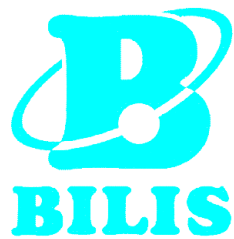


重度免疫不全マウスを用いた心筋梗塞モデル作製

Myocardial infarction model in severe combined immunodeficiency mice.



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Introduction

We created a myocardial infarction model in C57BL/6J-mice and attempted to measure cardiac function by echocardiography. Next, we created a myocardial infarction model using immunodeficient mice and confirmed the characteristic changes of the myocardial infarction model with and without immune function (NOD/ShiJic-ScidJcl-mice) compared with the myocardial infarction model of C57BL/6J-mice.

Methods

Materials

<Animals>

NOD/ShiJic-ScidJcl-mice : Male, Modeling: 12 weeks old

C57BL/6J-mice : Male, Modeling: 10 weeks old

<Experimental Groups>

Strain	Group	Number
NOD/ShiJic-ScidJcl-mice	Sham	3
NOD/ShiJic-ScidJcl-mice	Myocardial infarction	27
C57BL/6J-mice	Sham	6
C57BL/6J-mice	Myocardial infarction	31

This study was conducted as approved by the Institutional Animal Experiment Committee of NISSEI BILIS Co., Ltd., Shiga Laboratory.

Methods

Preparation

<Anesthetics>

Medetomidine hydrochloride 0.15 mg/kg+Midazolam 2.0 mg/kg
+Butorphanol tartrate 2.5 mg/kg, s.c.

<Antagonist>

Atipamezole hydrochloride 0.15 mg/kg, s.c.

Methods

Model making method

1. Under anesthesia, an incision was made in the skin of the mice to expose the larynx, and an 18 or 20 G indwelling needle was orally inserted to ventilate with a small animal ventilator (Tidal volume: 200 μ L/stroke, respiratory rate: 140 strokes/min) was started.
2. The chest side wall was opened to expose the heart, and the left anterior descending coronary artery was occluded using a 7-0 threaded suture needle.
3. The open intercostal space was closed with 6-0 silk thread and the skin at the incision was sutured.
4. In the sham group, the side wall of the chest was opened, the pericardium was incised to expose the heart, and then the chest was closed in the same manner as in the myocardial infarction group.

Methods

Inspection item

<Echocardiographic>

- Machine : Universal ultrasonic diagnostic imaging apparatus
(ViVid S6, GE Healthcare Japan)
- Inspection period : Day 1, 7, 14 and 28 after myocardial infarction
- Measurement method : Under isoflurane anesthesia
- Measurement item : EF, %FS, LVIDd, LVIDs, LVAWd, LVPWd, LVEDV, LVESV

<Myocardial infarction size>

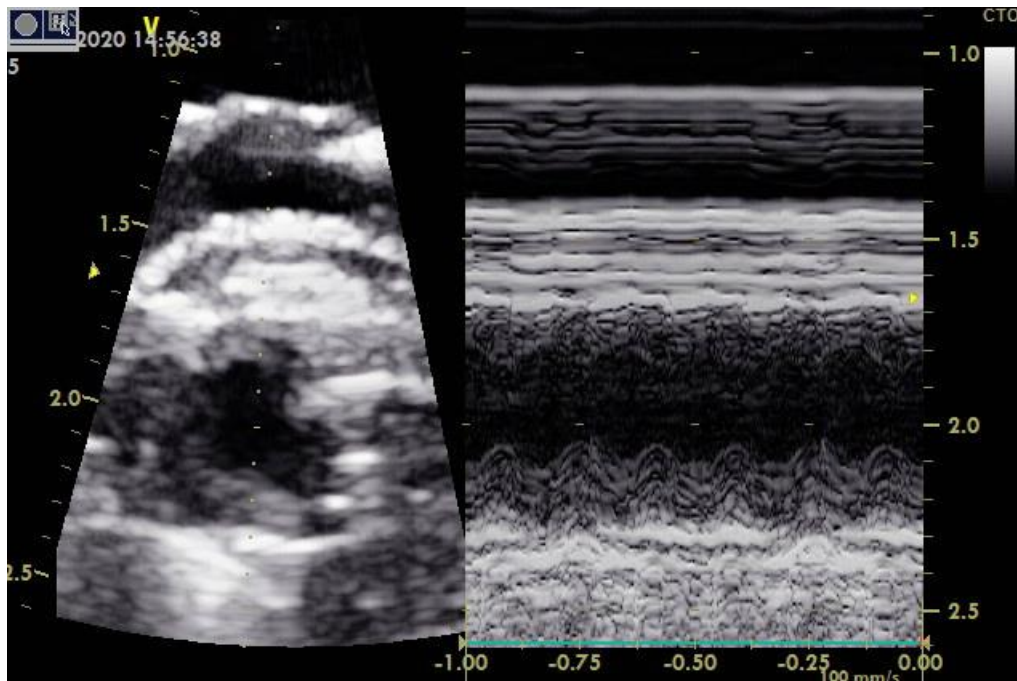
- Inspection period : Day 28 after myocardial infarction
- Preparation specimen : Masson's trichrome (MT)-stained specimens
- Calculation method : Calculate the area ratio (%) of myocardial fibrosis to the entire left ventricular wall

Results

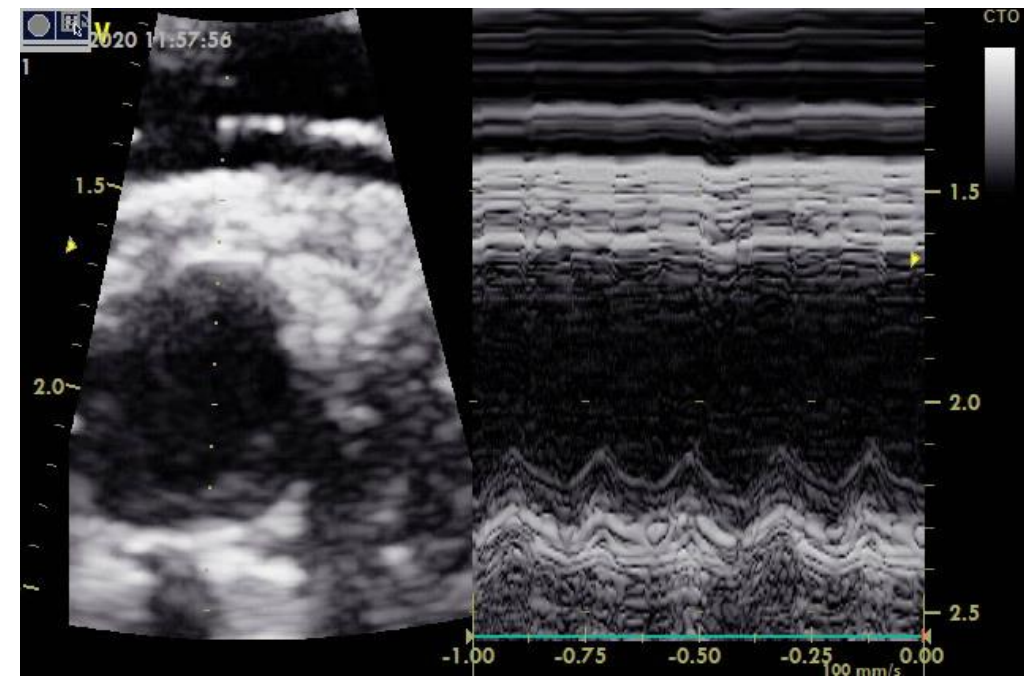
Typical example of echocardiographic

<NOD/ShiJic-ScidJcl-mice>

Day 28
Sham group



Day 28
Myocardial infarction group



Results

Echocardiographic parameters

<NOD/ShiJic-ScidJcl-mice>

Group	No. of animals	EF (%)				%FS				LVIDd (mm)				LVIDs (mm)			
		Day1	Day7	Day14	Day28	Day1	Day7	Day14	Day28	Day1	Day7	Day14	Day28	Day1	Day7	Day14	Day28
Sham	3	72.7 ±1.8	59.9 ±10.7	73.9 ±4.7	79.0 ±1.6	35.3 ±1.4	27.7 ±7.4	36.6 ±3.9	40.7 ±1.5	3.7 ±0.2	3.9 ±0.1	3.9 ±0.2	3.7 ±0.1	2.4 ±0.1	2.8 ±0.3	2.5 ±0.2	2.2 ±0.1
Myocardial infarction	25	49.0 # ±2.9	41.8 ±2.8	47.1 ## ±2.4	35.8 ## ±2.0 (24)	21.0 ## ±1.7	17.1 # ±1.4	19.7 ## ±1.4	14.0 ## ±0.9 (24)	3.7 ±0.1	4.4 ±0.1	4.6 # ±0.1	4.9 ## ±0.1 (24)	2.9 ±0.1	3.7 # ±0.1	3.7 ## ±0.1	4.2 ## ±0.1 (24)

Group	No. of animals	LVAWd (mm)				LVPWd (mm)				LVEDV (ml)				LVESV (ml)			
		Day1	Day7	Day14	Day28	Day1	Day7	Day14	Day28	Day1	Day7	Day14	Day28	Day1	Day7	Day14	Day28
Sham	3	0.8 ±0.0	0.7 ±0.1	0.8 ±0.1	0.8 ±0.1	1.0 ±0.1	0.8 ±0.1	0.8 ±0.0	1.0 ±0.0	0.054 ±0.006	0.061 ±0.003	0.062 ±0.008	0.054 ±0.004	0.014 ±0.002	0.025 ±0.007	0.016 ±0.004	0.011 ±0.001
Myocardial infarction	25	0.7 ±0.0	0.5 ±0.0	0.5 ## ±0.0	0.5 ## ±0.0 (24)	0.8 ±0.0	0.8 ±0.0	0.8 ±0.0	0.9 ±0.0 (24)	0.052 ±0.004	0.088 ±0.006	0.100 # ±0.006	0.124 ## ±0.008 (24)	0.027 ±0.003	0.053 ±0.006	0.054 # ±0.005	0.081 ## ±0.007 (24)

Each value represents the mean±S.E.

Each figure in parenthesis represents the number of animals.

EF: ejection fraction, %FS: % fractional shortening, LVIDd: diastolic left ventricular internal dimension,

LVIDs: systolic left ventricular internal dimension, LVAWd: diastolic left ventricular anterior wall, LVPWd: diastolic left ventricular posterior wall,

LVEDV:left ventricular end-dyastolic volume, LVESV: left ventricular end-systolic volume

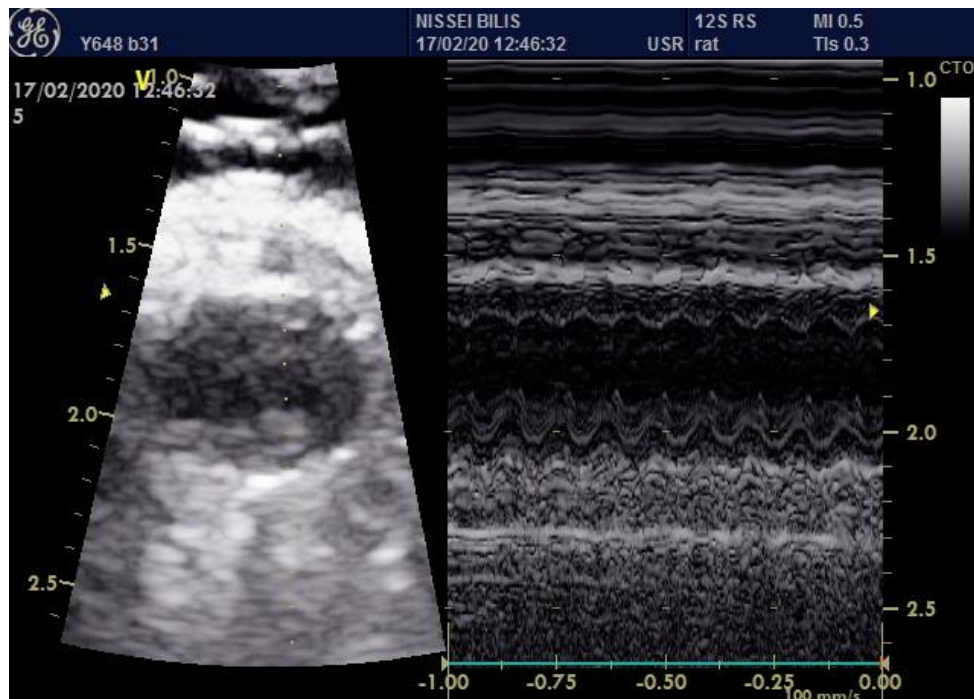
and ##: significant difference from sham at $p<0.05$ and $p<0.01$, respectively (Student's t -test or Aspin-Welch's t -test).

Results

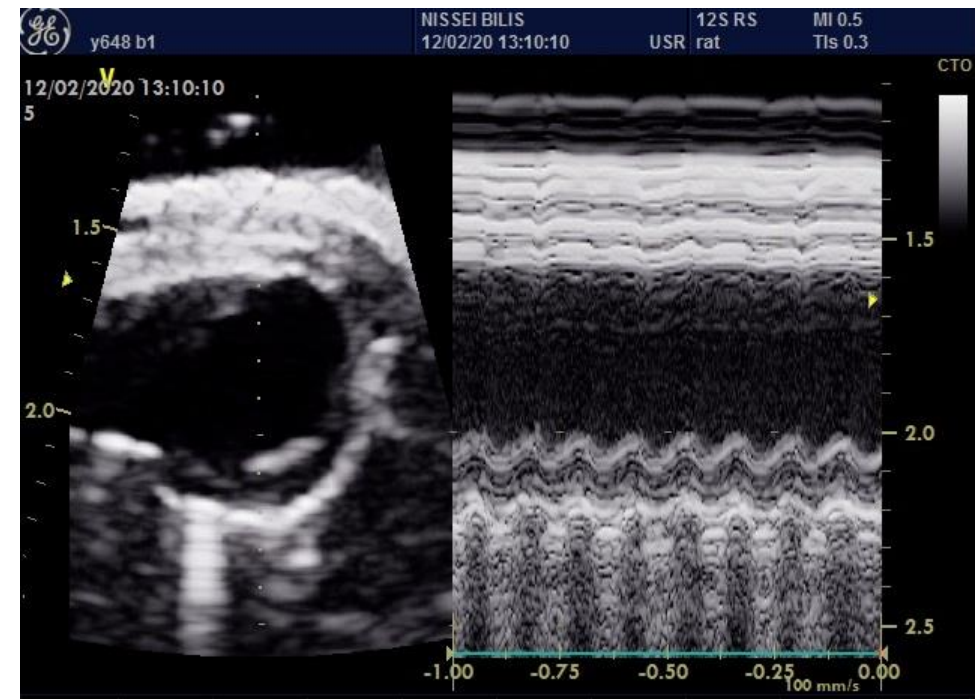
Typical example of echocardiographic

<C57BL/6J-mice>

Day 28
Sham group



Day 28
Myocardial infarction group



Results

Echocardiographic parameters

<C57BL/6J-mice>

Group	No. of animals	EF (%)				%FS				LVIDd (mm)				LVIDs (mm)			
		Day1	Day7	Day14	Day28	Day1	Day7	Day14	Day28	Day1	Day7	Day14	Day28	Day1	Day7	Day14	Day28
Sham	6	78.5 ±2.3	84.2 ±1.2	82.5 ±1.6	83.0 ±1.2	40.4 ±2.1	46.1 ±1.4	44.3 ±1.7	44.8 ±1.4	3.6 ±0.0	3.7 ±0.1	3.7 ±0.1	3.6 ±0.2	2.1 ±0.1	2.0 ±0.1	2.0 ±0.1	2.0 ±0.1
Myocardial infarction	26	54.4 ## ±2.3	42.6 ## ±3.1 (25)	40.9 ## ±3.0 (25)	39.2 ## ±3.6 (25)	23.6 ## ±1.4	17.8 ## ±1.8 (25)	16.8 ## ±1.6 (25)	16.2 ## ±1.8 (25)	3.8 ±0.1	4.7 ## ±0.1 (25)	4.9 ## ±0.2 (25)	5.2 ## ±0.2 (25)	2.9 ## ±0.1	3.9 ## ±0.2 (25)	4.1 ## ±0.2 (25)	4.4 ## ±0.2 (25)

Group	No. of animals	LVAWd (mm)				LVPWd (mm)				LVEDV (ml)				LVESV (ml)			
		Day1	Day7	Day14	Day28	Day1	Day7	Day14	Day28	Day1	Day7	Day14	Day28	Day1	Day7	Day14	Day28
Sham	6	0.9 ±0.1	1.1 ±0.0	1.1 ±0.0	1.2 ±0.0	1.0 ±0.0	1.1 ±0.0	1.1 ±0.0	1.2 ±0.0	0.046 ±0.002	0.053 ±0.006	0.050 ±0.006	0.048 ±0.006	0.010 ±0.001	0.009 ±0.002	0.009 ±0.002	0.008 ±0.001
Myocardial infarction	26	0.8 ±0.0	0.7 ## ±0.0 (25)	0.7 ## ±0.0 (25)	0.7 ## ±0.1 (25)	1.0 # ±0.0	0.9 ±0.0 (25)	1.0 # ±0.0 (25)	0.9 ## ±0.0 (25)	0.055 ±0.003	0.107 ## ±0.008 (25)	0.125 ## ±0.012 (25)	0.151 ## ±0.016 (25)	0.026 ## ±0.002	0.066 ## ±0.008 (25)	0.079 ## ±0.010 (25)	0.103 ## ±0.015 (25)

Each value represents the mean±S.E.

Each figure in parenthesis represents the number of animals.

EF: ejection fraction, %FS: % fractional shortening, LVIDd: diastolic left ventricular internal dimension,

LVIDs: systolic left ventricular internal dimension, LVAWd: diastolic left ventricular anterior wall, LVPWd: diastolic left ventricular posterior wall,

LVEDV:left ventricular end-diastolic volume, LVESV: left ventricular end-systolic volume

and ##: significant difference from sham at $p<0.05$ and $p<0.01$, respectively (Student's t -test or Aspin-Welch's t -test).

Results

Echocardiographic parameters

- NOD/ShiJic-ScidJcl-mice : Sham (n=3)
- ▲ NOD/ShiJic-ScidJcl-mice : MI (n=25)
- C57BL/6J-mice : Sham (n=6)
- ▲ C57BL/6J-mice : MI (n=26)

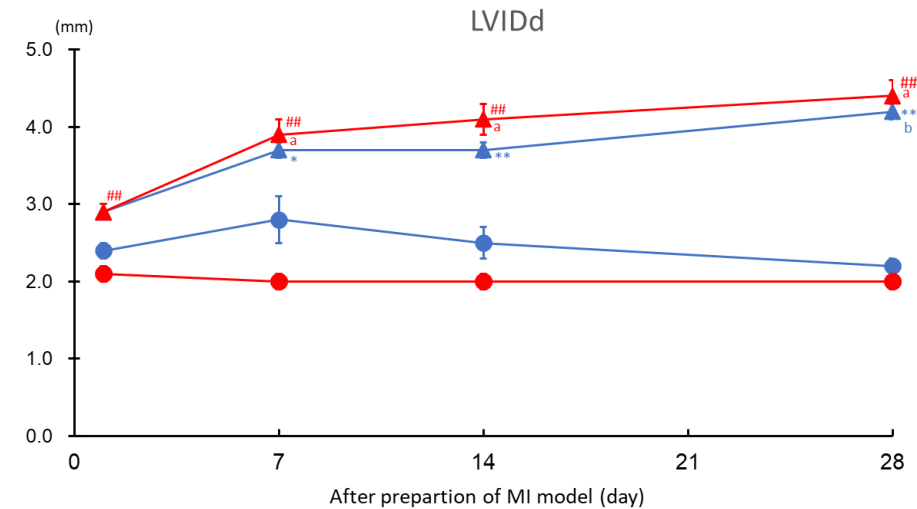
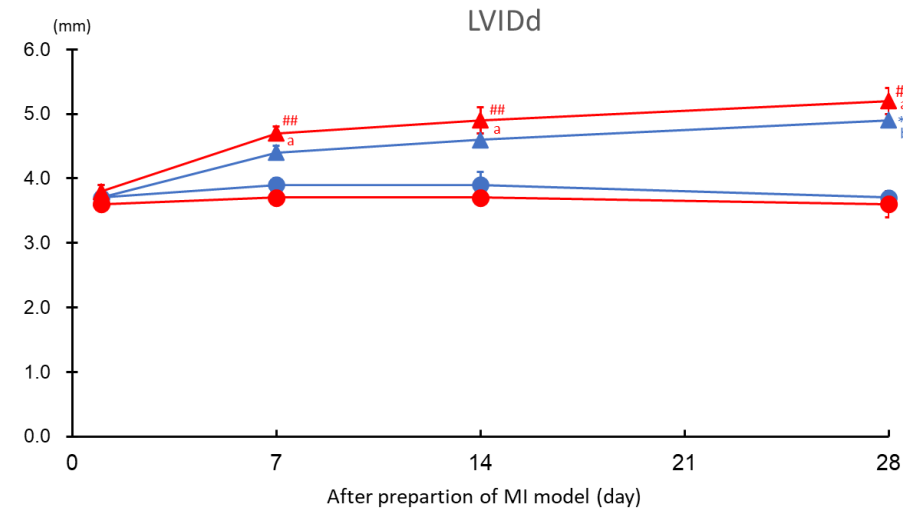
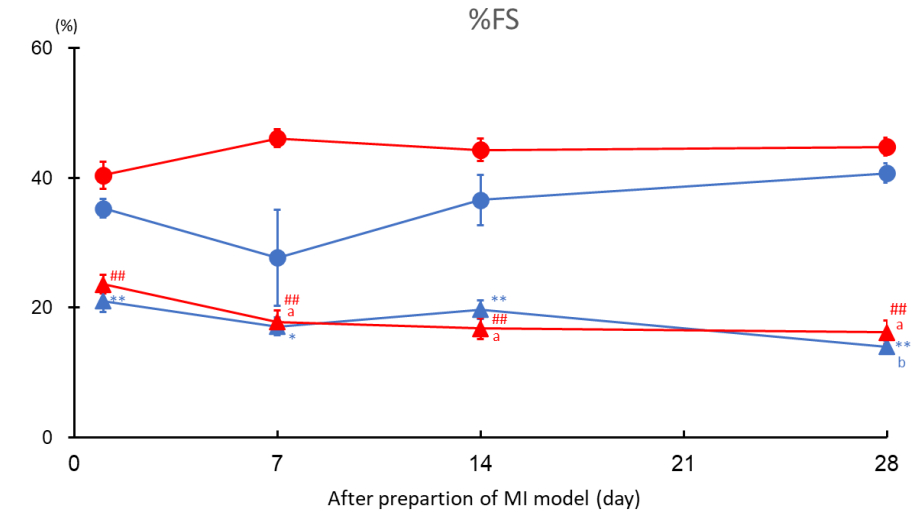
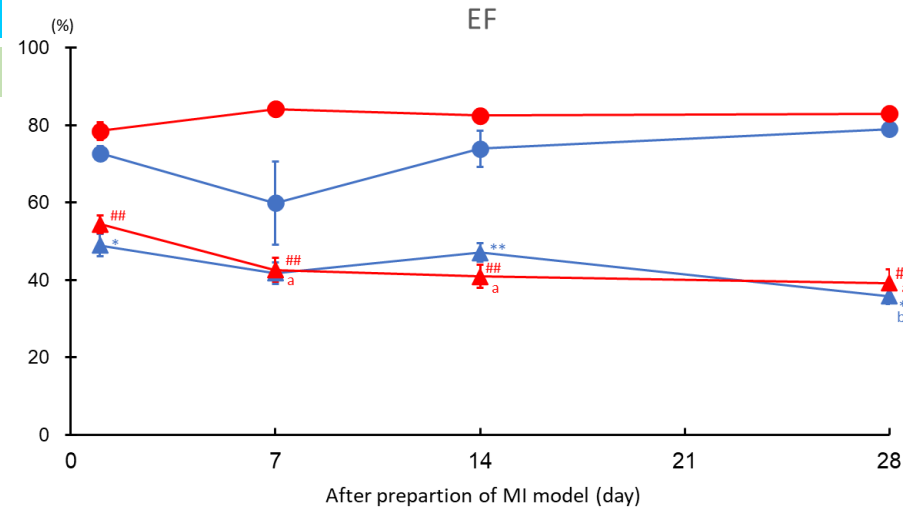
MI : Myocardial infarction

Each value represents the mean \pm S.E.

a : n=25 b : n=24

* $p < 0.05$, ** $p < 0.01$ vs. NOD/ShiJic-ScidJcl-mice:sham
(Student's t-test or Aspin-Welch's t-test)

$p < 0.05$, ## $p < 0.01$ vs. C57BL/6J-mice:sham
(Student's t-test or Aspin-Welch's t-test)



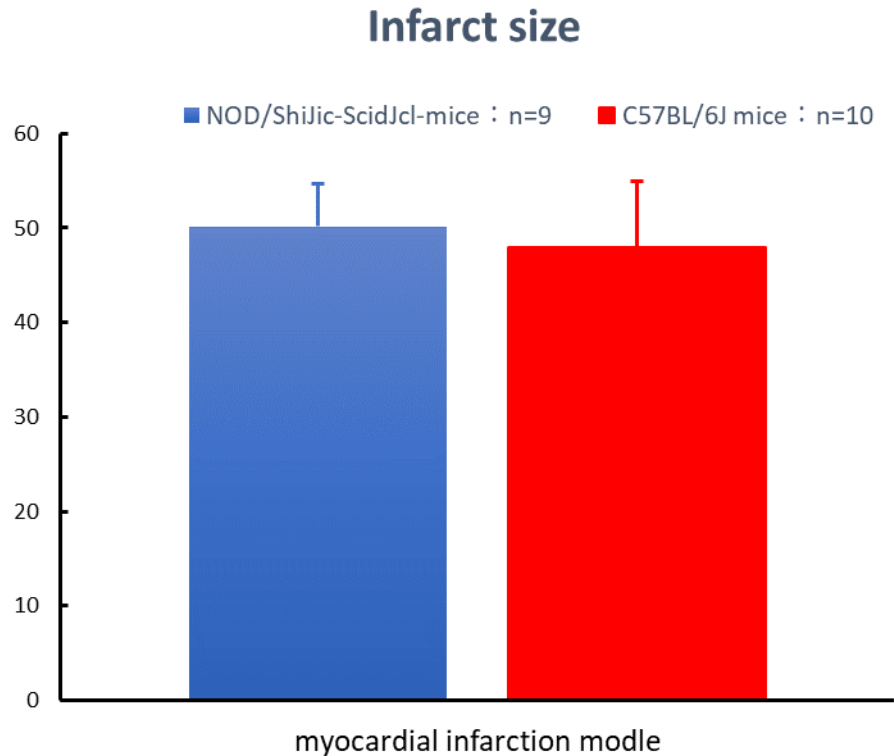
EF and %FS of echocardiography of myocardial infarction model preparation group of C57BL/6J mice and NOD/ShiJic-ScidJcl-mice showed lower value compared with sham group from the first day of myocardial infarction model preparation.

In addition, EF, %FS, LVIDd and LVIDs remained approximately the same as on modeling day 7 to day 28.

Results

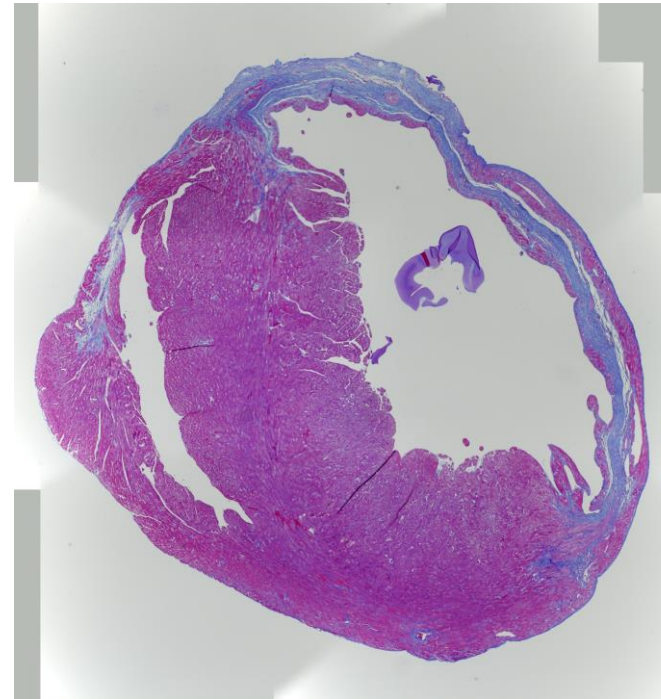
Infarct size of LV

<parameter>

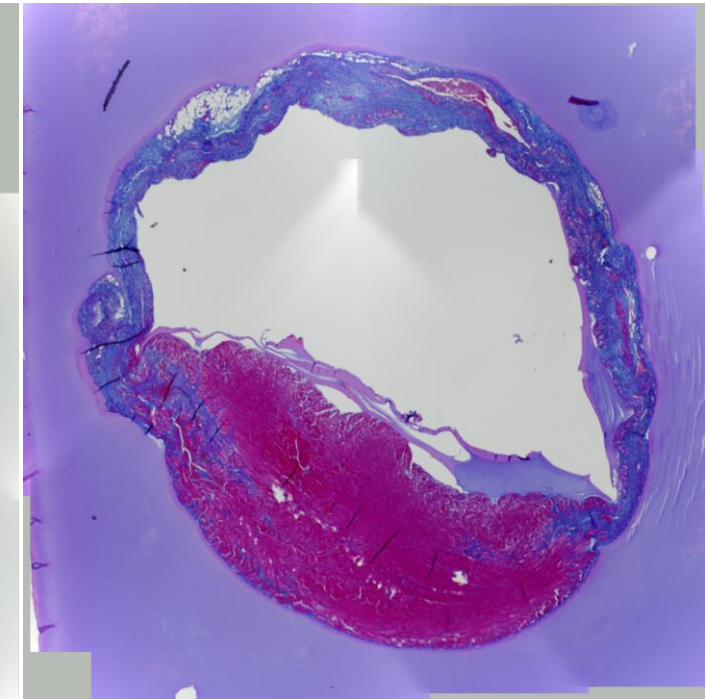


Each value represents the mean \pm S.E.

<Typical example of specimen>



NOD/ShiJic-ScidJcl-mice



C57BL/6J-mice

Myocardial fibrosis area/Left ventricular area ratios were $50.2 \pm 4.5\%$ in NOD/ShiJic-ScidJcl-mice and $48.0 \pm 7.0\%$ in C57BL/6J-mice .

Discussion

It was possible to capture changes in cardiac function of myocardial infarction model mice over time.

In addition, there were no differences between NOD/ShiJic-ScidJcl-mice and C57BL/6J-mice in echocardiographic cardiac function measurements and cardiac fibrosis area ratio of the myocardial infarction model.