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Taurine Alleviates Sympathetic Innervation by Inhibiting NLRP3 Inflammasome in Postinfarcted Rats

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Abstract: The NLRP3 inflammasome is activated by myocardial infarction and then induces the activation of inflammatory caspase-1 activation and maturation of IL-1β, a regulator of synthesis of the nerve growth factor (NGF). Here, we studied whether taurine, 2-aminoethanesulphonic acid, can attenuate cardiac sympathetic reinnervation by modulating NLRP3 inflammasome-mediated NGF in a rat model of myocardial infarction. Male Wistar rats were subjected to coronary ligation and then randomized to either saline or taurine for 3 days or 4 weeks. Postinfarction was associated with activation of NF-κB (p65) and NLRP3 inflammasome component and increased the protein and expression of IL-1β. Macrophages at the border zone were shown to be positive for IL-1β 3 days postinfarction. Compared with vehicle, infarcted rats treated with taurine significantly attenuated myocardial messenger RNA and protein levels of NF-κB, NLRP3 inflammasome, mature caspase-1, and IL-1β. Immunofluorescent analysis, real-time quantitative reverse transcription polymerase chain reaction, and Western blotting of NGF showed that sympathetic hyperinnervation was blunted after administering taurine. Arrhythmia vulnerability in the taurine-treated infarcted rats was significantly improved than those in vehicle. *Ex vivo* studies showed that taurine infusion reduced myocardial IL-1β level at the extent similar to either pyrrolidine dithiocarbamate or CP-456,773, inhibitors of NF-κB and NLRP3 inflammasome, implying the key axis of NF-κB/NLRP3 inflammasome in mediating taurine-related anti-inflammation. Furthermore, administration of anti-IL-1β antibody reduced NGF levels. Taurine attenuated sympathetic innervation mainly by NLRP3 inflammasome/IL-1β–dependent pathway, which downregulated expression of NGF in infarcted rats. These findings may provide a new insight into the anti-inflammation effect of taurine.

Key Words: interleukin-1β, nerve growth factor, NLRP3 inflammasome, myocardial infarction, sympathetic innervation

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INTRODUCTION

Taurine (2-aminoethanesulphonic acid) is the most abundant organic molecule in the human body.[1] Although it can be synthesized endogenously, mammals cannot adequately synthesize taurine and depend on dietary sources to satisfy requirements. Taurine is found in very high concentration in the heart.[2] Taurine has been shown to be involved in many diverse pathophysiological functions of the heart, including the balance of neurotransmitters and anti-inflammation[3]; however, the mechanisms of these actions are not yet understood. During myocardial infarction (MI), neutrophils secrete latent forms of proteolytic enzymes, and these enzymes are then activated by myeloperoxidase-produced hypochlorous acid.[4] Taurine undergoes halogenation in phagocytes and is converted to taurine chloramines, a more stable and less toxic anti-inflammatory mediator.[4] By contrast, in a rat model, Allo et al[5] showed that taurine deficiency protected the heart against ischemia/reperfusion-induced injury. Therefore, further studies are warranted to investigate the potentially beneficial effects of taurine in ischemic heart injury.

Cardiac sympathetic hyperinnervation post-MI has been correlated with an increased incidence of fatal arrhythmias. Fatal ventricular arrhythmias are a common complication after MI, accounting for almost 50% of cases of sudden cardiac death in survivors of MI, and increased sympathetic nerve density has been shown to be the main cause.[6] Cardiac remodeling post-MI is an intricate inflammatory process that involves many signaling pathways. In addition, inflammation induced by MI has been shown to upregulate nerve growth factor (NGF), which plays an important role in sympathetic sprouting and sympathetic hyperinnervation.[7] The proinflammatory IL-1β has been shown to regulate the synthesis of NGF in peripheral nerves[8] and fibroblasts.[9] Thus, the inflammatory response after MI may contribute to sympathetic nerve by a cytokine/neurotrophin axis.

Inflammation plays an important role in the wound healing process after injury. An exaggerated inflammatory response is believed to be responsible for the increased morbidity and mortality after MI. Inflammasomes are innate immune signaling pathways and cytosolic multiprotein complexes that are present mainly in macrophages. The structure of all inflammasomes is similar, typically containing nucleotide-binding oligomerization

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domain-like receptor sensor (NLRP3), effector (caspase-1), adapter (Apoptosis-Associated Speck-Like Protein Containing CARD [ASC]), and substrate (proinflammatory cytokines pro-IL-1β/18) components. Of these components, the sensors recognize danger signals released during tissue injury such as damage-associated molecular pattern molecules (DAMPs) and stress (such as urate crystal, extracellular Adenosine triphosphate, cell debris, and β-amyloid). DAMPs are associated with activation of NF-κB.[10,11] The activation of NF-κB is extremely important for inflammatory signaling pathway after MI. The inhibition of NLRP3 by small interfering RNA has been shown to prevent the activation of inflammasomes and cardiac cell death, thereby ameliorating myocardial remodeling.[12,13] Therefore, the pharmacological inhibition of NLRP3 may be a new strategy to attenuate ischemia/reperfusion injury post-MI.

Recently, taurine has been shown to inhibit NLRP3 inflammasome in arsenic trioxide–induced liver diseases.[14] Furthermore, taurine inhibited NF-κB nuclear translocation,[15] an upstream regulator of the NLRP3 inflammasome. Thus, it is reasonable to speculate that besides formation of taurine haloamine, taurine may play an anti-inflammatory role by inhibition of NLRP3 inflammasome. MI was associated with decreased intracellular taurine content in the heart through attenuated expression of taurine transporters.[16] Exogenous taurine supplement upregulated taurine transporter expression and increased the intracellular content of taurine. However, whether taurine administration efficiently regulates sympathetic nerve remodeling after MI remained unclear. Given the regulation of NGF secretion is in a cell-specific and tissue-specific manner,[17] the effect of IL-1β on NGF secretion in myocardium should be clarified. Thus, we assessed (1) the effect of chronic administration of taurine on sympathetic reinnervation and ventricular arrhythmias, (2) the relative contribution of taurine haloamine and NLRP3 inflammasome inhibition in taurine-mediated anti-inflammation, and (3) the effect of IL-1β on myocardial NGF expression in a rat MI model.

METHODS

All animal experiments were approved by the local ethical review committee on animal care and conducted in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

Animals

Experiment 1 (in vivo)

The anterior descending arteries of male Wistar rats (250–300 g) were ligated as previously described,[18] leading to left ventricular (LV) free-wall rupture. In brief, after adequate anesthesia with Zoletil (20 mg/kg body weight) and xylazine (9 mg/kg) intraperitoneally, the rats were intubated with a 14-gauge polyethylene catheter and ventilated with room air using a small animal ventilator (model 683, Harvard Apparatus, Boston, MA). The rats were randomly assigned to a vehicle group or taurine group (100 mg/kg per day; Sigma Aldrich, St. Louis, MO) so that there was approximately the same number of survivors in each group. The dose of taurine was chosen as previous studies.[16] Given the NLRP3 inflammasome was formed in the myocardium within 24 hours of the AMI,[14] the drug was started 24 hours after MI by daily oral gavage. The study was conducted over 4 weeks, as most myocardial remodeling in rats (70%–80%) takes place within 3 weeks.[19] The control group underwent a sham operation. The administration of taurine was stopped approximately 24 hours before the end of the experiments so as to terminate its pharmacological actions. The hearts of the rats were excised 3 or 28 days post-MI to represent the early and late stages of MI, respectively.

Experiment 2 (Ex Vivo)

The mechanisms underlying taurine-mediated anti-inflammation included 2 pathways: formation of taurine haloamine and inhibition of NLRP3 inflammasome. To discern the relative contribution of 2 anti-inflammation pathways to IL-1β levels, we performed ex vivo experiments by using a specific inhibitor of the NLRP3 inflammasome. Three days after MI had been induced by coronary ligation, the hearts of the rats were isolated and divided into the following groups: no treatment (vehicle), taurine (150 μM), taurine + pyrrolidine dithiocarbamate (PDTC, a NF-κB inhibitor, 20 μM, Sigma-Aldrich Corporation, St. Louis, MO), and taurine + CP-456,773(20 nM; IC50 for NLRP3: 7.5 nM). CP-456,773 specifically blocks NLRP3-induced ASC oligomerization, but not other inflammasome-forming molecules, such as NLRP1.[20] The doses of taurine,[14] PDTC,[21] and CP-456,773[20] have been shown to be effective in modulating biological activities. A noncirculating modified Tyrode's solution was used to perfuse the hearts, which contained (in mM) $NaHCO_3$ 23.0, NaCl 117.0, NaH_2PO_4 0.8, $CaCl_2$ 2.0, $MgCl_2$ 1.0, KCl 4.6, and glucose 5.5 and was equilibrated at 37°C and oxygenated with a 95% O_2 to 5% CO_2 gas mixture. The drugs were infused for 30 minutes. At the end of the study, the hearts (n = 5 in each group) were subjected to Western blotting for the detection of IL-1β protein at the remote zone (>2 mm outside the infarct).

Experiment 3 (*Ex Vivo*)

To assess the IL-1β effects on regulating NGF levels, we performed ex vivo experiments. Three days after MI had been induced by coronary ligation, the hearts of the rats were isolated and divided into the following groups: no treatment (vehicle) and IL-1β antibody (20 μg/mL; Invitrogen, Carlsbad, CA). The dose of IL-1β antibody[22] has been shown to be effective in modulating biological activities. The perfusion settings were the same as Experiment 2. At the end of the study, all hearts (n = 5 in each group) were subjected to Western analysis for the detection of NGF protein at the remote zone.

Hemodynamics and Infarct Size Measurements

At the end of the study, the size of the infarcts and hemodynamic parameters were measured (see **online Supplementary material**, **Supplemental Digital Content**

1, http://links.lww.com/JCVP/A627). Only rats with clinically important MIs (>30%) were selected for analysis.

Ex vivo Electrophysiological Studies

We then performed programmed electrical stimulation to evaluate the possible arrhythmogenic risk of sympathetic innervation. We used the Langendorff heart perfusion technique to avoid any potential confounding effects of post-MI hormonal activation on pacing-induced ventricular arrhythmias as previously reported.[23] For detailed information, please refer to the Supplemental Digital Content 1 (see Supplementary material online, http://links.lww.com/JCVP/A627).

Real-Time Reverse Transcription Polymerase Chain Reaction (RT-PCR) of NF-κB (p65), NLRP3, Caspase-1, IL-1β, and NGF

Real-time RT-PCR was performed from samples obtained from the border zone (<2 mm within the infarct) at day 3 for *NF-κB (p65)*, *NLRP3*, *caspase-1*, and *IL-1β* and from the remote zone at day 28 for *NGF* with the TaqMan system (Prism 7700 Sequence Detection System, PE Biosystems) as previously described.[18] *Cyclophilin* messenger RNA (mRNA) was used as the internal standard as it is present at a reasonably constant level in most tissues. In the quantification experiments, the expression of target genes was normalized to the housekeeping gene *cyclophilin*. The reaction conditions were implemented for 40 cycles of the amplification step using a computer that was connected to the detector. The primers used were listed in Table 1.

Western Blot Analysis of NF-κB, NLRP3, Caspase-1, IL-1β, and NGF

Protein levels of NLRP3, NF-κB, caspase-1, and IL-1β were measured in the myocardium from the border zone at day 3, and the protein level of NGF was measured in the myocardium from the remote zone at day 28. Antibodies to NF-κB (p65) (nuclear fraction; 1:1000, Cat # sc-166748; Santa-Cruz Biotechnology), NLRP3 (1:1000, Cat # sc-66846; Santa-Cruz Biotechnology), cleaved caspase-1 (1:1000, Cat # sc-56036; Santa-Cruz Biotechnology), cleaved IL-1β (1:1000, Cat # sc-23460; Cell Signaling Technology), NGF (1:1000, Cat # AB1528; Chemicon), and β-actin (1:1000, Cat # A1978; Sigma-Aldrich) were used. Western blotting procedures were described previously.[18] Experiments were replicated 3 times and results expressed as the mean value.

Immunohistochemical Studies of NF-κB, NLRP3, CD68, IL-1β, Tyrosine Hydroxylase, Growth-Associated Factor 43, and Neurofilament

To better understand NF-κB functionality, immunohistochemical staining was used using an antibody (Santa-Cruz Biotechnology) against the nuclear localization sequence of the p65 subunit at the day 3 from the border zone. We counted 200 cells in each field of view to measure the ratio of positively stained cells.

To confirm inflammasome activation in the myocardium, double-staining with NLRP3 and IL-1β was performed at the day 3 from the border zone.

During the inflammatory phase of wound healing, macrophages have been shown to be a major source of IL-1β.[24] Therefore, we then double-stained macrophages with IL-1β to evaluate the function of macrophages from the border zone at day 3. Five-μm thick cryosections were prepared and incubated with antibodies against CD68 (a general marker of all macrophages; 1:200, Cat # ab201340; Abcam) and IL-1β (1:100, Cat # sc-23460; Cell Signaling Technology). These antibodies have been tested for specificity in rats. Directly conjugated antibodies with identical isotypes were used as negative controls. The average of 10 random scans per section was used for analysis. The results were quantified as the ratio of positively stained area to the total area at ×400 magnification.

Myocardium from the remote zone at day 28 was subjected to immunofluorescence staining to evaluate the quantification and spatial distribution of sympathetic nerve fibers. For a detailed information regarding staining for tyrosine hydroxylase (1:200, Cat # AB152; Chemicon), growth-associated factor 43 (1:400, Cat # AB5220; Chemicon), and neurofilament (1:1000, Cat # AB5539; Chemicon), please refer to the **Supplemental Digital Content 1** (see **Supplementary material online**, http://links.lww.com/JCVP/A627).

Laboratory Measurements

IL-1β in plasma or myocardial homogenates from the border zone at day 3 after MI was detected with an IL-1β ELISA kit (BD Biosciences, San Diego, CA) according to the manufacturer's instructions.

To assess sympathetic nerve function, norepinephrine levels were measured in the myocardium from the remote

TABLE 1. Forward and Reverse Sequences of RT-PCR Primers

	Primer Sequence ((5′-3′)	
Target Gene	**Forward**	**Reverse**
NF-κB (p65)	CTGGCCATGGACGATCTGTT	GCACTTGTAACGGAAACGCA
NLRP3	CTGCATGCCGTATCTGGTTG	GCTGAGCAAGCTAAAGGCTTC
Caspase-1	AGGAGGGAATATGTGGG	AACCTTGGGCTTGTCTT
IL-1β	GCAATGGTCGGGACATAGTT	AGACCTGACTTGGCAGAGGA
NGF	CAGCTTTCTATCCTGGCCACTC	GAGTCTCCCTCTGGGACATTG
Cyclophilin A	ATGGTCAACCCC ACCGTGTTCTTCG	CGTGTGAAGTCA CCACCCTGACACA

zone. The total level of norepinephrine was assessed using a commercial ELISA kit (Noradrenalin ELISA, IBL Immuno-Biological Laboratories Co, Hamburg, Germany).

Statistical Analysis

The results are presented as mean ± SD. All statistical analyses were conducted using SPSS software (SPSS, version 19.0, Chicago, IL). A 2-way analysis of variance (ANOVA) with infarct size and treatment as factors was performed to compare groups, followed by the Scheffe's method for multiple comparisons. Electrophysiological data (programmed electrical stimulation-induced arrhythmia scores) were compared using the Kruskal–Wallis test followed by the Mann–Whitney test. The significance level was assumed at a P value of <0.05.

RESULTS

Part 1: Acute Stage (Day 3)

There were no significant differences in infarct size between the 2 groups at the acute stage. Please refer to the **Supplemental Digital Content 1** (see **Supplementary Table online**, http://links.lww.com/JCVP/A627).

Effect of Taurine on NF-κB

The effect of taurine on NF-κB activation was examined in the nuclear fraction of the myocardium at the border zone. The nuclear level of NF-κB protein was significantly increased in the vehicle-treated infarcted group compared with sham (Fig. 1A). Compared with vehicle, taurine administration in the infarcted group significantly reduced NF-κB protein but not statistically significant reduction of NF-κB protein in the taurine-sham group compared with that in the sham-saline group. Interaction between taurine and MI was significant ($P < 0.001$, two-way ANOVA). Similarly, the changes of NF-κB mRNA expression showed the same trend to the protein (Fig. 1B).

Immunohistochemically, activation of NF-κB can be visualized by the translocation of p65 from the cytoplasm to the nucleus. NF-κB was scantly expressed in the myocardial cell nuclei in the sham. After MI, cardiomyocytes were stained positively for NF-κB in the nucleus (Fig. 1C). By contrast, taurine administration significantly reduced the cell ratio of positively stained cells.

Effect of Taurine on NLRP3 Inflammasome

We evaluated the protein and mRNA expression of NLRP3 inflammasome components by Western blot and RT-PCR. In the vehicle-treated rats, the levels of NLRP3 protein and caspase-1 protein (Fig. 2A) were significantly increased compared with that of the sham, indicating that the NLRP3 inflammasome was activated. Both NLRP3 and caspase-1 levels were inhibited by taurine treatment.

NLRP3 and *caspase-1* mRNA were significantly increased in the vehicle-treated infarcted group compared with sham (Fig. 2B). The expression of *NLRP3* and *caspase-1* mRNA was significantly reduced after adding taurine compared with vehicle.

In this study, plasma and myocardial IL-1β levels assessed by ELISA were significantly increased after MI, which can be reduced after administering taurine (Figs. 2C, D). Accordingly, the protein levels of the active form of IL-1β showed a robust decrease after administering taurine in the

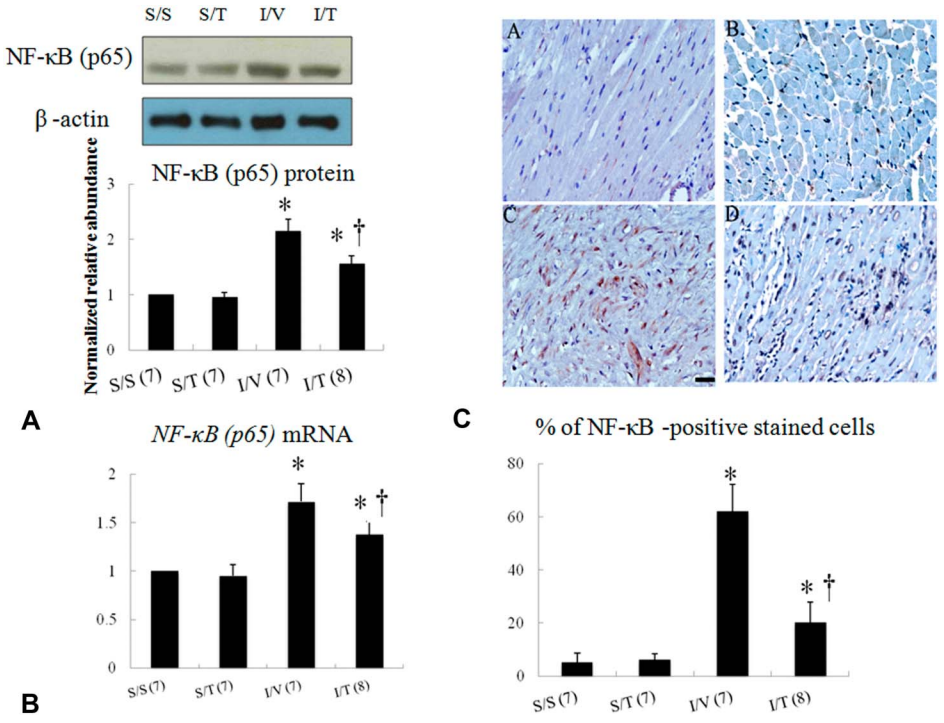

FIGURE 1. Effect of taurine on NF-κB(p65) at day 3 from the border zone. The protein (A) and mRNA (B) of NF-κB(p65) were significantly decreased after adding taurine. Relative abundance was obtained against that of β-actin. Results are mean ± SD of 3 independent experiments. C. The nuclear translocation of NF-κB(p65) was semi-quantitatively measured by counting 200 cells in each field of view for the ratio of positively stained cells (brown). The number of animals in each group is indicated in parentheses. The line length corresponds to 50 μm. Each column and bar represents mean ± SD. S/S, sham treated with saline; S/T, sham treated with taurine; I/V, infarcted rats treated with saline; I/T, infarcted rats treated with taurine. *$P < 0.05$ compared with S/S and S/T; †$P < 0.05$ compared with I/V by 2-way ANOVA followed by the Scheffe's method.

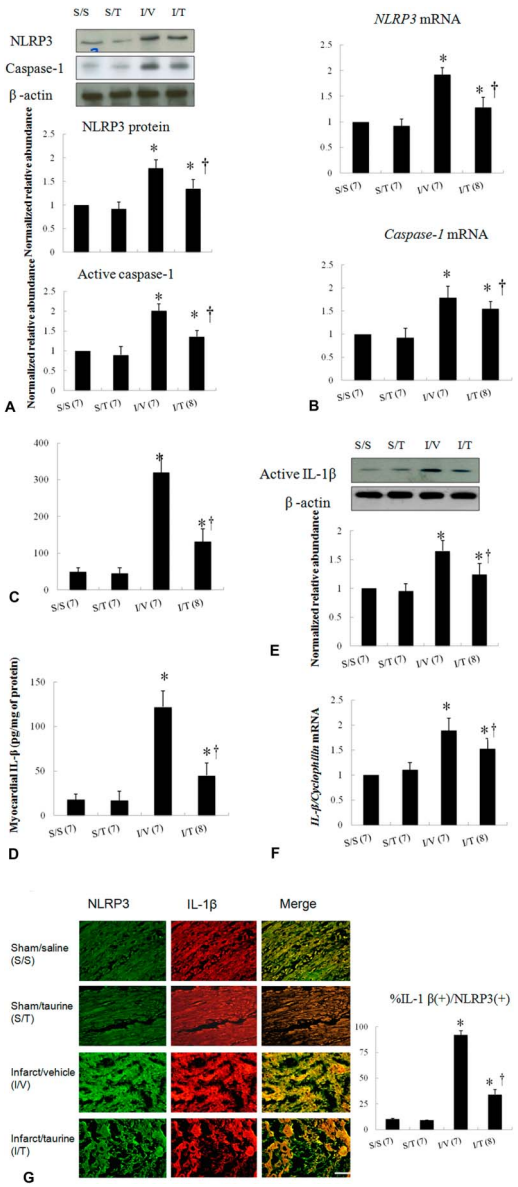

FIGURE 2. Effect of taurine on NLRP3 inflammasome at day 3 from the border zone. The protein (A) and mRNA (B) of NLRP3 and cleaved caspase-1 p20 were significantly increased after infarction, which can be inhibited after taurine administration. MI resulted in an increase in plasma (C) and myocardial (D) IL-1β. Treatment with taurine significantly reduced the active IL-1β protein (E) and mRNA levels (F). Relative abundance was obtained against that of β-actin. Results are mean ± SD of 3 independent experiments. G, immunofluorescence analysis of the expression of NLRP3 and IL-1β. Representative immunostaining images show the NLRP3/IL-1β double labeling. The intensity of the NLRP3/IL-1β double labeling is significantly reduced in taurine-treated infarcted rats. Quantitative analysis of the ratio of NLRP3/IL-1β colabeling areas to total areas. The number of animals in each group is indicated in parentheses. The line length corresponds to 20 μm. Each column and bar represents mean ± SD. S/S, sham treated with saline; S/T, sham treated with taurine; I/V, infarcted rats treated with saline; I/T, infarcted rats treated with taurine. $*P < 0.05$ compared with S/S and S/T; $\dagger P < 0.05$ compared with I/V.

infarcted group, as demonstrated by Western blot analysis (Fig. 2E). Taurine treatment also reduced the mRNA levels of IL-1β (Fig. 2F).

To assess whether the NLRP3 inflammasome is involved in the IL-1β regulation, we used immunohistochemistry with NLRP3 and IL-1β. Figure 2G shows more intense NLRP3 and IL-1β costaining in infarcted hearts, implying that IL-1β mediates most of the downstream effects of activated NLRP3 inflammasomes. A subsequent analysis of the effect of taurine indicated that double-labeling of NLRP3 and IL-1β can be significantly reduced compared with vehicle. These data mirrored the results of Western blot.

Effect of Taurine on Macrophage Infiltration

To assess the role of macrophage in IL-1β release, infarct sections were colabeled for IL-1β and the macrophage marker CD68 at day 3 after infarction (Fig. 3). The infarcted rats treated with vehicle showed a significant increase in the number of infiltrating macrophages in the infarct. Furthermore, the vehicle-treated hearts displayed marked IL-1β increase in areas of macrophage infiltration, whereas taurine-treated hearts had significant decrease in the number of infiltrating macrophages and IL-1β levels in the infarct area. The results indicated that the NLRP3 inflammasome may play an important role in the recruitment and chemotaxis of infiltrating macrophages during MI, and this was partially mediated by the release of IL-1β.

Part 2: Chronic Stage (Day 28)

There were no significant differences in mortality between the infarcted groups throughout the study. The relative weights of the hearts at the end of the experimental period (12 weeks of age) corrected for body weight are shown in Table 2. Four weeks after infarction, the LV infarcted areas were very thin and had been completely replaced by fully differentiated scar tissue. There was no significant change in LV weight inclusive of the septum among the infarcted groups during the 4 weeks of the study. However, the ratio of lung weight to body weight, a measure of lung edema, was significantly lower in the taurine group compared with the vehicle group. In addition, $+dp/dt$ and $-dp/dt$ were significantly higher in the taurine group than in the vehicle group. There were no significant differences in infarct size, LV end-diastolic pressure, or LV end-systolic pressure between the infarcted groups.

Effect of Taurine on NGF Protein and mRNA

NGF regulation in the rat hearts was investigated in the in vivo study 28 days after infarction. Western blot analysis showed significantly upregulated NGF levels by 2.15-fold at the remote zone in the vehicle-treated infarcted rats compared with the sham-operated rats ($P < 0.001$, Fig. 4A). Compared with the vehicle group, the NGF levels at the remote zone in the taurine group were significantly lower. PCR amplification of the cDNA revealed that the NGF mRNA levels at the remote zone were upregulated by 2.23 fold in the vehicle group compared with the sham-operated group ($P < 0.001$, Fig. 4B). In addition, the NGF mRNA levels were significantly lower in the taurine group compared with the vehicle group.

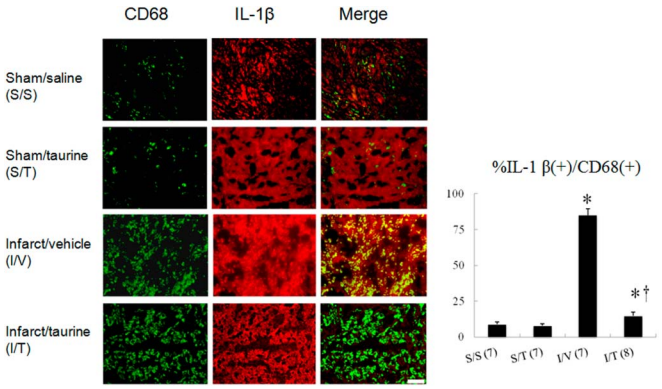

FIGURE 3. Effect of taurine on macrophage infiltration. Immunohistochemical staining of CD68 and IL-1β at day 3 after MI from the border zone. IL-1β–expressing CD68 (+) macrophages were observed in infarcted myocardium treated with vehicle (I/V) but were significantly reduced by taurine (I/T). The IL-1β-expressing CD68 (+) macrophages were calculated and expressed as bar graphs. The number of animals in each group is indicated in parentheses. The line length corresponds to 20 μm. Each column and bar represents mean ± SD. *$P < 0.05$ compared with vehicle. S/S, sham treated with saline; S/T, sham treated with taurine; I/V, infarcted rats treated with saline; I/T, infarcted rats treated with taurine. *$P < 0.05$ compared with S/S and S/T; †$P < 0.05$ compared with I/V.

Effect of Taurine on Cardiac Sympathetic Innervation

To investigate cardiac sympathetic hyperinnervation after infarction, we assessed the sympathetic nerve anatomy and function using immunofluorescence analyses and analyzing levels of myocardial norepinephrine. The results showed that tyrosine hydroxylase–immunostained nerve fibers were oriented along the longitudinal axis of adjacent myofibers (Fig. 5A). In addition, the density of tyrosine hydroxylase–positive nerves was significantly higher in the vehicle group than in sham-operated group. Moreover, the taurine group had a significantly lower nerve density at the remote regions than the vehicle group after infarction (0.25 ± 0.10% in the taurine group vs. 0.42 ± 0.06% in the vehicle group, $P < 0.001$). Similar to the tyrosine hydroxylase results, growth-associated protein 43-positive (Fig. 5B) and neurofilament-positive (data not shown) nerve densities were significantly higher in the taurine group compared with the vehicle group.

The administration of taurine did not influence the basal tissue concentrations of norepinephrine in the sham-operated group (data not shown). In addition, the levels of norepinephrine in the left ventricle were significantly higher by 2.56 fold at the remote zone in the vehicle group than in the sham-operated group (2.94 ± 0.25 vs. 1.15 ± 0.21 μg/g protein, $P < 0.0001$, Table 2). Moreover, the levels of norepinephrine in the left ventricle were significantly lower at the remote regions in the taurine group than in the vehicle group. These results were consistent with those of the immunofluorescent studies.

Effect of Taurine on Arrhythmias

We then performed ventricular pacing to further investigate the physiological effect of attenuated sympathetic hyperinnervation. The arrhythmia score in the sham-operated group was very low (0.2 ± 0.3) (Fig. 5C), whereas ventricular tachyarrhythmias including ventricular fibrillation and tachycardia could be inducible by programmed stimulation in the vehicle group. Compared with vehicle treatment, the administration of taurine significantly reduced the inducibility of ventricular tachyarrhythmias.

Part 3: Ex vivo

To differentiate the relative contribution of 2 anti-inflammation pathways to taurine-mediated inhibition of

TABLE 2. Cardiac Morphology, Hemodynamics, and Tissue NE Concentrations at the End of Study (28 Days After MI)

	Sham		Infarction		
Parameters	**Saline**	**Taurine**	**Vehicle**	**Taurine**	***p* (interaction)**
No. of rats	10	9	10	12	
Body weight, g	372 ± 15	388 ± 16	389 ± 16	383 ± 15	0.725
Heart rate, bpm	388 ± 20	391 ± 15	398 ± 19	405 ± 19	0.261
LVESP, mm Hg	105 ± 7	106 ± 6	102 ± 8	104 ± 7	0.352
LVEDP, mm Hg	5 ± 1	5 ± 2	18 ± 4*	15 ± 6*	<0.001
+dp/d*t*, mm Hg/s	7864 ± 294	7642 ± 323	2543 ± 228*	3054 ± 269*†	<0.001
-Dp/d*t*, mm Hg/s	6589 ± 265	6894 ± 282	2242 ± 254*	2699 ± 252*†	<0.001
Infarct size, %	—	—	42 ± 3	41 ± 4	0.792
LVW/BW, mg/g	2.05 ± 0.18	2.15 ± 0.25	3.25 ± 0.42*	2.99 ± 0.31*	0.024
RVW/BW, mg/g	0.59 ± 0.12	0.63 ± 0.08	0.87 ± 0.12*	0.62 ± 0.11†	0.041
LungW/BW, mg/g	4.25 ± 0.33	4.45 ± 0.38	5.95 ± 0.48*	4.58 ± 0.58†	0.038
LV NE, μg/g protein	1.15 ± 0.21	1.24 ± 0.22	2.94 ± 0.25*	1.65 ± 0.32*†	<0.001

Values are mean ± SD. Two-way ANOVA was performed followed by Bonferroni's post-hoc test.
*$P < 0.05$ compared with respective sham.
†$P < 0.05$ compared with the vehicle-treated infarcted group.
BW, body weight; LungW, lung weight; LVEDP, left ventricular end-diastolic pressure; LVESP, left ventricular end-systolic pressure; LVW, left ventricular weight; RVW, right ventricular weight.

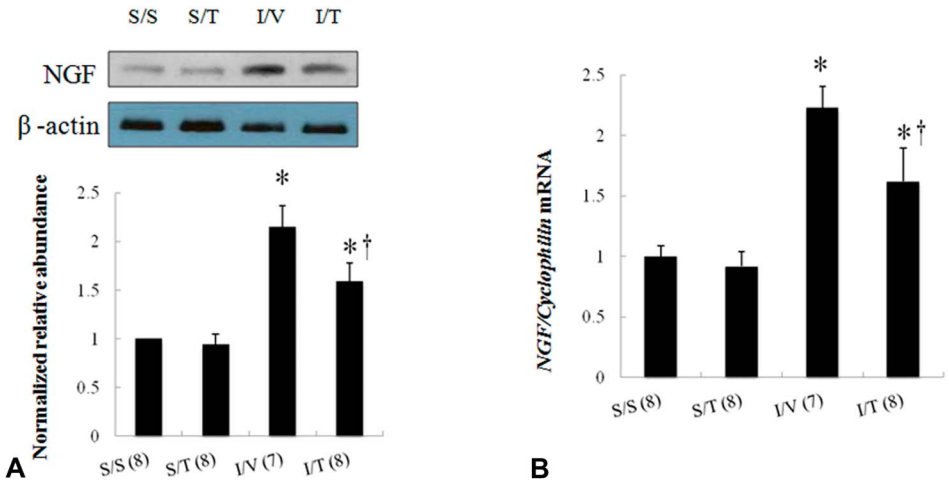

FIGURE 4. Effect of taurine on NGF protein (A) and mRNA (B) at day 28 from the remote zone in the in vivo experiment. The number of animals in each group is indicated in parentheses. Each column and bar represents mean ± SD. S/S, sham treated with saline; S/T, sham treated with taurine; I/V, infarcted rats treated with saline; I/T, infarcted rats treated with taurine. *$P < 0.05$ compared with S/S and S/T; †$P < 0.05$ compared with I/V.

IL-1β expression, we performed ex vivo experiments (Experiment 2). As shown in Figure 6A, addition of either PDTC or CP-456,773 did not have additionally attenuated IL-1β effects compared with taurine alone.

The requirement of IL-1β in the regulation of NGF was shown in the ex vivo study (Experiment 3). As shown in Figure 6B, the administration of IL-1β antibody significantly attenuated NGF levels compared with vehicle.

DISCUSSION

To the best of our knowledge, this is the first report on taurine to evaluate the effect of ventricular arrhythmias during ventricular remodeling after MI. Taurine administration after infarction inhibited NF-κB protein levels and resulted in attenuated NGF expression in a NLRP3 inflammasome/IL-1β–dependent pathway. Our results show that the association between NLRP3 inflammasome and ventricular arrhythmias is anatomically and functionally linked. These results suggest that antiarrhythmic effects of taurine are mostly, if not all, mediated by inhibition of the NLRP3/IL-1β axis. These results are consistent with the beneficial effects of taurine, as documented structurally by analysis of macrophage infiltration and cardiac nerve sprouting; molecularly by myocardial NF-κB, NLRP3, caspase-1, IL-1β, and NGF; biochemically by myocardial norepinephrine; pharmacologically by PDTC and CP-456,773; and functionally by improvement of fatal ventricular tachyarrhythmias. Our results were consistent with the notion that taurine influences inflammation-related markers at the transcriptional level.

The attenuated effect of taurine on sympathetic reinnervation was demonstrated by the inhibition of the NLRP3/IL-1β axis. Our conclusions are supported by 3 lines of evidence (Fig. 7).

1. The NLRP3 inflammasome was activated after infarction. This study demonstrates the NF-κB(p65) expression is enhanced after MI, in a correlation with activation of NLRP3 inflammasome. Our results showed that the components of NLRP3 inflammasome in the normal heart were low but significantly upregulated after infarction. In addition, the NLRP3 inflammasome was shown to be a key mediator of inflammatory responses and tissue injury after MI. Although evidence suggests chronic inflammation as an important pathogenetic factor in ventricular remodeling, the role of NLRP3 inflammasome activation in ventricular remodeling is a topic of longstanding debate. Sandanger et al[25] reported that the lack of NLRP3 resulted in a larger MI size after in vivo ischemia/reperfusion. In addition, Jong et al[26] reported that NLRP3 did not play a role in acute MI because of its low cardiac expression. However, our results showed that the activation of the inflammasome seems to be a major danger-sensing pathway to adverse ventricular remodeling after MI. The discrepancy could be explained by the fact that the effect of NLRP3 may vary according to the experimental conditions and model used, such as the degree of stress and inflammatory responses. Indeed, our results are consistent with previous studies which showed that depletion of either the sensor (NLRP3) or effector enzyme (caspase-1) component of the inflammasome complex could prevent its activation and protect the heart from adverse cardiac remodeling.[12]

The cellular sources of IL-1β, the downstream molecule of the NLRP3 inflammasome, within the heart remain unclear at present. IL-1β has been shown to be upregulated and activated soon after MI in various heart cell types and not only infiltrating macrophages, fibroblasts, and endothelial cells but also border zone cardiomyocytes.[10,12,27] Macrophages can detect "danger" signals and are one of the initial responders to tissue injury, consequently initiating inflammatory processes that can protect against injury.[28] Cardiac fibroblasts play crucial roles in both the myocardial fibrosis process and also in the development of cardiac disease because of the production of autocrine/paracrine factors (such as IL-1β). Our findings are consistent with a recent study, showing the formation of the inflammasome mainly in macrophages.[29] Indeed, our results are consistent with the notion that IL-1β

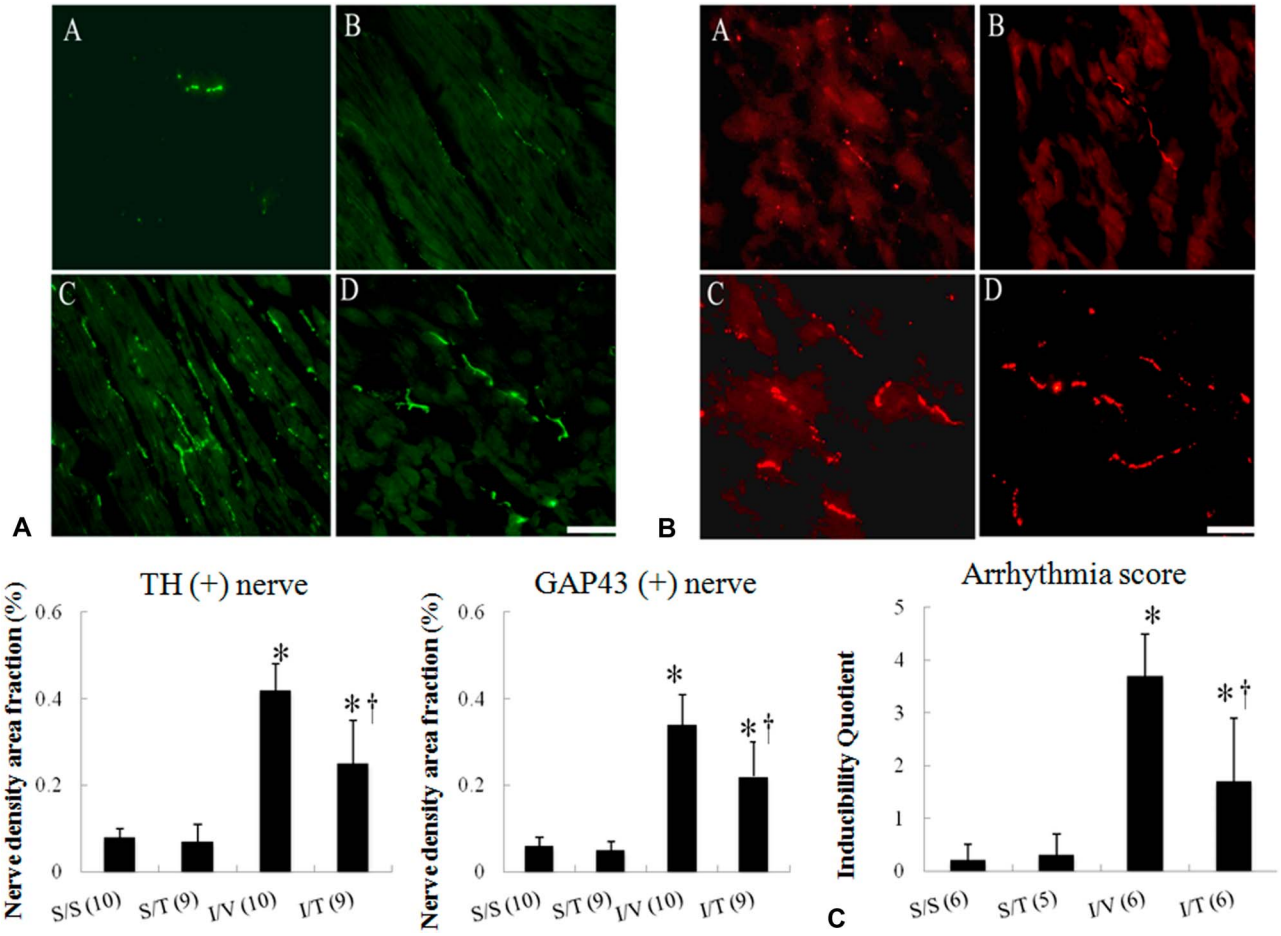

FIGURE 5. Immunofluorescent staining for tyrosine hydroxylase and growth associated protein 43 from the remote regions (magnification ×400) at day 28 after MI from the remote zone. A, Tyrosine hydroxylase. Tyrosine hydroxylase–positive nerve fibers were located between myofibrils and were oriented in a longitudinal direction as with the myofibrils. B, Growth-associated protein 43. Bar = 50 μm. S/S (A), sham treated with saline; S/T (B), sham treated with taurine; I/V (C), infarcted rats treated with saline; I/T (D), infarcted rats treated with taurine. C, An ex vivo model showing the inducibility quotient of ventricular arrhythmias by programmed electrical stimulation 4 weeks after MI. The number of animals in each group is indicated in parentheses. Each column and bar represents mean ± SD. *$P < 0.05$ compared with S/S and S/T; †$P < 0.05$ compared with I/V.

promotes the polarization of macrophages toward the M1 proinflammatory phenotype thereby increasing inflammatory responses.[30] Our results explained at least in part the ability of M1 macrophage depletion by clodronate liposomes to prevent sympathetic hyperinnervation.[31]

2. NLRP3 inflammasome inhibition ameliorated sympathetic sprouting by the IL-1β pathway. Experimental studies have demonstrated the postinfarction causative effects of inflammation on sympathetic reinnervation, whereas inhibition of inflammation with the use of anti-inflammatory agents has been shown to impede this process and improve nerve sprouting and sympathetic hyperinnervation.[6] Previous studies have shown that the inhibition of inflammatory responses with the use of dexamethasone[30] and resveratrol[32] can attenuate sympathetic hyperinnervation after MI. However, these drugs only block certain inflammatory factors, and other inflammatory factors or pathways may be involved. Accordingly, the upstream blockade of the inflammatory cascade caused by the NLRP3 inflammasome may be a feasible and effective method to improve sympathetic hyperinnervation after MI. The role of NLRP3 inflammasome was highlighted because the increase in NGF production could be attenuated by treatment of the hearts with anti–IL-1β blocking antibody.
3. Taurine administration was associated with attenuated sympathetic reinnervation and pacing-induced arrhythmias. Our results showed that the combined treatment with CP-456,773 and taurine did not further attenuate IL-1β levels over taurine alone, suggesting that both share a common pathway to regulate IL-1β after MI, and the role of NLRP3 inflammasome inhibition may be more important than taurine haloamine in reducing taurine-mediated IL-1β levels. Increasing clinical evidence has suggested that patients with arrhythmia after MI have

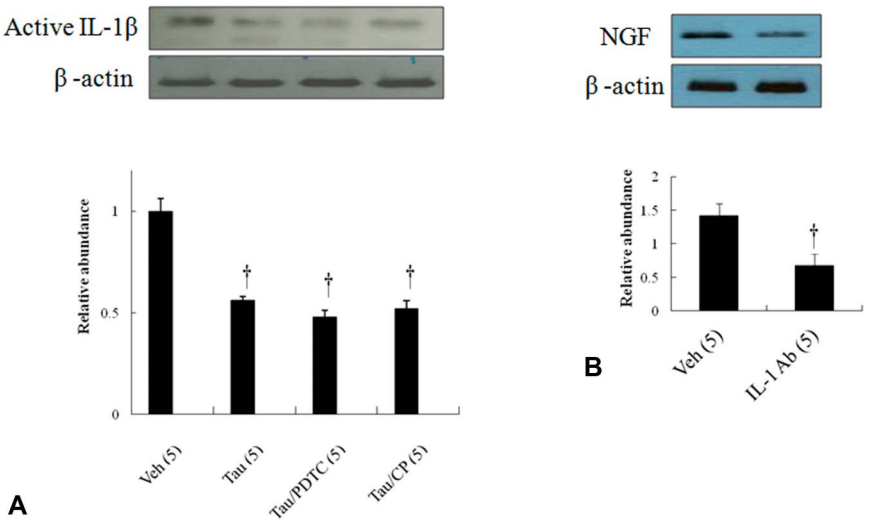

FIGURE 6. A, (Experiment 2). Western blot shows that addition of either PDTC or CP-456,773 did not have additionally attenuated IL-1β effects compared with taurine alone. B, (Experiment 3). Western blot shows that the administration of IL-1β antibody significantly attenuated NGF levels. Relative abundance was obtained against that of β-actin. Results are mean ± SD of 3 independent experiments. The number of animals in each group is indicated in parentheses. $*P < 0.05$ compared with the vehicle-treated infarct group.

higher circulating levels of inflammatory cytokines compared with patients without arrhythmia after MI.[33,34] In addition, even in situations without MI or structural heart diseases, systemic inflammation has been associated with a significantly higher risk of ventricular tachyarrhythmias.[35] Our results support these clinical observations and suggest possible mechanisms between electrophysiological remodeling and the inflammatory phase after MI.

Other Mechanisms

Although our results suggest that the taurine-induced reduction in arrhythmias may be due to a reduction in inflammation and the expression of NGF, other mechanisms may also contribute the beneficial effects of taurine, such as the direct effect of IL-1β on arrhythmias. The direct electrophysiological effects of the downstream molecule of NLRP3 inflammasome, IL-1β, are well characterized in isolated cardiac myocytes from normal hearts and from those of mice treated with the IL-1 receptor antagonist.[27,36] Overexpression of IL-1β prolongs the action potential duration, reduces repolarizing K^+ currents, and increases calcium sparks in cardiomyocytes, which induces electrical vulnerability.[27,36] In addition, the role of IL-1β in slow conduction and cell–cell uncoupling after MI through the degradation of the ventricular gap junction protein connexin 43 has been proposed.[37] Both important factors contribute to arrhythmogenesis.

Clinical Implications

From a clinical standpoint, taurine may represent a completely novel approach in the treatment of postinfarction arrhythmias. Although taurine is presently not used in the treatment of cardiac arrhythmias, our results are important given that around one-third of cardiovascular-related post-MI deaths have been attributed to sudden cardiac death because of ventricular arrhythmias.[38]

First, ion channel blockers have been used to prevent arrhythmias in the post-MI setting which have been largely unsuccessful in treating ventricular arrhythmias and are discontinued because of dangerous proarrhythmic effects.[39] Furthermore, this approach is focused on treating the symptom (arrhythmia) rather than treating the underlying cause

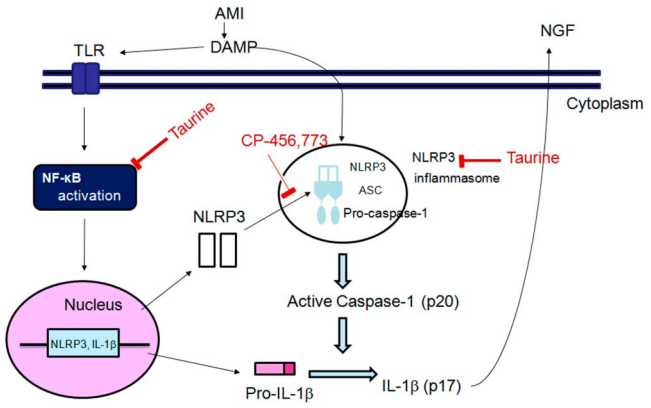

FIGURE 7. Schematic representation illustrates the involvements of taurine in NGF-related sympathetic innervation in postinfarcted rats. Activation of the NLRP3 inflammasome, which is composed of NLRP3, ASC, and procaspase-1, is tightly controlled by 2 signals. The first "priming" signal, includes MI-mediated DAMP and results in enhanced expressions of inflammasome components and target proteins by activating the transcription factor NF-κB. The second "activation" signal activates NLRP3, which then recruits the ASC scaffolding protein and procaspase-1 leading to homodimerization and the autocatalytic activation of caspase-1. Active caspase-1 cleaves pro–IL-1β into the active isoform. Taurine suppresses the NLRP3 protein, thus preventing assembly of the inflammasome. Taurine attenuates NGF expression through IL-1β inhibition. Inhibition of these signaling pathways by their respective inhibitors is indicated by the vertical lines. TLR, toll-like receptor; DAMPs, danger-associated molecular patterns.

(electrophysiological and structural remodeling). Targeting the post-MI reparative phase or inflammation initially may be a more appropriate antiarrhythmic approach.

Second, considering the strong proinflammatory activity of IL-1β, the activation of inflammasomes must be tightly regulated. The inflammasome occupies a central role in the inflammatory response after MI. The degree of the inflammatory response during MI predicts the clinical outcome in patients with MI, with those patients having more inflammation showing higher rates of heart failure or death. However, nonsteroidal anti-inflammatory drug treatment has been associated with detrimental clinical outcomes, and current guidelines advise that they should not be used in patients after MI.[40] Therefore, more specific inflammatory signaling targeting seems to be required to avoid suppressing beneficial functions.

Third, patients remain at a high-risk of recurrent cardiovascular events and mortality after acute MI.[41] The role of IL-1β in mediating in postinfarction remodeling may explain in part that in the trial Canakinumab Anti-inflammatory Thrombosis Outcomes Study (CANTOS). Ridker et al[42] demonstrated that administration of canakinumab limits recurrent events in stable post-MI patients who have residual inflammation.

STUDY LIMITATIONS

In this study, we explored the potential clinical efficacy of taurine in the treatment of patients after MI. However, its clinical application may be limited by several factors. First, our post-MI remodeling and arrhythmogenesis experiments were performed in otherwise healthy animals, which did not reflect the underlying systemic inflammation and clinical pathophysiology in patients after MI. Previous studies have shown that IL-1β is upregulated ~3-fold in post-MI atherosclerotic versus post-MI wild-type mice.[43] Second, inflammation-related signaling pathways significantly differ between small and larger mammals.[44] Thus, small animal models used in this study could not be applied to humans. Finally, all of our experiments were conducted using pharmacological inhibitors, which potentially have many nonspecific targets. It would be interesting to further evaluate the in vivo relationship between the NLRP3 pathway and the antiarrhythmic effect of taurine using an *NLRP3*-knockout animal model.

CONCLUSIONS

This study may provide new insight into the mechanisms by which taurine reduces the inducibility of ventricular arrhythmias after infarction, including attenuating sympathetic reinnervation and targeting the NLRP3/IL-1β response. Therefore, signaling associated with NLRP3/IL-1β transactivation in vivo and further regulation of NGF protein may be an appropriate approach to develop therapeutic strategies for antiarrhythmias after infarction.

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