Transient Receptor Potential Vanilloid Gene Deletion Exacerbates Inflammation and Atypical Cardiac Remodeling After Myocardial Infarction

Wei Huang, Jack Rubinstein, Alejandro R. Prieto, Loc Vinh Thang, Donna H. Wang

Abstract—The transient receptor potential vanilloid (TRPV1) channels expressed in sensory afferent fibers innervating the heart may be activated by protons or endovanilloids released during myocardial ischemia (MI), leading to angina. Although our previous in vitro data indicate that TRPV1 activation may preserve cardiac function after ischemia-reperfusion injury, the underlying mechanisms are largely unknown. To test the hypothesis that TRPV1 modulates inflammatory and early remodeling processes to prevent cardiac functional deterioration after myocardial infarction, TRPV1-null mutant (TRPV1−/−) and wild-type (WT) mice were subjected to left anterior descending coronary ligation or sham operation. The infarct size was greater in TRPV1−/− than in WT mice (P<0.001) 3 days after MI, and the mortality rate was higher in TRPV1−/− than in WT mice (P<0.05) 7 days after MI. The levels of plasma cardiac troponin I; cytokines, including tumor necrosis factor-α, interleukin-1β, and interleukin-6; chemokines, including monocyte chemoattractant protein-1 and macrophage inflammatory protein-2; and infiltration of inflammatory cells, including neutrophils, macrophages, and myofibroblasts; as well as collagen contents, were greater in TRPV1−/− than in WT mice (P<0.05) in the infarct area on days 3 and 7 after MI. Changes in left ventricular geometry led to increased end-systolic and -diastolic diameters and reduced contractile function in TRPV1−/− compared with WT mice. These data show that TRPV1 gene deletion results in excessive inflammation, disproportional left ventricular remodeling, and deteriorated cardiac function after MI, indicating that TRPV1 may prevent infarct expansion and cardiac injury by inhibiting inflammation and abnormal tissue remodeling. (Hypertension. 2009;53:243-250.)

Key Words: transient receptor potential vanilloid subtype ■ myocardial infarction ■ inflammation ■ early remodeling ■ transgenic animal model

The transient receptor potential vanilloid (TRPV1) receptor is a ligand-gated nonselective cation channel, primarily expressed in sensory nerves innervating the heart and blood vessels.1,2 TRPV1 may function as a molecular integrator of multiple chemical and physical stimuli, including protons, noxious heat, endovanilloids, and capsaicin.3,4 Myocardial ischemia causes the release of protons and bradykinin, which may activate or sensitize TRPV1 expressed in cardiac sensory nerve terminals, including unmyelinated C-fibers and thinly myelinated Aδ-fibers, to cause angina.5,6 Indeed, ischemic stimulation of cardiac afferent nerves has been shown to be mediated by activation of TRPV1.1

Using the isolated, perfused Langendorff heart preparation, we showed that TRPV1 protects the heart from postischemic reperfusion injury possibly via increasing substance P release from sensory nerve terminals.7 Moreover, TRPV1 contributes to the beneficial effects of preconditioning of the heart against ischemia-reperfusion injury via triggering the release of substance P and/or calcitonin gene-related peptide.8 It has been shown that patients with preinfarction angina have a better prognosis after acute infarction than those without, a phenomenon ascribed to ischemic preconditioning.9 However, it is unknown whether TRPV1, a molecular transmitter of pain, plays a protective role against myocardial infarction (MI) in vivo.

MI is accompanied by an inflammatory process, which is a prerequisite for healing and scar formation.10 The degree of the inflammatory response is also a key determinant of the host’s outcome.11 The initial healing phase of the acute MI is characterized by mononuclear and fibroblast cell infiltration in the absence of polymorphonuclear leukocytes, and the later healing and remodeling processes are intertwined.12 TRPV1 has been shown to play a protective role against endotoxin-induced inflammation and to facilitate wound healing in the cornea after injury.13,14 TRPV1-induced neuropeptide release, including calcitonin gene-related peptide and substance P, has also been shown to be involved in inflammation in a manner mediating distinct effects.15,16 However, it is unknown whether TRPV1 plays a role in the cardiac inflammatory process after MI, and, if so, how it affects cardiac healing and remodeling.

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Patients with asymptomatic MI show a higher mortality than those with symptoms. Increased understanding of the role of cardiac TRPV1-positive sensory nerves in MI would help in defining whether TRPV1 merely transmits a warning sign that a more severe attack is about to happen or whether its activation protects the heart from MI injury by modulating cardiac inflammatory and healing processes. This study tests the hypothesis that TRPV1 regulates the inflammatory process and early remodeling to prevent cardiac functional deterioration after MI.

Methods

Animals and Surgical Procedures
Ten-week-old male TRPV1 knockout (TRPV1−/−) or wild-type (WT) mice (Jackson Laboratory, Bar Harbor, Maine) were subjected to either a proximally left anterior descending ligation or a sham operation. Mice were anesthetized with pentobarbital (50 mg/kg IP) and ventilated with room air using a MiniVent mouse ventilator (Hugo Sachs Elektronik). Incision was made in the fourth intercostal space to open the pericardium. MI was induced by a permanent left anterior descending ligation with a 7-0 Prolene suture (Ethicon) and confirmed under a dissecting microscope (Olympus SZ30) by the discoloration of the ischemic area. Sham-operated animals underwent the same procedure except for left anterior descending ligation. After surgery, mice were inspected ≥3 times daily to check for mortality and causes of death. The surviving animals were randomly measured for cardiac function and subsequently killed on day 3 or 7 after surgery.

Risk Area and Infarct Size
Evans blue dye (1%) was perfused into the aorta and coronary arteries and stained the nonischemic area blue. Hearts were excised, sliced into 5 cross-sections below the ligature, weighed, incubated in a 1% triphenyltetrazolium chloride solution, and photographed for infarct size calculation. The total left ventricular (LV) area, risk area, and infarct area were determined using computer assisted software (National Institutes of Health Image) and multiplied by the weight of the section. Three ratios were obtained: infarct area:area at risk, infarct area/left ventricle, and area at risk/left ventricle.

Immunohistochemical Studies
The mouse hearts were dissected, fixed in 4% formaldehyde solution, and embedded in paraffin. Sections (4 to 5 µm) were stained with the following antibodies: rat antimonocle neutrophil antibody (AbD Serotec) or rat antimonocle macrophages antibody (Cedarlane). After rinsing, slides were incubated with either antineutrophil (1:75) or anti–Mac-2 (1:100) and incubated with a biotinylated rabbit antirabbit secondary antibody (1:100, Vector Laboratories). For the staining of myofibroblasts, slides were incubated with primary mouse antimonytoblast mixture (primary antibody, secondary antibody, and normal mouse serum at a 1:200, 1:1000, and 1:50 dilution, respectively, Vector Laboratories), avidin alkaline phosphatase solution (KPL, Kierkegaard Perry Laboratories), and then vector fast red (substrate kit 1, Vector Laboratories). Masson’s trichrome was performed according to a standard protocol.

Quantitative Analysis
All of the quantitative analyses were performed by ≥2 independent investigators on blind specimens. Neutrophils, macrophages, and myofibroblasts were counted in stained sections. To determine the average cellular density (cells per millimeter squared), the stained cells in the peri-infarction zone were counted in 10 different fields of 40× objective.

Determination of Plasma Cardiac Troponin I
Before each mouse was euthanized, each was injected with heparin, and the blood was collected. The plasma concentration of cardiac troponin I (cTnI) was measured as an index of cardiac cellular damage by using the quantitative rapid assay kit (Life Diagnostics).

Determination of Tissue Cytokines and Chemokines by ELISA Assay
Protein levels of cytokines, including tumor necrosis factor (TNF)-α, interleukin (IL)-6, and IL-1β, and chemokines, including monocyte chemotactic protein 1 and macrophage inflammatory protein-2 in the myocardium, were determined using various ELISA kits (R&D Systems). Tissue samples of myocardium were homogenized in ice-cold PBS buffer containing protease inhibitor mixture (50 mg wet weight per milliliter), and total proteins were extracted using NE-PER Cytoplasmic Extraction Reagents (Pierce). Total protein concentration (picograms per milligram of total tissue protein) was determined using a Bio-Rad Protein Assay (Bio-Rad Laboratories).

Hydroxyproline Assay for Collagen Content
The collagen content of the myocardial tissue was determined by the hydroxyproline assay. Tissue was freeze-dried, weighed, homogenized in 0.1 mol/L of NaCl and 5 mmol/L of NaHCO3, washed 5 times with the same solution, and hydrolyzed in 0.5 mL of 6 N HCl. Samples were filtered, vacuum dried, and then dissolved in distilled water. The hydroxyproline content was determined with a colorimetric assay by the protocol described in Peng et al using 0.5 to 5.0 µg of hydroxyproline as a calibrated curve, and the data were expressed as micrograms of collagen per milligram of dry weight, assuming that collagen contains an average of 13.5% hydroxyproline.

Transthoracic Echocardiography
Three or 7 days after MI, the mice were reanesthetized with pentobarbital (50 mg/kg IP) and placed on a heating pad. Echocardiography was performed using a GE Vivid 7/3Color ultrasound machine (General Electric) with a 10-MHz transducer applied parasternally to the shaved chest wall. The following parameters were measured as indicators of function and remodeling: LV internal diameter in diastole (LVIDd), LV internal diameter in systole (LVIDs), and posterior wall thickness, and the ejection fraction was directly calculated with integrated software using the Teicholz formula derived from a fractional shortening measurement (LVIDd−LVIDs/LVIDd×100).

Statistical Analysis
All of the values were expressed as means±SEs. The differences among groups for the percentages of survival rate and the area at risk were analyzed using 1-way ANOVA, followed by Bonferroni adjustment for multiple comparison. The data from the echocardiography studies; the plasma cardiac troponin I; the levels of inflammatory cells; and cytokine, chemokine, and collagen content were analyzed by 2-way ANOVA, followed by Bonferroni adjustment for multiple comparisons. Differences were considered statistically significant at P<0.05.

Results

Lower Survival Rate in TRPV1−/− Mice After MI
All of the sham-operated mice in both strains survived until the end of the experimental period. In contrast, as shown in Figure 1, TRPV1−/− mice had a significantly lower survival rate after MI compared with that of WT mice. The most frequent cause of death in both TRPV1−/− and WT mice was LV rupture.

Increased Infarct Size in TRPV1−/− Mice After MI
Figure 2 shows that left anterior descending ligation produced a larger infarct size in the LV wall in TRPV1−/− mice compared with WT mice (Figure 2A). Quantitative analyses
plasma cTnI levels remained elevated in TRPV1−/− mice compared with WT mice, as determined with the Kaplan-Meier method. Log-rank P<0.0001.

Augmented Inflammation Infiltration in TRPV1−/− Mice After MI

Figure 3 shows that plasma cTnI levels were increased 3 days after MI in both TRPV1−/− and WT mice, with a higher level in the former than latter. On day 7 after MI, plasma cTnI levels remained elevated in TRPV1−/− but not WT mice.

Augmented Expression of Inflammatory Cytokines and Chemokines in TRPV1−/− Mice After MI

Figure 7 shows upregulated cytokines, including TNF-α, IL-1β, and IL-6, and Figure 8 shows elevated chemokines, including monocyte chemotactic protein-1 and macrophage inflammatory protein-2, in the infarct regions on day 3 or 7 days after MI was increased in both TRPV1−/− and WT hearts, with the former mice having even greater elevation. In contrast to the clearance of neutrophilic infiltrate on day 7, macrophage density was higher in both strains on day 7 compared with day 3 after MI. Enhanced inflammatory cell infiltration observed on days 3 or 7 after MI in TRPV1−/− mice suggests that TRPV1 deficiency might prolong the time course of resolution of the inflammatory process in healing infarct.

Figure 6 shows that cardiac infarcts 3 or 7 days after MI were infiltrated with myofibroblasts and characterized with spindle-shaped α-smooth muscle actin–expressing cells. TRPV1−/− hearts, compared with WT hearts, showed significantly increased myofibroblast density in the peri-infarct myocardium. Similar to changes in macrophage density, myofibroblast density was higher in both strains on day 7 compared with day 3 after MI.
or 7 post-MI in TRPV1−/− and WT mice. The increases in cytokines and chemokines at both time points were significantly higher in TRPV1−/− than in WT mice, with the exception of no significant difference in macrophage inflammatory protein-2 between TRPV1−/− and WT mice on day 7 post-MI (Figure 8B). Cytokine and chemokine levels were attenuated on day 7 compared with day 3 after MI in both TRPV1−/− and WT Hearts.

Enhanced Collagen Deposition in TRPV1−/− Mice After MI

Figure 9 shows that, in both strains, collagen continued to accumulate at the site of infarction from day 3 to day 7 after MI. In the infarct zone itself, the collagen deposition was not different between the 2 strains. However, conspicuously increased collagen deposition was observed in peri-infarct sites in TRPV1−/− compared with WT mice (Figure 9A). Quantitative analysis also showed increased collagen contents in the peri-infarct region in TRPV1−/− compared with WT mice (Figure 9B), and the increases were enhanced on day 7 compared with day 3 post-MI in both strains.

Enhanced Remodeling and Deterioration of Cardiac Function in TRPV1−/− Mice After MI

As shown in the Table, there was no significant difference in body weight or heart rate on days 3 or 7 post-MI among 4 groups. An increase in LV internal dimension and a decrease in ejection factor were evident on day 3 in both TRPV1−/− and WT MI groups compared with sham-operated animals, and they were worse in TRPV1−/− compared with WT mice in the MI groups. Enhanced thickening of posterior wall thickness in diastole/posterior wall thickness in systole and a further increase in LV internal dimension were observed in TRPV1−/− compared with WT mice 7 days after MI, suggesting of exaggerated progression of remodeling and LV dilation in TRPV1−/− MI mice. These geometric changes were accompanied by a further functional deterioration in TRPV1−/−-MI mice, as indicated by progressive decreases in the ejection factor compared with the corresponding WT MI mice on day 7.

Discussion

We demonstrate for the first time that TRPV1 deficiency results in increased mortality, aggravated inflammatory re-
response, enhanced cardiac fibrosis, exaggerated progression of LV remodeling, and deteriorated cardiac function after acute MI. The molecular basis underlying deteriorated cardiac functional and structural changes post-MI may involve intensified inflammatory cell infiltration, cytokine/chemokine production/release, and collagen deposition in TRPV1/−/− mice.

A note of caution that the mechanisms involved in the post-MI inflammation and myocardial remodeling may include complex pathways other than the TRPV1-mediated process.6,9,10 The data generated from global knockout mice may need to be interpreted with caution in that compromised systemic changes may occur, which may affect the disease process or outcome.

Two essential prognostic factors that assess MI recovery deal with the infarct size and LV remodeling. We found that the increase in infarct size in TRPV1/−/− mice was accompanied by a higher mortality rate because of LV ruptures and/or congestive heart failure. Cardiac rupture is an acute fatal complication in the early days after MI, which is primarily associated with matrix metalloproteinase–induced collagen degradation.21 TRPV1 deletion may induce an earlier degradation of existing collagen, contributing to cardiac rupture. Given that collagen contents in the heart are the results of a dynamic balance between collagen synthesis and degrada-

tion,21,22 we are not certain whether increased collagen contents on days 3 and 7 after MI in TRPV1/−/− mice were a compensatory response to an accelerated degradation of collagen or a direct stimulating effect on collagen synthesis.

Consistent with the increased infarct size, TRPV1/−/− mice also presented a higher plasma cTnI level up to day 7 after MI, whereas it returned to the baseline in WT mice. In comparison with higher mammals, mice show a more rapid and transient response, with conversion of necrotic myocardium to granulation tissues mostly completed within a week.23,24 Our results of the plasma cTnI level in WT mice are consistent with these reports, whereas it takes more than a week after MI for the plasma cTnI level to return to normal in human.24

Enlarged infarct size induced by TRPV1 deficiency may involve several cell death pathways. It has been shown that TRPV1 activation inhibits TNF-dependent activation of nuclear factor-κB (NF-κB).25 NF-κB is an ubiquitously expressed dimeric transcription factor involved in a number of biological processes, including inflammation, cell adhesion,
Activation of NF-κB increases tissue injury after acute MI. One possible mechanism contributing to enlarged infarct size is that TRPV1 deletion would remove the inhibition of NF-κB activation, resulting in NF-κB–induced enhancement of inflammatory response and subsequent myocardial damage. Indeed, several cytokines, including TNF-α, IL-1β, and IL-6, as well as chemokines, such as monocyte chemoattractant protein-1, have a κB-binding domain in their promoter sites, and expression of these molecules is governed by NF-κB.28

The consequences of inflammatory processes after MI can be favorable or harmful, with the former leading to healing processes, leading to persistent tissue damage.29,36 It seems paradoxical that TRPV1-deficient mice displayed both excessive collagen deposition in the posterior wall and enlarged LV after MI, when considering that the scar is relatively nondistensible and resistant to deformation.37 The process of ventricular enlargement can be influenced by ≥3 interdependent factors, ie, infarct size, degree of healing, and ventricular wall stresses.38 Given that the infarct area was mostly in the anterior wall, the posterior wall may have compensated with increased thickness in the TRPV1−/− hearts and may contribute to the ventricular enlargement seen. Moreover, in light of the fact that the infarct region is relatively nondistensible and resistant to deformation, the presence of an excess necrosis and apoptosis.11 We observed remarkable tissue remodeling in the TRPV1−/− mice.34,35 Unsuppressed inflammatory response after granulation tissue formation and unlimited expansion of fibrosis to the noninfarct myocardium would prevent the switch from inflammatory to healing processes, leading to persistent tissue damage.29,36

Figure 8. Chemokine expression in infarcted zone. Monocyte chemoattractant protein (MCP-1; A) and macrophage inflammatory protein (MIP-2; B) expression analyzed by ELISA. Results are expressed as means±SEMs; n=8. *P<0.05 vs corresponding sham; #P<0.05 vs WT MI; +P<0.05 vs corresponding day 3.

Figure 9. Collagen deposition in the peri-infarct zone. Top, Masson’s trichrome staining for collagen in the infarct region (×200, blue-stained). Bottom, Quantitative collagen in various groups. n=8; *P<0.05 vs corresponding sham; #P<0.05 vs WT MI; +P<0.05 vs corresponding day 3.
enlarged left ventricle as early as 3 days after MI may exacerbate LV expansion, as noted by an increase in the end-systolic diameter (a powerful predictor of death).38

The extent of LV dilation after MI is an important determinant of risk for major adverse cardiovascular events, including ventricular arrhythmias, heart failure, and death.39

Patients with more extensive LV remodeling are at greater risk for cardiovascular fatalities, including sudden death attributable to ventricular arrhythmias because of slower pulse propagation velocities through myocardium partially replaced by fibrosis where anisotropic re-entry occurs.40

Furthermore, increased collagen deposition changes ventricular compliance, resulting in increased stiffness and altered LV performance, which contributes to increased incidence of congestive heart failure, aneurysm formation, and mortality.21

Thus, uncontrolled cardiac remodeling observed in TRPV1−/− mice would predict a worse prognosis after MI.

Clinical Perspectives

A higher mortality rate occurs among silent MI, particularly in aging and diabetic populations,17,18 where defects in TRPV1-positive sensory nerve function are known.41,42 In contrast, patients with preinfarction angina have a better prognosis after MI than those without, and TRPV1 has been implicated in mediating the pain sensation of angina and the beneficial effect of preconditioning because of preinfarct ischemia. However, direct evidence of TRPV1 playing a key role in inflammation and healing after MI is lacking. This study indicates that TRPV1 may play a protective role in postinfarction healing, possibly via the anti-inflammation mechanism, and may serve as a new target for improving clinical outcomes after acute MI.

Sources of Funding

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Disclosures

None.

References


Table. Echocardiographic Results After MI in WT and TRPV1−/− Mice

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Data are expressed as means±SEMs unless otherwise specified. HR indicates heart rate; BW, body weight; PWthd, posterior wall thickness in diastole; PWths, posterior wall thickness in systole; LVIdt, LV internal diameter in diastole; LVIDs, LV internal diameter in systole; EF, LV ejection fraction.

*P<0.05 vs sham.
†P<0.05 vs WT.
‡P<0.05 vs corresponding day 3.


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