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MODULATION OF LTCC PATHWAYS BY A MELUSIN MIMETIC INCREASES VENTRICULAR CONTRACTILITY DURING LPS-INDUCED CARDIOMYOPATHY

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ABSTRACT—**Aim:** Sepsis-induced cardiomyopathy is commonplace and carries an increased risk of death. Melusin, a cardiac muscle-specific chaperone, exerts cardioprotective function under varied stressful conditions through activation of the AKT pathway. The objective of this study was to determine the role of melusin in the pathogenesis of lipopolysaccharide (LPS)-induced cardiac dysfunction and to explore its signaling pathway for the identification of putative therapeutic targets. **Methods and results:** Prospective, randomized, controlled experimental study in a research laboratory. Melusin overexpressing (MelOV) and wild-type (MelWT) mice were used. MelOV and MelWT mice were injected intraperitoneally with LPS. Cardiac function was assessed using trans-thoracic echocardiography. Myocardial expression of L-type calcium channel (LTCC), phospho-Akt and phospho-Gsk3-b were also measured. In separate experiments, wild-type mice were treated post-LPS challenge with the allosteric Akt inhibitor Arq092 and a mimetic peptide (R7W-MP) targeting the LTCC. The impact of these therapies on protein–protein interactions, cardiac function, and survival was assessed. MelOV mice had limited derangement in cardiac function after LPS challenge. Protection was associated with higher Akt and Gsk3-b phosphorylation and restored LTCC density. Pharmacological inhibition of Akt activity reversed melusin-dependent cardiac protection. Treatment with R7W-MP preserved cardiac function in wild-type mice after LPS challenge and significantly improved survival. **Conclusions:** This study identifies AKT/Melusin as a key pathway for preserving cardiac function following LPS challenge. The cell-permeable mimetic peptide (R7W-MP) represents a putative therapeutic for sepsis-induced cardiomyopathy.

KEYWORDS—Acute cardiomyopathy, calcium channel, lipopolysaccharide, melusin, sepsis

ABBREVIATIONS—LPS—lipopolysaccharide; LTCC—L-type calcium channel; MelOV—Melusin overexpressing mice; MelWT—Melusin wild type mice; R7W-MP—R7W-Mimetic Peptide; SIC—Sepsis-induced cardiomyopathy

INTRODUCTION

Sepsis-induced cardiomyopathy (SIC) is an acute, potentially reversible complication that occurs in nearly 60% of critically ill patients with sepsis (1). Its presence is associated with an increased risk of death (2–5). SIC is clinically recognized by echocardiographic evidence of ventricular dilatation, impaired cardiac contractility, and diastolic dysfunction in the absence of underlying cardiac disease (3). Nevertheless, effective SIC-specific treatments are currently lacking. Management therapies such as vasopressors and inotropes are mainly supportive and carry a significant risk of side effects.

Mechanisms of myocardial depression following a septic insult include downregulation of adrenergic pathways, mitochondrial dysfunction, impaired electromechanical coupling at the myofibrillar level, and disturbed intracellular calcium trafficking (6–9). In preclinical studies, mice exposed to bacterial lipopolysaccharide (LPS) showed a diminished calcium-induced calcium release (CiCr) mechanism and, consequently, decreased intracellular calcium handling and muscle contractility. This effect has been causally linked to a reduced cardiomyocyte sarcolemmal density of the L-type calcium channel (LTCC) complex, a trigger of cardiac muscle contraction during the action potential (8–10). An imbalance of calcium handling and alterations in

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Independent data access and analysis: Corresponding authors have full access to all the data in the study and take responsibility for its integrity and the data analysis. All data, code, and materials used in the analysis are available upon corresponding author request.

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subcellular localization of the LTCC may result from alterations in the protein kinase B (Akt) signaling transduction pathway, which plays a key role in physiological functioning of the heart (11–13).

We previously showed that melusin, a chaperone protein highly expressed in cardiac muscle, plays a prominent cardio-protective role in cardiac remodeling following pathological injuries, including ventricular pressure overload and myocardial ischemia (11, 14–16). This melusin-dependent effect occurs following activation of the Akt myocardial signaling pathway through direct phosphorylation of Akt, Gsk3beta, and Erk1/2 (17–19). In line with this evidence, we examined whether the Akt/Gsk3/melusin signaling pathway may offer a protective effect on LTCC alterations and cardiac contractility in a mouse model of LPS-induced SIC (Fig. 1A). Our second aim was to test a cell-permeable mimetic peptide (R7W-MP), which acts downstream of Akt signaling and targets LTCC protein density at the plasma membrane (20, 21), as a putative therapeutic strategy for SIC.

MATERIALS AND METHODS

Ethics statement

All experiments were conducted in accordance with the guidelines from Directive 2010/63/EU of the European Parliament on the protection of animals and approved by the University of Turin Ethical Committee for animal research and by the Italian Ministry of Health (license no. 22/2017-UT). The investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85–23, revised 1985).

Animal breeding and preparation

Three-month-old male FVB, Melusin Wild-Type (MelWT), and Melusin Overexpressing (MelOV) mice (17), weighing 23 to 27 g were used in the study. Mice were obtained from the Molecular Biotechnology Center, University of Turin, Italy. Studies followed institutional animal welfare guidelines and legislation, approved by the local Animal Ethics Committee.

Induction of LPS model

LPS powder derived from *Escherichia Coli* 055:B5 (Sigma–Aldrich, Saint Louis, MO, USA) was reconstituted in sterile n-saline solution at a concentration of 1 mg/mL. Under anesthesia using 2% isoflurane, LPS was injected intraperitoneally into the right iliac fossa, with either 20 mg/kg of LPS or an equivalent volume of n-saline solution given as a control. Subsequently, after sepsis induction, 1 mL n-saline solution was administered intra-peritoneally into the left iliac fossa as a fluid replacement (Fig. 1B).

Echocardiography

Measurements were performed following recognized guidelines for mouse heart assessment (22) using high-resolution trans-thoracic M-mode and two-dimensional echocardiography (Vevo 2100 High-Definition Ultrasound, FujiFilm VisualSonics Inc., Toronto, ON, Canada). Measurements were acquired at baseline and 6 h after LPS injection (Fig. 1B). Mice were briefly anesthetized with 1.5% to 2% of isoflurane to maintain an optimal sedation level throughout the procedure and placed on a heating platform to maintain body temperature at $36^{\circ}C \pm 0.5^{\circ}C$. Cardiac function was assessed when heart rate was in the range of 400 to 600 bpm. The first scan was performed in two-dimensional brightness echocardiography (B–Mode) to obtain a comprehensive picture from the aortic valve to the cardiac apex. Subsequently, the probe was rotated through 90° to obtain a B-Mode short-axis view of the left ventricle and switched to time-motion mode (M–Mode). Scanning was performed with the acquisition of at least three complete cardiac cycles. Fractional shortening was calculated using the formula:

$$FS = 100 \times ((LVIDd - LVIDs))/LVIDd$$

Each scanning session required up to 10 min to obtain the recordings. These measures were acquired at baseline and at 6 h post-LPS injection. Afterwards, mice were euthanized in a carbon dioxide (CO_2) chamber.

Western blotting analysis

Both treated and control hearts from MelWT and MelOV mice were frozen and powdered in liquid nitrogen with a pestle. Powders were homogenized in TBS 1% Triton X-100, in the presence of phosphatase inhibitors (10 mM NaF, 1 mM Na_3VO_4), 1 mM PMSF, and a Roche complete protease inhibitor cocktail. Fifty micrograms of total cardiac extract was separated by SDS-page gel and blotted on a nitrocellulose membrane. For protein analysis, primary antibodies for Akt and phospho-Akt (1:1,000, Cell Signaling), Gsk3β and phospho-Gsk3β (1:1,000, Cell Signaling), Vinculin (1:1,000, Sigma), and CACNA1C (1:1,000 Abcam) for the subunit Cavα1.2, were used. These were diluted in TBS plus 1% BSA and incubated overnight at 4°C in agitation. Anti-mouse or anti-rabbit secondary antibodies 1:10.000 in TBS plus 0.3% were incubated for 1 h at RT before the detection of UV signals using Chemidoc machinery (Biorad).

Akt inhibitor (Arq092)—miransertib

The selective allosteric Akt inhibitor (Arq092, Miransertib, ArQule, Burlington, MA) binds to and inhibits Akt activity in a non-ATP competitive manner (23). Miransertib, or n-saline solution as control, was administered by intravenous injection of 5 mg/kg in 0.2 mL of n-saline solution into MelOV mice at 2 h following LPS injection (Fig. 1B).

Treatment with the cell-permeable mimetic peptide (R7W-MP)

R7W-MP (RRRRRRRWDQRPDREAPRS) and R7M-Scramble (RRRRRRRWYPYDVPDYA) were synthesized by Genscript (Tokyo, Japan). In the LPS mouse model, peptides were injected as a single dose (5 mg/kg in 0.2 mL saline solution) intra-peritoneally 3 h after LPS injection (Fig. 1B).

Cell cultures

HL-1 cells were cultured in Claycomb medium (Sigma) with 10% FBS (Sigma), 100 U/mL penicillin, 0.1 mg/mL streptomycin (Euroclone), 1% Ultra-glutamine 1 (Lonza), and 0.1 M Norepinephrine (Sigma) in a gelatin/fibronectin pre-coated flask. Transfection of plasmids (Cavα1.2-pNLF1-N and CavB2-pFN21A) was performed in serum-free medium (Opti-MEM I reduced-serum medium, Life Technologies) using Lipofectamine 2000 (Life Technologies).

DNA constructs and NanoBRET assay

For the BRET Assay, all constructs were previously generated (21). Briefly, coding sequences for Cavα1.2 and Cavβ2 were cloned into the pNLF1-N and the HaloTag-pFN21A vectors (from Promega). All cloning steps were performed using the In-Fusion HD Cloning Plus Kit (Clonotech). Briefly, Cavα1.2 and Cavβ2 were cloned into the pNLF1-N vector and the HaloTag- pFN21A vector (from Promega), respectively. HL-1 were transfected with plasmids in serum-free medium (Opti-MEM I reduced-serum medium, Life Technologies) using Lipofectamine 2000 (Life Technologies). After 6 h, the optimum medium was replaced with Claycomb complete medium (Sigma). After 24 h transfection HL-1 cells were starved and cells were challenged with LPS (5 μg/mL) for 16 h. The day after R7W-MP were added as indicated. For protein–protein interaction assays, HL-1 transfected cells were re-seeded at a density of 2×10^5 cells/mL into a White 96-well Microplate with Clear Bottom and incubated with 100 nM of NanoBRET 618 Ligand (Promega) for experimental samples or DMSO (Sigma–Aldrich) for no ligand-control samples. Signals were detected 5 h after treatment using a GloMax® Discover Microplate Reader (Promega) by measuring donor signal (460 nm) and the acceptor signal (610 nm). Raw NanoBRET values were calculated by dividing the acceptor emission value by the donor emission value for each sample to generate raw NanoBRET ratio values. The final corrected BU was obtained using the following formula:

$$\text{Mean BU experimental} - \text{Mean BU no} - \text{ligand control} = \text{Mean corrected BU}$$

Survival analysis

Three-month-old MelWT male mice were anesthetized with 2% isoflurane before undergoing subcutaneous nape implantation of an osmotic minipump (ALZET® Osmotic Pump, 24 h to 200 μL volume-pump, Cupertino, CA). The pump was used to enable continuous, slow release of either R7W-MP (10 mg/kg/day) or n-saline solution as a control. Immediately after implantation, mice were injected intraperitoneally with 20 mg/kg of LPS and, 3 h later, with a

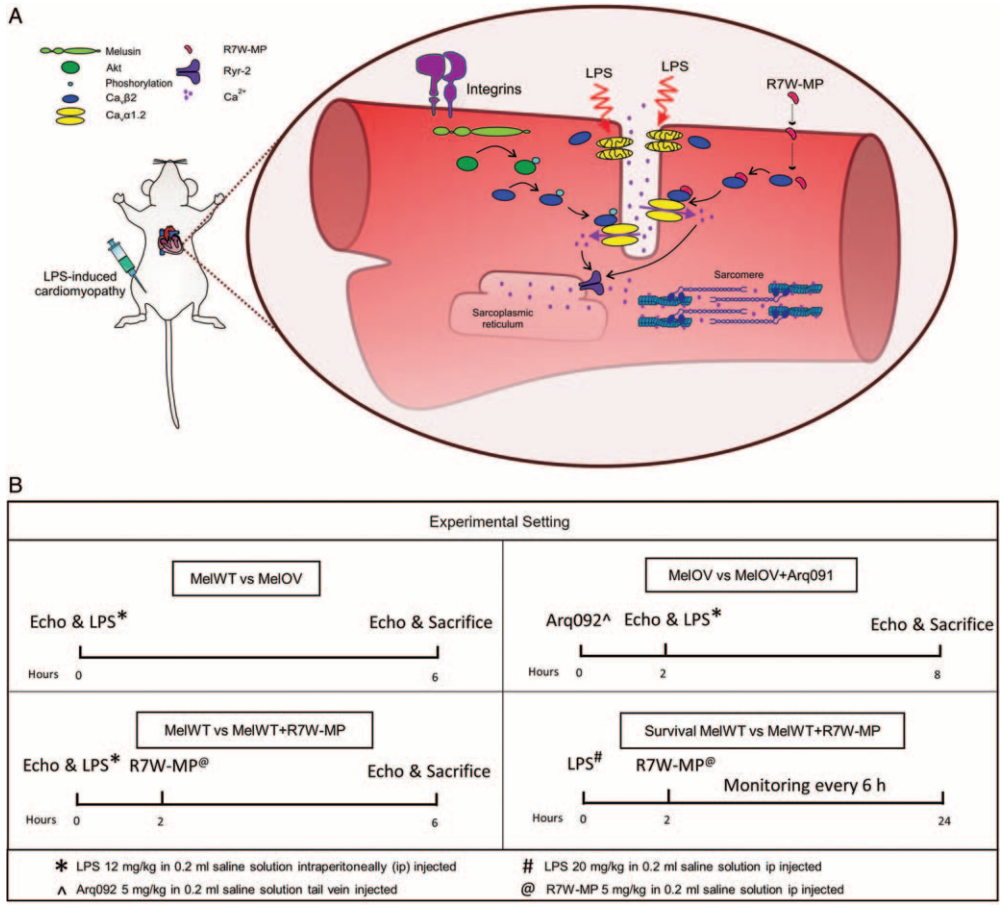

FIG. 1. **Graphical abstract and experimental setting.** (A) Graphical description of the mice model and cardiomyocyte protein involved in the study. To modulate the expression of calcium channels in vivo murine septic model. LPS injection leads to decreased expression of LTCC in cardiomyocytes. Melusin and R7W-MP independently protected LTCC from degradation via activation of the LTCC beta subunit (Cavß2) which positively modulated the expression and functionality of the alpha subunit (Cavα1.2—main poring unit forming LTCC). (B) Experimental setting used with LPS dosage and timing of measurements and drugs administration. LTCC, L-type calcium channel.

3 mg/kg bolus of R7W-MP. Mice were then monitored for survival over the following 24 h (Fig. 1B).

Statistics

All data are expressed as median and interquartile range (IQR). The Shapiro–Wilk Normality Test was used to determine normal distribution. A Kruskal–Wallis test with post hoc Dunn's Multiple Comparison testing was performed to assess the differences between and within groups. Wilcoxon–Mann–Whitney test was used to assess differences between the two groups. Survival was evaluated by Kaplan-Meyer analysis. Differences were considered statistically significant when the probability value was less than 0.05. (GraphPad Prism 9.2.0, GraphPad Software, San Diego, CA).

RESULTS

Melusin overexpression protects from LPS induced cardiomyopathy

To investigate whether melusin can protect cardiac muscle, transgenic mice with cardiac-selective overexpression of melusin (MelOV) were challenged with LPS and analyzed by echocardiography. Melusin WT mice (MelWT) served as controls. Compared to baseline, fractional shortening (FS) of MelOV mice was preserved 6 h after LPS treatment compared to a 50% decrease in MelWT mice ($P < 0.05$) (Fig. 2A). Both groups showed significantly increased heart rate (HR) after LPS challenge (Fig. 2B).

Consistent with published data, sham MelOV mice had higher p-Akt/Vinculm expression compared to sham MelWT controls (Fig. 2C–E). Although LPS challenge induced a significant increase in p-Akt in both mouse strains, MelOV mice expressed significantly more compared to their MelWT counterparts ($P < 0.05$) (Fig. 2D–F). In line with the expected positive effect of Akt on LTCC levels, sham MelOV mice had a significantly greater amount of the LTCC pore subunit Cavα1.2 compared to sham MelWT mice (Fig. 2F). This effect was also maintained with the LPS challenge; despite both groups showing a significant reduction in Cavα1.2 expression, LPS-treated MelOV mice retained more channel protein. Taken together, these data indicate that melusin over-expression maintains increased levels of p-Akt with a concomitant increase in Cavα1.2 expression, and this appeared to be protective against LPS-induced cardiomyopathy.

Inhibition of Akt phosphorylation decreases the protective effect of melusin over-expression in LPS-induced cardiomyopathy

To confirm the importance of Akt phosphorylation in protecting against LPS-induced cardiomyopathy, three mice per group were treated with the selective pan-Akt inhibitor ARQ

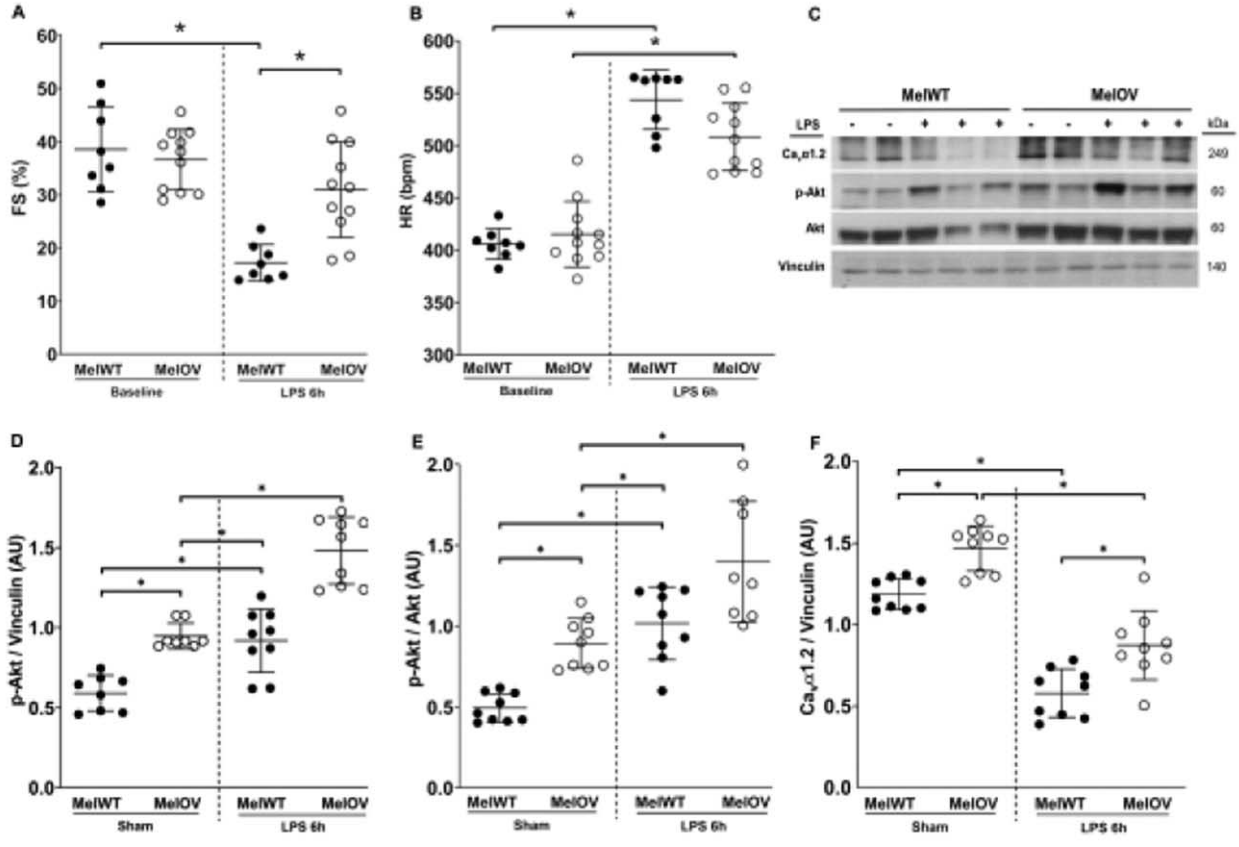

FIG. 2. **Melusin overexpression protects mice from LPS induced cardiomyopathy.** (A) FS at baseline and 6 h after LPS challenge in MelWT (n = 8) and MelOV (n = 11); $^*P < 0.05$; (B) HR at baseline and 6 h after LPS challenge. Groups same as in (A); $^*P < 0.05$; (C) Western blot analysis of Cavα1, pAkt, Akt in sham and LPS-treated MelWT and MelOV mice; (D) Densitometric ratio of p-Akt/Vinculin, groups as in (C); $^*P < 0.05$; (E) Densitometric ratio of p-Akt/Akt, groups as in (C); $^*P < 0.05$; (F) Densitometric ratio of $Ca_v\alpha1.2$/Vinculin, groups as in (D); $^*P < 0.05$. FS, fractional shortening; HR, heart rate; MelOV, melusin over-expressed mice; MelWT, melusin wild-type mice.

092 (miransertib). MelOV mice treated with ARQ092 showed a significant decrease of FS at 6 h post-LPS compared to non-treated animals (Fig. 3A), suggesting that melusin exerts its cardioprotective effect through Akt phosphorylation. Both groups showed increased HR after LPS challenge (Fig. 3B). At the protein level, treated endotoxic MelOV mice (N = 3) showed a significant reduction in phosphorylation levels of Akt and its target GSK-3ß, thus confirming the effectiveness of inhibition (Fig. 3C–E).

R7W-MP administration protects MelWT mice from LPS-induced cardiomyopathy

R7W-MP is a cell-permeable peptide that mimics the downstream Akt-dependent effect on LTCC protein density and restores the plasma membrane density of deregulated Cavα1.2 by directly binding to Cavß2 (21). We investigated whether direct modulation of LTCC density by R7W-MP could prevent cardiac dysfunction in LPS-challenged MelWT mice, using MelWT sham mice as model control and Scramble mice as an injection control. Compared to MelWT sham mice or those treated with scramble peptide, MelWT mice receiving R7W-MP did not show any decrease in FS 6 h after the LPS challenge (Fig. 4A). The rise in heart rate following LPS challenge was attenuated in R7W-MP treated mice compared to all other groups (Fig. 4B).

Administration of R7W-MP counteracts the negative effect of LPS on LTCC activity

We tested whether the interaction between the pore subunit Cavα1.2 and its cytoplasmic chaperone Cavß2 is affected by LPS administration as assembly and function of the LTCC at the plasma membrane are governed by a proper association between Cavα1.2 and its chaperone (24). Using the Nano-BRET protein interaction assay, LPS administration significantly reduced interaction between Cavα1.2 and Cavß2 in HL-1 cardiomyocytes, thereby negatively modulating the level of LTCC at the plasma membrane (Fig. 4C). On the other hand, administration of R7W-MP promptly restored interaction between the two LTCC subunits in a time-dependent manner (Fig. 4C). Administration of R7W-MP thus completely reverses the negative effects of LPS on LTCC assembly.

Treatment with R7W-MP improves survival in MelWT mice

To test the *in vivo* protective effect of R7W-MP we treated LPS-challenged MelWT-mice with a bolus of R7W-MP followed by continuous infusion MelWT sham mice were used as control. Treatment with R7W-MP significantly prolonged survival of MelWT-treated mice compared to controls after a higher dose of LPS (Fig. 4D).

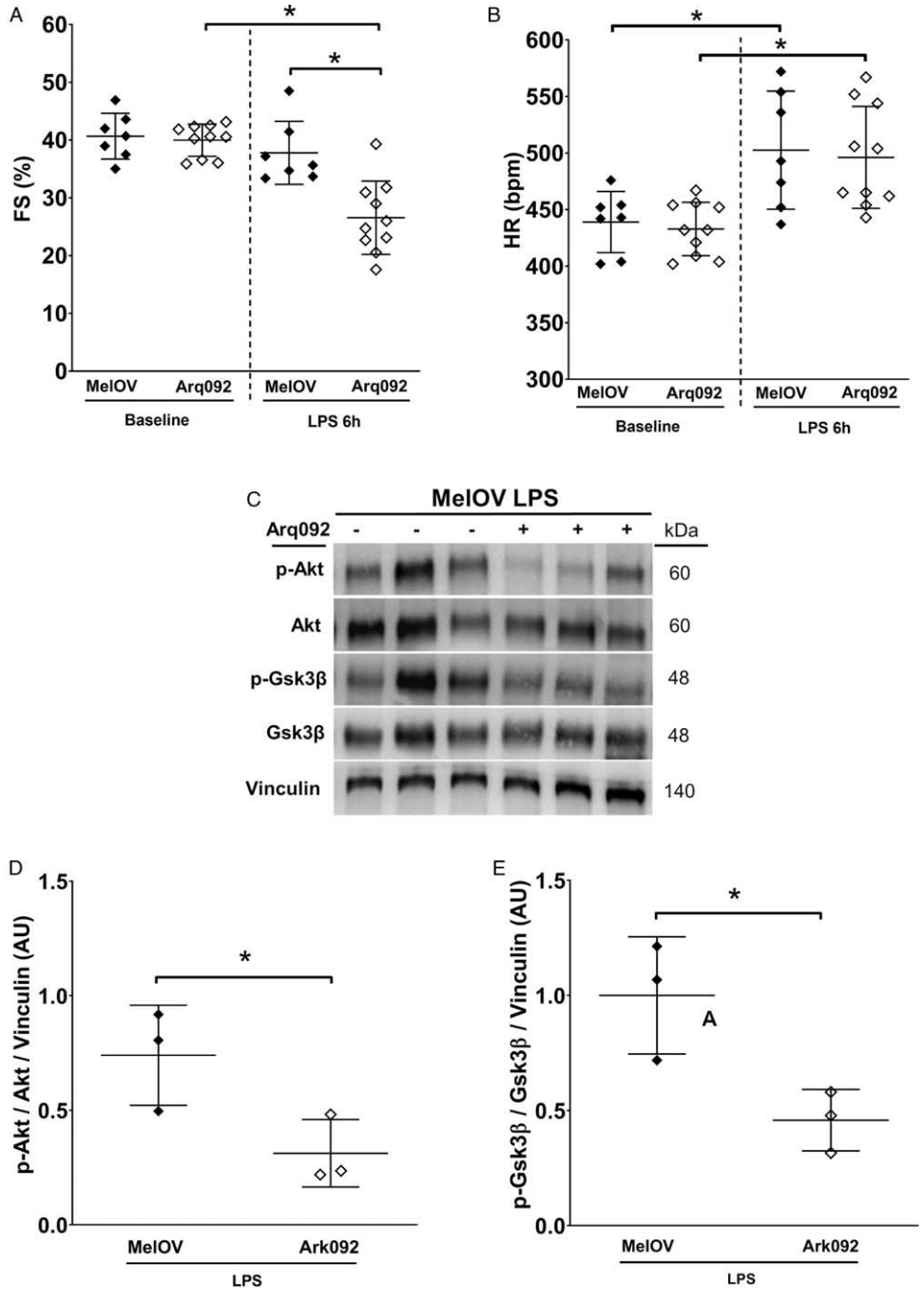

Fig. 3. **Inhibition of Akt phosphorylation decreases the protective effect of Melusin over-expression against LPS induced cardiomyopathy.** (A) FS at baseline and 6 h after LPS challenge in MelOV (N = 7) and melusin over-expressed mice treated with Arq092 (Arq092, N = 10); $^{*}P < 0.05$; (B) HR at baseline and 6 h after LPS challenge. Groups same as in (A); $^{*}P < 0.05$; (C) Western Blot analysis of pAkt, Akt, pGsk3β, and Gsk3β in LPS-treated MelOV mice and those treated with Arq092 (N = 3 animal per group); (D) Densitometric ratio of pAkt/Akt/Vinculin, groups as in (C); $^{*}P < 0.05$; (E) Densitometric ratio of pGsk3β/Gsk3β/Vinculin, groups as in (C); $^{*}P < 0.05$. FS, fractional shortening; HR, heart rate; MelOV, melusin over-expressed mice.

DISCUSSION

The main findings of this study are that melusin overexpression protects against LPS-induced cardiomyopathy; the protective effect of melusin acts through activation of the Akt signaling pathway and targets the LTCC complex; and that treatment with an LTCC-targeting R7W-MP peptide, which has an Akt-based mechanism of action, preserves cardiac function, and extends survival of LPS-challenged mice by maintaining LTCC density at the plasma membrane.

Mechanisms of myocardial depression after septic insult include downregulation of adrenergic pathways, mitochondrial dysfunction, impaired electromechanical coupling at the myofibrillar level, and disturbed intracellular calcium trafficking (1, 6–9). Calcium dysregulation is considered a major player contributing to the development of cardiac dysfunction in both animal models and patients with SIC (25). Recent preclinical studies have shown a causal relationship between the survival process in SIC and restoration of myocardial calcium

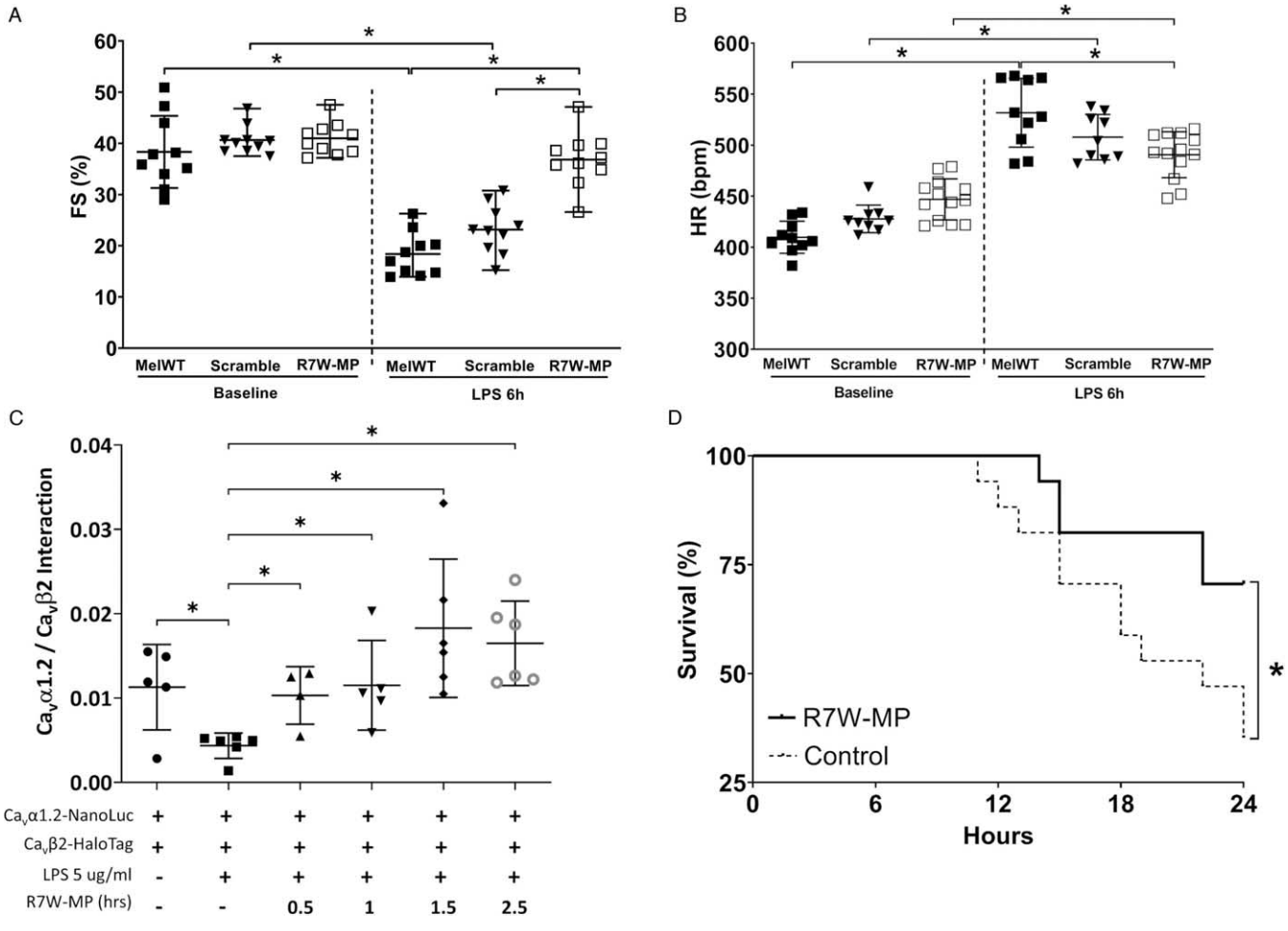

FIG. 4. **Administration of R7W-MP protects MelWT mice from LPS-induced cardiomyopathy, restored the interaction between CaVα1.2 and CaVβ2 in HL1 cardiac cells after treatment of LPS, and increase the survival over 24 h in an endotoxemic mice model.** (A) FS at baseline and after 6 h after LPS challenge in melusin wild-type mice (MelWT, n = 10), melusin wild-type mice treated with R7W scramble peptide (Scramble, n = 10), melusin wild-type mice treated with active R7W MP (peptide, n = 10); (B) HR at baseline and 6 h after LPS challenge. Groups same as in (A); $^*P < 0.05$; (C) CaVα1.2/CaVβ2 protein interaction measured by BRET assay in HL-1 cells treated as indicated (n = 5). Peptide (R7W-MP) dose 1.3 μmol/L; $^*P < 0.05$; (D) 24-h Kaplan–Meier survival curve comparing melusin wild-type mice (MelWT, n = 17) and melusin wild-type mice treated with active R7W-MP (peptide, N = 17); $^*P < 0.05$. BRET, bioluminescence resonance energy transfer; FS, fractional shortening; HR, heart rate; MelWT, melusin wild-type mice.

trafficking (26). Therapies directly targeting calcium handling could have important clinical implications.

The LTCC complex is the triggering component at the surface of myofibrillar cells for the first calcium influx after the depolarization wave, leading to activation of calcium induced-calcium release (CICR) and myocardial contraction. Notably, alterations of the plasma membrane density of LTCCs are causally associated with multiple pathological cardiac phenotypes (27, 28). LTCC membrane density is negatively affected after exposure to LPS (8–10). In this process, multiple studies from our group and others have demonstrated involvement of the PI3K-Akt signaling pathway in improving cardiac function (11, 12, 29, 30).

The molecular chaperone melusin, highly expressed in cardiac muscle, plays a prominent cardioprotective role in cardiac remodeling following pathological injuries. Analysis of signaling pathways indicated that melusin over-expression was related to increased basal phosphorylation of Akt, Gsk3beta, and Erk1/2. Moreover, Akt, Gsk3beta, and Erk1/2 were hyper-phosphorylated following ventricular pressure overload in melusin-overexpressing compared with wild-type mice (17). In the current study, we investigated the putative cardioprotective role of melusin in a sepsis model and investigated the molecular pathways and targeted proteins involved. Increased Akt phosphorylation and higher p-Akt/Akt ratios were found in MelOV mice compared to MelWT, both in unstimulated and LPS-challenged conditions. These findings correlated with an increase in Cavα1.2 expression in MelOV mouse heart tissue. The link between p-Akt and LTCC expression is well described (12), and likely explains the increased amount of Cavα1.2 found in melusin-overexpressing hearts. Indeed, the increased level of LTCC expression in MelOV mice present at baseline could explain the protective effect observed at 6 h following LPS challenge, at which timepoint LTCC was reduced in LPS-treated WT mice. Inotropism is linked to the CICR mechanism (31–33) which, in turn, relies on an LTCC-dependent calcium current towards the intracellular space and subsequent activation of a greater calcium release from the sarcoplasmic reticulum (34). As such, cardiomyocyte contractility may be directly related to the density of LTCCs on the surface of the T-tubule of cardiomyocytes.

Of note, LPS-challenged MelWT mice showed a marked decrease in Cavα1.2 protein levels compared to basal levels and LPS-challenged MelOV. We thus demonstrate a counterbalancing mechanism in the MelOV mice by which Cavα1.2 concentration is increased due to augmented activation of Akt. Indeed, treatment of MelOV mice with Arq092 (Miransertib), an inhibitor of Akt phosphorylation could inhibit, at least in the short-term, melusin-dependent effects that protect LTCCs from degradation during sepsis. In line with both Akt inhibition and a decrease in LTCC levels, LPS-challenged MelWT mice had a similar decrease in FS as observed in MelWT mice. On the other hand, we also found a possible link between inhibition of Akt phosphorylation in MelOV mice and loss of protective function after LPS challenge, with short-term protection offered by p-Akt with respect to LTCC concentration. p-Akt plays a fundamental role in phosphorylation and activation of the beta subunit of Cav1.2 which contributes to correct folding, splicing, and trafficking of the alpha subunit to the membrane surface (12). We have previously described the importance of the beta subunit over trafficking of the alpha subunit to modulate the CiCR (12) (Fig. 1).

In the present study, we investigated the effect of R7W-MP, a short mimetic peptide that directly modulates the affinity of Cavß2 chaperone subunit for the Cav1.2 pore unit and promotes its localization at the plasma membrane. Treatment with R7W-MP significantly improved the phenotype of endotoxic MelWT mice, attenuating the fall in FS. By contrast, a significant decrease in FS was seen in WT control mice and WT mice treated with an R7W-scramble peptide. To investigate this process further we used an *in vitro* NANOBRET assay which indirectly demonstrates the ability of the peptide to prevent dissociation of the beta subunit from the alpha subunit. When the two subunits are separated, there is an increase in alpha subunit degradation, as occurs after LPS challenge. Our results show that peptide treatment can affect this dissociation both *in vitro* and *in vivo*. Finally, administration of R7W-MP after LPS challenge was associated with a decrease in 24 h mortality, indicating a potential protective effect against LPS-induced cardiomyopathy. Overall, our findings show that the decrease in myocardial contractility seen in this endotoxic sepsis model could be modified by increasing calcium channel density.

Our study presents some limitations. First, we used LPS to induce a septic state. While reliable and reproducible, it does not precisely mimic the human septic phenotype. The model was also short-term, and treatment was administered shortly after the endotoxin insult. Further studies should be conducted using a more realistic, long-term model such as fluid-resuscitated fecal peritonitis. Secondly, hemodynamic investigation and data were limited due to the limitations of the mouse model. Further studies assessing cardiac output, and preload and afterload-independent indices of cardiac contractility should be performed in larger animal models. Monitoring of arterial blood pressure should be used to assess the vascular effect of these treatments, and biochemical markers of organ dysfunction measured. Pharmacokinetic and pharmacodynamic studies are also needed to determine an optimal dosing regimen of the peptide, its biodistribution, and its efficacy in improving myocardial function and survival following delayed administration, that is, after SIC has become established.

In conclusion, our findings indicate that the melusin-Akt signaling pathway plays an important role in increasing cardiac contractility and offering cardiac protection. As the administration of R7W-MP peptide could attenuate cardiac dysfunction following an LPS insult, targeting LTCC density may be a potential novel therapeutic for SIC.

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