Effects of trehalose on recurrence of remodeling after ventricular reconstruction in rats with ischemic cardiomyopathy

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Short title: Trehalose attenuates LV remodeling after SVR

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Abstract

Recurrence of left ventricular (LV) remodeling after surgical ventricular reconstruction (SVR) for ischemic cardiomyopathy has been reported to be partially attributed to autophagy. We aimed to examine the effects of trehalose, an autophagy inducer, on the recurrence of LV remodeling after SVR. After SVR in rats with ICM, trehalose was orally administered. The changes in LV end-diastolic dimension (LVEDD) and fractional shortening (FS) were evaluated. The activation of myocardial autophagy was also estimated by autophagy markers: microtubule-associated light chain 3 II (LC3-II) and p62; the former usually increases and the latter decreases if autophagy is activated. Significant LV reverse remodeling was observed early after SVR. On the other hand, the 28th postoperative day SVR + trehalose was associated with smaller LVEDD and better FS than SVR alone (LVEDD, $P = 0.043$; FS, $P < 0.01$). LC3-II increased comparably in both groups, while p62 was significantly lower in the SVR + trehalose group than in the SVR alone group ($P < 0.01$). In conclusion, trehalose attenuated the recurrence of LV remodeling and changed autophagy markers after SVR in rats with ICM. Trehalose may be a candidate for adjuvant therapy to retain the effects of SVR.

Keywords: autophagy・ ischemic cardiomyopathy・ surgical ventricular reconstruction・ trehalose

Introduction

The left ventricle (LV) dilates after functional deterioration due to myocardial infarction (MI), which is called LV remodeling and develops in 20% of patients with MI [1]. LV dilatation results from the compensation of reduced stroke volume after MI [2,3]. LV dilatation and impaired LV function due to chronic myocardial ischemia are described as ischemic cardiomyopathy (ICM). In patients with ICM, LV remodeling is a risk factor for mortality with or without revascularization [4,5].

To reduce LV size in ICM, surgical ventricular reconstruction (SVR) has been invented [6,7]. There would be three major effects of SVR: (1) scar exclusion, (2) restoration of a conical LV shape, and (3) reduction of LV size [8]. These factors would reduce LV wall stress and myocardial oxygen consumption. Thus far, several non-randomized studies have reported that SVR improves LV function and the New York Heart Association functional class in the midterm [6,9]. Furthermore, SVR would be effective in selected patients [10].

However, a randomized clinical trial, the Surgical Treatment for Ischemic Heart Failure (STICH) trial, showed no beneficial effects of SVR on cardiac symptoms, and exercise tolerance, and survival compared with coronary artery bypass grafting alone in the midterm [11]. The reason for the noneffective result of the trial has not been clear. In other studies, one of the concerns has been reported to be recurrence of LV remodeling after SVR [12,13]. These studies demonstrated that LV size increased again at 1 year after SVR. Furthermore, the hemodynamic values returned to the preoperative level in patients with recurrence of LV remodeling [12-15]. We speculate that the recurrence of LV remodeling is related to the nonbeneficial effects of SVR demonstrated in the STICH trial. Thus, if the mechanism of the recurrence of LV remodeling could be identified, we might improve the results of SVR.

We have investigated the mechanism of recurrence of LV remodeling after SVR using a rat model of ICM from the point of view of 'autophagy'. Autophagy is an intracellular degradation system that has two important roles: energy supply and intracellular quality control [15]. Myocardial autophagy has been reported to be activated during postinfarction cardiac remodeling [16]. We demonstrated that one of the autophagy markers decreased early after SVR but increased late after SVR as the recurrence of LV remodeling occurred [17]. Furthermore, the administration of an autophagy inhibitor after SVR exaggerated LV remodeling. Taken together, autophagy may play an adaptive role in the recurrence of LV remodeling after SVR.

This study aimed to examine the effects of pharmacological activation of autophagy using trehalose, an autophagy inducer, on the recurrence of LV remodeling after SVR. We hypothesized that the administration of trehalose could attenuate the recurrence of LV remodeling after SVR.

Materials and methods

Experimental design

Fig. 1 shows the experimental protocol. Ten-week-old male Sprague Dawley rats were randomized into the following four groups (n = 4 per group): sham, SVR, sham + trehalose, and SVR + trehalose groups. All rats underwent MI operation (first operation) to create a model of ICM. Twenty-eight days after the first operation, a sham or SVR operation (second operation) was performed. The rats were euthanized 28 days after the second operation. Trehalose rats were orally administered 10% trehalose via a drinking bottle for 21 days after the second operation until euthanization. The first, second operation were performed under general anesthesia using a single intramuscular

injection of ketamine (90 mg/kg: Ketalar, Daiichi Sankyo Pharmaceutical, Tokyo, JP) and xylazine (10 mg/kg: Selactar, Bayer Yakuhin, Tokyo, JP). For euthanization, in addition to the general anesthesia, an intraperitoneal injection of pentobarbital (150mg/kg: pentobarbital sodium salt, Nacalai Tesque, Tokyo, JP) was performed. To evaluate the early effect of SVR, other rats were randomly grouped into two groups (n = 4 per group): sham and SVR groups. The rats were euthanized 2 days after the second operation.

Echocardiography was performed before each operation and euthanization. Echocardiographic examiners were blinded to the use of trehalose. Lung and heart weights were measured at euthanization. Assessment of autophagy markers, histological examination, and reverse transcription polymerase chain reaction (RT-PCR) were performed using the LV myocardium which was excised at euthanization. All procedures were conducted according to the Hokkaido University Manual for Implementing Animal Experimentation and were consistent 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). This study was approved by the institutional animal ethics committee.

MI and SVR

MI and SVR operations were performed under the general anesthesia with intubation and mechanical ventilation as previously reported [17]. Briefly, MI was created by ligation of the left anterior descending artery by with 7-0 polypropylene (Ethicon, Somerville, NJ) via left lateral thoracotomy. Twenty-eight days after MI operation, the rats developed a large scar, but not an aneurysm, on the anterior LV wall with hypokinesis in the non-ischemic lesion. SVR was performed by plication of the akinetic

scar area using three mattress sutures with pledgetted 6-0 polypropylene (Ethicon) through a horizontal sternotomy. An over-and-over suture was then performed on the plicated scar tissue (video in ref. 16). The mortality rates calculated by the number of rats (n=86) operated in our laboratory including those in the other studies were 35%, 54%, and 11% after MI, SVR, and sham surgery, respectively.

Echocardiography

After the rats were anesthetized, transthoracic echocardiography was performed using a Sonos 5500 ultrasound system with a 12-MHz phased-array transducer (Philips Medical Systems, Andover, MA, USA). LV end-diastolic dimension (LVEDD), LV end-systolic dimension (LVESD), and fractional shortening (FS) were measured using M-mode tracings recorded from the long-axis view of the LV. Stroke volume and cardiac output were measured using pulse Doppler on the LV outflow tract. Mitral E wave and deceleration time (DCT) were evaluated using pulse Doppler of trans-mitral flow as diastolic functional parameters. The scar ratio was defined as follows: the circumferential length of the akinetic endocardium divided by that of the total endocardium at the papillary muscle level by a short-axis view.

Assessment of autophagy markers

To assess myocardial autophagy, the protein expressions of microtubule-associated light chain 3 II (LC3-II) and p62 were evaluated using the LV myocardium excised at euthanization. LC3-II and p62 are the two major autophagy markers [15]. Autophagy has the following four steps (autophagic flux): (1) induction, (2) formation of autophagosomes, (3) fusion of autophagosomes and lysosomes, and (4) autophagic body breakdown and recycling. LC3-II is converted from microtubule-associated light

chain 3 I (cytosolic form) and binds to the surface of autophagosome membranes. Therefore, the amount of LC3-II reflects the number of autophagosomes and is commonly used to estimate autophagy activation. However, an increase in LC3-II could overestimate the autophagy activation by suppressing its breakdown pathway. Alternatively, p62, which is selectively incorporated into the autophagosome and degraded during autophagic body breakdown, serves as a readout of autophagic degradation; a decrease in the expression of p62 means an increase in autophagic flux.

A semidry Western blot apparatus (Mini-PROTEAN Tetra Cell; Bio-Rad, Hercules, CA, USA) was used for electrophoresis and blotting. After sodium dodecyl sulphate polyacrylamide gel electrophoresis (12% Mini-PROTEAN TGX; Bio-Rad), the proteins were blotted onto a polyvinylidene difluoride membrane and incubated with primary (LC3B; Abcam, Cambridge, UK, p62; Cell Signaling Technology, Danvers, MA, USA) and secondary (anti-rabbit IgG; Cell Signaling Technology) antibodies. The bands were semiquantified by chemiluminescence using JustTLC (Sweday, Sodra Sandby, Sweden). The membranes were then dyed with naphthol blue black solution to normalize the band intensity.

Histological examination

The mid walls of the ventricles were fixed in 10% paraformaldehyde in phosphate-buffered saline, embedded in paraffin, and sectioned at 5-mm intervals. The cardiomyocyte area was examined using hematoxylin-eosin–stained sections. In the septal lesion (remote area from infarction), randomly chosen 100 oval-shaped cardiomyocytes with a nucleus in each group were analyzed to measure the cross-sectional cardiomyocyte area (μm^2) using ImageJ software (http://rsb.info.nih.gov/ij/; NIH, Bethesda, MD, USA). The percentage of the fibrotic area to the whole tissue area

was examined using Masson's trichrome staining as the fibrosis fraction. In the septal lesion, randomly chosen 10 frames from each slice were analyzed using ImageJ (NIH).

RT-PCR

To evaluate myocardial pathological hypertrophy, the following gene expressions were assessed using the LV myocardium which was excised at euthanization: α-myosin heavy chain (MHC), βMHC, brain natriuretic peptide (BNP), and α-skeletal actin (αSA). Myocardial total RNA was isolated from frozen tissue samples using the High Pure RNA Tissue Kit (Roche, Penzberg, Germany). Total RNA was reverse transcribed into cDNA using the Transcriptor First Strand cDNA Synthesis Kit (Roche). Quantitative real-time RT-PCR was performed with FastStart Essential DNA Probes Master (Roche) and RealTime ready assay (Roche Assay ID, 501294 for αMHC, 500524 for βMHC, 100102866 for BNP, and 05532957001 for αSA). Polymerase chain reaction amplification was performed in a volume of 20 μL using LightCycler Nano (Roche) according to the manufacturer's instructions. The results were normalized to S29 transcription as a housekeeping gene, which was comparable among the groups.

Statistical analysis

All data are presented as mean ± standard error of the mean for continuous variables. One-way analysis of variance with the post hoc Tukey–Kramer test was used to test for significant differences among the groups. To test the significant difference between the two groups, Student's t-test was used. Differences were considered statistically significant at $P < 0.05$. Minimum sample size was determined to be 16 by G-POWER using an alpha of 0.05 and a power of 0.80 for FS. Statistical analysis was performed using JMP Pro Statistical software (version 15.0; SAS Institute Inc., Cary, NC, USA).

Results

Echocardiographic parameters before the first and second operation

Table 1 and **2** show echocardiographic parameters before the first and second operation, respectively. All rats had a postinfarction myocardial scar in the anterior wall, dilated LV, and reduced LV systolic function before the second operation. All parameters were comparable among groups. LVEDD was larger, and FS was smaller before the second operation than the baseline values before the first operation.

Lung and heart weights at euthanization

Table 3 shows lung and heart weights at euthanization. Total heart weight could not be compared among the groups because pledgets used in SVR could bias the result. Body weight 2 days after the second operation was significantly lower in the SVR group than in the sham group. Lung and atrial weights were comparable among the groups.

Echocardiographic parameters before euthanization

Figure 2 shows representative M-mode pictures (**Fig. 2a**) and M-mode parameters (**Fig. 2b, 2c, 2d**) before euthanization. LVEDD and LVESD were smaller and FS was higher in the 2-day SVR group than in the 2-day sham group (**Fig. 2b, 2c, 2d**). On the other hand, 28 days after the second operation, no difference was found in those parameters between the SVR and sham groups. Additionally, LVEDD and LVESD 28 days after SVR were larger, and FS was smaller than those 2 days after SVR. Thus, the recurrence of LV remodeling and systolic functional deterioration after SVR were observed.

In contrast, LVEDD was smaller in the SVR + trehalose group than in the SVR and sham + trehalose groups (**Fig. 2b**); LVESD was smaller in the SVR + trehalose

group than in the other groups (**Fig. 2c**); FS was higher in the SVR + trehalose group than in the other groups (**Fig. 2d**). Furthermore, LVESD and FS 28 days after SVR with trehalose were comparable to those 2 days after SVR. These findings suggest that trehalose preserved LV size and systolic function after SVR. No difference was found in all parameters between the sham + trehalose and sham groups.

Protein expressions of LC3-II and p62

Figure 4 shows the myocardial protein expressions of LC3-II (**Fig. 4a**) and p62 (**Fig. 4b**) at euthanization. The expression of LC3-II was lower, and that of p62 was higher in the 2-day SVR group than in the 2-day sham group, suggesting a decrease in autophagic flux early after SVR. On the other hand, 28 days after SVR, no difference was found in the expressions of LC3-II and p62 between the SVR and sham groups. Furthermore, the expression of LC3-II was higher, and that of p62 was lower 28 days after SVR than those 2 days after SVR. These findings suggest that autophagic flux decreased early after SVR and increased late after SVR. In the SVR + trehalose group, the expression of p62 was lower than that in the SVR group, while the expression of LC3-II was comparable between the groups. This finding suggests that autophagic flux was increased more in the SVR + trehalose group than in the SVR group. No difference in p62 was found between the sham + trehalose and sham groups.

Myocardial gene expressions related to pathological hypertrophy

Table 4 shows the expression of genes in the LV myocardium which was excised at euthanization. BNP was significantly smaller in the 2-day SVR group than that in the 2-day sham group. No significant difference was observed in αMHC, βMHC, and BNP 28

days after the second operation. αSA, a fetal gene, was significantly higher in the sham + trehalose group than in the sham and SVR groups.

Myocyte area and myocardial fibrosis

Figure 5 shows the histological findings of the LV. The hearts sectioned and stained with Masson's trichrome are shown in **Fig. 5a**. Two days after SVR, the myocyte area was smaller in the SVR group than in the sham group. Twenty-eight days after SVR, the myocyte area was smaller in the SVR + trehalose group than in the sham group. The myocyte area was larger in the sham and sham + trehalose groups than in the 2-day sham group (**Fig. 5b**). No significant difference existed in the fibrosis fraction among the groups at each time point (**Fig. 5c**). Fibrosis fraction was larger in the sham + trehalose group than in the 2-day sham group.

Discussion

We demonstrated that the administration of trehalose after SVR attenuated the recurrence of LV remodeling and was associated with changes in autophagy markers.

Attenuation of recurrence of LV remodeling after SVR

We demonstrated that SVR reduced LV size early after SVR. However, late after SVR, LV remodeling recured as the level of the rats without SVR. These findings do not conflict with the clinical reports of SVR [12,13].

There is no clear strategy to attenuate LV remodeling after SVR. Previous experimental reports demonstrated that postoperative administration of an angiotensin-converting enzyme inhibitor and beta blocker attenuated LV remodeling after SVR in a

rat model of ICM [18,19]. However, extremely high doses of the drugs (lisinopril, 10 mg/kg; carvedilol, 50 mg/kg) were required to attenuate LV remodeling and would be difficult to use clinically. Additionally, these drugs have already been widely used in the clinic for patients with ICM even before SVR. Thus, we cannot expect the additive effects of these medications after SVR in the clinic.

We demonstrated that oral administration of 10% trehalose attenuated the recurrence of LV remodeling after SVR. Trehalose is a natural disaccharide that is ubiquitous in a wide variety of organisms, for example, bacteria, yeast, and fungi, while it is not synthesized by mammals. Substantial quantities of trehalose are present in modern food sources [20]. Trehalose has been reported to be safe for humans. Adverse effects of trehalose have not been reported at concentrations of up to 10% of the diet. Oral administration of 10% trehalose can be safely used in the clinic to attenuate LV remodeling after SVR.

Autophagy: a mechanism of trehalose effect?

Trehalose has been used as an autophagy inducer in several studies, including hepatocytes (hepatic steatosis), neurons (Huntington disease, Parkinson's disease), and the heart (MI) [21-23]. The mechanisms of autophagy activation by trehalose have been reported to be mammalian targets of rapamycin-independent pathways or inhibition of glucose transporters mimicking starvation. In an acute MI mouse model, trehalose has been reported to activate autophagy and attenuate LV remodeling in the late phase [23]. Moreover, trehalose did not attenuate LV remodeling in heterozygous Beclin 1 knockout mice, which are insensitive to autophagy inducers. These findings suggest that the effect of trehalose on myocytes is mediated by activating autophagy. Thus far, the effects of autophagy after MI have been reported to be attenuation of myocardial injury

and LV remodeling by the following mechanisms: (1) preservation of energy content, (2) improvement of mitochondrial function, and (3) clearance of misfolded proteins and damaged intracellular organelles [23-25]. Of these, we speculate that selective mitochondrial autophagy, known as 'mitophagy', plays an important role in attenuation of LV remodeling after SVR in patients with ICM. Damaged mitochondria have been reported to accumulate due to pressure overload and leak reactive oxygen species and apoptogenic proteins [26]. It has also been reported that the accumulation of mitochondria was observed in patients with recurrence of LV remodeling after SVR, although the mechanism was unclear [13]. Taken together, the possible roles of autophagy in preventing LV remodeling after SVR in patients with ICM are to promote the degradation of damaged mitochondria and to improve mitochondrial function.

The cardiac function improved two days after SVR even with increased p62, suggesting a decrease in autophagic flux. Autophagy would adaptively change dependent on the size or wall stress of the LV; we speculate that autophagy was attenuated due to the systolic functional improvement and the LV size reduction, that is, wall-stress reduction two days after SVR. On the other hand, p62 decreased in the SVR + trehalose group even if cardiac function was comparable to that in the 2-day SVR group. Trehalose administration after SVR may have affected the change in autophagy.

The present study does not confirm that the autophagic flux was stimulated in the SVR + trehalose group compared with the other groups. To precisely examine the autophagy flux, we tried to use chloroquine (CQ), an autophagy inhibitor that blocks the binding of the autophagosome to the lysosome, for several SVR rats (data not shown). However, all rats died a few days after injection of CQ i.p. and we were unable to obtain any data. The use of a lysosome inhibitor is an important topic in our future experiments to confirm the mechanisms of the effects of trehalose.

Trehalose alone did not attenuate LV remodeling and activate autophagy in ICM rats without SVR. These findings were different from those of a previous study using the acute MI model [23]. We speculate that the reasons would be as follows: insufficient amount of trehalose for ICM without SVR and late administration timing after MI. To answer the former question, the experiments on the serum levels of trehalose after changing the concentration and amount of trehalose are required. As for the latter, it might be too late to exert the effects of trehalose after completion of the myocardial scar, if SVR is not performed.

Limitations

This study has several limitations. First, the causal relationship between autophagy activation and the attenuation of LV remodeling was unknown in our study. To clarify the causal relationship, further experiments using autophagy inhibitors or those using knockout mice are required. However, the former experiment was not successful in this in-vivo study due to the high mortality. For the latter, the mouse hearts would be too small to perform the SVR procedure. Second, the anti-inflammatory and antioxidant effects of trehalose were not examined. Third, we did not measure blood pressure and serum glucose levels, which may influence the size of MI. Because trehalose has not been reported to influence them in humans and spontaneously hypertensive rats [27,28], the effects would also be small in the present study. Fourth, the results of this study cannot be directly applied to humans because the animals were not administered any guideline-directed medications for heart failure; the technique of SVR is different from that in clinical setting. However, a catheter-based SVR called LIVE therapy (BioVentrix Revivent TC system) has been recently invented and has something in common with our procedure [29]. Lastly, trehalose did not affect histology. The

incomplete wall-stress reduction in the SVR + trehalose group may be a reason for the insignificance. The variation of the data would also be another reason.

Conclusion

Trehalose attenuated the recurrence of LV remodeling SVR in a rat ICM model. Trehalose may be a candidate adjuvant therapy to retain the effects of SVR.

Conflicts of interest

The authors declare that they have no conflict of interest.

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Author contributions

All authors contributed to the conception and design of the study. Material preparation, date collection and analysis were performed by all authors. The first draft of the manuscript was written by Tetsuya Hieda, and all authors commented on previous version of the manuscript. All authors read and approved the final manuscript.

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Figure captions

Fig. 1 Experimental protocol. Myocardial infarction operation was performed for 10-week-old male SD rats. Twenty-eight days after the myocardial infarction, 16 rats underwent the second operation: sham or SVR operation. In the sham + trehalose and SVR + trehalose groups, 10% trehalose was given orally via a drinking bottle for 21 days until euthanization. To examine the early effect of SVR, other eight rats were euthanized two days after the second operation. Echocardiography was performed before each operation and euthanization. RT-PCR, Western blotting, and histological examination were performed after euthanization. RT-PCR, real time polymerase chain reaction; p.o., per os; SD, Sprague-Dawley; SVR, surgical ventricular reconstruction.

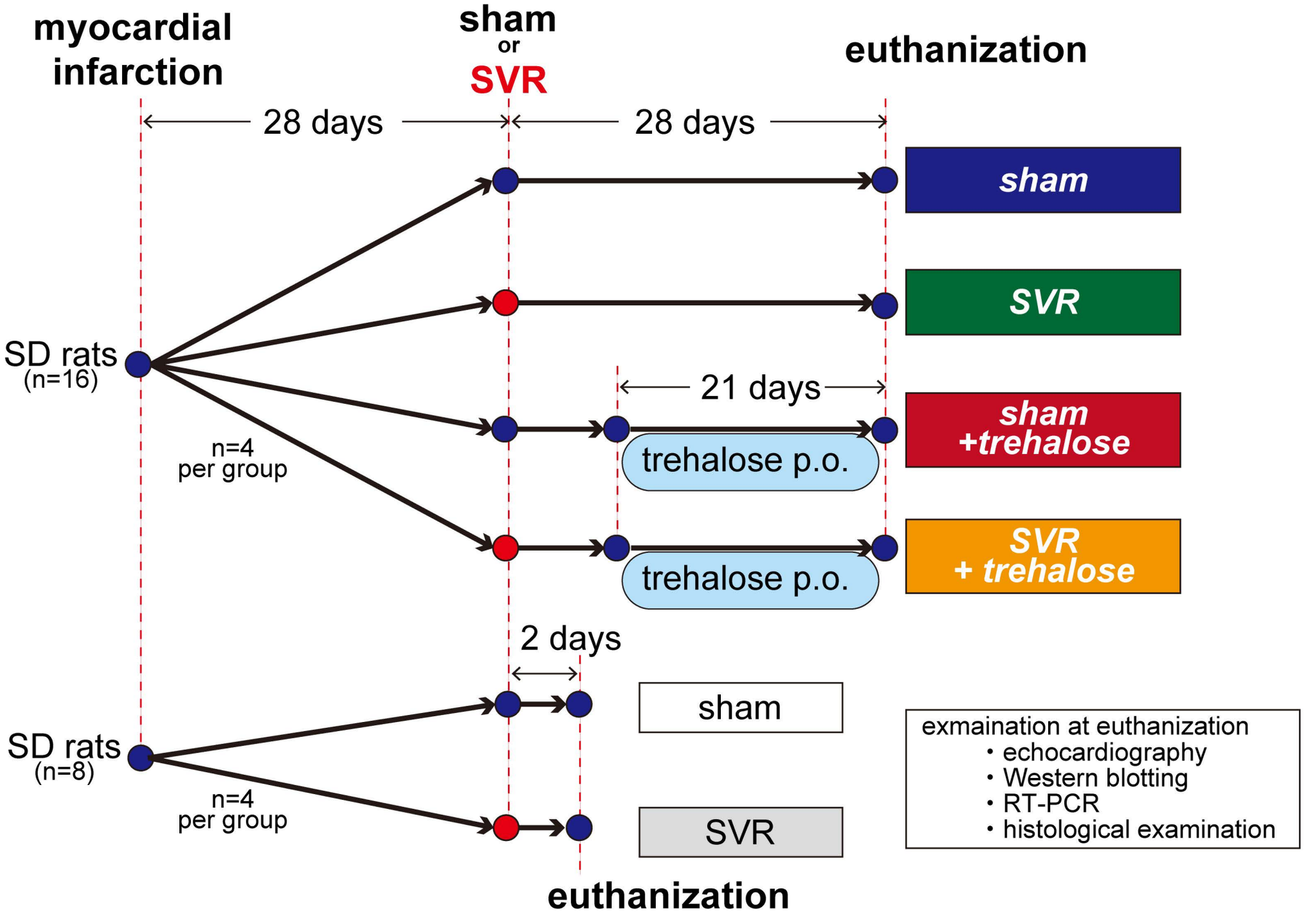
Fig. 2 Echocardiographic M-mode parameters before euthanization (four rats per each group) (**a**). Representative pictures of M-mode. Scale bar = 0.2 s. The solid and dotted double-headed arrows indicate LVEDD and LVESD, respectively. (**b**) LVEDD; (**c**) LVESD; (**d**) FS. The bars represent the mean. The whiskers represent the values of standard error of the mean. One-way analysis of variance with the post hoc Tukey–Kramer was used to test for significant differences among the groups. Student's t-test was used to test the significant difference between the two groups. *P < 0.05, **P < 0.01; # P < 0.05, ## P < 0.01, vs. 2-day SVR. FS, fractional shortening; LVEDD, left ventricular end-diastolic dimension; LVESD, left ventricular end-systolic dimension; SVR, surgical ventricular reconstruction; Tre, trehalose.

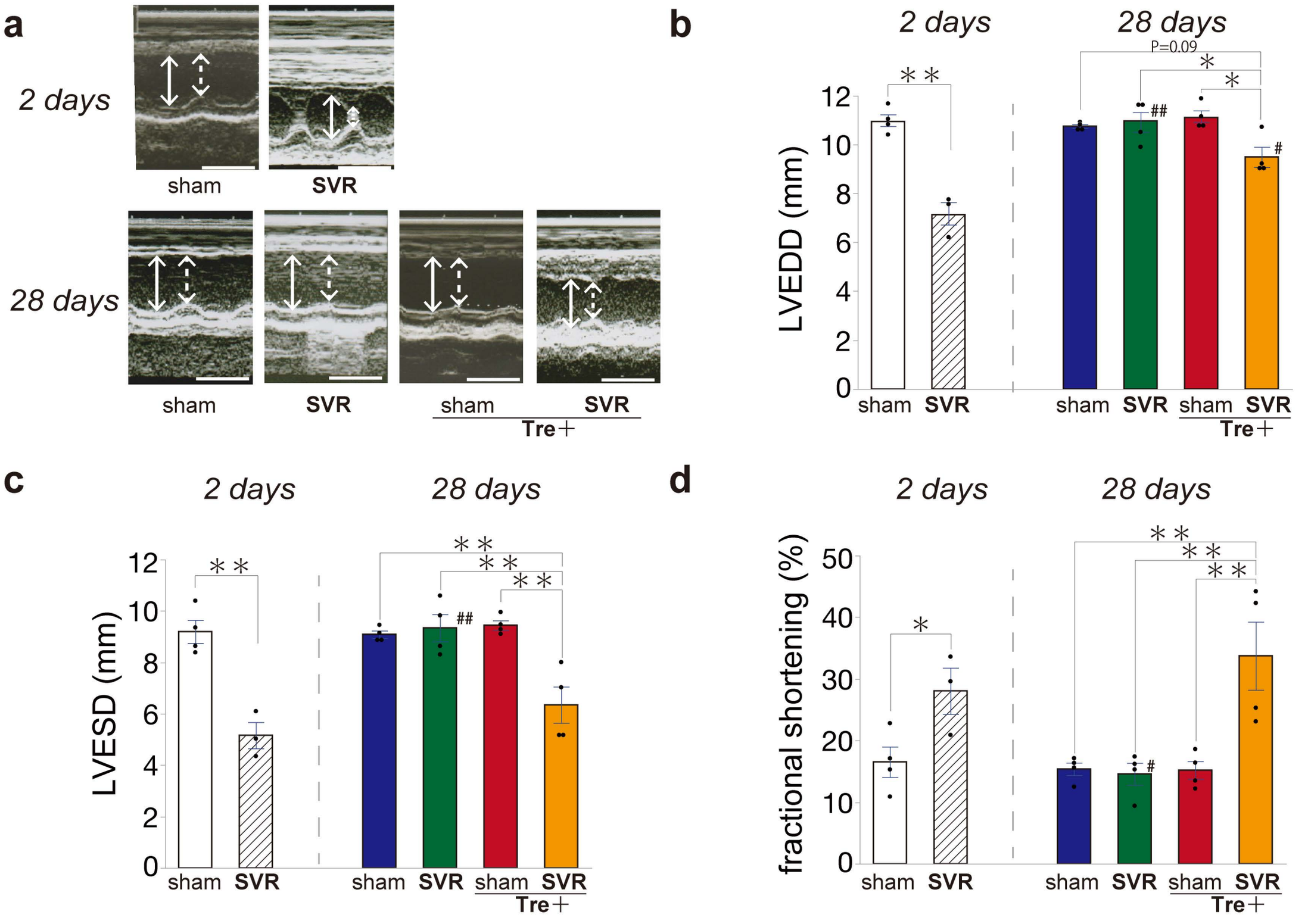
Fig. 3 Echocardiographic Doppler parameters before euthanization (four rats per each group): (**a**) cardiac output; (**b**) stroke volume; (**c**) E wave; (**d**) deceleration time. The bars represent the mean. The whiskers represent the values of standard error of the

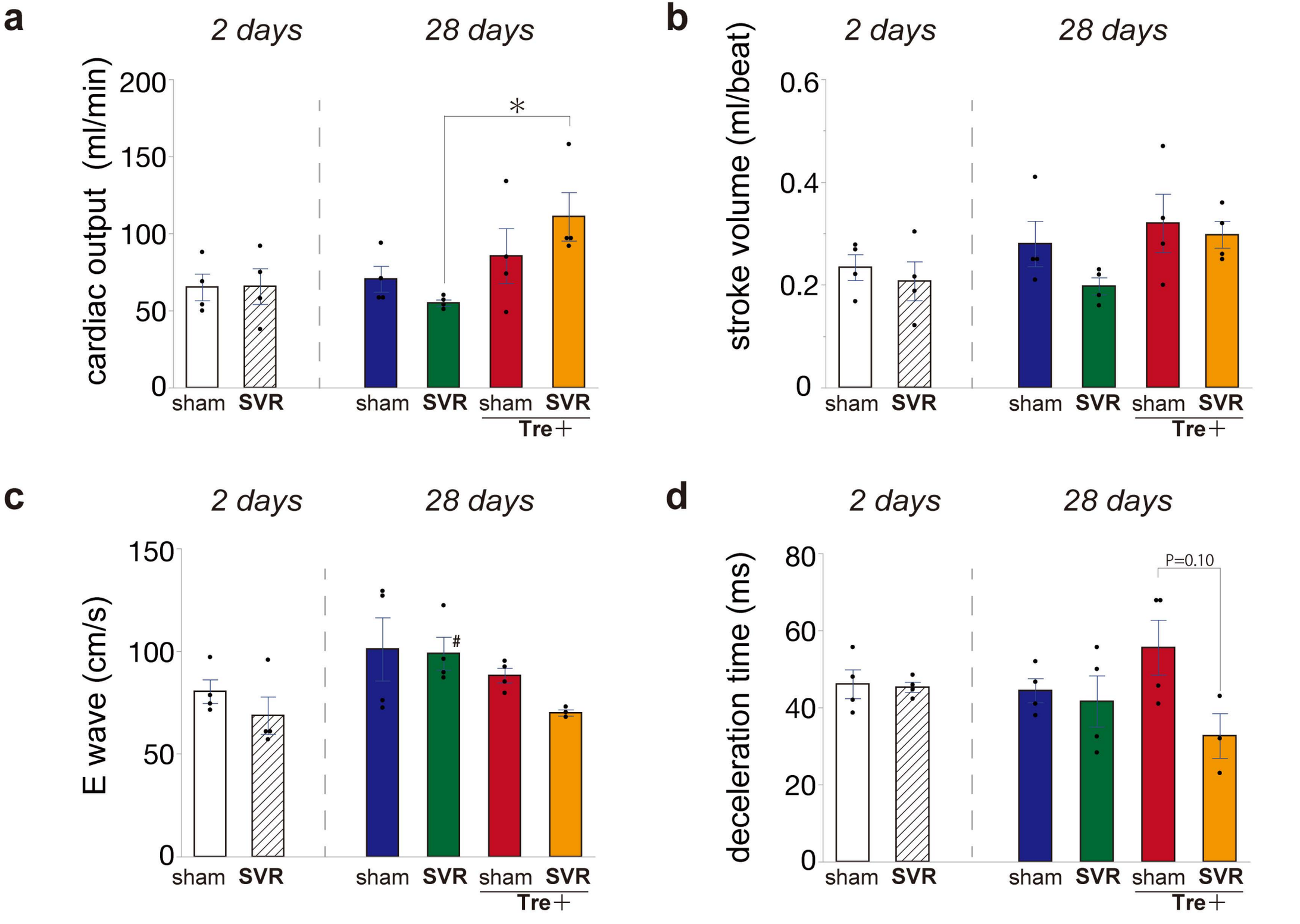
mean. One-way analysis of variance with the post hoc Tukey–Kramer test was used to test for significant differences among the groups. Student's t-test was used to test the significant difference between the two groups. *P < 0.05; # P < 0.05 vs. 2-day SVR. CO, cardiac output; DCT, deceleration time; SV, stroke volume; SVR, surgical ventricular reconstruction; Tre, trehalose.

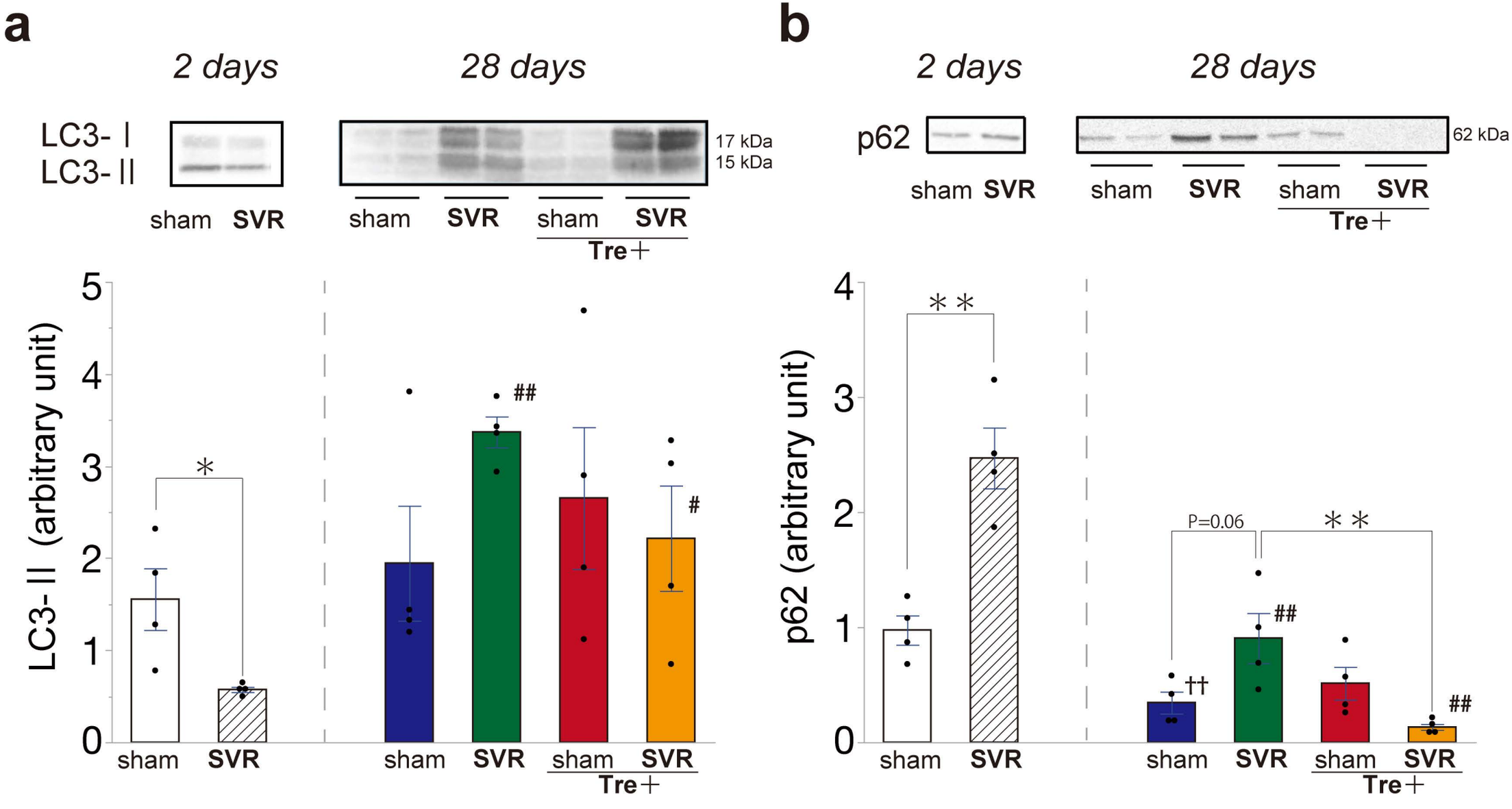
Fig. 4 Protein expression of LC3-II (**a**) and p62 (**b**) in the myocardium at euthanization (four rats per each group). The bars represent the mean. The whiskers represent the values of standard error of the mean. One-way analysis of variance with the post hoc Tukey–Kramer was used to test for significant differences among the groups. Student's t-test was used to test the significant difference between the two groups. *P < 0.05, **P < 0.01; †† < 0.01 vs. 2-day sham; # P < 0.05, ## P < 0.01 vs. 2-day SVR. LC3-II, microtubule-associated Light Chain 3 II; SVR, surgical ventricular reconstruction; Tre, trehalose.

Fig. 5 Cardiomyocyte area and fibrosis fraction at euthanization (four rats per each group) (**a**). Representative Masson's trichrome-stained transverse sections of the ventricles at euthanization. The allows indicate the plegdets used during SVR. Scale bar = 2000 μm. (**b**) Cardiomyocyte area in the hematoxylin-eosin-stained sections in the septal lesion. Randomly chosen 100 oval-shaped cardiomyocytes per a rat with a nucleus were analyzed. Scale bar = 25 μm. (**c**) Fibrosis fraction in the Masson's trichrome-stained sections in the septal lesion. Randomly chosen 10 frames per a rat were analyzed. Scale bar = 100 μm. The bars represent the mean. The whiskers represent the values of standard error of the mean. *P < 0.05; † P < 0.05, †† P < 0.01 vs. 2-day sham. SVR, surgical ventricular reconstruction; Tre, trehalose.


myocardial infarction
sham or SVR
euthanization
28 days
28 days
sham
SVR
SD rats (n=16)
21 days
sham +trehalose
trehalose p.o.
n=4 per group
SVR + trehalose
trehalose p.o.
2 days
sham
SD rats (n=8)
n=4 per group
SVR
euthanization
exmaination at euthanization
• echocardiography
• Western blotting
• RT-PCR
• histological examination


a
2 days
sham
SVR
28 days
sham
SVR
sham
SVR
Tre+
b
2 days
28 days
P=0.09
LVEDD (mm)
0
2
4
6
8
10
12
sham
SVR
sham
SVR
sham
SVR
Tre+
c
2 days
28 days
LVESD (mm)
0
2
4
6
8
10
12
sham
SVR
sham
SVR
sham
SVR
Tre+
d
2 days
28 days
fractional shortening (%)
0
10
20
30
40
50
sham
SVR
sham
SVR
sham
SVR
Tre+


a
2 days
28 days
cardiac output (ml/min)
200
150
100
50
0
*
sham
SVR
sham
SVR
sham
SVR
Tre+
b
2 days
28 days
stroke volume (ml/beat)
0.6
0.4
0.2
0
sham
SVR
sham
SVR
sham
SVR
Tre+
c
2 days
28 days
E wave (cm/s)
150
100
50
0
#
sham
SVR
sham
SVR
sham
SVR
Tre+
d
2 days
28 days
deceleration time (ms)
80
60
40
20
0
P=0.10
sham
SVR
sham
SVR
sham
SVR
Tre+


a
2 days
28 days
LC3- I
LC3- II
17 kDa
15 kDa
sham
SVR
sham
SVR
sham
SVR
Tre+
LC3- II (arbitrary unit)
0
1
2
3
4
5
*
##
#
sham
SVR
sham
SVR
sham
SVR
Tre+
b
2 days
28 days
p62
62 kDa
sham
SVR
sham
SVR
sham
SVR
Tre+
p62 (arbitrary unit)
0
1
2
3
4
**
P=0.06
**
††
##
##
sham
SVR
sham
SVR
sham
SVR
Tre+

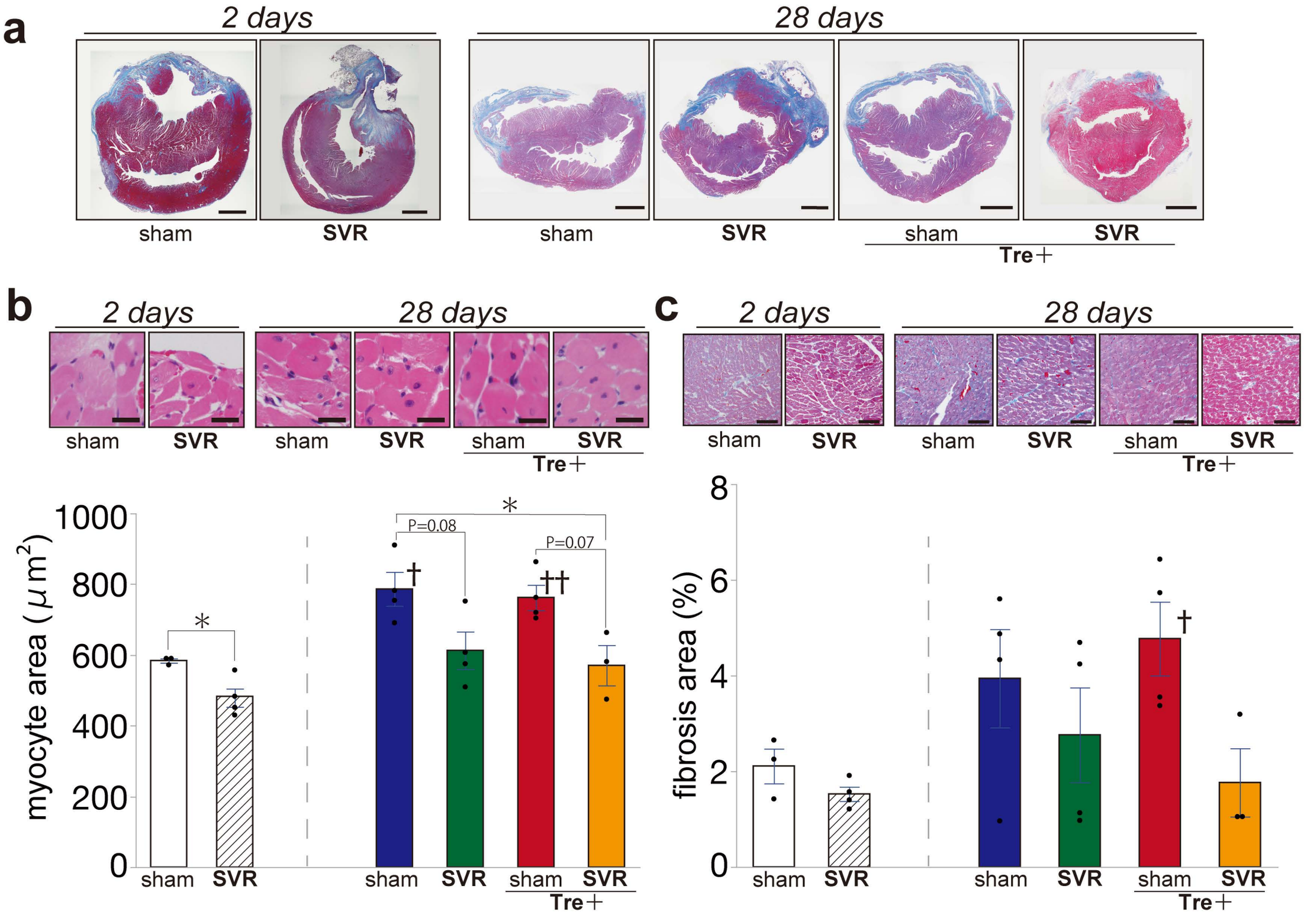

a
2 days
28 days
sham
SVR
sham
SVR
sham
SVR
Tre+
b
2 days
28 days
sham
SVR
sham
SVR
sham
SVR
Tre+
c
2 days
28 days
sham
SVR
sham
SVR
sham
SVR
Tre+
myocyte area (μm²)
1000
800
600
400
200
0
*
P=0.08
*
P=0.07
†
††
sham
SVR
sham
SVR
sham
SVR
Tre+
fibrosis area (%)
8
6
4
2
0
†
sham
SVR
sham
SVR
sham
SVR
Tre+

Table 1 Echocardiographic parameters before the first operation

	2-day group			28-day group				
	sham (n = 4)	SVR (n = 4)	P values	sham (n = 4)	SVR (n = 4)	sham + Tre (n = 4)	SVR + Tre (n = 4)	P values
BW, g	333 ± 10	342 ± 7	0.88	358 ± 12	350 ± 4	350 ± 11	345 ± 3	0.78
LVEDD, mm	7.7 ± 0.1	7.9 ± 0.1	0.81	8.0 ± 0.4	7.5 ± 0.1	7.4 ± 0.6	7.7 ± 0.1	0.52
LVESD, mm	4.4 ± 0.2	4.9 ± 0.2	0.89	5.0 ± 0.4	4.6 ± 0.5	4.2 ± 0.3	4.5 ± 0.2	0.53
FS, %	43 ± 2	37 ± 2	0.74	42 ± 6	40 ± 6	43 ± 2	42 ± 2	0.94

Values ± standard error of the mean. Four rats per each group. One-way analysis of variance was used to test for significant differences among the 28-day groups. Student's t-test was used to test the significant difference between the 2-day groups. BW, body weight; FS, fractional shortening; LVEDD, left ventricular end-diastolic dimension; LVESD, left ventricular end-systolic dimension; SVR, surgical ventricular reconstruction; Tre, trehalose.

Table 2 Echocardiographic parameters before the second operation

	2-day group			28-day group				
	sham (n = 4)	SVR (n = 4)	P values	sham (n = 4)	SVR (n = 4)	sham + Tre (n = 4)	SVR + Tre (n = 4)	P values
scar ratio, %	32 ± 3	29 ± 2	0.51	31 ± 2	36 ± 1	29 ± 2	33 ± 3	0.19
LVEDD, mm	10.9 ± 0.3	10.7 ± 0.5	0.76	10.3 ± 0.1	10.0 ± 0.3	10.7 ± 0.4	9.7 ± 0.2	0.14
LVESD, mm	9.1 ± 0.6	8.5 ± 0.7	0.48	8.6 ± 0.4	8.2 ± 0.6	8.9 ± 0.4	7.6 ± 0.5	0.30
FS, %	16 ± 4	22 ± 4	0.34	18 ± 4	18 ± 4	17 ± 1	22 ± 4	0.70
E wave, m/s	108 ± 10	97 ± 7	0.41	118 ± 8	97 ± 7	98 ± 14	102 ± 7	0.43
DCT, ms	48 ± 2	48 ± 2	0.85	43 ± 5	49 ± 7	58 ± 9	46 ± 6	0.47
CO, ml/min	76 ± 8	88 ± 7	0.31	79 ± 5	73 ± 2	92 ± 13	91 ± 16	0.47
SV, ml/beat	0.29 ± 0.02	0.33 ± 0.03	0.29	0.29 ± 0.02	0.25 ± 0.01	0.34 ± 0.05	0.34 ± 0.06	0.23

Values ± standard error of the mean. Four rats per each group. One-way analysis of variance was used to test for significant differences among the 28-day groups. Student's t-test was used to test the significant difference between the 2-day groups. BW, body weight; CO, cardiac output; DCT, deceleration time; FS, fractional shortening; LVEDD, left ventricular end-diastolic dimension; LVESD, left ventricular end-systolic dimension; SV, stroke volume; SVR, surgical ventricular reconstruction; Tre, trehalose.

Table 3 Body, lung, and atrial weights at euthanization

	2-day group			28-day group				
	sham (n = 4)	SVR (n = 4)	P values	sham (n = 4)	SVR (n = 4)	sham + Tre (n = 4)	SVR + Tre (n = 4)	P values
BW, g	423 ± 15	368 ± 17	0.049	485 ± 17	453 ± 22	483 ± 21	473 ± 10	0.59
LW, mg	2034 ± 126	1630 ± 114	0.85	3093 ± 648	3286 ± 196	2740 ± 429	3155 ± 724	0.90
AW, mg	315 ± 56	277 ± 60	0.91	609 ± 202	389 ± 77	331 ± 39	261 ± 21	0.19
LW/BW, mg/g	4.8 ± 0.2	4.3 ± 0.4	0.88	6.3 ± 1.3	7.3 ± 0.4	5.8 ± 1.1	6.8 ± 1.7	0.85
AW/BW, mg/g	0.7 ± 0.1	0.7 ± 0.1	0.89	1.2 ± 0.4	0.9 ± 0.2	0.7 ± 0.1	0.6 ± 0.1	0.23

Values ± standard error of the mean. Four rats per each group. One-way analysis of variance was used to test for significant differences among the 28-day groups. Student's t-test was used to test the significant difference between the 2-day groups. AW, atrial weight; BW, body weight; LW, lung weight; SVR, surgical ventricular reconstruction; Tre, trehalose.

Table 4 Expression of genes in the LV myocardium which was excised at euthanization

	2-day group			28-day group				
	sham (n = 4)	SVR (n = 4)	P values	sham (n = 4)	SVR (n = 4)	sham + Tre (n = 4)	SVR + Tre (n = 4)	P values
αMHC	0.12 ± 0.06	0.02 ± 0.01	0.16	0.06 ± 0.01	0.18 ± 0.10	0.31 ± 0.08	0.25 ± 0.11	0.24
βMHC	1.63 ± 0.29	0.83 ± 0.16	0.073	1.42 ± 0.23	0.73 ± 0.33	1.40 ± 0.32	0.78 ± 0.08	0.16
BNP	2.06 ± 0.59	0.54 ± 0.19*	0.028	1.43 ± 0.07	1.58 ± 0.22	1.80 ± 0.39	2.50 ± 1.25	0.68
αSA	0.96 ± 0.76	1.36 ± 1.15	0.78	0.65 ± 0.20	0.70 ± 0.11	1.51 ± 0.09**, #	1.03 ± 0.16	0.005

Values ± standard error of the mean. Four rats per each group. Units are arbitrary units. One-way analysis of variance with the post hoc Tukey-Kramer was used to test for significant differences among the 28-day groups. Student's t-test was used to test the significant difference between the 2-day groups. *P < 0.05, **P < 0.01 vs. sham; # P < 0.05 vs SVR. BNP, brain natriuretic peptide; LV, left ventricle; MHC, myosin heavy chain; SA, skeletal actin; SVR, surgical ventricular reconstruction; Tre, trehalose.