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Establishment of an echocardiographic evaluation system for right ventricular function in a monocrotaline-induced pulmonary hypertension model in rats

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Summary in Japanese

本研究では，超高解像度超音波画像システム Vevo F2（FUJIFIL VisualSonics, Inc.）を用いて，モノクロタリン誘発肺高血圧症モデルラットの右室機能を評価した．ボセンタンおよび SGLT2 阻害薬の一種であるトホグリフロジンの経口投与し，このモデルラットに対する薬効を評価した．エコーによる測定は，病態惹起から 1，2，3 および4週目に行った．また，エンドポイントの評価として右心室内圧を右心室内腔にカテーテルを挿入することで測定した．ボセンタンおよびトホグリフロジンは病態の進行に統計学的に有意な効果を示さなかったが，時間経過とともに悪化する病態の評価を行うことができた．病態進行に伴い，右室自由壁厚および等容拡張時間の有意な上昇，三尖弁輪収縮期移動距離および肺動脈血流の加速時間の有意な減少を観察した．

これらの結果から，本試験系はラット肺高血圧症モデルにおいて右心室機能の評価を可能にし，肺高血圧症治療薬の開発に有用であることが示唆された．

Objective

We evaluated the right ventricular function (RVF) in a rat pulmonary hypertension model (PH-model) induced by monocrotaline (MCT), using the Vevo F2 high-resolution ultrasound imaging system (FUJIFILM VisualSonics, Inc.).

As the indicators to validate the model animals, we observed

(1) The right ventricular pressure measured by inserting a catheter into the right ventricular cavity as an endpoint evaluation item.
(2) RV free wall thickness
(3) Pulmonary acceleration time (PAT)
(4) Isovolumic time (IVT)
(5) Velocity of the tricuspid valve annulus
(6) Endocardial global longitudinal strain (EndoGLS) of right ventricular free wall

In addition, efficacy of bosentan (BOS), endothelin receptor antagonists used as a drug for PH, and tofogliflozin (TOFO), a SGLT2 inhibitor in the MCT-induced PH model rats were evaluated by echocardiogram at the 1st, 2nd, 3rd and 4th weeks after MCT administration.

Materials and Methods

● Animal

Male rat, Crl:DC(SD), 7-week-old

● Model preparation

Single administration of Monocrotaline (60mg/kg, s.c.)

● Administration

Bosentan (200 mg/kg),
Tofogliflozin (10mg/kg)
Both p.o., q.d.

Vevo F2
Fujifilm VisualSonics, Inc.

● Echocardiography

20MHz probe connected to Vevo F2 (FUJIFILM VisualSonics, Inc.).
Rats were anesthetized by a 1.5% inspiratory concentration of isoflurane (1 L/min of air) on a heated pad.
The heart rate and body temperature were monitored continuously.

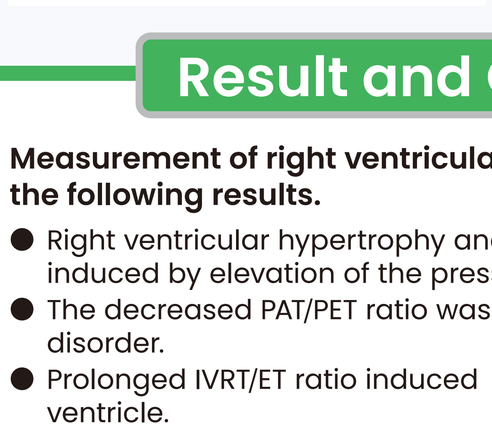
● RVEDP: right ventricular end systolic pressure measurements

A PE-50 filled with water was used to measure RVP in rats.
To measure RVESP, the catheter was inserted into the right ventricle through a hole made with a needle in the apex.

● RVMWR: right ventricular myocardial weight ratio

Sampled hearts and measured mass at 4 weeks.
RVMWR was calculated using the following formula:
Right ventricular free wall weight/(left ventricular weight + interventricular septum weight)×100

● Schedule



Group	Route	Dose	n
Normal	p.o.	0	6
Control (MCT)	p.o.	0	10
Bosentan (BOS)	p.o.	200 mg/kg	10
Tofogliflozin (TOFO)	p.o.	10 mg/kg	10

Result and Conclusion

Measurement of right ventricular function using VevoF2 revealed the following results.

- Right ventricular hypertrophy and myocardial hypertrophy were induced by elevation of the pressure in the right ventricle.
- The decreased PAT/PET ratio was induced by pulmonary circulatory disorder.
- Prolonged IVRT/ET ratio induced by diastolic dysfunction of the right ventricle.
- Reduction of velocity of the tricuspid valve annulus indicated right ventricular systolic dysfunction.
- Myocardial strains were strongly associated with alterations in right ventricular function parameters.

These results showed that the rats in MCT group developed PH after MCT administration by a remarkable increase in RVESP and RVMWR, indicating that MCT successfully induced PH in rats.

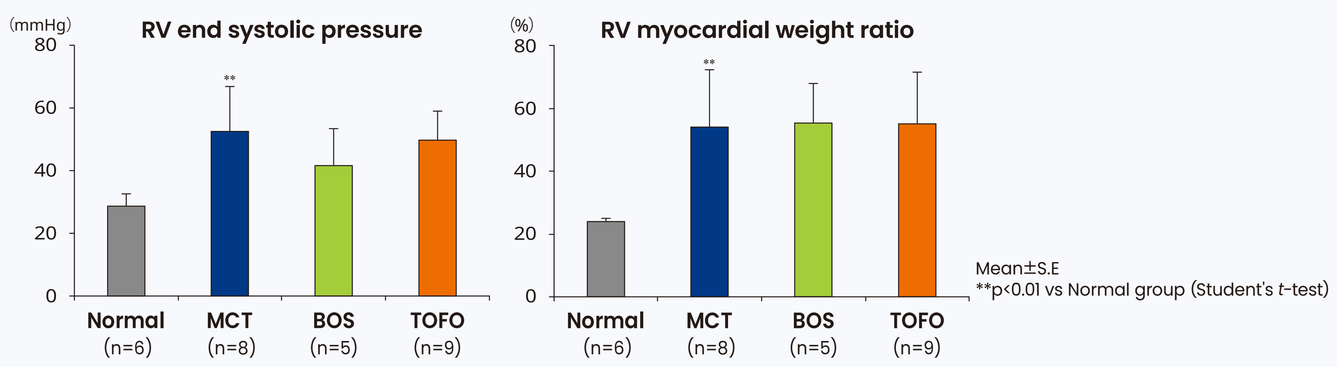
BOS and TOFO was not effective on PH in rats under the current protocol. Regarding BOS, efficacy may be demonstrated by verifying the optimal concentration of MCT and BOS used in the PH model. On the other hand, efficacy of TOFO against cardiovascular damage caused by heart failure needs to be verifying in other heart failure models as well.

Sign of PH	Indicator	Clinical PH	Rat PH model	BOS	TOFO
RV hypertrophy	RV free wall thickness	↑	↑	↑	↑
Pulmonary circulatory disorder	PAT/PET	↓	↓	↓	↓
Diastolic dysfunction of RV	IVRT/ET	↑	↑	↑††	↑††
Systolic dysfunction of RV	s' wave velocity	↓	↓	↓	↓
Decrease in RV myocardial excursion	EndoGLS	↑	↑	↑	↑

††: Significant effects were observed up to the second week of model preparation

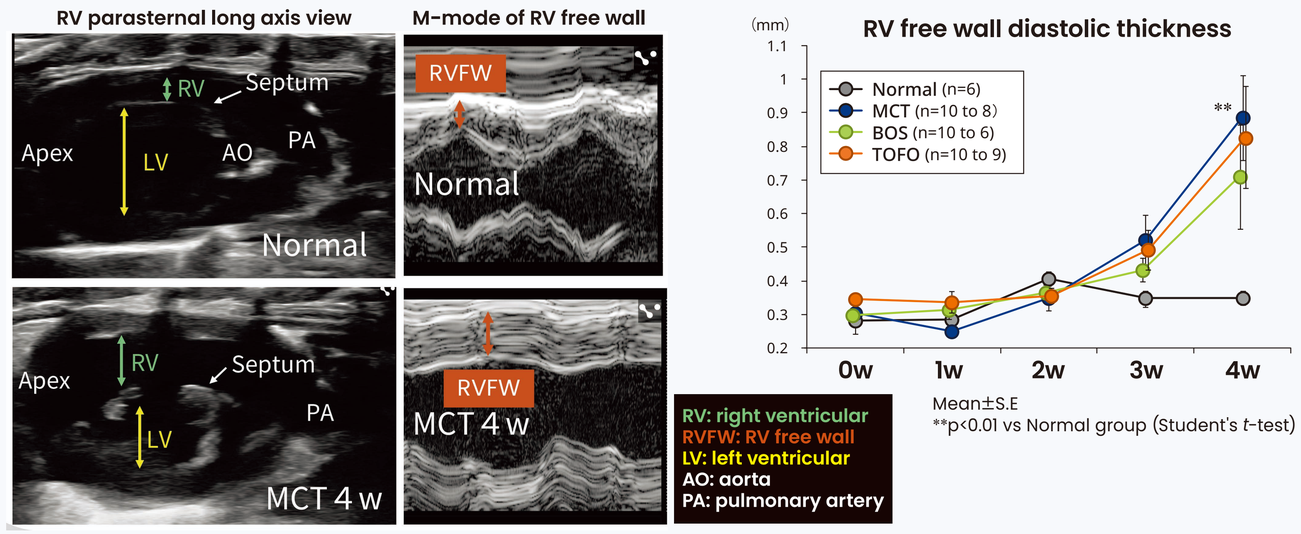
Results

RV end systolic pressure, RV myocardial weight ratio

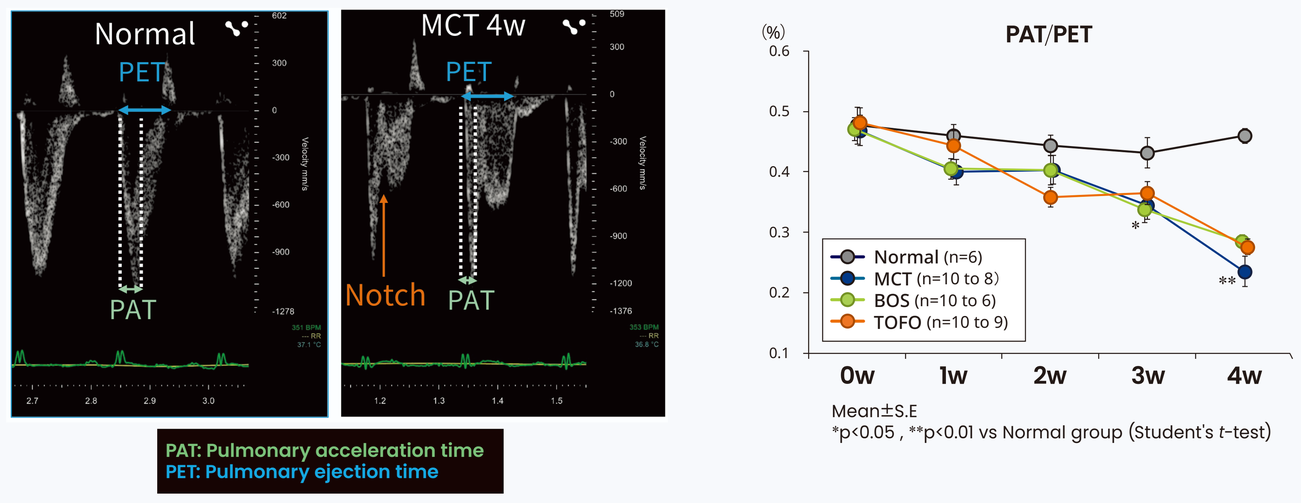


Results of Echocardiography

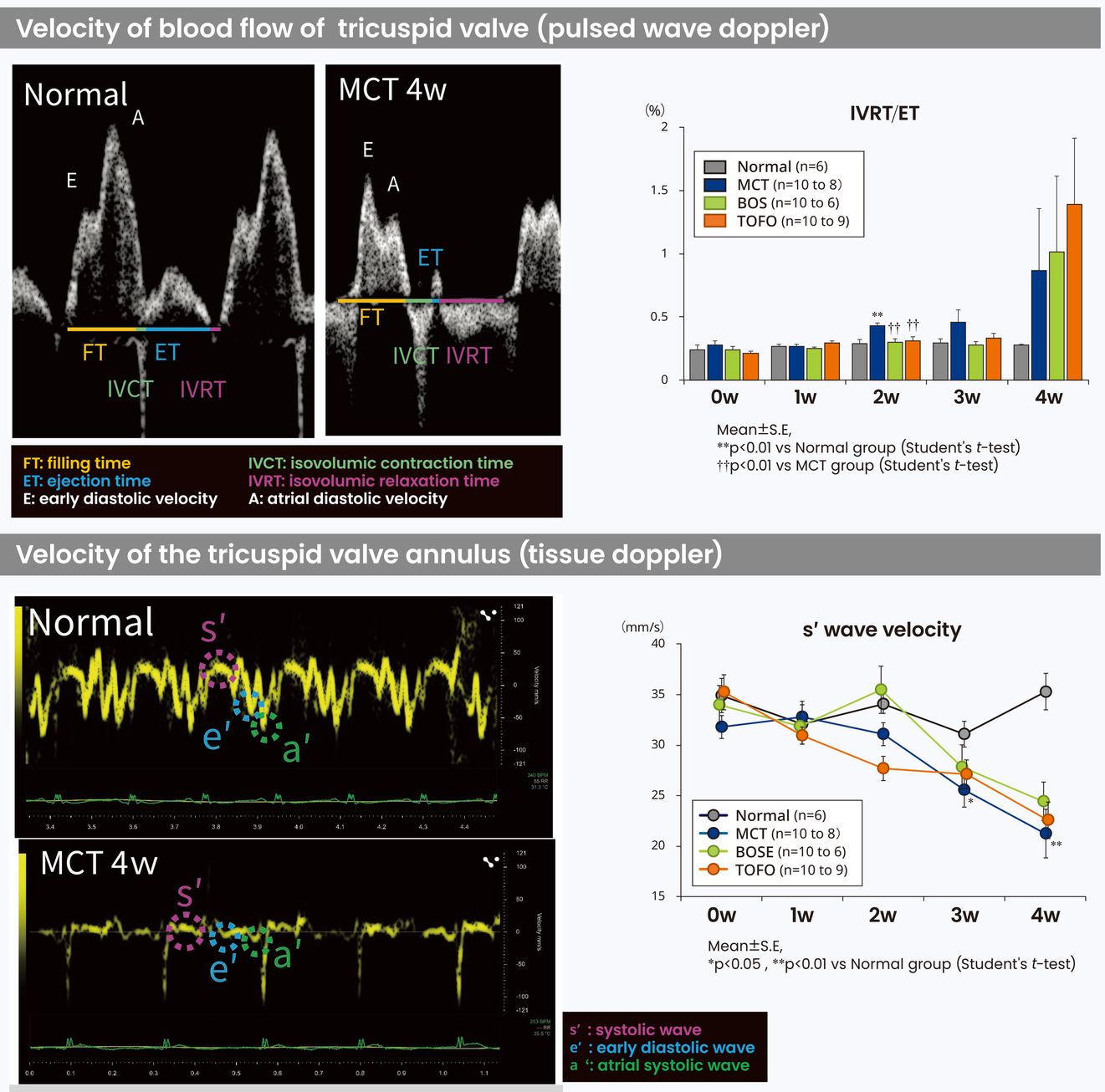
RV free wall diastolic thickness



Velocity of blood flow of pulmonary artery (pulsed wave doppler)

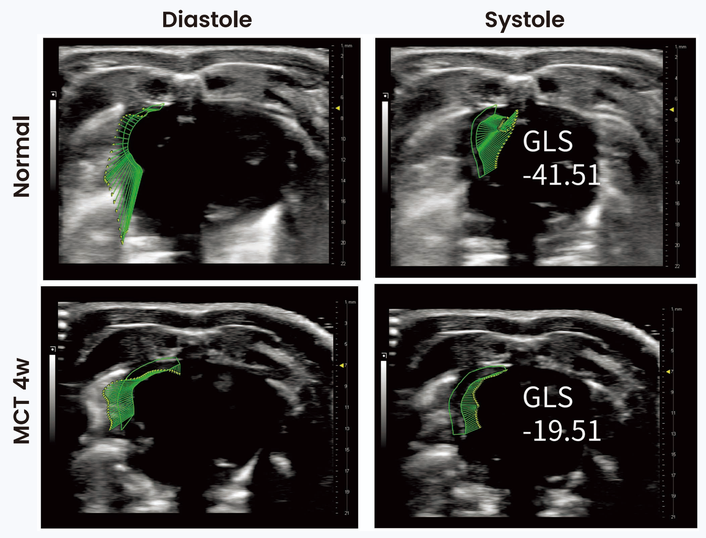


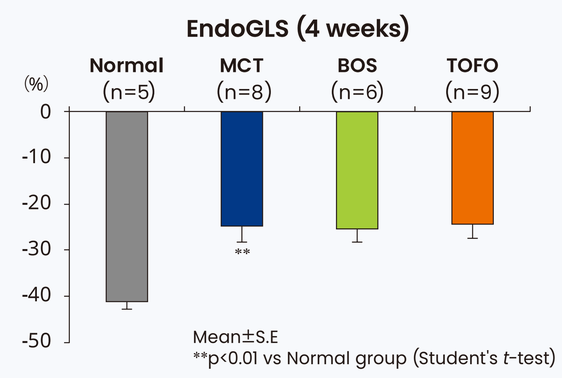
Velocity of blood flow of tricuspid valve (pulsed wave doppler)



Velocity of the tricuspid valve annulus (tissue doppler)

Endocardial global longitudinal strain (EndoGLS) of right ventricular free wall





**EndoGLS quantifies the longitudinal shortening of the heart in each cardiac phase, expressed as a negative percentage relative to the heart's initial length in end-diastole.
RV free wall trace tendency is expressed as velocity vectors around the endocardium in diastole (left panels) and systole (right panels) .**

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