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An Orally Available NLRP3 Inflammasome Inhibitor Prevents Western Diet–Induced Cardiac Dysfunction in Mice

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Abstract

Background: A diet rich in saturated fat and sugars (Western diet, WD) induces myocardial expression of the NLRP3 inflammasome and dysfunction in mice. We therefore hypothesized that a diet enriched with an orally available NLRP3 inflammasome inhibitor could prevent WD-induced cardiac dysfunction in mice.

Methods: Ten-week-old CD-1 male mice were fed WD or standard diet (SD) for 8 weeks. The compound 16673-34-0, an orally active NLRP3 inhibitor, was added to the diet at a concentration of 100 mg/Kg. The plasmatic levels of the NLRP3 inflammasome inhibitor were measured. Food intake, body weight, and glucose tolerance were assessed. Cardiac systolic and diastolic functions were measured by Doppler echocardiography at baseline, 4 weeks, and 8 weeks.

Results: WD induced a significant increase in body weight (+14%, $P = 0.02$), impaired glucose tolerance (+34%, $P = 0.03$), and a significant increase in isovolumetric relaxation time (+129%, $P = 0.03$) and reduction in left ventricular ejection fraction (–10%, $P = 0.03$), as compared to standard chow diet (SD). The treatment with NLRP3 inhibitor in the diet prevented cardiac systolic and diastolic dysfunction ($P < 0.05$ for left ventricular ejection fraction, isovolumetric relaxation time, and myocardial performance index in WD with drug vs. WD without drug), without significant changes in heart rate and metabolic parameters.

Conclusions: An orally available NLRP3 inhibitor prevented WD-induced cardiac dysfunction in obese mice.

Keywords

inflammation; diet; heart failure

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INTRODUCTION

Obesity and, indirectly, unhealthy diet are associated with the development of heart failure (HF). Diet can modulate cardiac function in preclinical animal models. A diet rich in sugars and saturated fat [ie, Western diet (WD)] induces cardiac systolic and diastolic dysfunction, and returning to a low-sugar and low-saturated fat diet can partially reverse the cardiac abnormalities.¹ The mechanisms underlying the WD-induced cardiac dysfunction are largely unknown. In vivo^{2,3} and in vitro^{4–10} studies suggest that specific nutrients such as saturated fat and sugars can induce the formation and the activation of the Nod-like receptor pyrin domain-containing protein (NLRP)-3 inflammasome, responsible for the production of pro-inflammatory cytokines such as interleukin (IL)-1 and IL-18, with well-known cardiodepressant properties.

We have recently shown that WD increases the systemic- and cardiac-specific expression of interleukin(IL)-18, and its deletion resulted in improved cardiac function.¹¹ The specific role of the NLRP3 inflammasome in the development and progression of cardiac dysfunction induced by WD is largely unknown. Recently, NLRP3 in-flammasome inhibitors with documented cardioprotective properties have been developed.¹²

We hypothesized that the treatment with an orally available NLRP3 inhibitor would prevent the cardiac dysfunction in mice with diet-induced obesity.

METHODS

Animals

All experiments were conducted in 8–10-week-old male CD1 wild-type mice (Envigo RMS, Inc, Indianapolis, IN) with unlimited access to food and water. All studies were approved by the Virginia Commonwealth University Institutional Animal Care and Use Committee and conducted according to the NIH guidelines.

Measurement of IL–18 Plasma Levels

We used ELISA and real-time PCR to measure IL–18 plasma levels 8 weeks after beginning dietary and pharmacologic interventions. Blood was collected with a heart puncture at the time of sacrifice, incubated for 15 minutes, and then centrifuged for 10 minutes at 4°C at 2000 rpm to obtain serum. Samples were stored at –80°C. A specific ELI-SA for murine IL-18 was used to detect IL-18 expression. A BioTek plate reader (Quant, BioTek, Winooski, VT) at 450 nm was used to read absorbance.

Dietary and Pharmacologic Intervention With the NLRP3 Inflammasome Inhibitor

Mice were randomly assigned to 4 groups for 8 weeks:(1) standard diet (SD—Teklad LM–485, Envigo); (2) WD (TD.88137, Envigo); (3) SD with NLRP3 inhibitor (16673-34-0); and (4) WD with NLRP3 inhibitor. The NLRP3 inhibitor was added to the diet at a concentration of 100 mg/kg. Nutritional characteristics of the diets have been previously described.^{1,13}

Measurement of the NLRP3 Inflammasome Inhibitor Plasma Levels

To determine absorption and plasma distribution of the inhibitor, high-performance liquid chromatography with tandem mass spectrometry (LC–MS/MS) was used to measure levels of NLRP3 inflammasome inhibitor in the plasma collected at 8 weeks. Briefly, plasma (30 μ L) from NLRP3 inhibitor–treated mice was precipitated with 250 μ L of 1% formic acid in acetonitrile. After vortex mixing and centrifugation, the supernatant was dried under a nitrogen stream and each sample was reconstituted with 10% methanol. Ten microliters of each sample were injected into the LC-MS/MS with reversed-phase chromatography. Multiple reaction monitoring transitions were used for quantitative analysis, and results were processed using MassLynx V4.1 software. The details of the methodology are described in a separate publication.

Metabolic Assessments

Food intake was measured daily, whereas body weight was assessed weekly using a digital weight scale ($N = 5$ – 8). Glucose tolerance was evaluated with an oral glucose tolerance test at baseline, 4 weeks, and 8 weeks, using a glucometer (Clarity Diagnostics, FL). After an overnight of fasting (8–10 hours), baseline glycemia was assessed followed by an oral gavage of 20% glucose solution 2 g/kg. Glycemia was measured after 15, 30, 60, and 120 minutes from the tail.

Echocardiography

Mice underwent transthoracic Doppler echocardiography using a Vevo770 imaging system (VisualSonics Inc, Toronto, Ontario, and 30–MHz probe) under light anesthesia (30–50 mg/kg pentobarbital), at baseline, 4 weeks, and 8 weeks, as previously described ($N = 5$ – 8).^{1,11,12} We measured left ventricular dimensions, mass, ejection fraction, isovolumetric contraction time and isovolumetric relaxation time (IRT), ejection time, myocardial performance index (MPI) or Tei index, and heart rate (HR). A single trained operator blinded to the dietary and pharmacologic intervention performed all echocardiographic measurements.

Statistical Analysis

Values were expressed as the mean and standard error of the mean. The statistical analysis was performed using SPSS 24.0 package for Mac (Chicago, IL). Differences within each group were analyzed using Student's T test for paired data, whereas differences between groups were assessed using analysis of variance followed by Student's T test for unpaired data with Bonferroni's correction for multiple groups. We estimated that a sample size of 6 or more for each group would provide a power >80% ($\alpha = 0.05$) to detect a "meaningful" difference, defined as a difference between means that exceeded the standard deviation in each group.

RESULTS

NLRP3 Inflammasome Inhibitor Plasma Levels

After 8 weeks of study, the group receiving the pharmacologic treatment with NLRP3 inflammasome inhibitor showed detectable levels of the drug in both the WD-fed group ($N = 5$) and the SD-fed group ($N = 5$) [$P < 0.05$ and $P < 0.05$ vs. corresponding no inhibitor group ($N = 3$ and $N = 3$, respectively)] (Fig. 1A), reflecting drug absorption in the gut and into the blood stream.

Effects of Western Diet on IL-18 Plasma Levels and Treatment With the NLRP3 Inhibitor

After 8 weeks, mice fed with WD had significantly higher IL-18 plasma levels compared to SD-fed mice (188.7 ± 16.5 vs. 107.4 ± 20.3 pg/mL; $P < 0.05$). Moreover, WD-fed mice treated with the NLRP3 inhibitor had significantly less IL-18 plasma levels compared to WD-fed mice not treated (66.8 ± 2.9 vs. 188.7 ± 16.5 pg/mL; $P < 0.05$) (Fig. 1B).

Metabolic Effects of Western Diet and Treatment With the NLRP3 Inhibitor

WD-fed mice had significantly higher 8-week cumulative caloric intake compared to SD-fed mice (1170 ± 27 vs. 948 ± 51 kcal; $P < 0.001$) (Fig. 1C) and corresponding higher body weight (43 ± 1.1 vs. 37 ± 1.4 g; $P = 0.02$) (Fig. 1D), higher fasting glycemia (102 ± 8 vs. 80 ± 6 mg/dL; $P = 0.048$), and impaired glucose tolerance measured at 8 weeks with oral glucose tolerance test (2-hour glycemia: 174 ± 14 vs. 125 ± 13 mg/dL; $P = 0.03$), compared to SD-fed mice (Fig. 1E). No significant differences were seen in mice treated with the NLRP3 inhibitor compared to the control groups (WD and SD) on food intake, body weight, and glucose tolerance (all $P > 0.05$) (Figs. 1C, D, and E), demonstrating a neutral effect of the drug on all metabolic parameters.

Treatment With the NLRP3 Inhibitor and Cardiac Function

WD-fed mice showed a mild but significant worsening of systolic function compared to SD-fed mice, measured as left ventricular ejection fraction (LVEF) ($53.4 \pm 1.4\%$ vs. $60.0 \pm 1.7\%$; $P = 0.03$) (Fig. 1F). Cardiac diastolic function was also significantly impaired in the WD-fed mice showing a higher IRT compared to SD (21 ± 3 vs. 8 ± 1 ms; $P = 0.001$) (Fig. 1G) and a higher MPI (0.52 ± 0.03 vs. 0.16 ± 0.01 ms; $P = 0.006$) (Fig. 1H). No significant differences in heart rate were found (349 ± 47 vs. 410 ± 36 bpm; $P = 0.33$) (Fig. 1I). Treatment with the NLRP3 inhibitor prevented the impairment in cardiac function seen with the WD as compared to the untreated WD-fed mice: LVEF was preserved ($64.5 \pm 3.4\%$ vs. $53.4 \pm 1.4\%$; $P = 0.03$), IRT was not prolonged (9.5 ± 1.6 vs. 14 ± 1.1 ms; $P = 0.01$), and the MPI was not increased (0.31 ± 0.02 vs. 0.19 ± 0.02 ms; $P = 0.007$) (Fig. 1I).

Of note, the LVEF in the NLRP3 inhibitor treated group was not statistically different compared to the SD group (Fig. 1F). Similarly, treatment with the NLRP3 inhibitor in the diet had no effects on LVEF, IRT, and MPI in the SD-fed mice (all $P > 0.05$).

DISCUSSION

This study shows that blockade of the NLRP3 inflammasome with an orally available inhibitor preserves diet-induced cardiac systolic and diastolic function in the mouse model. Epidemiological studies have associated WD with increased risk of obesity, diabetes, and indirectly of HF, although studies showing a direct detrimental effect of diet on cardiac function were limited. We and others had shown that WD-fed mice develop a cardiac systolic and diastolic dysfunction within 8 weeks of dietary intervention, and that switching to a diet low in sugars and saturated fat restored cardiac function.¹ Similarly, despite similar caloric intake, a diet rich in unsaturated fat, also known as “healthy fats,” can prevent weight gain and cardiac dysfunction in mice, and may drive exercise capacity in patients with obesity and HF with preserved LVEF (HFpEF).¹³ However, the underlying mechanism(s) of WD-induced cardiac phenotype are still incompletely understood. Undoubtedly a WD rich in saturated fat and sugars leads to obesity, but a disconnect seems to exist between the direct effects of diet on cardiac function and adiposity, suggesting that the effects of WD on cardiac function seem to be independent of those on increased risk of obesity.

Systemic inflammation contributes to exercise intolerance, predicts higher hospitalization rate, and finally worse outcomes in patients with HF.^{11,14–17} Dietary sugars and saturated fat are major components of the WD and are known to initiate proinflammatory pathways, particularly by priming and triggering the NLRP3 inflammasome,^{4,8,9} responsible for the production of interleukin-1 β (IL-1 β) and IL-18, proinflammatory cytokines with potent cardiodepressant properties. IL-18 also directly induces cardiac dysfunction when administered to mice and mediates the cardiac dysfunction induced by IL-1 in preclinical animal models of cardiac dysfunction.¹⁶ Mice with genetic deletion of IL-18 have preserved cardiac function after feeding with WD for 8 weeks, despite the fact that the deletion of IL-18 does not prevent obesity and actually even worsens obesity in that model as a result of the lack of IL-18 in the brain resulting in increased food intake.¹¹ We herein describe that the inhibition of the NLRP3 inflammasome results in preservation in cardiac function but without preventing the development of WD-induced obesity. Such results reinforce the concept that obesity and cardiac dysfunction are 2 independent consequences of WD.¹⁷ This concept may hold true also in patients with obesity and HF in whom obesity and adiposity parameters are associated with worse cardiorespiratory fitness, but not with cardiac function abnormalities,¹⁸ suggesting a disconnect between the role of obesity and metabolism on cardiac function and cardiorespiratory fitness.^{19,20} The same disconnect is apparent for other metabolic parameters such as fasting and postprandial glucose. The NLRP3 inhibitor did not prevent nor ameliorate the detrimental effects of WD on glucose metabolism, suggesting that the protective effects on cardiac function are independent of those on glycemic control. These results in many ways challenge the glucose-centric quest of cardiovascular protection therapies in patients with diabetes and provide a different light to interpret the large clinical benefit seen in cardiovascular outcomes despite modest reductions in glucose lowering with novel classes of agents such as the sodium-glucose cotransporter 2 inhibitors.²¹

Within the limitations of comparisons between different studies, it is tempting to speculate that the lack of excess weight gain with NLRP3 inflammasome inhibition in this study, or NLRP3 deletion in other studies, as opposed to IL-18 gene deletion, suggests that the

NLRP3 inflammasome is not responsible for the baseline production of IL-18 required to maintain metabolic homeostasis and that basal IL-18 may be derived from a different inflammasome, NLRP1 inflammasome.²² The beneficial effects of the drug we tested in this study on cardiac remodeling after ischemic and nonischemic injury as well as reduced myocardial injury in an ischemia-reperfusion model have been reported previously.²³

This study is, however, not without limitations. We only included male mice that were fed WD for 8 weeks and, as such, long-term effects of WD and of the NLRP3 inhibitor have not been tested. Although we measured the NLRP3 inflammasome inhibitor levels and we have shown to achieve plasma levels known to be active, we did not provide evidence for the inhibition of the target. However, the NLRP3 inhibitor resulted in reduced plasma IL-18, without the potential confounding of differences in adiposity and food intake, thus providing supporting of the inhibition of the NLRP3 inflammasome. However, the mechanism through which IL-18 may directly affect cardiac function is still a subject of debate¹⁶ and requires further mechanistic study. Finally, the echocardiographic examination requires highly skilled and trained personnel for an accurate and reproducible assessment. In our study, a single highly trained operator blinded to the intervention group assignments performed and interpreted the echocardiographic examination.

A recently completed pilot phase II study with anakinra, an IL-1 blocker, showed a reduction in inflammatory biomarkers and in a specific marker of myocardial strain (N-terminal pro-B type natriuretic peptide) and an improvement in treadmill exercise time and quality of life in obese patients with HF and preserved ejection fraction,²⁴ providing promising results for future larger clinical trials.

CONCLUSIONS

In conclusion, we show that an orally available NLRP3 inflammasome inhibitor prevents the WD-induced cardiac dysfunction in obese mice, with neutral effects on body weight and glucose tolerance. Further study is needed to determine whether the use of the NLRP3 inflammasome inhibitor is safe and effective to improve symptoms and functional capacity in patients with HF, particularly in those who are obese and have HFpEF and diastolic dysfunction.

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FIGURE 1.

Treatment with the NLRP3 inhibitor for 8 weeks resulted in increased plasma concentration of the drug (A). Interleukin-18 plasma levels were significantly reduced in Western diet (WD)-fed mice and concomitantly treated with the NLRP3 inhibitor (B). Caloric intake (C) and body weight (D) were greater in WD-fed mice, and treatment with NLRP3 inhibitor did not affect these parameters. Similarly, glucose tolerance was impaired in WD-fed mice, without significant changes with NLRP3 inhibitor treatment (E). Cardiac systolic function measured as LVEF was significantly reduced in WD-fed mice; however, LVEF remained greater than 50% (F). Treatment with NLRP3 inhibitor prevented the decline in LVEF

induced by WD (F). Cardiac diastolic function, measured as IRT (G) and MPI (H), was also impaired in WD-fed mice; however, treatment with NLRP3 inhibitor prevented such dysfunction. WD with and without treatment with NLRP3 inhibitor induced no significant changes on heart rate (I).

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