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Tocilizumab does not ameliorate inflammation-induced left ventricular dysfunction in a collagen-induced arthritis rat model

Ashmeetha Manilall [a,*], Lebogang Mokotedi [b], Sulè Gunter [b], Regina Le Roux [a], Serena Fourie [a], Aletta ME Millen [b]

[a] School of Physiology, Faculty of Health Sciences, University of the Witwatersrand, 2193 Johannesburg, South Africa
[b] Wits Integrated Molecular Physiology Research Initiative, Wits Health Consortium (PTY) Ltd, School of Physiology, Faculty of Health Sciences, University of the Witwatersrand, 2193 Johannesburg, South Africa

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ABSTRACT

Background: Interleukin-6 (IL-6) is an attractive therapeutic target due to its diverse roles in the pathogenesis of conditions characterized by systemic inflammation. IL-6 has also been implicated in the pathophysiology of heart failure. This study aimed to investigate the impact of IL-6 receptor blockade with tocilizumab on the molecular pathways underlying systemic inflammation-induced left ventricular (LV) dysfunction in a collagen-induced arthritis (CIA) rat model.

Methods: Seventy-three Sprague-Dawley rats were divided into three groups: control (n=28), CIA (n=29), and CIA+IL-6 blocker (n=16). Inflammation was induced in the CIA and CIA+IL-6 blocker groups using bovine type II collagen emulsified in incomplete Freund's adjuvant. After arthritis onset, the CIA+IL-6 blocker group received tocilizumab for six weeks. Circulating inflammatory markers, relative LV mRNA gene expressions, and LV systolic and diastolic function were assessed.

Results: CIA rats developed LV diastolic and early-stage LV systolic dysfunction, which was not ameliorated by IL-6 blocker administration ($p > 0.05$). IL-6 blocker administration did not impact circulating inflammatory markers (all $p > 0.05$) or LV mRNA expression of inflammatory markers compared to the CIA group, nor did it reverse inflammation-induced increases in LV mRNA expression of genes involved in fibrosis and collagen turnover, regulation of titin phosphorylation, Ca^{2+} handling, mitochondrial function, or apoptosis (all $p > 0.05$). However, LV mRNA expressions of *CD68* and *LOX*, genes involved in macrophage infiltration and collagen cross-linking, were increased in the CIA group ($p = 0.03$, $p = 0.0004$), but not in the CIA+IL-6 blocker group compared to controls ($p > 0.05$).

Conclusion: These results suggest that although IL-6 blockade by tocilizumab may prevent inflammation-induced collagen cross-linking, the current treatment regimen may not protect against inflammation-induced LV dysfunction. Therefore, the role of IL-6 in the molecular mechanisms of inflammation-induced LV dysfunction remains inconclusive.

1. Introduction

Despite the considerable pathophysiological variability in heart failure, an increasing body of evidence highlights the pivotal role of inflammation in developing left ventricular (LV) diastolic and systolic dysfunction [1]. A recent study assessing a broad range of biomarkers associated with various phenotypes of heart failure, including heart failure with preserved ejection fraction (HFpEF), found that inflammatory markers were most closely linked to heart failure cases leading to hospital admissions and mortality [2]. In the aforementioned study, tumour necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) were identified as two key cytokines among the independent predictors of adverse cardiac outcomes due to their elevated circulating concentrations [2]. IL-6, a multifunctional cytokine involved in immune regulation, haematopoiesis, acute phase response, and inflammation, was notably ranked among the top four predictive biomarkers for heart failure-related hospitalizations and mortality [2]. Although there is a strong association between elevated circulating IL-6 levels and adverse

* Corresponding author.
E-mail address: Ashmeetha.Manilall@wits.ac.za (A. Manilall).

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cardiovascular outcomes [3,4], the role of IL-6 in cardiac health remains controversial [5]. IL-6 signalling has been proposed to have cardio-protective effects in response to acute injury or inflammatory conditions. Increased IL-6 signalling reportedly reduces oxidative stress, and activates anti-apoptotic mechanisms to safeguard against myocardial damage, thereby preserving heart tissue in acute injury [6–8]. However, chronic IL-6 signalling is linked to an increased risk of cardiac hypertrophy and the development of heart failure [5]. Furthermore, in patients with autoimmune diseases such as rheumatoid arthritis, elevated IL-6 levels are independently associated with left ventricular diastolic dysfunction, a subclinical condition that often precedes HFpEF [2]. Given its role in inflammation-related disease pathogenesis and heart failure, targeting IL-6 has been proposed as a promising therapeutic strategy for managing HFpEF in inflammatory conditions [9].

Clinical studies on the cardiovascular effects of blocking circulating IL-6 are scarce and controversial. In this regard, some have cautioned against using IL-6 blockers in RA patients at risk for cardiovascular diseases, as it may increase the incidence of adverse cardiovascular events [10], while others suggested it was safe [11,12]. Moreover, in patients with other inflammatory conditions including peripheral artery disease and COVID-19, tocilizumab increased the risk for heart failure [13,14].Nevertheless, the molecular mechanisms whereby IL-6 receptor blockers impact cardiac structure and function in patients with LV dysfunction or HFpEF warrant investigation. In this regard, it has been suggested that IL-6 plays a crucial role in regulating cellular processes related to extracellular matrix remodelling, which may contribute to cardiac fibrosis and hypertrophy, common features of LV diastolic dysfunction [15]. Indeed, Mendelez et al. (2010) showed that intraperitoneal IL-6 infusion in rats increased LV stiffness, a feature of LV diastolic dysfunction, which was largely attributed to LV fibrosis and concentric hypertrophy [16]. Others have supported the role of IL-6 in regulating collagen production and myocardial fibrosis [5,6]. Conversely, both *in vivo* [17,18] and *in vitro* [19] studies have indicated that cardiac hypertrophy or LV dysfunction may be independent of IL-6. Pre-clinical studies using IL-6 blockers have reported reduced fibrotic remodelling following myocardial infarction [20] and viral-induced myocarditis [21], while others have observed no significant effects of IL-6 blockade on cardiac remodelling and fibrosis [22]. These controversies highlight the need for a better understanding of the mechanisms of IL-6 blockade on cardiac remodelling in chronic inflammation.

Although extracellular matrix remodelling is a well-established mechanism in the development of LV diastolic dysfunction, cardiomyocyte compliance, regulated by the phosphorylation of the sarcomeric protein titin, is another major contributor to inflammation-induced LV passive stiffness [23]. One study demonstrated that blocking IL-6 receptors following viral-induced myocarditis improved titin phosphorylation, leading to reduced myocyte stiffness [21]. However, these findings have not been replicated in models of chronic systemic inflammation. Moreover, IL-6 has been implicated in molecular pathways that lead to dysregulated Ca^{2+} handling, mitochondrial dysfunction, and apoptosis, which are key active processes underlying impaired LV relaxation and contribute to LV diastolic and systolic dysfunction [24–26]. In contrast, blocking IL-6 may have adverse effects, as LV systolic function was found to worsen in animals treated with IL-6 blockers compared to those not receiving treatment [22]. Furthermore, loss of IL-6 signalling has been associated with increased myocardial apoptosis [6,7]. Beyond its role in apoptosis, IL-6 is also involved in regulating the expression of sarcoplasmic/endoplasmic reticulum Ca^{2+} ATPase 2a (SERCA2a) in ventricular myocytes [25–27]. However, it remains uncertain whether these effects of IL-6 blockade translate into altered myocardial relaxation *in vivo*.

In summary, although IL-6 is strongly linked to adverse cardiovascular function in chronic inflammation, the therapeutic benefits of blocking IL-6 signalling on the cellular processes involved in LV dysfunction remain controversial. In this study, we hypothesized that blocking IL-6 signalling with tocilizumab in the collagen-induced arthritis (CIA) rat model could potentially modulate the expression of genes that regulate the cellular processes involved in both passive and active relaxation of inflammation-induced LV systolic and diastolic dysfunction.

2. Experimental methods and design

2.1. Animal treatment and sample collection

All experimental procedures received approval from the Animal Ethics Screening Committee of the University of the Witwatersrand (AESC approval: 2017/03/21C) and were conducted following the Guide for the Care and Use of Laboratory Animals. Three-month-old Sprague-Dawley rats were divided into three groups at random. Two groups underwent induction of systemic inflammation using a modified Chondrex collagen inoculation protocol [28]. Rats received a subcutaneous injection at the base of the tail with Bovine type II collagen (2 mg/ml in 0.05 M acetic acid, Chondrex catalogue #20021, Redmond, WA, USA) emulsified in incomplete Freund's adjuvant (1:1 volume, 0.2 ml, Chondrex catalogue #7002, Redmond, WA, USA). Booster injections (0.1ml) were given seven and twenty-one days after the initial immunization. The collagen-inoculated groups included the collagen-induced arthritis (CIA) group (n = 29) with no further treatment, and the CIA+IL-6 blocker group (n = 16), which received the IL-6 receptor blocker tocilizumab (8 mg/kg) by intraperitoneal injection [29], administered weekly for six weeks. The tocilizumab dose was selected and adapted based on the European League Against Rheumatism's recommended dose of 8 mg/kg [30]. All collagen-inoculated rats received daily doses of 1–4 mg/kg Tramazec for pain management. The control (con) group (n=28) received a subcutaneous saline injection (0.2ml). Tail cuff blood pressure was measured biweekly at midday using non-invasive blood pressure amplifiers (Biopac Systems, Santa Barbara, CA, USA) as described previously [31]. Arthritis scores and paw thickness were assessed in the rats' hind paws using a 0–4 grading scale [32] and a digital calliper, respectively, as previously described [31]. The rats used in this study are part of a larger ongoing investigation to determine the cardiovascular effects of collagen-induced systemic inflammation [31]. In compliance with ethics committee guidelines to reduce animal usage, echocardiography data for a subset (n = 63) of the rats have been previously published. In this study, 73 rats (con, 28; CIA, 29; CIA+IL-6 blocker, 16) provided measurable STE and gene expression data.

2.2. Echocardiography

Rats were anesthetized with an intramuscular injection of ketamine (100 mg/kg) and xylazine (5 mg/kg). Echocardiography was then conducted using an ultrasound system (Acuson SC 2000, Siemens Medical Solutions, USA, Inc.) to assess left ventricular (LV) diastolic and systolic functions, as previously described [31,33]. LV diastolic function was evaluated using pulsed wave Doppler and Tissue Doppler Imaging. In brief, LV geometry was assessed with two-dimensional M-mode images. Concentric LV remodelling was determined by calculating relative wall thickness. Mitral inflow velocity was measured in the apical four-chamber view during early (E) and late diastole (A). Tissue Doppler Imaging measured tissue lengthening at the mitral annulus of the lateral wall during early (e') and late (a') diastole. As mitral inflow velocity (E) depends on loading conditions and heart rate, whereas e' is relatively preload-independent, the E/e' ratio was calculated to estimate left ventricular filling pressures [34]. Systolic function was assessed by calculating the ejection fraction using the Teicholz method and fractional shortening [35]. Speckle-tracking echocardiography (STE), a more sensitive measure of early-stage LV systolic dysfunction, was used to determine myocardial deformation (LV peak systolic strain and strain rate) and myocardial motion (LV velocity and displacement) in the apical four-chamber view. B-mode video loops were captured and selected based on video quality and well-defined endocardial and

epicardial borders. To minimize beat-to-beat variability, five consecutive cardiac cycles were analysed. Automatic border detection software traced the endocardial and epicardial borders, with manual adjustments to ensure optimal tracking frame-by-frame. Strain, strain rate, velocity, and displacement were calculated in the longitudinal plane across six anatomical regions. Global longitudinal strain, strain rate, displacement, and velocity were computed as the average of the six anatomical segments over three consecutive cardiac cycles [36].

Following echocardiography, rats were terminated by thoracotomy whilst still under anesthesia. A blood sample was collected, the heart was removed and LV tissue was stored (-80°C) in RNAlater® (catalogue # AM7021, Ambion, Sigma Aldrich, Germany).

2.3. Circulating inflammatory markers

Serum concentrations of IL-6 (catalogue # E-EL-R0015, Elabscience Biotechnology, USA) and C-reactive protein (catalogue # E-EL-R0022, CRP, Elabscience Biotechnology, USA) were measured in duplicate by sandwich enzyme-linked immunosorbent assay (ELISA).

2.4. Extraction of mRNA and preparation of cDNA

Rat LV samples were homogenized by sonication and total RNA was extracted using an Illustra™ Mini Spin RNA extraction kit (catalogue # 45001163, GE Healthcare, Buckinghamshire, UK), according to the manufacturer's instructions. The resulting RNA (2 µl) was used to reverse-transcribe messenger RNA to produce cDNA using SuperScript™ IV VILO™ cDNA synthesis master mix (18 µl, catalogue # 11756050, Thermo Fisher Scientific, Life Technologies, Carlsbad, USA).

2.5. Comparative gene expression RT-PCR

Comparative gene expression RT-PCR was carried out using a StepOne Plus thermocycler (Thermo Fisher Scientific, Life Technologies, Carlsbad, USA). The reaction mixture included cDNA (~2 µg), pre-designed TaqMan® probe mixes for the reference gene (0.25 µl, Applied Biosystems), pre-designed TaqMan® probe mixes for each target gene (0.5 µl, TaqMan® gene expression assay, Applied Biosystems), and TaqMan® fast advanced PCR master mix (5 µl, Applied Biosystems) in a total volume of 10 µl. The cycling protocol began with an initial hold at 50 °C for 2 min, followed by a hot start at 95 °C for 10 min, and then 40 cycles of 95 °C for 15 s and 60 °C for 1 min. Relative gene expression fold-change was calculated using the delta-delta Ct ($2^{-\Delta\Delta Ct}$) method [37]. RefFinder [38] software determined the optimal reference genes for RT-PCR from a selected panel, identifying hypoxanthine guanine phosphoribosyl-transferase 1 (*Hprt1*) as the most appropriate reference gene.

For the assessment of extracellular matrix remodelling and fibrosis, comparative gene expression of the following genes was assessed using TaqMan® probes (Applied Biosystems): *Col1* which codes for collagen I protein (Rn01463848_m1), *TGFβ* (Rn99999016_m1), *LOX* which codes for lysyl oxidase protein (Rn01491829_m1), *TNF-α* (Rn00562055_m1), *IL-6* (Rn01410330_m1), α smooth muscle actin (*αSMA,* Rn01759928_g1), *CD68* (Rn01495634_g1), *MMP2* (Rn01538170_m1) and *MMP9* (Rn00579162_m1).

For the assessment of the pathways involved in titin phosphorylation, comparative gene expression of the following genes was assessed using TaqMan® probes (Applied Biosystems): *VCAM1* (Rn00563627_m1), *PTX3* which codes for pentraxin-3 protein (Rn01769850_m1), *iNOS* (Rn00561646_m1), *PP1γ*, which codes for protein phosphatase 1 (Rn04339209_m1), *PP5*, which codes for protein phosphatase 5 (Rn00669044_g1), *sGCα2* (Rn00675050_m1) and *sGCβ2* (Rn01636981_m1), which code for the sGC alpha 2 and sGC beta 2 subunits respectively.

To assess the contribution of active, energy dependent mechanisms of LV relaxation comparative gene expression of the following genes were assessed in LV tissues using TaqMan® probes (Applied Biosystems): *Myh6* which codes for α-MyHC (Rn00568304_m1), *Myh7* which codes for β-MyHC (Rn00568328_m1), *iNOS* (Rn00561646_m1), *SOD2* which codes for superoxide dismutase 2 (Rn00690588_g1), *SERCA2a* which codes for SERCA protein (Rn00568762_m1), *BAX* which codes for Bcl-2-associated X-protein (Rn01480161_g1) and *Bcl-2* which codes for B-cell lymphoma 2 protein (ID: Rn99999125_m1).

2.6. Data analysis

Statistical analyses were conducted using SAS software, version 9.4 (SAS Institute Inc.,). *A priori* calculation showed that 45 animals were required to achieve 80% power at an alpha level of 0.05 and a small effect size. Data are presented as mean ± SEM or median interquartile range (IQR), as appropriate. Rats with gene expression levels for 70 % or more of the target genes outside the detection limits of the gene expression assays were excluded from the analyses. Differences in normally distributed variables between groups were assessed using mixed model analysis of variance (ANOVA) with group and sex as the main effects, followed by Tukey's *post-hoc* tests. For non-normally distributed variables, group differences were determined using Kruskal–Wallis tests. As there were no differences in the molecular markers between males and females, results were combined. Results were considered statistically significant if $p \leq 0.05$.

3. Results

3.1. The effect of IL-6 blocker treatment on body mass, blood pressure, and circulating and left ventricular tissue inflammatory markers

The characterization of the control and CIA rats is reported elsewhere [31]. Briefly, for the partially overlapping subset of animals included in this study, body weight was lower in the CIA+IL-6 blocker group compared to the control ($p < 0.001$) and CIA ($p = 0.01$, Table 1)

Table 1
Body mass, blood pressure and circulating and tissue inflammatory markers in control, CIA and CIA+IL-6 blocker (tocilizumab) groups.

	Control (n = 28)	CIA (n = 29)	CIA+IL-6 blocker (n = 16)
Female n (%)	12 (43)	16 (55)	8 (50)
Body weight (g)	459.8 ± 9.5	425.5 ± 8.8*	384.1 ± 11.4**#
SBP (mm Hg)	128 ± 2	130 ± 2	129 ± 2
DBP (mm Hg)	87 ± 1	88 ± 1	91 ± 2
Paw score	0	4 (3 – 6)****	4.5 (2 – 7)****
Ankle joint thickness (mm)	8.6 ± 0.1	9.2 ± 0.1**	9.2 ± 0.2*
Tarsometatarsal joint thickness (mm)	5.0 ± 0.1	5.5 ± 0.1**	5.6 ± 0.2*
Circulating inflammatory marker concentrations			
IL-6 (pg/ml)	16.42 ± 2.08	29.89 ± 2.05**	31.29 ± 2.74**
CRP (ng/ml)	0.10 ± 0.04	0.56 ± 0.03***	0.66 ± 0.05***
Tissue inflammatory marker expressions (mRNA)			
IL-6	0.85 (0.65 – 0.93)	1.53 (0.85 – 2.06)**	1.64 (0.76 – 2.03)*
CD68	0.92 ± 0.05	1.12 ± 0.05*	0.97 ± 0.07
PTX-3	1.18 (0.43 – 1.30)	2.40 (1.74 – 4.06)***	2.22 (0.92 – 2.97)**

Data presented as means ± SEM or median (interquartile range). CIA, collagen-induced arthritis, SBP, systolic blood pressure, DBP, diastolic blood pressure, IL-6, interleukin 6, CRP, C-reactive protein.
* $p < 0.05$ *versus* controls.
** $p < 0.01$ *versus* controls.
*** $p < 0.0001$ *versus* controls.
**** $p < 0.00001$ *versus* controls.
# $p < 0.05$ *versus* CIA.

groups. There were no differences in systolic or diastolic blood pressure between the groups (all $p > 0.05$). Arthritis scores and ankle and tarsometatarsal thickness were increased in the CIA+IL-6 blocker group compared to controls (all $p < 0.05$) and were not different from the CIA group (all $p > 0.05$). Tocilizumab had no effect on serum concentrations of IL-6 or CRP in the CIA+IL-6 blocker group compared to the control or CIA groups (both $p > 0.05$). Blocking circulating IL-6 did not prevent CIA-induced local LV tissue inflammation, as the mRNA expression of *IL-6* and *PTX-3* were greater in the CIA+IL-6 blocker group compared to the control group ($p = 0.04$ and $p = 0.006$, respectively) and were not different from the CIA group ($p = 0.92$ and $p = 0.64$, respectively; Table 1). The local mRNA expression of *CD68*, a marker of leukocyte and macrophage infiltration, was higher in the CIA group ($p = 0.03$) but not in the CIA+IL-6 blocker group ($p = 0.83$) compared to the controls.

3.2. The effect of IL-6 blocker treatment on left ventricular geometry, diastolic and systolic function

Blocking circulating IL-6 had no effect on the inflammation-induced changes in echocardiographic measures of LV geometry (Table 2). In the CIA+IL-6 blocker group, similar to the CIA group, the relative wall thickness (RWT), an indicator of LV remodelling and hypertrophy, was increased compared to the control group. This indicates that blocking circulating IL-6 did not inhibit LV concentric remodelling induced by collagen inoculation (Table 2). Similar to the CIA group, LV filling pressure (E/e') was increased, while lateral e', global longitudinal strain, and global longitudinal velocity were impaired in the CIA+IL-6 blocker group compared to the control group (Table 2). Interestingly, the e'/a' ratio, a measure of LV stiffness, was impaired in the CIA group ($p = 0.03$) but not in the CIA+IL-6 blocker group compared to the control group ($p = 0.10$). Ejection fraction and midwall fractional shortening, markers of systolic function, were not different between the groups (all $p > 0.05$). These results indicate that blocking circulating IL-6 may have improved inflammation-induced LV stiffness, but did not prevent impairments in LV relaxation or subclinical systolic dysfunction.

Table 2
Echocardiographic measures of left ventricular myocardial remodelling, diastolic function and systolic function in control, CIA and CIA+IL-6 blocker (tocilizumab) groups.

	Control (n = 25)	CIA (n = 27)	CIA+IL-6 blocker (n = 16)
Myocardial remodelling			
Relative wall thickness	0.62 ± 0.02	0.73 ± 0.02**	0.71 ± 0.03*
LV diastolic function			
Lateral e' (cm/s)	4.52 ± 0.10	3.61 ± 0.10***	3.62 ± 0.14***
e'/a'	1.45 ± 0.06	1.23 ± 0.06*	1.28 ± 0.08
E/e'	25.12 ± 1.01	31.39 ± 0.98**	29.86 ± 1.27*
LV systolic function			
Ejection fraction (%)	83.0 ± 1.3	82.3 ± 1.3	81.0 ± 1.7
Midwall fractional shortening (%)	22.9 ± 0.7	22.1 ± 0.7	21.9 ± 0.9
Global longitudinal strain (cm/s)	-19.4 ± 0.8	-16.9 ± 0.8*	-16.3 ± 0.9*
Global longitudinal strain rate (%)	-3.43 ± 0.16	-3.33 ± 0.15	-3.06 ± 0.20
Global longitudinal velocity (cm/s)	0.58 ± 0.03	0.49 ± 0.03*	0.47 ± 0.04*
Global longitudinal displacement (mm)	0.28 ± 0.02	0.22 ± 0.02	0.21 ± 0.03

Data presented as means ± SEM. CIA, collagen induced arthritis; IL-6, interleukin-6; BW, body weight; LV, left ventricular; e', peak velocity during early diastole; e'/a', ratio of mitral annular lengthening in early and late diastole; E/e', ratio of early diastolic filling to peak velocity during early diastole.
* $p < 0.05$ compared to control.
** $p < 0.01$ compared to control.
*** $p < 0.001$ compared to control. Incomplete echocardiographic data profiles whereby all measurements could not be obtained were excluded from statistical analyses for control (n=3) and CIA (n=2) rats.

3.3. The effect of IL-6 blocker treatment on extracellular matrix gene expression

Treatment with tocilizumab did not offset the inflammation-induced increases in mRNA expression of pro-fibrotic genes. Compared to the control group, the CIA+IL-6 blocker group had significantly increased LV mRNA expression of *TGFβ* ($p = 0.01$, Fig. 1a), which was similar to the CIA group ($p > 0.05$). There were no differences in the LV mRNA expression of αSMA between the groups (Fig. 1b), indicating that neither the inflammatory protocol nor tocilizumab treatment resulted in the trans-differentiation of fibroblasts to myofibroblasts. Despite the unchanged expression of *αSMA*, the CIA+IL-6 blocker group had significantly increased LV mRNA expression of *Col1* ($p = 0.0004$, Fig. 1c) compared to the control group, similar to the CIA group ($p > 0.05$ for both). These results show that blocking circulating IL-6 did not prevent the inflammation-induced stimulation of fibrosis, consistent with the increases in relative wall thickness (RWT, Table 2). The CIA group showed an upregulation in *LOX* mRNA expression compared to controls ($p = 0.0004$, Fig. 1d), while in the CIA+IL-6 blocker group it was not different from controls ($p = 0.13$). This suggests that blocking circulating IL-6 may have partially offset the inflammation-induced collagen cross-linking, which may have contributed to improvements in the e'/a' ratio, and hence LV stiffness. Similar to the CIA group, LV relative mRNA expression of *MMP2* was increased in the CIA+IL-6 blocker group compared to the control group ($p = 0.005$, Fig. 1e), indicating that blocking circulating IL-6 did not prevent the inflammation-induced upregulation of collagen turnover. There were no differences in relative RNA expression of *MMP9* between the groups (all $p > 0.05$, Fig. 1f).

3.4. The effect of IL-6 blocker treatment on expression of genes involved in microvascular inflammation, immune cell infiltration and nitric-oxide (NO) signalling pathways

The inflammation-induced increased LV relative mRNA expression of *VCAM1* was not decreased by treatment with tocilizumab ($p = 0.02$ *versus* control, $p = 0.86$ *versus* CIA, Fig. 2a), indicating that inflammation-induced microvascular inflammation is not offset by IL-6 blocker treatment. Consistent with the increased *VCAM1* expression, LV relative mRNA expression of *iNOS* was greater in the CIA+IL-6 blocker group compared to the control group ($p = 0.001$) and not different from the CIA group ($p = 0.24$, Fig. 2b). Similar to the CIA group, the LV relative mRNA expression of *sGCα2* and *sGCβ2* were significantly decreased in the CIA+IL-6 blocker group compared to control group ($p = 0.02$ and $p = 0.04$, respectively; Fig. 2c and d), showing that blocking circulating IL-6 did not impact the inflammation-induced decreased in expression of the genes that code for subunits of sGC enzyme. These results are consistent with the impaired relaxation (e') and increased filling pressures (E/e') reported in the CIA+IL-6 blocker rats (Table 2).

The LV mRNA expression of *PP1γ*, the protein phosphatase previously implicated in titin phosphorylation, was not different between the three groups ($p > 0.05$; Fig. 3a). The LV mRNA expression of the protein phosphatase *PP5* was significantly higher in the CIA+IL-6 blocker group compared to the control group ($p = 0.02$) but was not different from the CIA groups ($p = 0.98$; Fig. 3b), suggesting that IL-6 may not be involved in the cellular pathways whereby PP5 contributes to the dephosphorylation of titin.

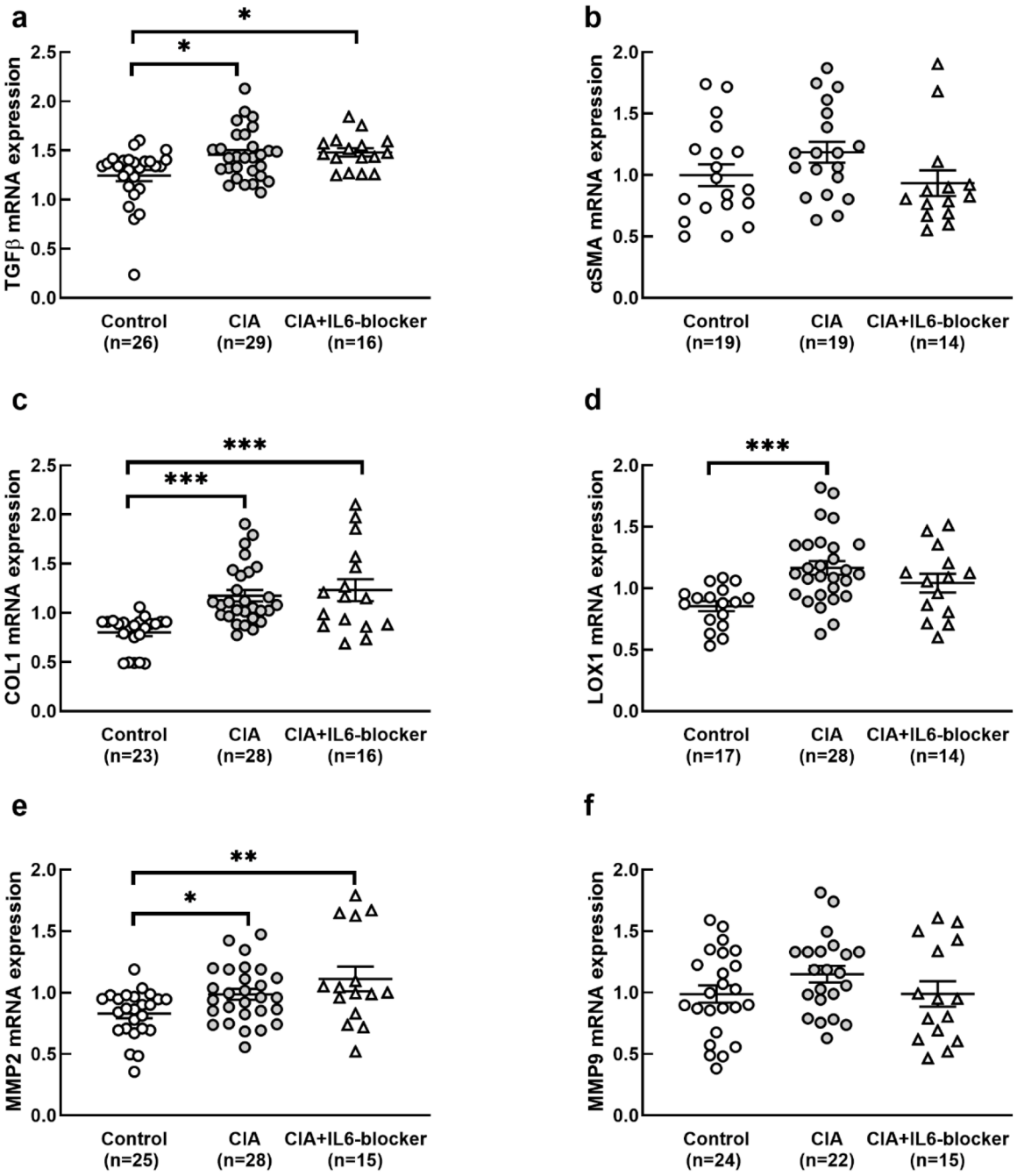

Fig. 1. LV expression of extracellular matrix genes. Rats were inoculated with collagen (CIA) and treated with an IL-6 blocker prior to measuring the relative mRNA expression of *TGFβ* (a), *αSMA* (b), *Col1* (c), *LOX* (d), *MMP2* (e), and *MMP9* (f) in left ventricular tissues of control, CIA, and IL-6 blocker-treated rats. The relative mRNA expression results are presented in arbitrary units, normalized to the expression of the endogenous reference gene *Hprt1*, using the $2^{-\Delta\Delta Ct}$ method. The data were obtained from duplicate real-time PCR experiments and are presented as means ± SEM. Rats with gene expression levels for 70 % or more of the target genes outside the detection limits of the gene expression assays were excluded from the analyses. Statistical significance is indicated as follows: * $p < 0.05$ vs. control; ** $p < 0.01$ vs. control; *** $p < 0.001$ vs. control.

3.5. The effect of IL-6 blocker treatment on expression of genes that play a role in Ca^{2+} handling, oxidative stress and apoptosis

The LV mRNA expression of *SERCA2a* and *S100A1* in the CIA+IL-6 blocker group were not different from the control ($p = 0.42$ and $p = 0.98$, respectively) or CIA groups ($p = 0.99$ and $p = 0.90$; Fig. 4a, and b), indicating that neither inflammation nor IL-6 blocker treatment impacted Ca^{2+} handling in LV tissues. The LV mRNA expressions of pro-apoptotic gene *BAX*, and anti-apoptotic gene *Bcl-2*, were not different between any of the groups (all $p > 0.05$, Fig. 4d, and e). Consistent with these findings, the ratio of *BAX* to *Bcl-2* was also not different between the groups (all $p > 0.05$, Fig. 4f), showing that systemic inflammation and blocking circulating IL-6 did not impact apoptosis in LV tissues. Consistent with these results, the LV mRNA expression of *SOD2*, a marker of mitochondrial oxidative stress, was not different between any of the groups (all $p > 0.05$, Fig. 4c).

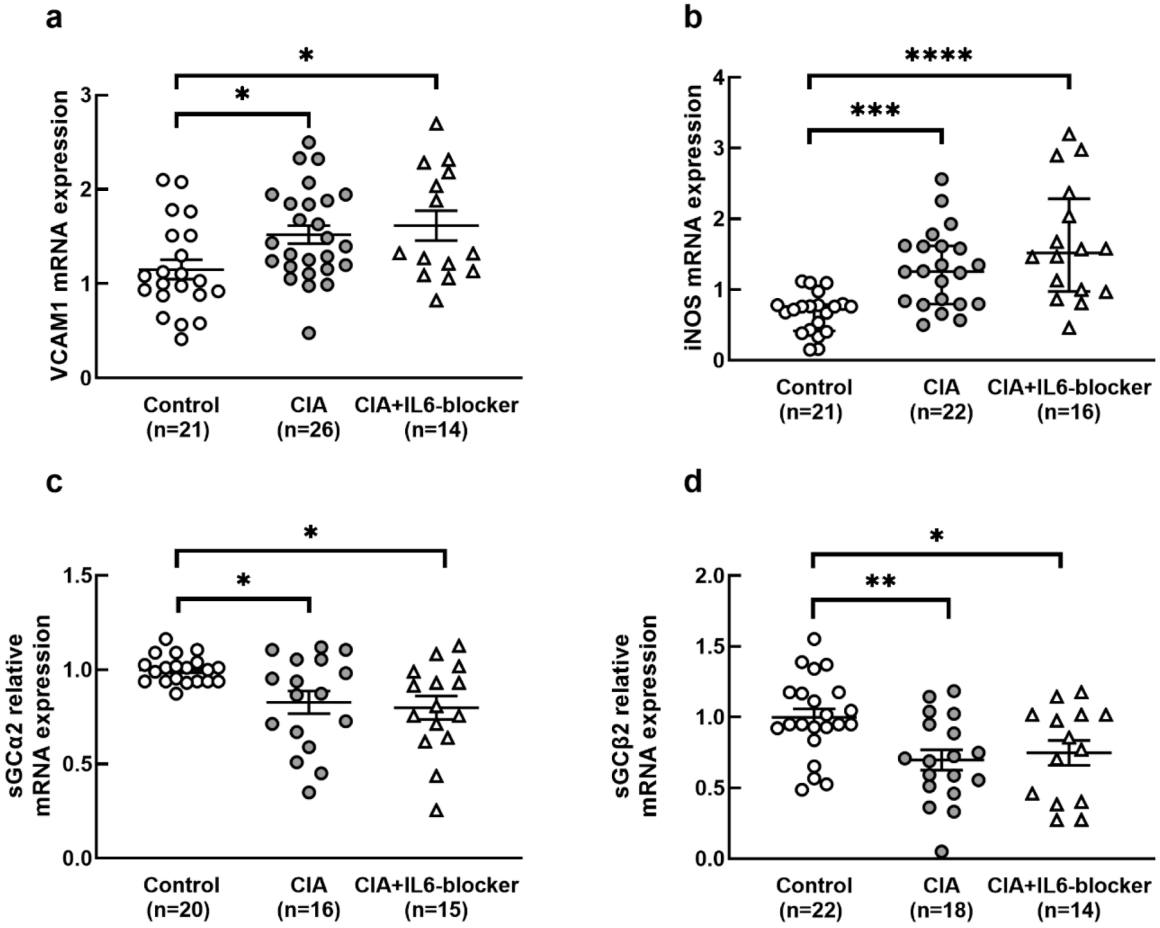

Fig. 2. LV expression of microvascular inflammation, immune cell activation and sGC subunit genes. Rats were inoculated with collagen (CIA) and treated with an IL-6 blocker prior to measuring the relative mRNA expression of *VCAM1* (a), *iNOS* (b), *sGCα2* (c), and *sGCβ2* (d) in left ventricular tissues of control, CIA, and IL-6 blocker-treated rats. Relative mRNA expression results are presented in arbitrary units, normalized to the expression of the endogenous reference gene *Hprt1*, using the $2^{-\Delta\Delta Ct}$ method. Data were obtained from duplicate real-time PCR experiments and are presented as means ± SEM for *VCAM1, sGCα2*, and *sGCβ2*, and as median and interquartile range for *iNOS*. Data for *sGCα2* and *sGCβ2* were normalized relative to the control group. Rats with gene expression levels for 70% or more of the target genes outside the detection limits of the gene expression assays were excluded from the analyses. Statistical significance is indicated as follows: * $p < 0.05$ vs. control; ** $p < 0.01$ vs. control; *** $p < 0.001$ vs. control.

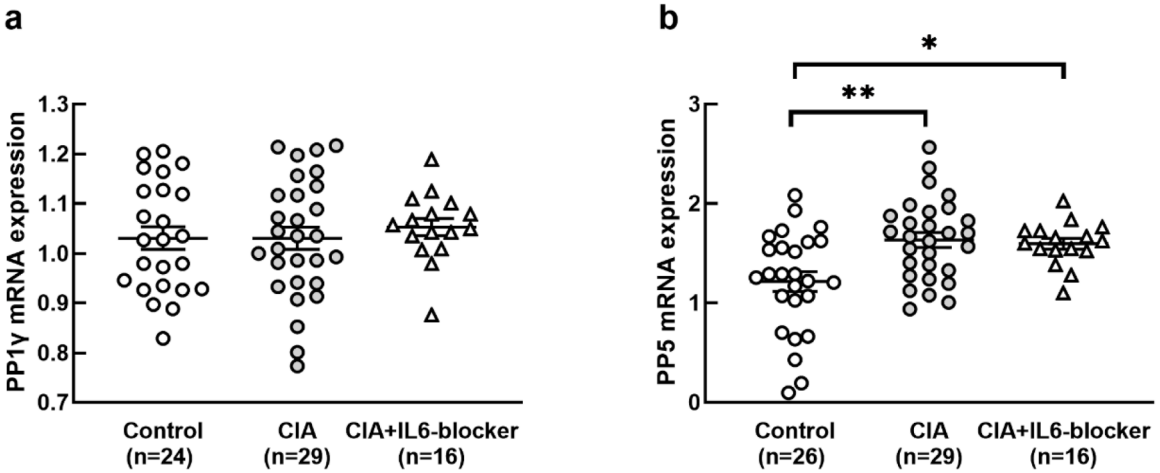

Fig. 3. LV expression of protein phosphatase genes. Rats were inoculated with collagen (CIA) and treated with an IL-6 blocker before measuring the relative mRNA expression of *PP1γ* (a) and *PP5* (b) in left ventricular tissues of control, CIA, and IL-6 blocker-treated rats. The relative mRNA expression results are presented in arbitrary units, normalized to the expression of the endogenous reference gene *Hprt1*, using the $2^{-\Delta\Delta Ct}$ method. Data were obtained from duplicate real-time PCR experiments and are presented as means ± SEM. Rats with gene expression levels for 70 % or more of the target genes outside the detection limits of the gene expression assays were excluded from the analyses. Statistical significance is indicated as follows: * $p < 0.05$ vs. control; ** $p < 0.01$ vs. control.

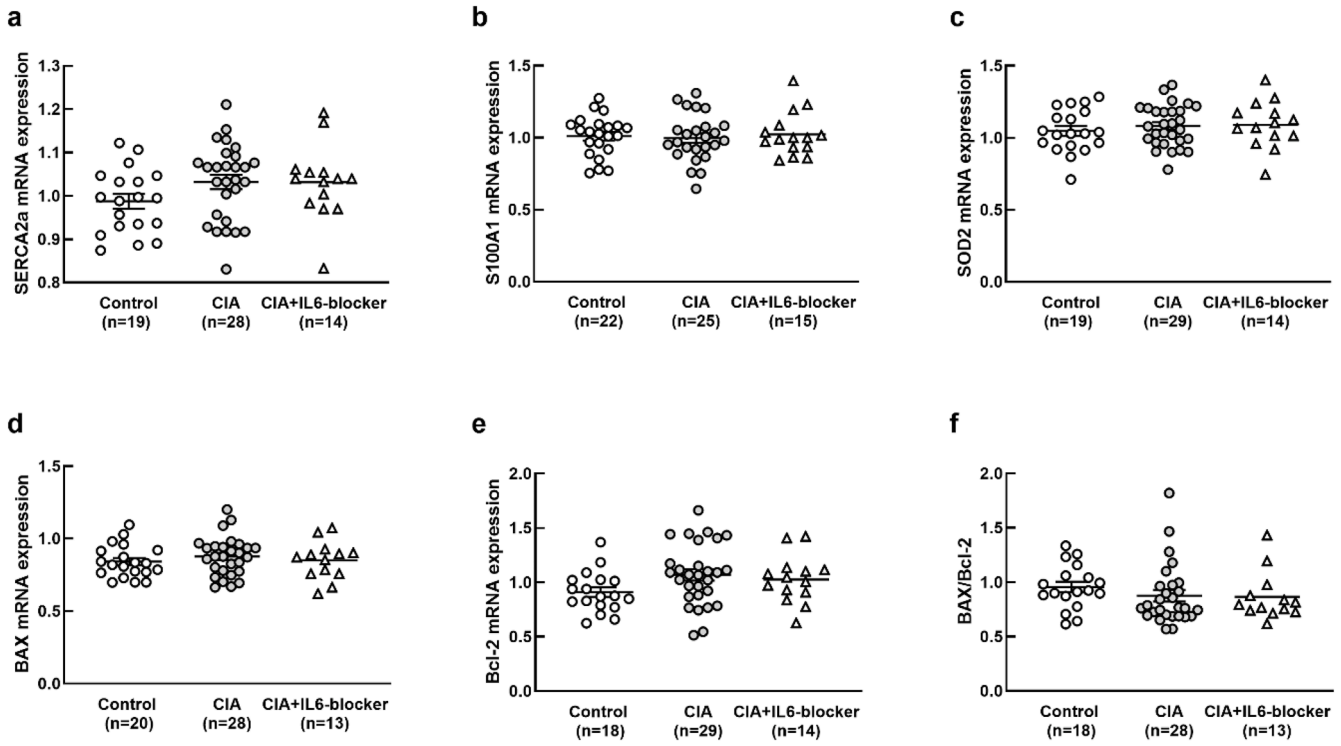

Fig. 4. LV expression of calcium handling, mitochondrial dysfunction and apoptotic genes. Rats were inoculated with collagen (CIA) and treated with an IL-6 blocker before measuring the relative mRNA expression of *SERCA2a* (a), *S100A1* (b), *SOD2* (c), *BAX* (d), *Bcl-2* (e), and the *BAX/Bcl-2* ratio (f) in left ventricular tissues of control, CIA, and IL-6 blocker-treated rats. The relative mRNA expression results are presented in arbitrary units, normalized to the expression of the endogenous reference gene *Hprt1*, using the $2^{-\Delta\Delta Ct}$ method. Rats with gene expression levels for 70 % or more of the target genes outside the detection limits of the gene expression assays were excluded from the analyses. Data were obtained from duplicate real-time PCR experiments and are presented as means ± SEM.

3.6. The effect of IL-6 blocker treatment on expression of myocardial contractility genes

The relative mRNA expression of *Myh6*, the gene that codes for the fast-twitch isoform of myosin, was decreased in the CIA+IL-6 blocker group compared to the control group ($p = 0.02$) and was not different from the CIA group ($p = 0.87$; Fig. 5a). The relative mRNA expression of *Myh7*, the gene that codes for the slow-twitch isoform of myosin, was increased in the CIA+IL-6 blocker group compared to the control group ($p = 0.002$) and was not different from the CIA group ($p = 0.74$, Fig. 5b). Consequently, similar to the CIA group, there was a decreased ratio of *Myh6/Myh7* in the CIA+IL-6 blocker group compared to the control group ($p = 0.003$; Fig. 5c). The increased expression of *Myh7* is consistent with the findings of increased pro-fibrotic gene expression and relative wall thickness in the CIA+IL-6 blocker group. Moreover, these findings confirm our previous reports that a switch in myosin isoform expression may contribute to impaired myocardial deformation, as we showed impaired global longitudinal strain and velocity in the CIA+IL-6 blocker group (Table 2).

4. Discussion

IL-6 exerts diverse effects on different types of cardiac cells, particularly cardiomyocytes and cardiac fibroblasts. In cardiomyocytes, IL-6 is

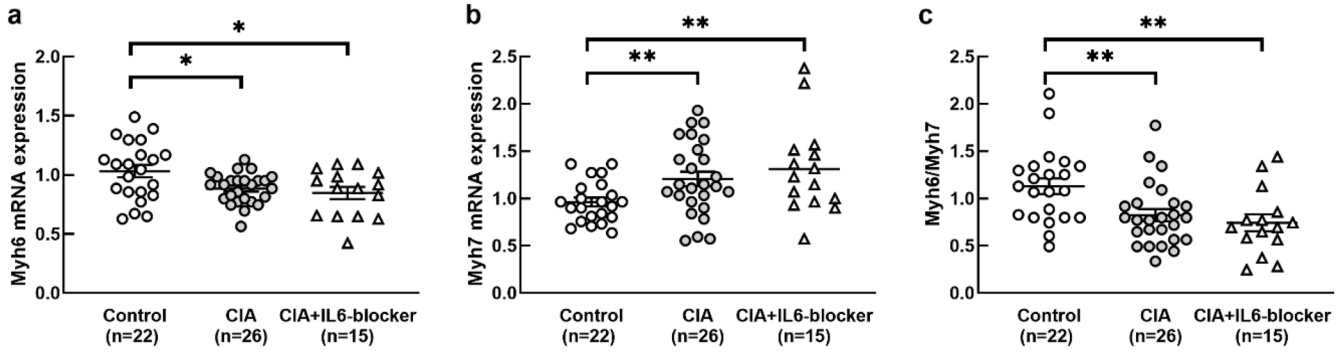

Fig. 5. LV expression of myocardial contractility genes. Rats were inoculated with collagen (CIA) and treated with an IL-6 blocker before measuring the relative mRNA expression of *Myh6* (a), *Myh7* (b), and the *Myh6/Myh7* ratio (c) in left ventricular tissues of control, CIA, and IL-6 blocker-treated rats. The relative mRNA expression results are presented in arbitrary units, normalized to the expression of the endogenous reference gene *Hprt1*, using the $2^{-\Delta\Delta Ct}$ method. Data were obtained from duplicate real-time PCR experiments and are presented as means ± SEM. Rats with gene expression levels for 70 % or more of the target genes outside the detection limits of the gene expression assays were excluded from the analyses. Statistical significance is indicated as follows: * $p < 0.05$ vs. control; ** $p < 0.01$ vs. control.

implicated in signaling pathways that lead to hypertrophy and survival, such as the p42/p44-MAPK and PI3K pathways, which are independent of JAK/STAT activation [39]. In contrast, IL-6 in cardiac fibroblasts plays an important role in stimulating cellular activities that contribute to fibrosis and remodeling [40]. Fibroblast-derived IL-6 has been shown to exacerbate adverse cardiac remodeling post-myocardial infarction by promoting the production of pro-fibrotic factors like IL-11[41]. Another study elucidated a signaling pathway where the Cadherin-11-IL-6 interaction between cardiac fibroblasts and cardiomyocytes promotes ventricular remodeling, highlighting the intricate interplay between these cell types in cardiac pathophysiology [42]. Despite several studies showing that IL-6 is a key stimulus for cardiac fibrosis in various animal and cell culture models [17,18,43,44], our results showed that blocking circulating IL-6 did not prevent the inflammation-induced upregulation of pro-fibrotic genes including *TGFβ, Col1*, and *MMP2*. Others have also reported that fibrosis may be independent of IL-6 signaling [16,22,43]. The increased expression of pro-fibrotic genes in the CIA and CIA+IL-6 blocker groups in the current study may have contributed to the increased RWT, indicative of LV concentric remodeling [16,22,43,45]. This increased pro-fibrotic gene expression is consistent with our previous report of increased cardiac collagen volume observed using histology [33], indicating the development of LV fibrosis in the CIA model. The LV fibrosis and increased RWT in the current study are consistent with the increased expression of *Myh7*, the gene that codes for β-MyHC, in both the CIA+IL-6 blocker and CIA groups. This upregulation of β-MyHC may have contributed to the impaired LV global longitudinal strain and velocity in these rats [33,46,47]. These changes in myocardial deformation and motion are indicators of early impairments in LV myocardial contractility, despite preserved ejection fraction. Moreover, none of the genes involved in Ca^{2+} handling, apoptosis, or oxidative stress were impacted by blocking circulating IL-6. As these genes were also not affected in the CIA group, it remains uncertain whether these processes are a cause or a consequence of LV diastolic or early-stage systolic dysfunction [47].

Evidence suggests that CD68 expression can be influenced by IL-6 signaling, indicating that IL-6 signaling could precede increased CD68 expression in inflammatory environments. Several studies have demonstrated a correlation between IL-6 levels and CD68 expression. For instance, IL-6 expression levels were positively correlated with tumor-associated macrophages that expressed CD68 on the cell surface, in patients with gastric cancer [48]. Similarly, in rats administered monocrotaline, which induces pulmonary arterial hypertension and systemic inflammation, IL-6 levels and CD68 expression were increased in lung tissues, further supporting the suggestion that IL-6 may drive macrophage recruitment or activation, which in turn express CD68 [49]. Although evidence for a co-regulation between LOX and IL-6 has been shown in pulmonary fibrosis, this has not previously been reported in cardiac tissue [50]. Nevertheless, we have previously shown a significant association between the mRNA expression of *CD68* and *LOX*, the gene that codes for the collagen cross-linking enzyme lysyl oxidase [51]. This suggests that increased LOX expression may be linked to immune cell infiltration. In the present study, we observed a partial attenuation of inflammation-induced upregulation of both *CD68* and *LOX* LV mRNA expression following IL-6 blocker treatment, suggesting that IL-6 signaling may indeed be involved in the regulation of macrophage infiltration and collagen cross-linking. The potential for IL-6 to enhance macrophage presence in inflamed tissues, as shown by previous studies, suggests that our findings of the partial reduction in *CD68* expression following IL-6 blockade could reflect diminished macrophage recruitment or activation. Furthermore, increased expression of LOX, and hence, increased collagen cross-linking [51], has been independently linked to increased myocardial stiffness [52]. However, the partial attenuation of *LOX* expression with blocking IL-6 was not sufficient to prevent the inflammation-induced decreases in LV relaxation (lateral e') or increases in filling pressures (E/e'), which, like the e'/a' ratio, are also indicators of LV diastolic dysfunction [53–55]. Considering we have previously shown that fibrosis is unlikely to be the main contributor to LV diastolic dysfunction [56], these results further support a central role for titin phosphorylation in the development of LV diastolic dysfunction. We showed that blocking circulating IL-6 did not prevent the inflammation-induced upregulation of the genes involved in the pathways of titin phosphorylation including *VCAM1, iNOS, sGCα2* or *sGCβ2*. These results suggest that tocilizumab did not impact the inflammation-induced NO-cGMP-PKG signalling pathway, previously implicated in titin hypophosphorylation [45]. Furthermore, blocking IL-6 also did not prevent the inflammation-induced upregulation of *PP5*, which we have reported as possibly having an important regulatory role in titin phosphorylation [57]. In contrast, a previous study showed improved titin phosphorylation when blocking circulating IL-6 in a model of Coxsackie virus B3 (CVB3)-induced myocarditis [21]. These authors reported that blocking circulating IL-6 reduced downstream LV tissue immune cell infiltration and activation [21], which may have disrupted signalling pathways involved in titin phosphorylation. In the present study, although *CD68* mRNA expression was reduced following IL-6 blocker treatment, it did not offset microvascular inflammation or LV tissue cytokine upregulation, which are the main drivers of titin hypo-phosphorylation [23].

Although treatment with the IL-6 blocker partially prevented collagen cross linking and LV stiffness in the present study, it was largely unable to prevent inflammation-induced LV diastolic and early systolic dysfunction. These negative results in the CIA+IL-6 blocker group may be explained by several reasons. IL-6 signalling is rather complex, and several studies have reported that it has both detrimental and protective effects in the heart [5]. These contrasting effects depend on whether IL-6 interacts with the soluble or the membrane-bound form of its receptor [1]. In this regard, IL-6 is a member of the gp130 cytokine family that includes several other cytokines that have been implicated in the pathophysiology of cardiovascular diseases, including IL-11, leukaemia inhibitory factor, and cardiotrophin-1. These cytokines signal via the common signalling receptor subunit gp130 [1,58,59], accounting for their overlapping biological functions [1,58–61]. Although the IL-6R was believed to be specific for IL-6, other cytokines belonging to the gp130 cytokine family may also bind to the IL-6R and induce signalling [60]. Indeed, activation of gp130 signalling, in the absence of IL-6, has been associated with myocardial remodelling [43].

Further, IL-6 may signal through different mechanisms. Classic IL-6 signalling involves the binding of IL-6 cytokines to their receptors (IL-6Rs) on the cell surface. This binding leads to the association of IL-6/IL-6R complexes with gp130, causing dimerization and the initiation of a signalling cascade [1,59]. However, IL-6 can also signal in the absence of cell surface IL-6R. Disintegrin and metalloprotease proteases cleave IL-6Rs from cell surfaces, generating a soluble form of the IL-6R (sIL-6R). Most soluble receptors in the body act as antagonists by competing with membrane-bound receptors for the ligand [1]. In contrast, sIL-6R acts as an agonist in cells that are not exclusively stimulated by IL-6. The sIL-6R binds IL-6, initiating gp130 signalling in cells that do not express IL-6R on their surface [1,62]. This mechanism, known as trans-signalling, extends the cellular actions of IL-6. The pro- and anti-inflammatory effects of IL-6 have been linked to these different signalling pathways, with trans-signalling implicated in pro-inflammatory signalling, while the classic signalling mechanism may be responsible for anti-inflammatory signals [58]. Therefore, it has been suggested that the *in vivo* role of IL-6 depends on the activation of different receptors in various experimental inflammatory models [1]. Indeed, IL-6 signalling via the gp130 pathway has been associated with the activation of cellular mechanisms that promote cardiac protection and prevent ischemia/reperfusion injury following myocardial infarction [63,64]. IL-6 has also been shown to exert protective anti-apoptotic actions that help maintain mitochondrial function in myocytes, suggesting a role in preserving cardiac tissue integrity [63–65]. Although the relative contributions of these IL-6 signalling pathways in heart failure have not been systematically studied, reports indicate that the adverse effects of

IL-6 on cardiac remodelling are dependent on the presence of sIL-6R [16,66].

It is therefore not surprising that the use of anti-IL-6 therapeutics is complicated by the classic- *versus* trans-signalling mechanisms of IL-6 action. Tocilizumab functions by inhibiting IL-6 interactions with both soluble and membrane-bound forms of its cognate receptor, thereby downregulating IL-6-mediated signalling [66,67]. This means that tocilizumab blocks both classic- and trans-signalling pathways and therefore, may block both pro- and anti-inflammatory activities of IL-6 [58,66,67]. Hence, in the current study, blocking IL-6 with tocilizumab may have impacted both the pro- and anti-inflammatory signalling pathways.

Another consideration is that tocilizumab is a human monoclonal antibody, and hence we cannot exclude drug-induced immunogenic effects in the current rodent model [20–22,68]. Nevertheless, several previous studies have successfully used tocilizumab in rodent models. In this regard, intraplanar injections of tocilizumab in the CIA model have shown improvements in synovitis and joint inflammation [69]. Other studies that have used chronic intraperitoneal administration of tocilizumab at the same dose as the current study (8mg/kg) have reported reduced inflammation-induced bone loss [70], ameliorated inflammation-induced cognitive performance and anxiety-like behaviour [70], a reduction in inflammation-induced endometriosis and improved metabolic profiles [33,71]. In the present study, the administration of the IL-6 blocker, tocilizumab, did not alter the inflammation-induced increases in serum IL-6 concentrations. However, it is well-accepted that IL-6 receptor inhibition is usually accompanied by increased serum activity of the cognate ligand [56,57]. Although IL-6 blockers disrupt IL-6 signal transduction by competitively binding membrane-bound and soluble receptors, when tocilizumab binds to IL-6 receptors, it hinders the clearance of IL-6 from circulation [58].

This study has further limitations. Despite reporting only mRNA expression of pro-fibrotic markers in LV tissues, our group has previously demonstrated increased collagen volume fraction in LV tissue using histology in the same CIA rat model [33]. Although mRNA expression may not reflect functional protein expression, echocardiography served as the measure of cardiac function in the current study. However, it would be interesting to investigate the correlation between echocardiographic measures and protein expression in future studies. Importantly, tocilizumab is usually not a first-line therapy in the management of RA, and is frequently used in combination with other anti-rheumatic or anti-inflammatory drugs. Therefore, future studies should investigate the impact of tocilizumab on cardiac function when used in combination with these other agents.

5. Conclusion

In conclusion, tocilizumab treatment prevented inflammation-induced molecular changes in markers of collagen cross-linking but did not affect LV fibrosis, titin phosphorylation, or calcium handling. Tocilizumab was not effective in reducing circulating cytokine concentrations and was largely ineffective in ameliorating the inflammation-induced upregulation of genes involved in the cellular pathways associated with LV diastolic dysfunction. These results suggest that at a dosage of 8 mg/kg with a treatment duration of once a week for 6 weeks the current treatment regimen, the IL-6 blockade by tocilizumab may not be protective against the molecular pathways underlying inflammation-induced cardiac LV systolic and diastolic dysfunction.

Ethics

No human studies were carried out by the authors for this article. This investigation was approved by the Animal Ethics Screening Committee of the University of the Witwatersrand (AESC approval: 2017/03/21C and 2019/02/10C).

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CRediT authorship contribution statement

Ashmeetha Manilall: Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation. **Lebogang Mokotedi:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation. **Sulè Gunter:** Writing – review & editing, Methodology, Investigation. **Regina Le Roux:** Writing – review & editing, Investigation, Data curation. **Serena Fourie:** Writing – review & editing, Investigation, Data curation. **Aletta ME Millen:** Writing – review & editing, Validation, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare no conflict of interest.

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References

[1] Hanna A, Frangogiannis NG. Inflammatory cytokines and chemokines as therapeutic targets in heart failure. Cardiovasc Drugs Ther 2020;34:849–63.
[2] Chirinos JA, Orlenko A, Zhao L, Basso MD, Cvijic ME, Li Z, et al. Multiple plasma biomarkers for risk stratification in patients with heart failure and preserved ejection fraction. J Am Coll Cardiol 2020;75:1281–95.
[3] Ahluwalia TS, Prins BP, Abdollahi M, Armstrong NJ, Aslibekyan S, Bain L, et al. Genome-wide association study of circulating interleukin 6 levels identifies novel loci. Hum Mol Genet 2021;30:393–409.
[4] Nakagomi A, Seino Y, Noma S, Kohashi K, Kosugi M, Kato K, et al. Relationships between the serum cholesterol levels, production of monocyte proinflammatory cytokines and long-term prognosis in patients with chronic heart failure. Internal Med 2014;53:2415–24.
[5] Fontes JA, Rose NR, Čihák ová D. The varying faces of IL-6: from cardiac protection to cardiac failure. Cytokine 2015;74:62–8.
[6] Tang P, Ma S, Dong M, Wang J, Chai S, Liu T, et al. Effect of interleukin-6 on myocardial regeneration in mice after cardiac injury. Biomed Pharmacother 2018;106:303–8.
[7] Banerjee I, Fuseler JW, Intwala AR, Baudino TA. IL-6 loss causes ventricular dysfunction, fibrosis, reduced capillary density, and dramatically alters the cell populations of the developing and adult heart. Am J Physiol-Heart Circul Physiol 2009;296:H1694–704.
[8] Peng Y, Yang Q, Gao S, Liu Z, Kong W, Bian X, et al. IL-6 protects cardiomyocytes from oxidative stress at the early stage of LPS-induced sepsis. Biochem Biophys Res Commun 2022;603:144–52.
[9] Kobayashi H, Kobayashi Y, Giles JT, Yoneyama K, Nakajima Y, Takei M. Tocilizumab treatment increases left ventricular ejection fraction and decreases left ventricular mass index in patients with rheumatoid arthritis without cardiac symptoms: assessed using 3.0 tesla cardiac magnetic resonance imaging. J Rheumatol 2014;41:1916–21.
[10] Giles JT, Sattar N, Gabriel S, Ridker PM, Gay S, Warne C, et al. Cardiovascular safety of Tocilizumab versus Etanercept in rheumatoid arthritis: a randomized controlled trial. Arthritis Rheumatol 2020;72:31–40.
[11] Castagné B, Viprey M, Martin J, Schott AM, Cucherat M, Soubrier M. Cardiovascular safety of tocilizumab: a systematic review and network meta-analysis. PLoS One 2019;14:e0220178.
[12] Zhang J, Xie F, Yun H, Chen L, Muntner P, Levitan EB, et al. Comparative effects of biologics on cardiovascular risk among older patients with rheumatoid arthritis. Ann Rheum Dis 2016;75:1813–8.
[13] Golledge J, Velu R, Quigley F, Jenkins J, Singh TP. Cohort study examining the association of immunosuppressant drug prescription with major adverse cardiovascular and limb events in patients with peripheral artery disease. Ann Vasc Surg 2022;78:310–20.

[14] Zou J, Jing F. Cardiovascular adverse events associated with monoclonal antibody products in patients with COVID-19. Pharmaceuticals (Basel) 2022;15(12):1472–87.
[15] Borlaug BA, Kass DA. Mechanisms of diastolic dysfunction in heart failure. Trends Cardiovasc Med 2006;16:273–9.
[16] Meléndez GC, McLarty JL, Levick SP, Du Y, Janicki JS, Brower GL. Interleukin 6 mediates myocardial fibrosis, concentric hypertrophy, and diastolic dysfunction in rats. Hypertension 2010;56:225–31.
[17] Kaminski K, Oledzka E, Bialobrzewska K, Kozuch M, Musial W, Winnicka M. The effects of moderate physical exercise on cardiac hypertrophy in interleukin 6 deficient mice. Adv Med Sci 2007;52:164–8.
[18] Lai NC, Gao MH, Tang E, Tang R, Guo T, Dalton ND, et al. Pressure overload-induced cardiac remodeling and dysfunction in the absence of interleukin 6 in mice. Lab Investig 2012;92:1518–26.
[19] Siwik DA, Chang DL-F, Colucci WS. Interleukin-1β and tumor necrosis factor-α decrease collagen synthesis and increase matrix metalloproteinase activity in cardiac fibroblasts in vitro. Circ Res 2000;86:1259–65.
[20] Kobara M, Noda K, Kitamura M, Okamoto A, Shiraishi T, Toba H, et al. Antibody against interleukin-6 receptor attenuates left ventricular remodelling after myocardial infarction in mice. Cardiovasc Res 2010;87:424–30.
[21] Savvatis K, Müller I, Fröhlich M, Pappritz K, Zietsch C, Hamdani N, et al. Interleukin-6 receptor inhibition modulates the immune reaction and restores titin phosphorylation in experimental myocarditis. Basic Res Cardiol 2014;109(6):449–63.
[22] Hartman MHT, Vreeswijk-Baudoin I, Groot HE, Van De Kolk KWA, De Boer RA, Mateo Leach I, et al. Inhibition of interleukin-6 receptor in a murine model of myocardial ischemia-reperfusion. PLoS One 2016;11:e0167195.
[23] Lewinter MM, Granzier HL. Cardiac titin and heart disease. J Cardiovasc Pharmacol 2014;63:207–12.
[24] Faria A, Persaud SJ. Cardiac oxidative stress in diabetes: mechanisms and therapeutic potential. Pharmacol Ther 2017;172:50–62.
[25] Gloss B, Villegas S, Villarreal FJ, Moriscot A, Dillmann WH. Thyroid hormone-induced stimulation of the sarcoplasmic reticulum Ca2+ ATPase gene is inhibited by LIF and IL-6. Am J Physiol Endocrinol Metab 2000;278:E738–43.
[26] Tanaka T, Kanda T, Takahashi T, Saegusa S, Moriya J, Kurabayashi M. Interleukin-6-induced reciprocal expression of SERCA and natriuretic peptides mRNA in cultured rat ventricular myocytes. J Int Med Res 2004;32:57–61.
[27] Wu C-K, Lee J-K, Chiang F-T, Yang C-H, Huang S-W, Hwang J-J, et al. Plasma levels of tumor necrosis factor-α and interleukin-6 are associated with diastolic heart failure through downregulation of sarcoplasmic reticulum Ca2+ ATPase. Crit Care Med 2011;39:984–92.
[28] Jasemian Y, Svendsen P, Deleuran B, F D-H. Refinement of the collagen induced arthritis model in rats by infrared thermography. J Adv Med Med Res 2011;22:469–77.
[29] Taskin MI, Gungor AC, Adali E, Yay A, Onder GO, Inceboz U. A humanized anti-interleukin 6 receptor monoclonal antibody, Tocilizumab, for the treatment of endometriosis in a rat model. Reprod Sci 2016;23:662–9.
[30] Smolen JS, Landewé R, Breedveld FC, Buch M, Burmester G, Dougados M, et al. EULAR recommendations for the management of rheumatoid arthritis with synthetic and biological disease-modifying antirheumatic drugs: 2013 update. Ann Rheum Dis 2014;73:492–509.
[31] Le Roux R, Mokotedi L, Fourie S, Manilall A, Gunter S, Millen AME. TNF-α inhibitors reduce inflammation-induced concentric remodelling, but not diastolic dysfunction in collagen-induced arthritis. Clin Exp Rheumatol 2022;40:24–32.
[32] Winter CA, Risley EA, Nuss GW. Carrageenin-induced edema in hind paw of the rat as an assay for antiiflammatory drugs. Proc Soc Exp Biol Med 1962;111:544–7.
[33] Mokotedi L, Michel FS, Mogane C, Gomes M, Woodiwiss AJ, Norton GR, et al. Associations of inflammatory markers with impaired left ventricular diastolic and systolic function in collagen-induced arthritis. PLoS One 2020;15:e0230657.
[34] Sharifov OF, Schiros CG, Aban I, Denney TS, Gupta H. Diagnostic accuracy of tissue doppler index E/e' for evaluating left ventricular filling pressure and diastolic dysfunction/heart failure with preserved ejection fraction: a systematic review and meta-analysis. J Am Heart Assoc 2016;5:e002530-e002549.
[35] Teichholz LE, Kreulen T, Herman MV, Gorlin R. Problems in echocardiographic volume determinations: echocardiographic-angiographic correlations in the presence of absence of asynergy. Am J Cardiol 1976;37:7–11.
[36] Weytjens C, Cosyns B, Dhooge J, Gallez C, Droogmans S, Lahoute T, et al. Doppler myocardial imaging in adult male rats: reference values and reproducibility of velocity and deformation parameters. Eur J Echocardiogr 2006;7:411–7.
[37] Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. Methods 2001;25:402–8.
[38] RefFinder. http://blooge.cn/RefFinder/.
[39] Fahmi A, Smart N, Punn A, Jabr R, Marber M, Heads R. p42/p44-MAPK and PI3K are sufficient for IL-6 family cytokines/gp130 to signal to hypertrophy and survival in cardiomyocytes in the absence of JAK/STAT activation. Cell Signal 2013;25:898–909.
[40] Blythe NM, Muraki K, Ludlow MJ, Stylianidis V, Gilbert HTJ, Evans EL, et al. Mechanically activated Piezo1 channels of cardiac fibroblasts stimulate p38 mitogen-activated protein kinase activity and interleukin-6 secretion. J Biol Chem 2019;294:17395–408.
[41] Li H, Bian Y. Fibroblast-derived interleukin-6 exacerbates adverse cardiac remodeling after myocardial infarction. Korean J Physiol Pharmacol 2024;28:285–94.
[42] Fang G, Li Y, Yuan J, Cao W, Song S, Chen L, et al. Cadherin-11-interleukin-6 signaling between cardiac fibroblast and cardiomyocyte promotes ventricular remodeling in a mouse pressure overload-induced heart failure model. Int J Mol Sci 2023;24:6549–68.
[43] Hirota H, Yoshida K, Kishimoto T, Taga T. Continuous activation of gp130, a signal-transducing receptor component for interleukin 6-related cytokines, causes myocardial hypertrophy in mice. Proc Natl Acad Sci 1995;92:4862–6.
[44] Zhao L, Cheng G, Jin R, Afzal MR, Samanta A, Xuan Y-T, et al. Deletion of interleukin-6 attenuates pressure overload-induced left ventricular hypertrophy and dysfunction. Circ Res 2016;118:1918–29.
[45] Franssen C, Gonzalez Miqueo A. The role of titin and extracellular matrix remodelling in heart failure with preserved ejection fraction. Neth Heart J 2016;24:259–67.
[46] England J, Loughna S. Heavy and light roles: myosin in the morphogenesis of the heart. Cell Mol Life Sci 2013;70:1221–39.
[47] Manilall A, Mokotedi L, Gunter S, Roux RL, Fourie S, Flanagan CA, et al. Tumor necrosis factor-A mediates inflammation-induced early-stage left ventricular systolic dysfunction. J Cardiovasc Pharmacol 2023;81:411–22.
[48] Su C, Fu X, Duan W, Yu P, Zhao Y. High density of CD68+ tumor-associated macrophages predicts a poor prognosis in gastric cancer mediated by IL-6 expression. Oncol Lett 2018;15(5):6217–24.
[49] Sánchez-Gloria JL, Martínez-Olivares CE, Rojas-Morales P, Hernández-Pando R, Carbó R, Rubio-Gayosso I, et al. Anti-inflammatory effect of allicin associated with fibrosis in pulmonary arterial hypertension. Int J Mol Sci 2021;22:8600.
[50] Nguyen X-X, Nishimoto T, Takihara T, Mlakar L, Bradshaw AD. Feghali-Bostwick C. Lysyl oxidase directly contributes to extracellular matrix production and fibrosis in systemic sclerosis. Am J Physiol-Lung Cell Mol Physiol 2021;320:L29–40.
[51] Páramo JA. New mechanisms of vascular fibrosis: role of lysyl oxidase. Clín Investig Arterioscler (Engl Ed) 2017;29:166–7.
[52] Gonzalez-Santamaria J, Villalba M, Busnadiego O, Lopez-Olaneta MM, Sandoval P, Snabel J, et al. Matrix cross-linking lysyl oxidases are induced in response to myocardial infarction and promote cardiac dysfunction. Cardiovasc Res 2016;109:67–78.
[53] Nishimura RA, Tajik AJ. Evaluation of diastolic filling of left ventricle in health and disease: doppler echocardiography is the clinician's Rosetta stone. J Am Coll Cardiol 1997;30:8–18.
[54] Smiseth OA. Evaluation of left ventricular diastolic function: state of the art after 35 years with Doppler assessment. J Echocardiogr 2018;16(2):55–64.
[55] Zerbib Y, Maizel J, Slama M. Echocardiographic assessment of left ventricular function. J Emerg Crit Care Med 2019;3:33.
[56] Manilall A, Mokotedi L, Gunter S, Le Roux R, Fourie S, Flanagan CA, et al. Inflammation-induced left ventricular fibrosis is partially mediated by tumor necrosis factor-α. Physiol Rep 2021;9:e15062.
[57] Manilall A, Mokotedi L, Gunter S, Le Roux R, Fourie S, Flanagan CA, et al. Increased protein phosphatase 5 expression in inflammation-induced left ventricular dysfunction in rats. BMC Cardiovasc Disord 2022;22(1):539.
[58] Rose-John S. IL-6 trans-signaling via the soluble IL-6 receptor: importance for the pro-inflammatory activities of IL-6. Int J Biol Sci 2012;8:1237–47.
[59] Rose-John S. Interleukin-6 Family cytokines. Cold Spring Harb Perspect Biol 2018;10:a028415.
[60] Garbers C, Hermanns HM, Schaper F, Müller-Newen G, Grötzinger J, Rose-John S, et al. Plasticity and cross-talk of interleukin 6-type cytokines. Cytokine Growth Factor Rev 2012;23:85–97.
[61] Garbers C, Spudy B, Aparicio-Siegmund S, Waetzig GH, Sommer J, Hölscher C, et al. An interleukin-6 receptor-dependent molecular switch mediates signal transduction of the IL-27 cytokine subunit p28 (IL-30) via a gp130 protein receptor homodimer. J Biol Chem 2013;288:4346–54.
[62] Zhang S, Li L, Shen A, Chen Y, Qi Z. Rational use of Tocilizumab in the treatment of novel coronavirus pneumonia. Clin Drug Investig 2020;40:511–8.
[63] Huang BT, Peng Y, Liu W, Zhang C, Huang FY, Wang PJ, et al. Increased interventricular septum wall thickness predicts all-cause death in patients with coronary artery disease. Intern Med J 2015;45:275–83.
[64] McGinnis GR, Ballmann CG, Peters B, Nanayakkara G, Roberts MD, Amin R, et al. Interleukin-6 mediates exercise preconditioning against myocardial ischemia reperfusion injury. Ajp Heart Circulat Physiol 2015;308:H1423–33.
[65] Wu J-W, Hu H, Li D, Ma L-K. Hypoxia-inducible factor 2-alpha-dependent induction of IL-6 protects the heart from ischemia/reperfusion injury. Aging 2021;13:3443–58.
[66] Chou C-H, Hung C-S, Liao C-W, Wei L-H, Chen C-W, Shun C-T, et al. IL-6 trans-signalling contributes to aldosterone-induced cardiac fibrosis. Cardiovasc Res 2018;114:690–702.
[67] Scott LJ. Tocilizumab: a review in rheumatoid arthritis. Drugs 2017;77:1865–79.
[68] Sheppard M, Laskou F, Stapleton PP, Hadavi S, Dasgupta B. Tocilizumab (Actemra). Hum Vaccin Immunother 2017;13:1972–88.
[69] Abdel-Maged AE-S, Gad AM, Abdel-Aziz AK, Aboulwafa MM, Azab SS. Comparative study of anti-VEGF Ranibizumab and interleukin-6 receptor antagonist Tocilizumab in adjuvant-induced arthritis. Toxicol Appl Pharmacol 2018;356:65–75.
[70] Poutoglidou F, Pourzitaki C, Manthou ME, Saitis A, Malliou F, Kouvelas D. Infliximab and Tocilizumab reduce anxiety-like behaviour and improve cognitive performance in a juvenile collagen–Induced arthritis rat model. Inflammation 2022;45:445–59.
[71] El-Zayadi AA, Mohamed SA, Arafa M, Mohammed SM, Zayed A, Abdelhafez MS, et al. Anti-IL-6 receptor monoclonal antibody as a new treatment of endometriosis. Immunol Res 2020;68:389–97.