

Original Article

## Establishment of a Model of Spontaneously-Running-Tokushima-Shikoku Rats with Left Atrial Thrombosis

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**Abstract:** Studies that investigate the underlying mechanisms of disease and treatment options typically require the use of a suitable animal model. Few suitable animal models exist for left atrial thrombosis. Here, we demonstrated that the Spontaneously-Running-Tokushima-Shikoku (SPORTS) rat — a Wistar strain known for its running ability—is predisposed to the development of thrombi in the left atrium. We investigated the incidence of left atrial thrombosis in male (n = 16) and female (n = 17) SPORTS rats and observed organized atrial thrombosis in 57% and 38% of males and female rats, respectively. In the male rats, systolic blood pressures and heart rates were significantly higher in SPORTS rats than in control Wistar rats. We could not find any evidence of arrhythmias, such as atrial fibrillation, during electrocardiographic examination of SPORTS rats. We believe that the SPORTS rat could serve as a new research model for left atrial thrombosis; further, it may be suitable for research investigating the development of new antithrombotic approaches for the control of atrial thrombosis or familial thrombophilia in humans. (DOI: 10.1293/tox.2012-0032; *J Toxicol Pathol* 2014; 27: 51–56)

**Key words:** rat, atrial thrombosis, heart

### Introduction

Atrial dilatation and fibrillation are well-known risk factors for the development of thrombosis within the atrium. Thrombosis develops easily when blood pooling occurs in the left atrium, often leading to cardio-embolic stroke (CES). Since atrial thrombosis is strongly associated with CES<sup>1</sup>, clarifying the mechanism responsible for thrombus formation in the left atrium would be valuable in the development of preventive measures and treatment strategies for CES.

Thrombophilia is a disorder associated with excessive

thrombosis<sup>2</sup>; further, familial thromboembolism results from abnormal or deficient coagulation control factors<sup>3–6</sup>, which leads to the development of idiopathic thrombophilia<sup>7</sup>. Understanding the mechanisms responsible for thrombosis in such patients would improve current treatment options, and the availability of a suitable animal model is expected to contribute greatly to current research endeavors in thrombosis. Therefore, our study aimed to establish an animal model for left atrial thrombosis.

In 1996, the male Spontaneously-Running-Tokushima-Shikoku (SPORTS) rat strain was identified in Wistar rats. SPORTS rats are able to spontaneously run long distances on an exercise wheel. These animals clock over 6,000 revolutions per day on an exercise wheel<sup>8</sup>; they are considered valuable in research investigating the effects of training and exercise in healthy individuals or persons with lifestyle-related diseases<sup>9</sup>. In an earlier study, we found that SPORTS rats had higher blood pressures than Wistar rats, although their blood pressures were not as high as those in spontaneous hypertensive rats (SHR) or stroke-prone spontaneous

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hypertensive rats (SHRSP). The SHR and SHRSP rats are well-known Wistar strains used in studies on hypertension and stroke, respectively<sup>10, 11</sup>. A related strain—the SHHF/Mcc rat—is a model for spontaneous hypertension, progressive renal dysfunction and congestive heart failure (CHF); left atrial thrombosis has been reportedly observed on necropsy of these SHHF/Mcc rats<sup>12</sup>. However, thus far, no reports have described a high incidence of atrial thrombosis in SHR, SHRSP or SHHF/Mcc rats. In this study, we examined the possibility of using the previously established Wistar strain of SPORTS rats, which develop spontaneous thrombi in the left atrium, as a model for atrial thrombosis.

## Materials and Methods

### Animals

A male rat that spontaneously ran long distances, over 6,000 revolutions per day on an exercise wheel, was discovered in an outbred strain of Wistar rats purchased from Charles River, Canada, in 1996. This strain was bred into a SPORTS rat strain at the Shikoku University (Tokushima, Japan)<sup>8</sup>. A colony of SPORTS rats was transferred to the University of Tokushima (Tokushima, Japan) and is currently being maintained there.

In this study, 28 male and 45 female SPORTS rats were used; 15 male and 16 female Wistar rats were used as controls; and 5 male SHR rats were purchased from Japan SLC, Inc. (Shizuoka, Japan). All the rats were housed in individual cages and maintained under specific pathogen-free conditions at the Tokushima University Animal Study Facilities (room temperature,  $23 \pm 1^\circ\text{C}$ ; lighting, 08:00–20:00). Rats were fed a standard non-purified diet (Oriental Yeast, Tokyo, Japan) and had *ad libitum* access to food and tap water. At the start of experimentation, all male and female SPORTS rats as well as all control rats were between 3 and 4 weeks of age, and they were housed in individual cages equipped with an exercise wheel (1.15 m/cycle). All the SPORTS rats clocked at least 6,000 revolutions per day on the exercise wheel<sup>8, 9</sup>. At the end of the experimentation, all rats were anesthetized with sodium pentobarbital (50 mg/kg) and then sacrificed by exsanguination.

The Institutional Animal Care and Use Committee approved this study prior to the start of experimentation, and we adhered to the policies and procedures outlined in the Guide for the Care and Use of Laboratory Animals published by the U.S. Department of Health and Human Services.

### Measurement of blood pressure and electrocardiography

We performed electrocardiography and measured the blood pressures and heart rates of 7 male and 3 female SPORTS rats, 7 male control rats and 5 male SHR rats at 10 weeks of age. Electrocardiograms (ECG) were recorded using an ECG processor analyzing system (SRV-2W, SBP-2000; Sotron, Tokyo, Japan), and we used the tail-cuff method and a noninvasive rat-mouse manometer transfer (TK-

370; Neuroscience, Tokyo, Japan) to record blood pressures and heart rates.

### Measurements of thyroid hormone and thyroid-stimulating hormone

We measured the levels of triiodothyronine (T3) and thyroxine (T4) levels to assess thyroid function and the levels of thyroid-stimulating hormone (TSH) to assess pituitary function in 6 male SPORTS rats and 6 male control rats at 16 weeks of age. Blood was collected from the abdominal aorta at the time of sacrifice, and the plasma T3, T4 and TSH levels were measured by radioimmunoassay (RIA; Immunotech Inc., Czech Republic).

### Pathology

Necropsy was performed when SPORTS rats were found dead or euthanized *in extremis* and terminally sacrificed at 80 weeks of age in males and at 100 weeks of age in females. The thoracic and abdominal cavities were opened; the heart, lungs, liver, kidneys, and spleen were removed; and the weight of each organ was recorded. Thereafter, the heart and lungs were fixed in 10% neutral buffered formalin, trimmed, embedded in paraffin, sectioned at a thickness of 4  $\mu\text{m}$ , stained with hematoxylin and eosin (H&E) and van Gieson's stain for elastin and examined microscopically.

### Statistical analysis

Data are expressed as means  $\pm$  SD. We used the two-tailed Student's *t*-test (Microsoft Excel, version 2007) to test for significance between groups.  $P < 0.05$  was considered statistically significant.

## Results

### Systolic pressures, heart rates and ECG

In the male rats, the average systolic blood pressure of the SPORTS, SHR and control rats were  $134.3 \pm 15.5$ ,  $197.9 \pm 27.3$  and  $115.2 \pm 10.8$  mmHg, respectively ( $P < 0.05$  for SPORTS vs. control rats,  $P < 0.01$  for SHR vs. control rats, and  $P < 0.05$  for SHR vs. SPORTS rats; Table 1). In the female SPORTS rats, the average systolic blood pressure was  $135.8 \pm 5.6$  mmHg.

In the male rats, the average heart rates of the SPORTS, SHR and control rats were  $458.8 \pm 21.3$ ,  $388.6 \pm 28.1$  and  $385.5 \pm 44.3$  beats/min, respectively ( $P < 0.01$  for SPORTS vs. control rats and  $P < 0.01$  for SPORTS vs. SHR rats, Table 1). In the female SPORTS rats, the average heart rate was  $446.0 \pm 21.6$  beats/min.

We did not observe any arrhythmias, such as atrial fibrillation, in the SPORTS or control rats during any of the ECG recordings.

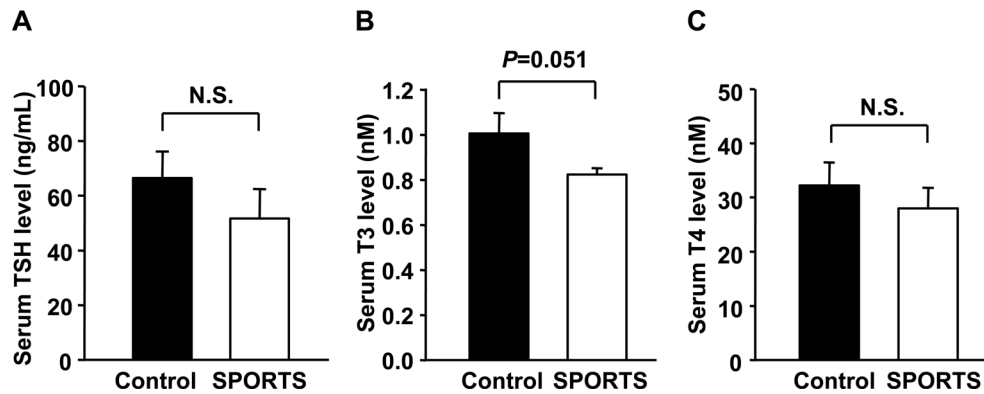
### Levels of T3, T4 and TSH

The average levels of TSH and T4 in 16-week-old rats did not differ significantly between the SPORTS and control rats (Fig. 1). The average levels of T3 in 16-week-old SPORTS rats tended to be lower than those in the control

**Table 1.** Systolic Blood Pressures and Heart Rates of Male SPORTS, Control, and SHR Rats

|                                | Control (n=7)             | SPORTS (n=7)                                  | SHR (n=5)                  |
|--------------------------------|---------------------------|---|----------------------------|
| Systolic blood pressure (mmHg) | 115.2 ± 10.8 <sup>a</sup> | 134.3 ± 15.5*<br>(135.8 ± 5.6) <sup>b</sup>   | 197.9 ± 27.3**,#           |
| Heart rate (beats/min)         | 385.5 ± 44.3              | 458.8 ± 21.3**<br>(446.0 ± 21.6) <sup>b</sup> | 388.6 ± 28.1 <sup>##</sup> |

<sup>a</sup> Mean ± SD. <sup>b</sup> Data for 3 female SPORTS rats. \*P<0.05 as compared with control rats; \*\*P<0.01 as compared with control rats. #P<0.05 as compared with SPORTS rats; ##P<0.01 as compared with SPORTS rats.

**Fig. 1.** TSH, T3 and T4 levels in SPORTS and control rats**Table 2.** Body and Relative Organ Weights for SPORTS and Control Rats

|                                     | Males          |                          | Females        |               |
|-------------------------------------|----------------|--------------------------|----------------|---------------|
|                                     | Control (n=15) | SPORTS (n=28)            | Control (n=16) | SPORTS (n=45) |
| Age (weeks)                         | 80             | 79.5 ± 26.7 <sup>a</sup> | 100            | 102.3 ± 28.4  |
| Body weight (g)                     | 463 ± 30       | 379 ± 74**               | 276 ± 27       | 264 ± 58      |
| Relative organ weights (g/100 g BW) |                |                          |                |               |
| Heart                               | 0.28 ± 0.09    | 0.57 ± 0.17**            | 0.31 ± 0.10    | 0.56 ± 0.18** |
| Lungs                               | 0.34 ± 0.15    | 1.09 ± 0.77**            | 0.42 ± 0.13    | 1.01 ± 0.60** |
| Liver                               | 2.65 ± 0.34    | 4.30 ± 1.04*             | 2.45 ± 0.28    | 4.92 ± 1.27*  |
| Spleen                              | 0.17 ± 0.05    | 0.26 ± 0.15*             | 0.19 ± 0.05    | 0.30 ± 0.20   |
| Kidneys                             | 0.71 ± 0.17    | 0.83 ± 0.17              | 0.68 ± 0.13    | 0.82 ± 0.30   |

<sup>a</sup> Mean ± SD. \*P<0.05 as compared with control rats; \*\*P<0.01 as compared with control rats. The average survival time in male and female SPORTS rats.

rats; however, this difference was not statistically significant ( $P = 0.051$ ).

#### *Survival period, body weight and relative organ weights*

The average survival periods of the male and female SPORTS rats were 79.5 ± 26.7 and 102.3 ± 28.4 weeks, respectively (Table 2). The average survival periods in the male and female SPORTS rats that developed atrial thrombosis were 71.1 ± 26.0 and 98.6 ± 22.4 weeks, respectively; further, the shortest life spans in the male and female SPORTS rats with macroscopic atrial thrombosis were 33 and 50 weeks, respectively (data not shown).

The body weights and relative organ weights in the male

and female SPORTS and control rats were compared (Table 2). The body and organ weights of 80-week-old male and 100-week-old female Wistar rats were used as controls. The average body weight of the male SPORTS rats was considerably lower than that of the control rats. The relative weights of the heart and lungs to the body weight in the SPORTS rats were significantly higher than those in the control rats ( $P < 0.01$ , Table 2). The relative weight of the liver to the body weight in the SPORTS rats was significantly higher than that in the control rats ( $P < 0.05$ , Table 2).

#### *Incidence of thrombi in the atria*

Atrial thrombosis occurred in 57.1% and 37.8% of male and female SPORTS rats, respectively (Table 3). Atrial

**Table 3.** Incidence Rates of Atrial Thrombi in SPORTS and Control Rats

|         | Male       |        | Female     |        |
|---------|------------|--------|------------|--------|
| Control | 0          | (n=15) | 0          | (n=16) |
| SPORTS  | 16 (57.1%) | (n=28) | 17 (37.8%) | (n=45) |

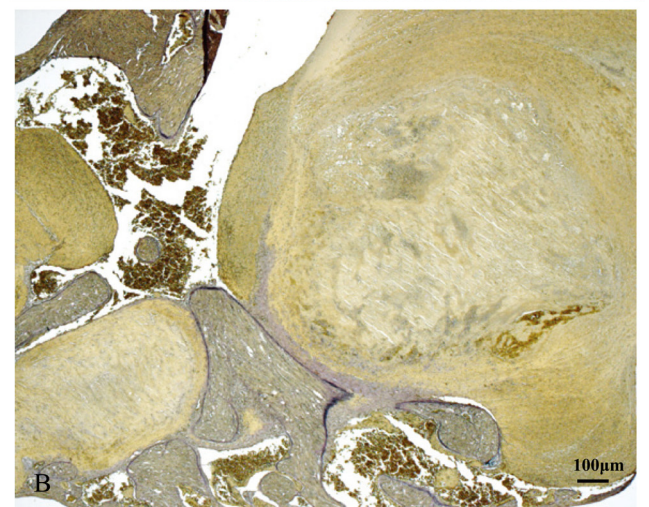
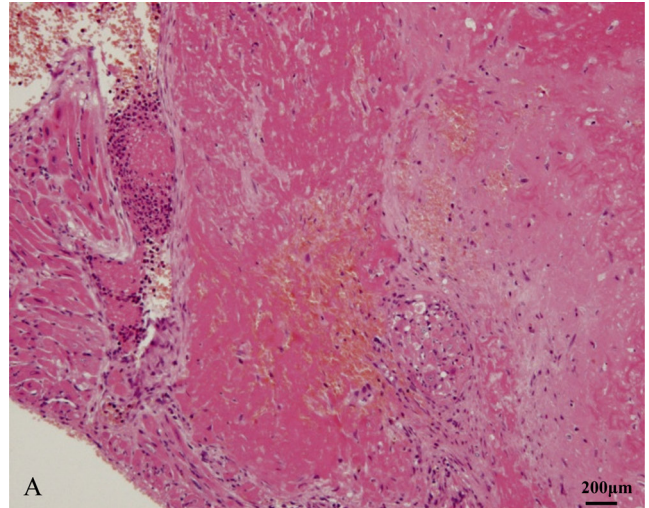
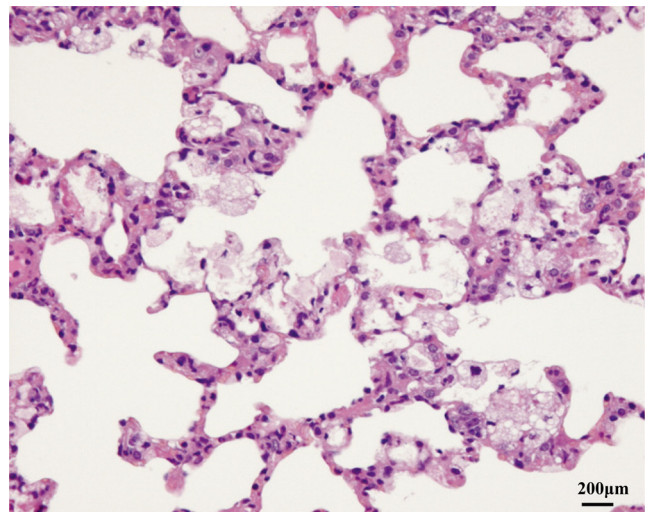
**Fig. 2.** Macroscopic findings in the heart of a SPORTS rat. A hard white thrombus in the left atrium.

thrombosis in the right atrium also occurred in 7.1% of male SPORTS rats; further, in one of these rats, thrombi developed in the hepatic veins. The necropsy results for the control rats did not show any evidence of macroscopic atrial thrombosis in any of the animals (Table 3). Further, necropsy of the 5 male SHR rats also did not show macroscopic atrial thrombosis (data not shown).

#### *Pathology findings*

Hard, white thrombi were observed in the left atria of the SPORTS rats on macroscopic examination (Fig. 2). Figure 3 shows the histological findings for the organized thrombi, with dense connective tissue in the left atrium stained with H&E (panel A) and van Gieson's stain for elastin (panel B). The organization of thrombi was clearly observed in the left atrium. Many neutrophils accumulated around each thrombus, and some neutrophils and lymphocytes were observed to infiltrate the atrial wall. No signs of degeneration, necrosis or fibrosis was observed in the valves, atria, or ventricles.

The histological findings for the lungs of the SPORTS rats are shown in Fig. 4; many foam cells were observed in the pulmonary alveoli.

**Fig. 3.** Light micrograph of thrombosis in the left atrium (panel A, hematoxylin and eosin staining; B, van Gieson staining). An organized thrombus is present in the left atrium.**Fig. 4.** Light micrograph of the lung of a SPORTS rat (hematoxylin and eosin staining). Many foam cells are present in the pulmonary alveoli.



## Discussion

Previous studies have investigated the characteristics of male and female SPORTS rats and reported the hyperactive wheel-running ability of these rats<sup>8</sup>. In the present study, we observed that SPORTS rats were predisposed to the development of atrial thrombosis. Previous studies have reported that atrial thrombosis can occur following exposure to certain chemicals<sup>13, 14</sup>. For instance, the peroxisome proliferator-activated receptor-gamma agonist troglitazone is known to induce atrial thrombosis in Wistar rats<sup>12</sup>. We could not find any evidence of macroscopic thrombosis in our control Wistar rats; further, the 2-year National Toxicology Program (NTP) for rodent studies reported the incidences of atrial thrombosis in male and female F344 rats to be 4.11% and 1.01%, respectively, and those in male and female B6C3F1 mice were reported to be 0.70% and 0.68%, respectively<sup>13</sup>. Thus, the incidence of atrial thrombosis was clearly higher in the SPORTS rats than in other strains.

SHR, SHRSP and SHHF/Mcc rats are derived from a single Wistar strain<sup>10, 11, 15</sup> and are useful models for studies concerning the mechanisms and complications of high blood pressure, such as stroke and CHF. Compared with SHR and SHHF/Mcc rats, SPORTS rats have a lower systolic blood pressure and a considerably higher heart rate<sup>16–18</sup>. Atrial fibrillation is considered a cause of thromboembolism<sup>19</sup>; however, the absence of arrhythmias, including atrial fibrillation, in the SPORTS rats used in the present study suggests that atrial fibrillation was not a cause of atrial thrombi in these animals. Although SPORTS, SHR, and SHHF/Mcc rats belong to the same strain of Wistar rats, thus far, the underlying cause of the faster heart rate in the SPORTS rats remains unknown. In a previous study, we observed that SPORTS rats showed a slightly higher blood pressure than other strains, although this difference was not statistically significant<sup>20</sup>. This lack of significance was possibly due to the small sample size used in the study and/or the decreased ejection fraction in SPORTS rats as compared with those in other strains (data is not shown).

In the present study, the average TSH, T3 and T4 levels showed no significant differences between the SPORTS and control rats. Therefore, the higher systolic pressures and faster heart rates in the SPORTS rats could not have resulted from abnormal levels of thyroid hormone. We believe that SPORTS rats have sympathetic hypertonia, which could increase the activity of coagulation factors. Moreover, we observed that the lungs and hearts of SPORTS rats weighed more than the same organs in aged-matched control Wistar rats (Table 2). We hypothesize that cardiac enlargement and the presence of thrombi in the left atria of SPORTS rats caused these differences in cardiac weight<sup>21</sup>. The differences in lung weights are thought to accompany the infiltration of foam cells in the alveoli<sup>22</sup>.

The mechanisms underlying the development of thrombosis in SPORTS rats may be relevant for studies investigating CHF. In addition, future studies should investigate the relationship between thrombosis and circulating

norepinephrine levels. The contribution of inflammation in the atria of young SPORTS rats to the development of atrial thrombosis cannot be ruled out without further studies<sup>23</sup>. In order to fully understand the causes underlying atrial thrombosis in SPORTS rats, further research, including genetic analyses for the identification of the genes responsible for atrial thrombosis, is necessary. Moreover, investigation of the hormones, cytokines and metabolic anomalies that may contribute to thrombosis is also essential<sup>24–26</sup>.

In conclusion, we have established that the SPORTS rat strain is predisposed to the development of atrial thrombi. SPORTS rats may thus be a useful new animal model for clarifying the causes of atrial thrombosis and familial thrombophilia in humans; further, this model could be utilized in the development of novel antithrombotic drugs.

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