardiac function by 96 h. The exacerbated and prolonged myocardial inflammation and cardiac dysfunction in old endotoxemic mice were associated with lower myocardial Klotho level and its further reduction by endotoxemia. Interestingly, recombinant IL-37 up-regulated myocardial Klotho level in old mice with or without endotoxemia, and treatment of old endotoxemic mice with IL-37 improved myocardial inflammation resolution and cardiac functional recovery. Similarly, recombinant Klotho suppressed myocardial inflammatory response and promoted inflammation resolution in old endotoxemic mice, leading to complete recovery of cardiac function by 96 h. **Conclusion:** Myocardial Klotho insufficiency in old mice exacerbates myocardial inflammatory response, impairs inflammation resolution and hinders cardiac functional recovery. IL-37 is capable of up-regulating myocardial Klotho level to promote myocardial inflammation resolution and cardiac functional recovery in old endotoxemic mice.

Keywords: Klotho; IL-37; inflammation resolution; endotoxemia; cardiac dysfunction

Introduction

Sepsis management is a major challenge for healthcare systems worldwide. In the United States, sepsis accounts for a significant portion of all hospital deaths [1]. Sepsis occurs mainly in people of advanced age, and old septic patients have greater comorbidities and worse prognoses [2]. With the prolongation of life expectancy, the impact of sepsis is becoming greater. Investigation of the mechanisms underlying the pathobiology of sepsis in the elderly will provide information for the development of therapeutic approaches.

Septic cardiomyopathy is a common complication of severe sepsis and worsens the prognosis of patients [3]. Accumulating evidence indicates that myocardial injury caused by sepsis is due to excessive inflammation [3], and several inflammatory mediators induced by bacterial products, such as endotoxin (lipopolysaccharide, LPS), are cardio depressants [4]. Thus, LPS contributes to the mechanism underlying the pathobiology associated with sepsis [5]. Our previous studies demonstrated that a low dose of LPS induces rapid cardiac contractile depression [6]. Further, we observed that old endotoxemic mice develop more severe cardiac dysfunction than young adult mice at 6-24 h following an exposure to LPS, along with greater elevation of cytokines and chemokines in the plasma and myocardium [6, 7]. A study found that old mice had a lower survival rate and exhibited excessive multi-organ dysfunction, as well as exaggerated inflammation during sepsis induced by cecal ligation and puncture (CLP) [8]. Observations made in laboratory animals are comparable to the clinical manifestation of older patients with sepsis. Therefore, the excessive inflammatory response may be the key risk factor for multi-organ dysfunction, particularly cardiac dysfunction, in old septic subjects.

Anti-aging protein Klotho was initially identified in the kidney [9] and has been found subsequently in the parathyroid gland, skeletal muscle and large blood vessels [7, 10, 11]. We have found that the myocardium and heart valve tissue also express Klotho, and the expression of this anti-aging protein in these cardiovascular tissues is down-regulated with aging [7, 12]. Several studies indicated that Klotho has an anti-inflammatory function [7, 13, 14]. In this regard, Klotho knockout mice display a pre-mature aging phenotype and have greater inflammation and more severe organ injury than wild-type mice when being subjected to infection [15]. More importantly, a clinical study found that renal Klotho levels were markedly reduced in septic patients [16], indicating a weakened anti-inflammatory capacity in patients with sepsis. Our previous study found that recombinant Klotho suppresses myocardial inflammatory response in the early phase of endotoxemia, up to 24 h [7]. Thus, Klotho insufficiency in aging heart likely exacerbates myocardial inflammation caused by endotoxemia and sepsis. Such pro-inflammatory effect of aging-related Klotho insufficiency may lead to worse and long-lasting cardiac dysfunction following endotoxemia or sepsis. Pharmacological up-regulation of Klotho levels in aging heart could be a feasible approach for cardiac protection in old subjects with endotoxemia or sepsis.

IL-37, a member of the IL-1 family of cytokines, was identified as an inhibitor of innate immunity [17]. Its expression has been identified in most human cell types, including monocytes, dendritic cells, endothelial cells and epithelial cells, and it acts as a natural regulator of the inflammatory responses [17]. IL-37 has repressive effects on LPS-stimulated cells, such as macrophages and endothelial cells [17]. A clinical study found that the level of IL-37 is increased in response to inflammation in old patients with sepsis [18]. In addition, our previous study found that IL-37 improves cardiac function in old mice at 24 h after an exposure to LPS [19]. Currently, the effect of IL-37 on cardiac functional recovery in the late phase of endotoxemia, at 48 to 96 h, is unclear, and the interaction of IL-37 with Klotho has not been investigated.

The purposes of this study were to determine the mechanistic role of Klotho insufficiency in myocardial inflammation and its resolution following endotoxemia in old mice and to explore the potential of IL-37 for up-regulation of myocardial Klotho level. Specifically, this study determined whether Klotho insufficiency in aging hearts leads to prolonged myocardial inflammation in endotoxemia and whether prolonged myocardial inflammation is responsible for long-lasting cardiac dysfunction. Further, this study examined whether IL-37 is capable of up-regulating Klotho level to promote myocardial inflammation resolution and cardiac function recovery in old endotoxemic mice.

Materials and methods

Ethics statement

The experiments were approved by the Institutional Animal Care and Use Committee of the University of Colorado Denver, and this investigation conforms to the Guide for the Care and Use of Laboratory Animals (National Research Council, revised 1996).

Chemicals and reagents

Antibodies against ICAM-1 (sc-8439) and GAPDH (sc-32233) were purchased from Santa Cruz Biotechnology, Inc (Dallas, TX). Antibody against Klotho (PA5-88303) was purchased from ThermoFhisher Scientific (Waltham, MA). Recombinant Klotho protein (1819-KL), recombinant IL-37 protein (7585-IL), and Enzyme-linked immunosorbent assay (ELISA) kits for IL-6 (DY206) were purchased from the R&D System (Minneapolis, MN). ELISA kit for Klotho (MBS2022324) was purchased from MyBioSource (San Diego, CA). Lipopolysaccharide (LPS, Escherichia coli 0111:B4) and all other chemicals and reagents were purchased from Sigma-Aldrich Chemical (St Louis, MO).

Animals and treatment

Male young adult (3 to 4 months) and old (18 to 22 months) C57BL/6 mice were obtained from the Jackson Laboratory (Bar Harbor, Maine) and National Institute on Aging (Bethesda, MD), respectively. Mice were acclimated for 14 days in a 12:12 h light-dark cycle with free access to water and a regular chow diet.

Young adult mice were assigned to LPS group or saline group. Old mice were assigned to saline, LPS + saline, LPS + IL-37 or LPS + Klotho group. All treatments were performed in the morning through tail vein injection. LPS (0.5 mg/kg) was delivered in 0.1 ml of sterile normal saline. Recombinant Klotho (10 μg/kg) and recombinant IL-37 (50 μg/kg), in 0.1 ml of sterile normal saline, was administered 30-60 min after administration of LPS. Control treatment was performed using the same volume of sterile normal saline. The current study used a small dose of LPS (0.5 mg/kg) utilized in our previous studies [6, 20]. This small dose of LPS induces reversible cardiac dysfunction in young adult mice without mortality, but causes more severe cardiac dysfunction in

old mice with a mortality rate approximately 10%. In addition, our previous studies show that the dosages of IL-37 (50 μg/kg) and Klotho (10 μg/kg) used in the current study are effective to inhibit inflammation in vivo [7, 21]. Experiments were terminated at 24, 48 and 96 h (n≥6 for each time point) after administration of LPS or saline. Blood, myocardium and kidney were collected following analysis of left ventricle (LV) function using a pressure-volume micro-catheter.

Measurement of cardiac function

Cardiac function was assessed as described previously [22]. Briefly, mice were anesthetized with isoflurane inhalation (5% for induction, 2% for maintenance) and anticoagulated with heparin (Elkins-Sinn, Cherry Hill, NJ; 1,000 units/kg, IP). Animals were laid supine on a heating blanket and core body temperature was maintained at 37±0.5°C. A pressure-volume microcatheter (Millar Instrument, Houston, TX; 1.0 F) was inserted into the left ventricle (LV) through the right common carotid artery. The pressure-volume loop and heart rate were recorded using the MPVS-400 system with the aid of PVAN software (Millar Instruments, Houston, TX). Heart rate, LV developed pressure, end-systolic and end-diastolic volume, ejection fraction, and cardiac output was analyzed.

Immunoblotting

Immunoblotting was applied to determine Klotho protein levels in the kidney and myocardium, as well as ICAM-1 levels in the myocardium. Proteins in tissue homogenate were separated on gradient (4%-20%) mini-gels and transferred onto nitrocellulose membrane (Bio-Rad Laboratories, Hercules, CA). The membranes were blocked with 5% skim milk solution for 1 hour at room temperature. The blocked membranes were incubated with a primary antibody against a protein of interest overnight at 4°C. After washing with phosphate-buffered saline (PBS) containing 0.05% Tween 20, the membranes were incubated with a peroxidase-linked secondary antibody (specific to the primary antibody) for 1 hour at room temperature. After further washing, the membranes were treated with enhanced chemiluminescence reagents. The membrane was then exposed to X-ray films. Image J (Wayne Rasband, National Institutes of Health, Bethesda, MD) was used to analyze band density.

ELISA

ELISA kits were utilized to quantify IL-6 and Klotho in myocardial tissue homogenate. Samples and standards were prepared according to manufacturers' instructions. The absorbance of standards and samples was determined spectrophotometrically at 450 nm, using a microplate reader (Bio-Rad Laboratories, Inc, Hercules, CA). Results were plotted against the linear portion of a standard curve.

Statistical analysis

Data are present as mean ± standard error (SE). Statistical analysis was performed using StatView software (Abacus Concepts, Calabasas, CA, USA). Analysis of variance (ANOVA) with Fisher post hoc test was used to analyze differences between experimental groups, and differences were confirmed using the Mann-Whitney U-test. Statistical significance was defined as $P \leq 0.05$.

Results

Persistent cardiac dysfunction in old endotoxemic mice is associated with exacerbated and prolonged myocardial inflammation (inflamm-aging)

In previous studies on the acute effect of endotoxemia, we observed that endotoxemia induces greater myocardial inflammatory responses and worse cardiac dysfunction at 24 h in old mice compared to young adult mice [7]. To understand the relationship of cardiac functional recovery with myocardial inflammation resolution, we prolonged the observation time to 96 h in the present study. We found that both young adult and old mice displayed cardiac dysfunction at 24 h after LPS challenge. As shown in (Figure 1A and Table 1), old mice had much lower ejection fraction (EF: 31 ± 4% vs. 49 ± 5%; $p<0.05$) and LV developed pressure (DP: 50 ± 5 mmHg vs. 62 ± 7 mmHg; $p<0.05$) at 24 h in comparison to young adult mice. EF and DP had recovered to baseline levels at 96 h after LPS challenge in young adult mice. However, these cardiac functional parameters remained significantly lower than baseline levels in old mice (EF: 43 ± 3% vs. 73 ± 8%; DP: 70 ± 7 mmHg vs. 85 ± 9 mmHg; $p<0.05$). These results show that endotoxin induces worse cardiac dysfunction in old mice and that the recovery of cardiac function is incomplete by 96 h in old mice. In order to determine the temporal recovery of cardiac function in endotoxemic old mice, we extended the experiment to 10 days. The results in Supplemental Data 1 show that cardiac function remained low 7 days after administration of LPS, and it was fully recovered 10 days after administration of LPS in old mice.

Table 1: Old mice display more severe cardiac dysfunction following endotoxemia

	Adult Ctrl	Adult LPS 24 h	Adult LPS 48 h	Adult LPS 96 h	OLd Ctrl	OLd LPS 24 h	OLd LPS 48 h	OLd LPS 96 h
Heart rate (bpm)	450 ± 38	560 ± 45*	520 ± 54	490 ± 41	445 ± 51	540 ± 52*	515 ± 51	470 ± 60
Developed Pressure (mmHg)	85 ± 6	53 ± 6*	72 ± 5*	83 ± 9	83 ± 8	43 ± 5*#	60 ± 5*#	65 ± 7*#
End-systolic volume (µl)	5.8 ± 0.6	11.5 ± 0.9*	8.5 ± 1.1*	5.8± 0.7	6.9 ± 0.7	18.0 ± 1.5*#	15.8 ± 1.2*#	12.1 ± 1.1#
End-diastolic volume (µl)	18.0 ± 2.0	20.0 ± 2.3	18.0 ± 1.9	17.5 ± 1.3	18.0 ± 1.3	25.0 ± 2.0*#	24.1 ± 2.3#	22.2 ± 1.7*#
Ejection Fraction (%)	73 ± 7	47 ± 5*	55 ± 6*	65 ± 7	68 ± 7	30 ± 4*#	37 ± 4*#	43 ± 5*#
Cardiac output (ml/min)	6.3 ± 0.5	4.8 ± 0.5*	5.1 ± 0.5*	5.8 ± 0.4	6.0 ± 0.4	3.3 ± 0.3*#	3.8 ± 0.3*#	4.4 ± 0.4*#

Data are expressed as mean±SE. n≥6 in each group; *P<0.05 vs. control, #P<0.05 vs. young adult at the same time point.

Interestingly, we found that more severe and persistent cardiac dysfunction in old endotoxemic mice is accompanied by myocardial "inflamm-aging", a term specifying exacerbated inflammation associated with aging [23]. As shown in Figure 1B, myocardial IL-6 levels increased in endotoxemic young adult and old mice at 24 h. Compared with young adult mice at 24 h, myocardial IL-6 levels in old mice were about 2 folds of that in young adult mice (505 ± 67 vs. 260 ± 30 ng/mg; p<0.05). Myocardial ICAM-1 levels were also much higher in old mice in comparison to young adult mice (Figure 1B). The levels of IL-6 and ICAM-1 in both young adult and old mice decline at 48 and 96 h. While the levels of these pro-inflammatory mediators returned to the baseline levels at 96 h in the hearts of young adult mice, they remained elevated in hearts of old mice (Figure 1B). These results demonstrate that exaggerated myocardial inflammatory response and impaired ability of resolution of myocardial inflammation are components of myocardial inflamm-aging, and both contribute to the mechanism of persistent cardiac dysfunction in old endotoxemic mice.

IL-37 up-regulates myocardial expression of Klotho in old mice and preserves myocardial Klotho level following endotoxemia

Figure 1C shows that Klotho levels were much lower in the heart and kidney of old mice compared with adult mice. With an exposure to endotoxin, Klotho levels markedly decreased at 24 h in the heart and kidney of both adult and old mice, and the reduction of Klotho level was greater in old mice. Interestingly, Klotho levels in the heart and kidney of young adult mice recovered to the baseline at 96 h while they remained low in the heart and kidney of old mice at this time point.

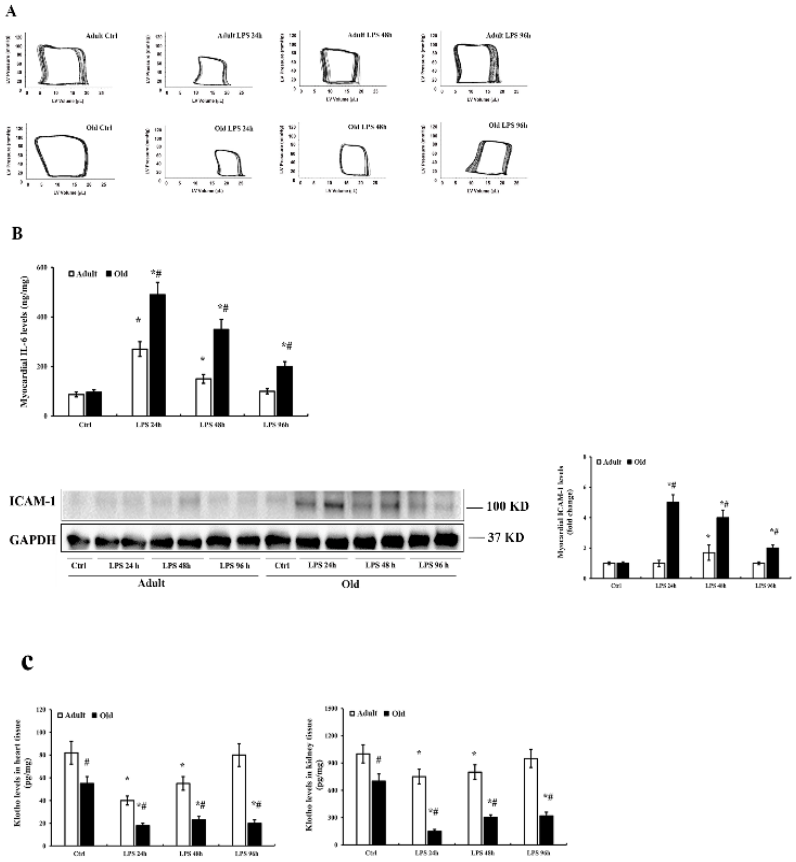


Figure 1: Endotoxemia induces exaggerated and prolonged myocardial inflammation and cardiac dysfunction in old mice that are associated with Klotho insufficiency. Young adult and old mice were treated with lipopolysaccharide (LPS, 0.5 mg/kg, iv) or normal saline for 24, 48 or 96 h. Left ventricle function was analyzed using a microcatheter. (A) Representative pressure-volume loops show that endotoxemia induced greater cardiac dysfunction in old mice compared with young adult mice. Cardiac function recovered over time in both young adult and old mice, and it was comparable to control value at 96 h in young adult mice but remained depressed in old mice at this time point. (B) Myocardial levels of IL-6 and ICAM-1 were assessed using ELISA and immunoblotting, respectively. Levels of these inflammatory mediators increased in the myocardium of young adult mice at 24 and 48 h, and they declined to the control level at 96 h. In old mice, myocardial levels of these inflammatory mediators were much higher at each time point than those in young adult mice and remained above control levels at 96 h. (C) Klotho levels in the tissue homogenate of heart and kidney were assessed using ELISA Klotho levels were lower in the old mice, and endotoxemia caused a greater reduction in Klotho levels in the heart and kidney of old mice. Data are expressed as mean ± SE, n≥6 , **P*< 0.05 vs. control, #*P*< 0.05 vs. young adult at the same time point.

To determine the effect of recombinant IL-37 on the expression of Klotho in old mice, we treated old mice with recombinant IL-37 for 24, 48 and 96 h in the absence of exposure to LPS. Surprisingly, Klotho levels in the heart and kidney were markedly elevated over time. Klotho levels in both heart and kidney at 96 h were about 5 times higher than the levels in the saline-treated control group (Figure 2). As LPS decreases Klotho levels in the heart and kidney of old mice at 24, 48, and 96 h, we administered recombinant IL-37 to the old endotoxemic mice. Interestingly, treatment with IL-37 prevented the decline of Klotho levels in the heart and kidney, resulting in the preservation and elevation of Klotho levels in old mice following endotoxemia (Figure 3).

IL-37 suppresses inflammation and promotes inflammation resolution to improve the recovery of cardiac dysfunction in old endotoxemic mice

IL-37 is a member of the interleukin-1 family and exerts an anti-inflammatory effect in several inflammatory diseases. A recent study found that IL-37 improves cardiac function in adult endotoxemia mice via inhibition of the inflammatory NF-κB signaling pathway at 6 h [19]. In the present study, we found that IL-37 administration markedly improved left ventricle function in old endotoxemic mice (Table 2). Compared with the LPS only group, the developed pressure was elevated by 38%, ejection fraction was increased by 55% and cardiac output was increased by about 63% in the LPS + IL-37 group at 24 h in old mice (Table 2). Although the cardiac function was slowly recovering in both LPS alone group and LPS + IL-37 group at 48 and 96 h, the recovery was markedly improved in LPS + IL-37 group in comparison with the LPS alone group at the same time point. The left ventricle function parameters were recovered the baseline levels at 96 h in the LPS + IL-37 group, but not in the LPS alone group at this time point.

Table 2: Recombinant IL-37 improves cardiac functional recovery in old mice following endotoxemia.

	Ctrl	LPS 24 h	LPS 48 h	LPS 96 h	LPS + IL-37 24 h	LPS + IL-37 48 h	LPS + IL-37 96 h
Heart rate (bpm)	480 ± 45	540 ± 60	510 ± 65	490 ± 36	510 ± 62	480 ± 42	485 ± 57
Developed Pressure (mmHg)	80 ± 7	50 ± 5*	58 ± 7*	68 ± 7*	69 ± 7*#	71 ± 8*#	76 ± 9#
End-systolic volume (ul)	7.0 ± 0.6	18.5 ±0.7*	14.5 ±1.3*	11.0±1.2*	10.0 ±1.2*#	8.9 ± 0.9*#	7.1 ± 0.5#
End-diastolic volume (ul)	18.0 ±2.0	25.0 ±2.6*	22.0 ± 2.1*	20.0 ± 1.9*	20.0 ± 1.8*#	19.0 ± 2.0#	18.2 ± 1.8#
Ejection Fraction (%)	65 ± 7	31 ± 4*	35 ± 3*	43 ± 3*	48 ± 6*#	50 ± 6*#	60 ± 7#
Cardiac output (ml/min)	5.5 ± 0.6	3.0 ± 0.4*	3.3 ± 0.4*	4.0 ± 0.5*	4.9±0.5*#	5.0 ± 0.6*#	5.3 ± 0.5#

Data are expressed as mean±SE. n≥8 in Ctrl and LPS alone groups; n≥6 in LPS + IL-37 groups; *P<0.05 vs. control, #P<0.05 vs. LPS alone at the same time point.

Myocardial levels of IL-6 and ICAM-1 were determined in the old endotoxemic mice with or without IL-37 treatment. ICAM-1 levels in old endotoxemic mice were reduced by 50% at 24 and 48 h by IL-37 treatment in comparison with the LPS only group. The two adhesion molecule levels returned to baseline at 96 h in the LPS + IL-37 group, but not in the LPS treatment only group. The IL-6 levels in old endotoxemic mice were decreased at 24 h and 48 h by IL-37-treatment and recovered to the baseline at 96 h (Figure 3).

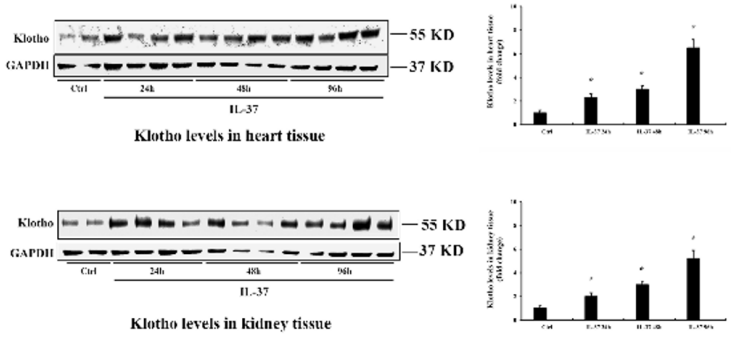


Figure 2: Recombinant IL-37 up-regulates Klotho expression in naive old mice. Immunoblots and densitometry data show that treatment of old naïve mice with recombinant IL-37 up-regulated the level of Klotho in heart and kidney in a time-dependent fashion. Data are expressed as mean ± SE, n≥6 , *P< 0.05 vs. control.

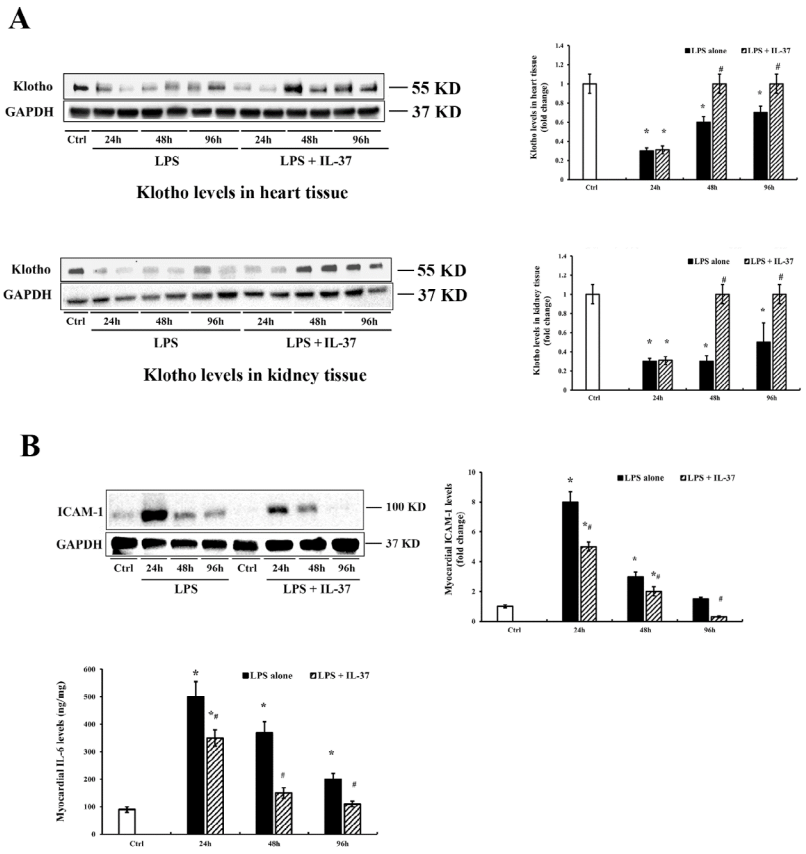


Figure 3: Recombinant IL-37 intensifies the restoration of Klotho levels, suppresses myocardial inflammatory responses and promotes myocardial inflammation resolution in old endotoxemic mice. Old mice were treated with LPS (0.5 mg/kg, iv) alone or LPS followed by recombinant IL-37 (50 µg/kg, iv) 30 min later. Control mice were treated with normal saline. IL-6 levels in myocardial homogenate were assessed using ELISA. ICAM-1 levels in the myocardium and Klotho levels in the myocardium and kidney tissue were determined by immunoblotting and densitometry. (A) Treatment with recombinant IL-37 30 min following LPS exposure resulted in the restoration of Klotho levels in the heart and kidney at 48 and 96 h after LPS exposure. (B) Treatment with IL-37 after LPS exposure reduced levels of inflammatory mediators in the myocardium at each time point in old mice, resulting in their levels being normalized at 96 h. Data are expressed as mean ± SE, n≥6 , *P< 0.05 vs. control, #P< 0.05 vs. LPS alone at the same time point.

Klotho promotes myocardial inflammation resolution and improves the recovery from cardiac dysfunction in old mice

To determine the effect of Klotho on the myocardial inflammation resolution in old mice, we evaluated and compared the levels of ICAM-1 and IL-6 in LPS alone group and LPS + Klotho group at 24, 48 and 96 h after administration of LPS. Although the levels of these inflammatory mediators decreased over time from 24 to 96 h, they remained significantly higher at 96 h in LPS only group than the baseline. In contrast, Klotho treatment resulted in the returning to the baseline levels for myocardial ICAM-1 and IL-6 at 96 h (Figure 4A). Thus, recombinant Klotho not only attenuates myocardial inflammation response in old endotoxemic mice, but also promotes their myocardial inflammation resolution.

The representative pressure-volume loops in Figure 4B show LV function at 24, 48, and 96 h after the exposure to LPS. Compared to the saline-treated old mice, old mice treated with LPS alone displayed greater reduction in LV function at 24 h. Although LV function was improved over time, it remained significantly lower at 96 h than the control level. However, old endotoxemic mice treated with Klotho had better LV function at each time point (Figure 4B and Table 3). At 96 h, these mice displayed significantly improved developed pressure (75 ± 6 mmHg vs. 68 ± 7 mmHg in LPS alone group; $P<0.05$, n≥6), ejection fraction (56 ± 7% vs. 43 ± 3% in LPS alone group; $P<0.05$, n≥6) and cardiac output (5.1 ± 0.6 ml/min vs. 4.0 ± 0.5 ml/min in LPS alone group; $P<0.05$, n≥6) in comparison to old mice treated with LPS alone (Figure 4B and Table 3).

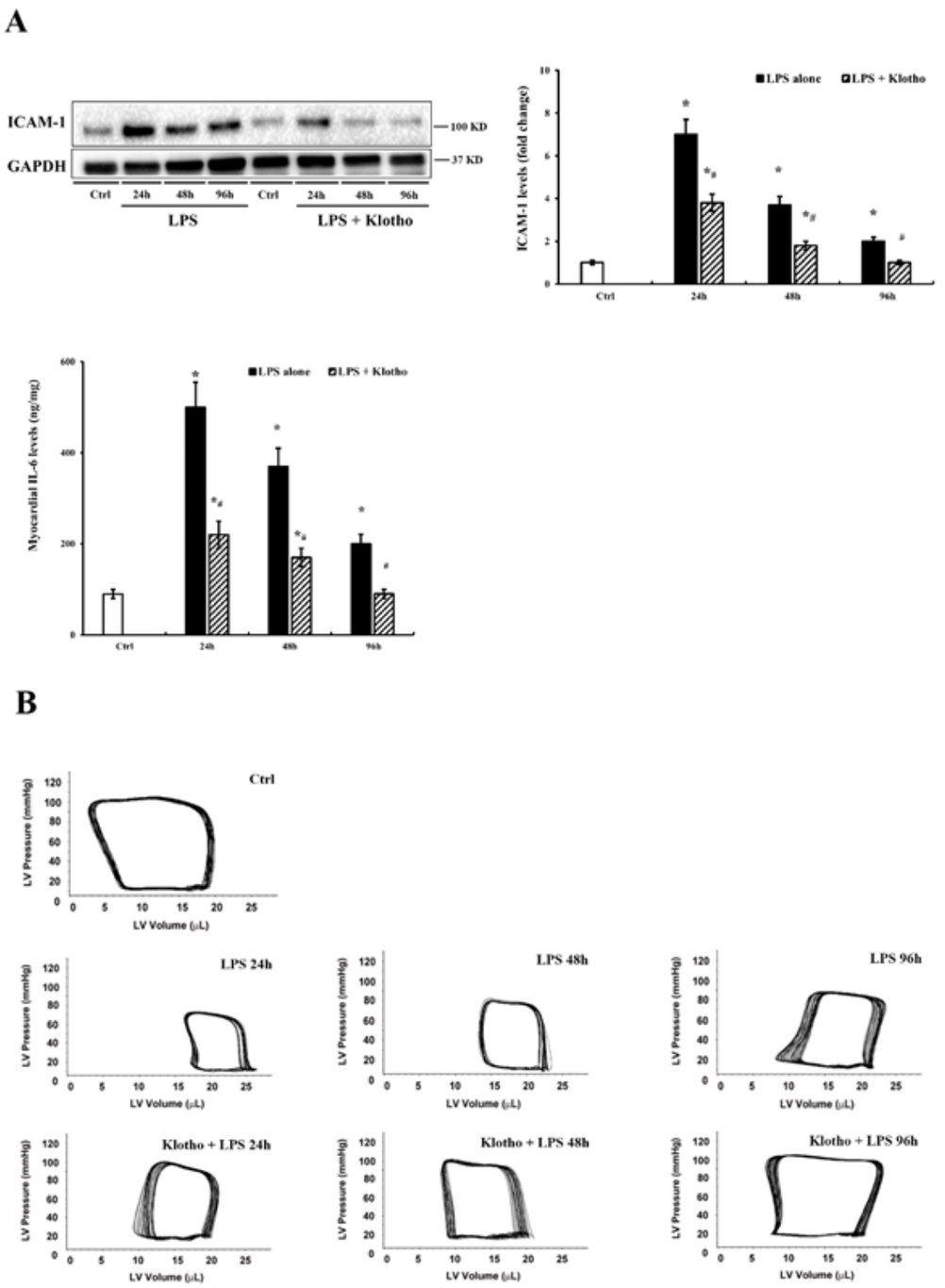


Figure 4: Recombinant Klotho down-regulates myocardial inflammatory responses, promotes myocardial inflammation resolution and attenuates cardiac dysfunction in old endotoxemic mice. Old mice were treated with LPS (0.5 mg/kg, iv) alone or LPS followed by recombinant Klotho (10 μg/kg, iv) 30 min later. Control mice were treated with normal saline. ICAM-1 levels in myocardial tissue were determined by immunoblotting and densitometry. IL-6 levels were assessed by ELISA. Left ventricle pressure-volume loops were recorded at 24, 48 and 96 h following LPS exposure. (A) Treatment with recombinant Klotho reduced myocardial levels of IL-6 and ICAM-1 in old mice at 24 and 48 h following the exposure to LPS and restored their levels to the baseline by 96 h. (B) Klotho markedly improved cardiac function in old endotoxemic mice at each time point. Data are expressed as mean ± SE, n≥6, *P< 0.05 vs. control, #P< 0.05 vs. LPS alone at the same time point.

Table 3: Recombinant Klotho improves cardiac functional recovery in old mice following endotoxemia

	Ctrl	LPS 24h	LPS 48h	LPS 96h	LPS+Klotho 24 h	LPS+Klotho 48 h	LPS+Klotho 96 h
Heart rate (bpm)	480 ± 45	540 ± 60	510 ± 65	490 ± 36	520 ± 49	490 ± 53	480 ± 38
Developed pressure (mmHg)	80 ± 7	50 ± 5*	58 ± 7*	68 ± 7*	65 ± 8*#	70 ± 9*#	75 ± 6
End-systolic volume (ul)	7.0 ±0.6	18.5 ± 0.7*	14.5 ± 1.3*	11.0 ± 1.2*	12.7 ± 1.3*#	9.0 ± 1.0*#	8.0 ± 0.7#
End-diastolic volume (ul)	18.0 ± 2.0	25.0 ± 2.6*	22.0 ± 2.1*	20.0 ± 1.9*	21.1 ± 1.8*#	19.0 ± 2.1	18.0 ± 2.0
Ejection Fraction (%)	65 ± 7	31 ± 4*	35 ± 3*	43 ± 3*	44 ± 5*#	50 ± 6*#	56 ± 7#
Cardiac output (ml/min)	5.5 ± 0.6	3.0 ± 0.4*	3.3 ± 0.4*	4.0 ± 0.5*	4.0 ± 0.3*#	5.0 ± 0.5*#	5.1 ± 0.6#

Data are expressed as mean±SE. n≥8 in Ctrl and LPS alone groups; n≥6 in LPS + Klotho groups; *P<0.05 vs. control, #P<0.05 vs. LPS alone at the same time point.

Discussion

Organ dysfunction is common in the elderly with sepsis or endotoxemia [24]. However, it remains unclear why advanced age makes the subject more vulnerable to severe organ dysfunction. In the present study, we found that the more severe and long-lasting cardiac dysfunction in old endotoxemic mice is due to excessive and prolonged myocardial inflammation. Klotho insufficiency in aging hearts contributes to the mechanism of exaggerated and prolonged inflammation, as well as impaired recovery of cardiac function in old endotoxemic mice. Recombinant Klotho improves the recovery of cardiac function in old endotoxemic mice by down-regulation of the myocardial inflammatory responses and promotion of inflammation resolution. Up-regulation of Klotho production in the aging heart is a novel function of anti-inflammatory cytokine IL-37 and constitutes an important mechanism by which IL-37 promotes myocardial inflammation resolution and cardiac function recovery.

The magnitude and duration of tissue inflammatory change in response to a noxious insult is determined by the level of initial inflammatory response and the ability to resolve the inflammation [25]. When the integrity of anti-inflammatory mechanism is affected by aging, inflammatory response is exaggerated and the ability of resolution of inflammation becomes attenuated, resulting in exacerbated and long-lasting inflammation. Such a phenomenon is termed as “inflamm-aging” [26]. In the present study, we observed that myocardial inflamm-aging is present in old endotoxemic mice, reflected as greater magnitude of myocardial inflammation in the early phase of endotoxemia (up to 24 h after exposure to LPS) and the inability to resolve myocardial inflammation in the subsequent phase (from 24 to 96 h). Clearly, the results of the present study demonstrate that inflamm-aging exacerbates myocardial inflammation following endotoxemia by elevating the inflammatory response and attenuating the ability of clearing the inflammation.

Inflammatory cytokines, such as TNF-α, IL-1β and IL-6, are cardiodepressants and contribute to cardiac dysfunction via their direct effects on the myocardium and/or through activation of other detrimental mechanisms in the myocardium, including generation of toxic oxygen species, mitochondrial dysfunction and the loss of calcium homeostasis, as well as up-regulation of the expression of adhesion molecules [6,7,27,28]. ICAM-1 is immunoglobulin-like adhesion molecules expressed on endothelial cells and many other cell types. They play a key role in mediating the recruitment of leukocytes to areas of inflammation and are typical biomarkers of inflammation. Our previous study found that ICAM-1 contribute to the mechanism of endotoxemic cardiac dysfunction [20]. Interestingly, ICAM-1 plays a role in endotoxemic cardiac dysfunction through a neutrophil-independent mechanism [20]. In the present study, we observed that in comparison to young adult mice, old mice display greater myocardial production of inflammatory mediators, i.e. IL-6 and ICAM-1 examined, following an exposure to LPS, and inflammation resolution is impaired in the hearts of old mice as levels of inflammatory mediators in the myocardium remain elevated 4 days after an exposure to LPS. The exacerbated and

prolonged myocardial inflammation in old endotoxemic mice is a characteristic manifestation of myocardial inflamm-aging, and it is closely associated with the more severe and long-lasting cardiac dysfunction in old endotoxemic mice. Ample evidence demonstrates the mechanistic role of inflammatory mediators in cardiac dysfunction and support the notion that inflamm-aging contributes to the mechanism underlying the more severe and persistent cardiac dysfunction caused by noxious insults in the old subjects. Currently, the mechanism of myocardial inflamm-aging is unclear.

Klotho is an anti-aging protein present in multiple tissues, and its level declines with aging. Decreased Klotho protein level correlates with reduced lifespan and aging-related disorders, such as osteoporosis, coronary artery disease, and stroke [29]. Indeed, we observed significantly lower levels of myocardial Klotho in old mice in comparison to young adult mice. Interesting observations of the present study include greater reduction of myocardial Klotho levels by endotoxemia in old mice and inability of aging hearts to restore their Klotho levels 4 days after in endotoxemia. A low level of inflammation associated with aging is one of the mechanisms of Klotho down-regulation [30]. The greater reduction of myocardial Klotho level and failure to restore the Klotho level after 4 days are likely due to exaggerated production of inflammatory mediators and persistent elevation of these mediators in the myocardium as inflammatory cytokines have been found to down-regulate Klotho expression in mice [31]. Several studies, in vitro and in vivo, demonstrated that Klotho has an anti-inflammatory function, and the exogenous administration of soluble Klotho or overexpression of Klotho can significantly inhibit the inflammatory responses, i.e. the NF-κB activity, and decrease the expression of TNF-α, IL-1β and IL-6 [7, 13, 32], which are well-known cardiodepressants and contribute to cardiac dysfunction. Moreover, previous study showed that Klotho administration can also induce anti-inflammatory mediators, such as IL-10, by activation of the JAK2/STAT3 signaling axis [33]. Particularly, recombinant Klotho has been found to inhibit TNF-α-induced NF-κB activation and attenuate the expression of ICAM-1 in HUVECs [34]. Based on the information, it is reasonable to hypothesize that Klotho insufficiency in aging hearts and further reduction myocardial Klotho level following endotoxemia play a mechanistic role in mediating myocardial hyper-inflammatory response and persistent myocardial inflammation observed in old endotoxemic mice.

We tested this hypothesis by evaluating the effect of recombinant Klotho on myocardial inflammation and cardiac function in old endotoxemic mice. Recombinant Klotho not only reduced the levels of inflammatory mediators in the myocardium at 24 h, but also promoted the resolution of myocardial inflammation, resulting in essentially normalized levels of inflammatory mediators in the myocardium after 4 days into endotoxemia. As a result, cardiac function recovery is markedly improved to a level slightly lower than control. The novel finding that Klotho promotes myocardial inflammation resolution and cardiac function recovery in old endotoxemic mice indicates that Klotho insufficiency is responsible for the impaired myocardial inflammation resolution and delayed cardiac function recovery. This finding also highlights the therapeutic potential of recombinant Klotho for cardiac protection in old subjects affected by endotoxemia or sepsis.

Inflammation associated with aging is believed to be a mechanism by which Klotho expression is down-regulated in old subjects [30]. However, it remains unclear whether Klotho expression in old subjects could be up-regulated. We explored the potential of anti-inflammatory approach for up-regulation of Klotho expression in old mice.

Our previous study demonstrates a potent anti-inflammatory effect of IL-37 on the cardiovascular tissue [19, 21]. Interestingly, we observed in the present study that administration of recombinant IL-37 to naïve old mice increases Klotho protein levels in the heart and kidney in a time-dependent fashion, with the maximal increase at 4 days. Further, treatment of old endotoxemic mice with recombinant IL-37 results in the restoration of Klotho levels in the heart and kidney at 4 days into endotoxemia. With the restoration of Klotho level in the heart, myocardial inflammation is completely resolved, and cardiac function is fully recovered in IL-37-treated old endotoxemic mice at 4 days into endotoxemia. It is noteworthy that old endotoxemic mice treated with recombinant IL-37 have better cardiac function than those treated with recombinant Klotho. It is likely that other mechanisms utilized by IL-37 in addition to the mechanism through up-regulation of Klotho have a moderate contribution to the full recovery of cardiac function.

Anti-inflammation might be the main mechanism by which IL-37 up-regulates Klotho levels. Previous study showed that inflammatory cytokines, such as TWEAK and TNF-α, downregulate Klotho expression through a NF-κB-dependent mechanism [35]. It appears that IL-37, as a fundamental inhibitor of innate immunity, reduces systemic inflammation in endotoxemic mice to elevate Klotho levels. Klotho levels are also increased in naïve old mice treated with IL-37. This observation is significant since it demonstrates that pharmacological up-regulation of myocardial Klotho level is achievable. The effect of recombinant IL-37 on Klotho expression in naïve old mice is especially interesting as it indicates that a specific pharmacological agent can be applied to up-regulation of Klotho expression for anti-aging and broader applications to mitigate aging-related health conditions. In this regard, clinical studies have demonstrated that higher plasma Klotho concentrations are associated with reduced risk of coronary artery disease, heart failure and stroke [36].

Noticeably, myocardial inflammation is completely resolved, and cardiac function is fully recovered in young adult mice 4 days after endotoxemia while myocardial inflammation is still present and cardiac function remains low in old mice at this time. While the full recovery of cardiac function took 10 days in old endotoxemic mice with no intervention, the recovery time was shortened to 4 days in old endotoxemic mice treated with either Klotho or IL-37. Thus, Klotho and IL-37 have therapeutic potential for cardiac protection in old subjects with endotoxemia.

It remains unclear from the current study what cardiac

cells are the main source of myocardial Klotho and whether IL-37 enhances their expression of Klotho to elevate myocardial level of this anti-aging protein. Further study will address these questions. It should be pointed out also that female mice were not included in the current study. However, no study suggests gender disparity in the response to IL-37 or Klotho. While a comparable outcome is expected from old female endotoxemic mice following administration of IL-37 or Klotho, future study needs to address potential gender difference.

Conclusion

The present study demonstrates that exacerbated and persistent inflammation in old endotoxemic mice contributes to the mechanism of exaggerated and prolonged cardiac dysfunction. Klotho insufficiency in the aging heart plays a mechanistic role in mediating myocardial hyper-inflammation and more severe cardiac dysfunction in old endotoxemic mice. Recombinant Klotho suppresses myocardial inflammatory response, promotes myocardial inflammation resolution and improves cardiac function recovery. Recombinant IL-37 is capable of up-regulating Klotho level in the heart and kidney of old naïve mice, abrogating the delay in inflammation resolution and cardiac function recovery in old endotoxemic mice (Figure 5). Both Klotho and IL-37 have the potential for cardiac protection in old subjects affected by endotoxemia.

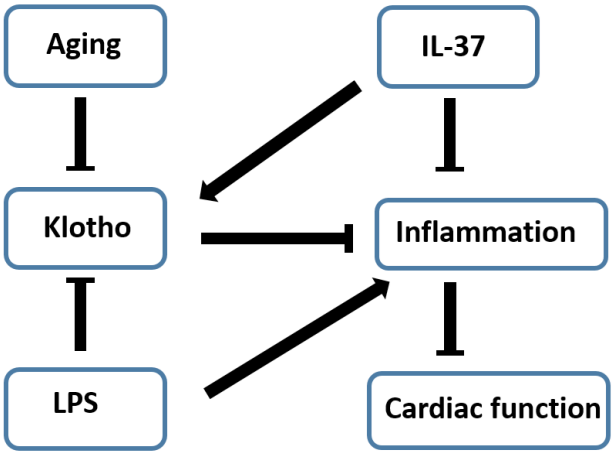


Figure 5: Schematic diagram summarizes the main findings of this study.

While aging down-regulates Klotho expression to augment myocardial inflammation and cardiac dysfunction in endotoxemia, IL-37 up-regulates Klotho expression to suppress myocardial inflammatory responses and to promote myocardial inflammation resolution. The direct effect of IL-37 on the inflammatory responses and indirect effect through induction of Klotho promote inflammation resolution and cardiac function recovery in old endotoxemic mice.

Abbreviations: **IL-37:** Interleukin-37, **ICAM-1:** Intercellular adhesion molecular-1, **IL-6:** Interleukin-6, **ELISA:** Enzyme-linked immunosorbent assay, **LPS:** Lipopolysaccharide

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author Contributions

Conceptualization, XM. Methodology, XL and YZ. Investigation, XL, YZ, QY, ET and LA. Data Acquisition and Analysis, XL, YZ, QY, ET and LA. Original manuscript draft preparation, XL, YZ and QY. Manuscript review and revision, XM, KY and DF; Supervision, XM and DF; Project administration, XM; Funding Acquisition, XM.

All authors have read and approved the final version of this manuscript.

Funding

This work was supported in part by the National Institute of Health grant R01GM129641 and Veterans Administration grant I01BX0051631.

Data availability

All data are available from the corresponding authors under reasonable conditions.

Declarations Ethics approval and consent to participate

The experiments were approved by the Institutional Animal Care and Use Committee of the University of Colorado Denver, and this investigation conforms to the Guide for the Care and Use of Laboratory Animals (National Research Council, revised 1996).

References

1. Paoli CJ, Reynolds MA, Sinha M, Gitlin M, Crouser E (2018) Epidemiology and costs of sepsis in the United States—an analysis based on timing of diagnosis and severity level. Crit Care Med. 46:1889-1897.
2. Martin S, Perez A, Aldecoa C (2017) Sepsis and immunosenescence in the elderly patient: a review. Front Med (Lausanne). 4:20.
3. Hollenberg SM, Singer M (2021) Pathophysiology of sepsis-induced cardiomyopathy. Nature Reviews Cardiology. 18:424-434.
4. Kumar A, Haery C, Parrillo JE (2000) Myocardial dysfunction in septic shock. Crit Care Clin. 16:251-287.
5. de Pádua Lúcio K, Rabelo ACS, Araújo CM, Brandão GC, de Souza GHB, et al. (2018) Anti-inflammatory and antioxidant properties of black mulberry (Morus nigra L.) in a model of LPS-induced sepsis. Oxid Med Cell Longev. 2018:5048031.
6. Slimani H, Zhai Y, Yousif NG, Ao L, Zeng Q, et al. (2014) Enhanced monocyte chemoattractant protein-1 production in aging mice exaggerates cardiac depression during endotoxemia. Crit Care. 18:527.
7. Hui H, Zhai Y, Ao L, Cleveland JC Jr, Liu H, et al. (2017) Klotho suppresses the inflammatory responses and ameliorates cardiac dysfunction in aging endotoxemic mice. Oncotarget. 8:15663-15676.

8. Stortz JA, Hollen MK, Nacionales DC, Horiguchi H, Ungaro R, et al. (2019) Old Mice Demonstrate Organ Dysfunction as well as Prolonged Inflammation, Immunosuppression, and Weight Loss in a Modified Surgical Sepsis Model. Crit Care Med. 47:e919-e929.

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

10. Olauson H, Mencke R, Hillebrands JL, Larsson TE (2017) Tissue expression and source of circulating alphaKlotho. Bone. 100:19-35.

11. Lim K, Groen A, Molostvov G, Lu T, Lilley KS, et al. (2015) Alpha-Klotho expression in human tissues. J Clin Endocrinol Metab. 100:E1308-1318.

12. Li F, Yao Q, Ao L, Cleveland JC, Dong N, et al. (2017) Klotho suppresses high phosphate-induced osteogenic responses in human aortic valve interstitial cells through inhibition of Sox9. J Mol Med (Berl). 95:739-751.

13. 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. 60:1907-16.

14. Junho CVC, González-Lafuente L, Neres-Santos RS, Navarro-García JA, Rodríguez-Sánchez E, et al. (2022) Klotho relieves inflammation and exerts a cardioprotective effect during renal ischemia/reperfusion-induced cardiorenal syndrome. Biomed Pharmacother. 153:113515.

15. Inoue S, Sato T, Suzuki-Utsunomiya K, Komori Y, Hozumi K, et al. (2013) Sepsis-induced hypercytokinemia and lymphocyte apoptosis in aging-accelerated Klotho knockout mice. Shock. 39:311-316.

16. Jou-Valencia D, Molema G, Popa E, Aslan A, van Dijk F, et al. (2018) Renal Klotho is Reduced in Septic Patients and Pretreatment With Recombinant Klotho Attenuates Organ Injury in Lipopolysaccharide-Challenged Mice. Crit Care Med. 46:e1196-1203.

17. Nold MF, Nold-Petry CA, Zepp JA, Palmer BE, Bufler P, et al. (2010) IL-37 is a fundamental inhibitor of innate immunity. Nat Immunol.11:1014-1022.

18. Wang Y-C, Weng G-P, Liu J-P, Li L, Cheng Q-H (2019) Elevated serum IL-37 concentrations in patients with sepsis. Medicine. 98:e14756.

19. Li J, Zhai Y, Ao L, Hui H, Fullerton DA, et al. (2017) Interleukin-37 suppresses the inflammatory response to protect cardiac function in old endotoxemic mice. Cytokine. 95:55-63.

20. Raeburn CD, Calkins CM, Zimmerman MA, Song Y, Ao L, et al. (2002) ICAM-1 and VCAM-1 mediate endotoxemic myocardial dysfunction independent of neutrophil accumulation. Am J Physiol Regul Integr Comp Physiol. 283:R477-R486.

21. Zeng Q, Song R, Fullerton DA, Ao L, Zhai Y, et al. (2017) Interleukin-37 suppresses the osteogenic responses of human aortic valve interstitial cells in vitro and alleviates valve lesions in mice. Proc Natl Acad Sci U S A. 114:1631-1636.

22. Pacher P, Nagayama T, Mukhopadhyay P, Bátkai S, Kass DA (2008) Measurement of cardiac function using pressure–volume conductance catheter technique in mice and rats. Nat Protoc. 3:1422-1434.

23. Dugan B, Conway J, Duggal NA (2023) Inflammaging as a target for healthy ageing. Age Ageing. 52:afac328.

24. Iwashyna TJ, Cooke CR, Wunsch H, Kahn JM (2012) Population burden of long-term survivorship after severe sepsis in older Americans. J Am Geriatr Soc. 60:1070-1077.

25. Feehan KT, Gilroy DW (2019) Is resolution the end of inflammation? Trends Mol Med. 25:198-214.

26. Franceschi C, Bonafè M, Valensin S, Olivieri F, De Luca M, et al. (2000) Inflamm□aging: an evolutionary perspective on immunosenescence. Ann N Y Acad Sci. 908:244-254.

27. Zanotti-Cavazzoni SL, Hollenberg SM (2009) Cardiac dysfunction in severe sepsis and septic shock. Curr Opin Crit Care. 15:392-397.

28. Laudes IJ, Guo R-F, Riedemann NC, Speyer C, Craig R, et al. (2004) Disturbed homeostasis of lung intercellular adhesion molecule-1 and vascular cell adhesion molecule-1 during sepsis. Am J Pathol. 164:1435-1445.

29. Arking DE, Atzmon G, Arking A, Barzilai N, Dietz HC (2005) Association between a functional variant of the KLOTHO gene and high-density lipoprotein cholesterol, blood pressure, stroke, and longevity. Circ Res. 96:412-418.

30. Ruiz-Andres O, Sanchez-Niño MD, Moreno JA, Ruiz-Ortega M, Ramos AM, et al. (2016) Downregulation of kidney protective factors by inflammation: role of transcription factors and epigenetic mechanisms. Am J Physiol Renal Physiol. 311:F1329-F1340.

31. Thurston RD, Larmonier CB, Majewski PM, Ramalingam R, Midura-Kiela M, et al. (2010) Tumor necrosis factor and interferon-γ down-regulate Klotho in mice with colitis. Gastroenterology. 138:1384-1394.

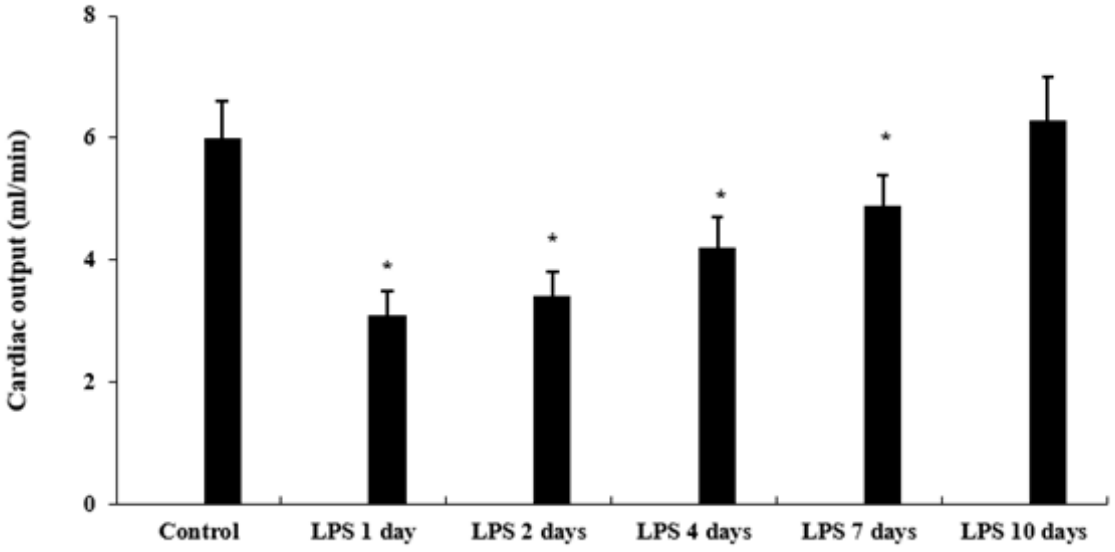
32. Fan Y, Cui C, Rosen CJ, Sato T, Xu R, et al. (2022) Klotho in Osx+-mesenchymal progenitors exerts pro-osteogenic and anti-inflammatory effects during mandibular alveolar bone formation and repair. Signal Transduct Target Ther. 7:155.

33. Mytych J, Romerowicz-Misielak M, Koziorowski M (2018) Klotho protects human monocytes from LPS-induced immune impairment associated with immunosenescent-like phenotype. Mol Cell Endocrinol. 470:1-13.

34. Maekawa Y, Ishikawa K, Yasuda O, Oguro R, Hanasaki H, et al. (2009) Klotho suppresses TNF-α-induced expression of adhesion molecules in the endothelium and attenuates NF-κB activation. Endocrine. 35:341-346.

35. Moreno JA, Izquierdo MC, Sanchez-Nino MD, Suarez-Alvarez B, Lopez-Larrea C, et al. (2011) The inflammatory cytokines TWEAK and TNFα reduce renal klotho expression through NFκB. Journal of the J Am Soc Nephrol: JASN. 22:1315-1325.

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



Supplemental data 1: Temporal recovery of cardiac dysfunction in old endotoxemic mice.

Old mice were treated with lipopolysaccharide (LPS, 0.5 mg/kg, iv) or normal saline. Cardiac output was analyzed using microcatheter 1, 2, 4, 7, 10 days after treatment. The lowest cardiac output was observed one day after LPS treatment. Cardiac function improved over time, and it was fully recovered 10 days after administration of LPS. Data are expressed as mean±SE. n=5 in each group; *P<0.05 vs. control (combined data from mice treated with normal saline for varied time).