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Preservation of Cardiac Function and Attenuation of Remodelling in Transient Receptor Potential Vanilloid 4 Knockout Mice Following Myocardial Infarction

Gregory H Turner[1], **Weike Bao**[2*], **Beat M Jucker**[2], **John J Lepore**[2], **Robert N Willette**[2], **and Kevin S Thorneloe**[2]

[1]*BNI-ASU Center for Preclinical Imaging, Barrow Neurological Institute, St. Joseph's Hospital and Medical Center, Phoenix, AZ, USA*

[2]*Heart Failure Discovery Performance Unit, Metabolic Pathways and Cardiovascular Unit, GlaxoSmithKline Pharmaceuticals, King of Prussia, PA, USA*

[*]**Corresponding author**: Bao W, Heart Failure Discovery Performance Unit, Metabolic Pathways and Cardiovascular Unit, GlaxoSmithKline Pharmaceuticals, King of Prussia, PA-19406, USA, Tel: 6109522564; E-mail: Weike.2.Bao@gsk.com

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Abstract

Transient receptor potential channel vanilloid 4 (TRPV4) is a non-selective Ca^{2+} permeable cation channel, recently implicated by computational methods as a key signalling component during myocardial infarction. We previously showed that pharmacological TRPV4 inhibition can prevent and resolve pulmonary edema in pre-clinical heart failure animal models. Here, we examined the impact of genetic deletion of TRPV4 ($TRPV4^{-/-}$) on cardiac function, both basally and following myocardial infarction. Cardiac function was similar in wild type and $TRPV4^{-/-}$ mice under normal conditions. By contrast, following myocardial infarction induced by permanent ligation of the left anterior descending coronary artery, left ventricular systolic and diastolic volumes were reduced and ejection fraction was significantly improved in $TRPV4^{-/-}$ when compared to wild type mice. Consistent with the differences in chamber volumes between $TRPV4^{-/-}$ and wild type mice, myocardial infarction induced a significant increase in heart weight and left ventricular mass index in wild type, but not in $TRPV4^{-/-}$ mice. In a separate cohort of mice, we also investigated the effect of genetic deletion of TRPV4 on infarct size after a 30 min myocardial ischemia and 24 hours reperfusion. There were no differences in myocardial infarct size or area at risk in $TRPV4^{-/-}$ and wild type mice after ischemia/reperfusion injury. These results suggest that TRPV4 does not mediate acute myocardial ischemic injury, but does play an important role in ventricular remodelling known to correlate with poor outcomes following acute myocardial infarction.

Keywords: TRPV4; Magnetic resonance imaging (MRI); Myocardial infarction; Cardiac function

Introduction

The transient receptor potential vanilloid 4 (TRPV4) channel is a polymodal-gated cationic channel that is expressed in a variety of tissues, including various epithelia, inflammatory cells, the heart, and blood vessels [1-5]. TRPV4 contributes to intracellular Ca^{2+} homeostasis and has been implicated in many physiologic mechanisms, including endothelial dependent vasodilation, cell volume regulation, and vascular permeability. TRPV4 activity has been associated with disruption of tight-junctions and increased permeability in cultured epithelial and endothelial cells [5-8]. Furthermore, activation of TRPV4 by increasing vascular [9] or airway [10] pressure in intact lungs promotes formation of pulmonary edema. These observations are consistent with the established pressure-sensitive activation property of the TRPV4 channel [1].

In our previous work, the integrated circulatory action of TRPV4 was explored in multiple species [7]. TRPV4 activation elicited a circulatory collapse associated with disruption of the endothelial permeability barrier in the lung leading to severe pulmonary edema, congestion and cardiac failure. Cardiac failure was hypothesized as secondary to formation of pulmonary edema, as TRPV4 activation was without effect in isolated hearts. However, TRPV4 expression has been detected in heart [11], with little to no expression in cardiac myocytes and localization to the coronary endothelium [7]. Furthermore, TRPV4 channel expression and activity, has been identified in cultured cardiac fibroblasts [12]. More recently, fibroblast TRPV4 activity has been shown to be increased in idiopathic pulmonary fibrosis patients, and is stimulated by enhanced stiffness with an ability to drive pulmonary fibrosis in mice [13].

We have demonstrated that blockade of TRPV4 with a pharmacological TRPV4 antagonist prevents pulmonary edema in rodent models of heart failure [5]. In that study TRPV4 blocker pre-treatment also resulted in a reduced left ventricular dilation post-myocardial infarction (MI), suggesting the potential for an effect of TRPV4 inhibition on the heart. Given these effects of pharmacological inhibition, and the implication that TRPV4 is a key signalling component in the setting of MI by computational approaches [14], here we used an alternative, genetic approach, TRPV4 gene deleting mouse model ($TRPV4^{-/-}$ mouse) to evaluate the role of TRPV4 in ventricular remodelling and myocardial injury.

Material and Methods

In vivo studies: Permanent coronary ligation and myocardial ischemia/reperfusion injury

All animal studies were performed in compliance with the guide for the care and use of laboratory animals as published by the US National Institutes of Health and were approved by the Institutional Animal Care and Use Committee of GlaxoSmithKline. Male $TRPV4^{+/+}$ (WT) and $TRPV4^{-/-}$ [15] mice, 25-30 g and age from 10 to 12 weeks, on a BALB/c AnNCrl background strain (Charles River Laboratories, Wilmington, DE), were utilized.

For permanent coronary ligation, the left anterior descending coronary artery was ligated with 8-0 nylon suture as previously reported [5]. Sham animals underwent a similar surgical procedure without ligation. Animals were anesthetized with Nembutal 60 mg/kg IP and supplemental doses were given as required. The pain reflex was examined by pinching the tail or toe to indicate an appropriate anaesthesia before skin incision. Artificial tear ointment was applied to the mice prior to the surgery to prevent corneal drying. Post-surgery, animals were given saline IP to replace the loss of fluid, were provided nasal oxygen, and kept warm in a 37°C incubator until fully ambulatory, at which point they were returned to their home cages in the animal facility.

To determine whether deletion of the TRPV4 gene would affect vascularization of the left anterior descending coronary artery territory, and/or acute sensitivity to ischemia injury, wild type and TRPV4$^{-/-}$ mice were subjected to a 30 min ischemia followed by 24 hour reperfusion and the area at risk and infarct size were determined by Evans blue and TTC staining respectively as previously described in detail [16].

MRI

One week post-MI (permanent ligation), scans were performed using a 9.4T vertical bore magnet and BioSpec spectrometer (Bruker, Billerica, MA) with an 89 mm imaging gradient set (100 gauss/cm) and a 30 mm whole-body mouse transmit/receive coil. Four groups of mice (WT sham; TRPV4$^{-/-}$ sham; WT with MI ; TRPV4$^{-/-}$ with MI) were induced and maintained under isoflurane (1-2%) anaesthesia in medical grade air while respiration was continually monitored via a pillow sensor positioned under the abdomen (SA Instruments, Stoney Brook, NY).

Fast gradient-echo scout images were acquired in three orthogonal planes covering the heart (TR/TE=137/2.7 ms, 128 × 128 matrix, FOV=6.0 cm, NEX=4) to determine the long axis of the left ventricle. Transverse, bright-blood, fast gradient-echo cine images (TR/TE=6.82/1.8 ms, 128 × 128 matrix, FOV=6.0 cm, 300 reps, 10 movie frames) were acquired in a single plane through the short axis of the left ventricle and were reconstructed using IntraGate (Bruker) retrospective cardiac gating software. Cardiac functional parameters for the left ventricle (left ventricular end diastolic volume LV-EDV; left ventricular end systolic volume LV-ESV; left ventricular ejection fraction LV-EF; and left ventricular mass index LVMI) were calculated from MR images using the Analyze (Biomedical Imaging Resource, Mayo Clinic, Rochester MN) software package. Upon completion of MRI, animals were sacrificed and heart and lung wet weights, and tibia lengths were measured.

Area at risk and infarct size measurement after myocardial ischemia reperfusion injury

In a separate cohort of mice, area at risk and infarct size were measured after a 30 min ischemia and 24 hour reperfusion (as described above) in TRPV4$^{-/-}$ and WT mice. Briefly, at the end of study the mouse heart was excised and perfused with saline to wash out residual blood through an aortic cannula. To delineate infarcted tissues from viable myocardium, the heart was then perfused with a 1% solution of 2,3,5-triphenyltetrazolium chloride (TTC) in phosphate buffer (pH 7.4, 37°C). The viable myocardium stained red, and the infarcted myocardium stained white. To delineate the area at risk (ischemic area), the coronary artery was then tied at the site of the previous occlusion using the same suture that was remained in site after a 30 min LAD occlusion before the aortic root was perfused with a 1% Evans blue dye (Sigma) in normal saline. As a result of this procedure, the portion of the LV supplied by the previously occluded coronary artery (area at risk) was identified by the absence of blue dye, whereas the rest of the LV was stained dark blue. The heart was frozen, after which all atria and right ventricular tissues were excised. The LV was cut into 4-5 transverse slices, which were fixed in 10% neutral buffered formaldehyde. Both sides of the LV slices were photographed using a digital camera. The borders of the infarct, area at risk and area of not at risk of heart images were traced and measured using image-pro plus, and from these measurements area at risk and infarct size were calculated using the following mathematical formulas: Area at risk=sum of area at risk/(sum of area at risk+sum of area not at risk) × 100%; infarct size=(sum of infarct area/sum of area at risk) × 100%.

Parameter	Sham		MI	
	WT	TRPV4$^{-/-}$	WT	TRPV4$^{-/-}$
EF (%)	59 ± 4	57 ± 4	27 ± 2$^{\wedge}$	37 ± 4$^{*\#}$
LVEDV (µl)	60 ± 4	74 ± 7	110 ± 5$^{\wedge}$	76 ± 11$^{\#}$
LVESV (µl)	25 ± 4	32 ± 5	81 ± 5$^{\wedge}$	51 ± 10$^{\#}$
LVMI (mg/g)	2.8 ± 0.1	3.1 ± 0.4	3.7 ± 0.2$^{\wedge}$	3.4 ± 0.2
Heart weight (HW; mg)	123 ± 4	138 ± 9	164 ± 7$^{\wedge}$	152 ± 6
HW/Tibia ratio (mg/mm)	6.9 ± 0.3	7.7 ± 0.5	9.2 ± 0.3$^{\wedge}$	8.7 ± 0.4
Lung weight (LW) wet (mg)	146 ± 2	144 ± 4	243 ± 24$^{\wedge}$	224 ± 29*
LW wet/Tibia ratio (mg/mm)	8.2 ± 0.2	8.0 ± 0.2	14 ± 1$^{\wedge}$	13 ± 2*
Body weight (mg)	27.2 ± 0.9	27.8 ± 0.8	22.6 ± 0.6$^{\wedge}$	23.6 ± 0.8*
Tibia length (mm)	17.8 ± 0.2	18.1 ± 0.2	17.8 ± 0.2	17.6 ± 0.1

Table 1: WT and TRPV4$^{-/-}$ mouse parameters 1 week post sham or MI surgery. Data are mean ± SEM. Unpaired t-tests with Welch's corrections were performed, $^{\#}p<0.05$ vs. MI WT; $^{\wedge}p<0.05$ vs. Sham WT; $^{*}p<0.05$ vs. Sham TRPV4$^{-/-}$.

Statistical analysis

Data are presented as mean ± SEM. Statistical differences were determined using two-tailed, unpaired Student's t-tests, with Welch's correction as appropriate. P values of <0.05 were considered statistically significant. Survival curves were compared using Log-rank (Mantel-Cox) test. All statistical analyses were performed using GraphPad Prism 6 (San Diego, CA).

Results

Myocardial infarction in TRPV4$^{-/-}$ mice

Ejection fraction, our primary end-point in this study, was examined by MRI to compare between WT and TRPV4$^{-/-}$ mice 1 week post-MI. In WT mice, MI reduced the ejection fraction and increased end systolic and end diastolic volumes (Figure 1 and Table 1); thinning of

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the anterior wall of the infarcted ventricle was clearly visible. In contrast, the reduction in EF (EF values: 27 ± 2% WT MI vs. 37 ± 4% TRPV4$^{-/-}$ MI, P<0.05) and chamber dilation (Table 1) induced by MI were significantly blunted in TRPV4$^{-/-}$ mice. There were no significant differences in these parameters between the WT and TRPV4$^{-/-}$ sham control groups.

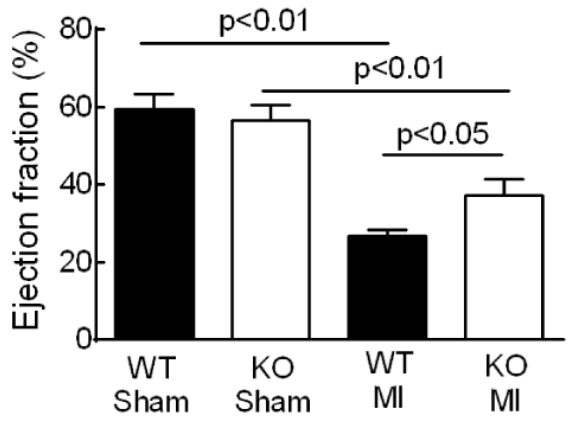

Figure 1: TRPV4$^{-/-}$ (KO) mice exhibit a better ejection fraction than wild type (WT) mice at 7 days post myocardial infarction. Data are mean ± SEM (n=6-9/group), with p-values shown from unpaired t tests performed with Welch's correction.

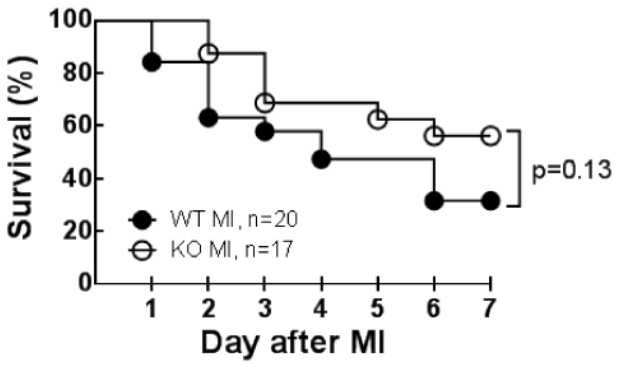

Figure 2: Survival curves of WT and TRPV4$^{-/-}$ (KO) mice up to 7 days post-MI. Twenty wild-type and seventeen TRPV4$^{-/-}$ mice were subjected to permanent left anterior descending (LAD) coronary artery ligation to induce myocardial infarction at day 0. The survival time course was analysed using the log-rank (Mantel-Cox) test.

MI induced a significant increase in both heart weight and left ventricular mass index (LVMI) in WT mice, but not in TRPV4$^{-/-}$ mice (Table 1). In the MI groups there were no significant differences between WT and TRPV4$^{-/-}$ raw lung wet weights, or when lung weights were normalized to tibia length (Table 1). Control mice (sham) demonstrated no significant differences between WT and TRPV4$^{-/-}$ for all cardiac and lung endpoints. Overall survival of the TRPV4$^{-/-}$ group over the period of 7 days post-MI was not significantly different than WT (Figure 2).

Area at risk and infarct assessment after ischemia/reperfusion (I/R) in TRPV4$^{-/-}$ mice

In separate groups of TRPV4$^{-/-}$ and WT mice, the infarcted myocardium and the area at risk were compared following a 30 min myocardial ischemia and 24 hour reperfusion. No significant differences in the areas at risk were noted (p=0.9), suggesting similar vascularization of the left anterior descending coronary artery territory in both WT and TRPV4$^{-/-}$. In addition, infarct size as a per cent of area at risk was similar in TRPV4$^{-/-}$ and WT mice (p=0.9) after a 30 min myocardial ischemia and 24 hour of reperfusion (Figure 3), suggesting a similar myocardial response to acute myocardial ischemic injury.

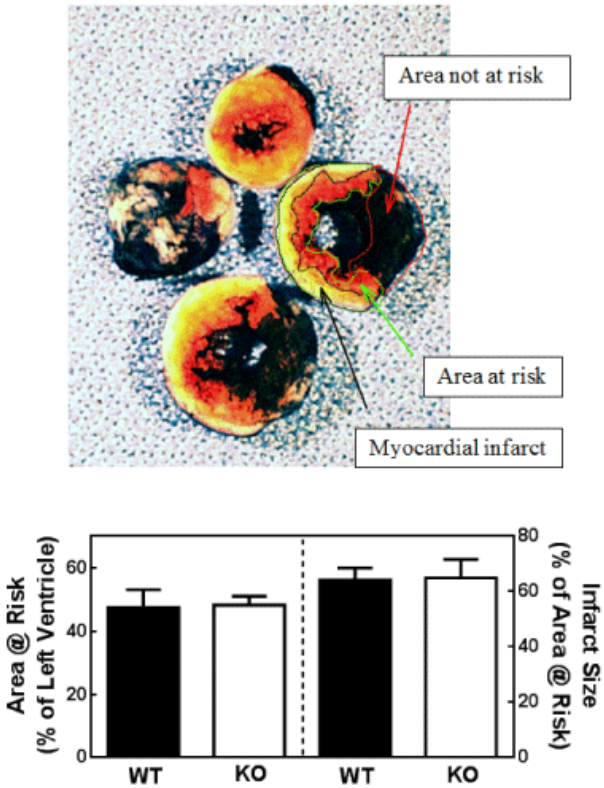

Figure 3: Area at risk and myocardial infarct size following 30 min myocardial ischemia and 24 hour reperfusion injury. A: Representative photograph of mouse heart sections stained with TTC and Evans blue dye after 30 min myocardial ischemia and 24 hour reperfusion; myocardial infarct (white), area at risk (white and red), and area not at risk (dark blue). B: Area at risk assessed as a percentage of the left ventricle area, and myocardial infarct size assessed as a percentage of area at risk. WT and TRPV4$^{-/-}$ (KO) mice (n=4/group) were compared by unpaired t-tests for both endpoints.

Discussion

TRPV4 has been recently implicated by computational approaches as a key signaling molecule in the setting of MI [13]. Our previous work has shown that pharmacological TRPV4 activation induces extensive pulmonary edema, along with a complex series of cardiovascular effects including endothelial breakdown and right sided cardiac failure [7]. Furthermore, we have shown that pharmacological TRPV4 inhibition can reduce pulmonary edema in experimental models of heart failure [5].

In the current study we focused on evaluating the cardiac effects of TRPV4 genetic deletion. MI induced a significant increase in heart weight in WT mice that was absent in TRPV4$^{-/-}$ mice 1 week post-MI. This effect was corroborated by MRI measurements of left ventricle mass index (LVMI), and indicates that TRPV4 is a mediator of cardiac hypertrophy and remodelling associated with MI. Consistent with the reduction in post-MI remodelling in TRPV4$^{-/-}$ mice there was a preservation of LV-EF, and LV chamber volumes in diastole and systole when compared to WT MI mice. Although this study was not powered to address a survival benefit in this severe permanent ligation model, there was a trend towards improved survival in TRPV4$^{-/-}$ mice following MI, a similar trend towards an increase in survival after myocardial infarction which was observed previously in response to pharmacological TRPV4 inhibitor pre-treatment [5].

The cardiac phenotype in the TRPV4$^{-/-}$ mice reported here is generally consistent with our prior observations using pharmacological TRPV4 blockade in MI mice [5], however there are important distinctions. When mice were pre-treated followed by the continued administration of TRPV4 blocker for 2 weeks post-MI, reductions in LV diameters were similar to that observed in the TRPV4$^{-/-}$ mice. However, the improved LV-EF observed in TRPV4$^{-/-}$ mice was absent with TRPV4 inhibitor pre-treatment. The LV-EF differences between the two studies may be attributed to the time at which cardiac function was evaluated (2 weeks post-MI for pharmacological inhibition, 1 week for TRPV4$^{-/-}$ mice), or differences in mouse background strains (C57Bl6 versus BALB/c). Alternatively, the LV-EF benefit could reflect the complete absence of channel protein in TRPV4$^{-/-}$ mice, which includes any functionally relevant protein/protein interactions that are independent of ionic conduction [17]. Taken together these studies suggest that TRPV4 inhibition may be required before an ischemic event to provide cardiac benefit, as initiation of pharmacological TRPV4 blockade 1 week post-MI provided no LV benefit [5].

There was no significant difference in LV structure and function by MRI in age-matched TRPV4$^{-/-}$ and WT sham control mice. These results suggest that the TRPV4$^{-/-}$ MI phenotype is unrelated to basal cardiac function. There were also no strain differences in area at risk or infarct size after 30 min ischemia and 24 hour reperfusion injury, indicating no differences in the vascularization of the left anterior descending territory, or acute sensitivity to ischemic injury. These data are consistent with the lack of effect on infarct magnitude in our prior MI studies with pharmacological TRPV4 inhibition [5].

When considered in the context of previous TRPV4 blocker studies, the present TRPV4$^{-/-}$ study provides evidence for distinct cardiac and pulmonary TRPV4 actions in the setting of MI. Several TRPV4 actions that are relevant to cardiac remodelling and dysfunction have been described. First, TRPV4 is expressed in cultured cardiac fibroblasts, and when inhibited suppresses fibroblast differentiation into myofibroblasts [12]. Myofibroblasts are abundant in infarct tissue and implicated in adverse remodeling [18]. Therefore, it has been hypothesized that stiffness induced by MI could activate pressure-sensitive TRPV4, and that TRPV4 inhibition could reduce myofibroblast formation, thereby inhibiting adverse remodelling and improving cardiac function [12]. This mechanism is potentially consistent with the cardiac benefits demonstrated here in TRPV4$^{-/-}$ mice and with the role for TRPV4 recently reported in the development of pulmonary fibrosis [13]. Furthermore, we cannot rule out non-cardiac, systemic benefits of TRPV4 inhibition as contributors to the cardiac functional benefit. This includes metabolic benefits [19,20], modulation of cytokine production and inflammatory cell function [10,21-23], as well as endothelial cell calcium and vascular reactivity [2].

The current study has several limitations with respect to a mechanistic understanding. These include the use of a whole-body TRPV4$^{-/-}$ mouse, which does not allow for an evaluation of the particular cell types in which TRPV4 is expressed that are driving the cardiac benefits described. The development and use of cell type specific TRPV4$^{-/-}$ mouse lines in future studies could help elucidate the role of cardiac myocytes, fibroblasts, endothelial and inflammatory cells. Furthermore, a cardiac histological evaluation at day 7 post-MI would help better understand the basis of the hypertrophy and functional differences at this time point. However, since there were no differences in area at risk and infarct size in our acute ischemia/reperfusion study, we did not measure infarct size in our chronic MI study. This may warrant future research.

In this study we have demonstrated a benefit of genetic deletion of TRPV4 in mice on the development of cardiac hypertrophy, chamber remodelling, and LV dysfunction in response to MI. These data further support the growing rationale for TRPV4 inhibition as a potential therapeutic strategy in the treatment of heart failure, where both pulmonary and cardiac benefit may be observed.

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