Original Article

Rescue of Pulmonary Hypertension with an Oral Sulfonamide Antibiotic Sulfisoxazole by Endothelin Receptor Antagonistic Actions

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Pulmonary hypertension (PH) is a disease of unknown etiology that ultimately causes right ventricle heart failure with a lethal outcome. An increase in circulating endothelin (ET)-1 levels may contribute to disease progression. This study aimed to examine the possible effects of an orally active ET receptor antagonist, sulfisoxazole (SFX), for the rescue of PH, right ventricular hypertrophy, and eventual right ventricular failure. PH rats (single injection of monocrotaline [MCT]) were treated with an ET antagonist, SFX, an orally active sulfonamide antibody. Effects of SFX on PH rats were assessed in terms of survival rate, pulmonary artery blood pressure (PABP), autonomic nerve activity, and atrial natriuretic peptide (ANP) concentration in right ventricular myocytes and plasma. SFX did not change systemic blood pressure, however, it significantly suppressed the elevation of PABP. SFX maintained the derangement of autonomic nerve control, blunted an increase in ANP in myocytes and plasma, and significantly improved survival in right heart failure and/or related organs dysfunction in PH rats. The ET antagonistic action of the antimicrobial agent, SFX, was experimentally confirmed for treatment of PH in rats. (*Hypertens Res* 2008; 31: 1781–1790)

***Key Words*: pulmonary hypertension, sulfisoxazole, endothelin, atrial natriuremic peptide, heart rate variability**

Introduction

Pulmonary hypertension (PH) is associated with endothelial dysfunction that may mediate or contribute to the disease process, including an increase in circulating endothelin (ET)-1 levels. ET is a potent vasoactive and mitogenic peptide that is formed from the corresponding big ETs through the action of ET converting enzymes in endothelial cells and released toward the vascular smooth muscle consistent with a paracrine role (*1*, *2*). Circulating ET-1 levels are increased in humans who have primary and secondary PH (*3*, *4*) with an increase in local pulmonary ET-1 expression (*5*), which suggests that this peptide may contribute to the pathogenic process. A non-specific ET receptor antagonist can prevent acute and chronic hypoxic PH, and reverse the structural remodeling of the pulmonary vasculature (*6*). Blockade of ET activity by using the specific ET_A-receptor antagonist, FR139317, ameliorates RVH in PH in the monocrotaline (MCT)-induced rat PH model (*7*). It has been reported that several mechanisms involved in the pathophysiology of PH may contribute to severely increased circulating atrial natriuretic peptide (ANP) levels, which suggests that ANP secretion is activated in response to hemodynamic impairment in severe PH (*8*).

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Sulfisoxazole (SFX), 4-amino-*N*-(3,4-dimethyl-5-isooxazolyl) benzenesulfonamide, is an orally active drug of the sulfonamide group that has a wide range of antimicrobial activity against both Gram-positive and Gram-negative bacteria, and is also recognized as an ET_A receptor antagonist (*9*). Additionally, SFX has excellent pharmacological properties characterized by rapid absorption and excretion (*10*). We demonstrate here the beneficial effects of SFX in an MCT-induced experimental PH model in rats in terms of survival rate, pulmonary artery blood pressure (PABP), cardiac autonomic function, and ANP secretion in cardiac myocytes.

Methods

Rats and Housing

Ten-week-old male Wistar rats weighing 340–370 g were used in this study. All rats were purchased from Seac Yoshitomi Ltd. (Tokyo, Japan). The rats were housed in individual cages in a lightproof room (TB181 or TB182; National, Osaka, Japan) with a light-dark cycle (LD 12:12; lights on at 08:00 AM), maintained under constant temperature (22±2°C). This study was conducted in accordance 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) and was approved by the Animal Use Committee of Oita University School of Medicine.

Animal Model and Study Design

Rats were divided into four groups: control (no MCT injection), MCT (MCT-injected without SFX treatment), $SFX_{0.3}$ (MCT-injected with 0.3 g/kg/d SFX treatment), and $SFX_{1.0}$ (MCT-injected with 1.0 g/kg/d SFX treatment), respectively. MCT (Sigma Chemical Co., St. Louis, USA), which causes pulmonary vascular damage leading to PH, right ventricular hypertrophy (RVH), and eventual chronic heart failure (CHF) (*11*), was dissolved in 1 mol/L HCl at a concentration of 40 mg/mL neutralized with 1 mol/L NaOH to a pH of 7.40, diluted with sterile distilled water, and then injected at a concentration of 10 mg/mL. A single dose of 80 mg/kg of MCT was injected subcutaneously into the right side of the trunk region of the rats. Rats received a standard food regimen (CE-2; CLER, Tokyo, Japan), 20 g/d with or without SFX. Oral administration of SFX was started 7 d after MCT injection. In order to evaluate whether SFX improves long-term survival in MCT-induced PH rats, we observed the survival rates in the MCT and SFX groups for 10 weeks after MCT injection. Five weeks after MCT injection, seven rats in each group were measured for their pulmonary artery blood pressure, were sacrificed, and the hearts were harvested to investigate the RVH index (ratio of right ventricular free wall weight [RV] divided by the sum of the septum [*S*] and left ventricular free wall weight [LV] [RV/(LV+*S*)]). Rats were weighed every day and their food intake was measured to allow adjustment of their SFX consumption in the food.

Recordings of Isometric Contraction

Male Wistar rats weighing 300 to 350 g were anesthetized by an intraperitoneal injection of pentobarbiturate (50 mg/kg), and ventilated *via* the trachea with a small rodent ventilator (Model No. 141; New England Medical Instruments Inc., Medway, USA). The heart was removed and placed in Krebs-Henseleit solution (K-H solution) with the following composition (mmol/L): 119 NaCl, 4.7 KCl, 1 $MgCl_2$, 2.5 $CaCl_2$, 25 $NaHCO_3$, 1.2 KH_2PO_4, 11 glucose (pH=7.4 with HCl). The main pulmonary artery was dissected and connective tissues were cleaned. The artery was cut into a 2–3 mm–wide ring, and the endothelium was gently removed with a cotton swab. The functional removal of endothelium was confirmed by the lack of relaxation induced by 3 μmol/L acetylcholine, where the artery was pre-contracted with 10 μmol/L phenylephrine (data not shown). One side of the ring was pinned and the other side was connected to a force transducer (TB-612T, Nihon Kohden, Tokyo, Japan) in a 500 μL chamber perfused with 95% oxygen–bubbled solution at 37°C. The ring was mounted in K-H solution for 45 min, and the ring was then exposed to high K^+ solution (modified K-H solution with 63.7 mmol/L NaCl, 60 mmol/L KCl). After obtaining the maximum tension, the rings were washed with K-H solution for 7 min and exposed to one of the following solutions: 1) vehicle (dimethyl sulfoxide [DMSO])+100 nmol/L ET in K-H solution, 2) 0.1 mmol/L SFX+100 nmol/L ET in K-H solution, or 3) 1.0 mmol/L SFX+100 nmol/L ET in K-H solution. Data were collected with a computerized acquisition system (Power Lab and Chart software; AD Instruments, Sydney, Australia). The contractile forces of rings by experimental solution were assessed as a ratio to the response obtained in high K^+ solution.

Measurement of PABP

Rats were anesthetized (50 mg/kg pentobarbital peritoneal injection), and the thoracic cavity was opened for the direct insertion of a pressure transducer (model TB-612T; Nihon Kohden) into the pulmonary artery, under the assistance of a ventilator (Model 141; New England Medical Instruments Inc.).

ECG Recordings and Heart Rate Variability

ECG recordings and data analysis were performed as described previously (*12*). Briefly, a telemetric ECG radio transmitter (TA11CTA-F40; Data Sciences International, St. Paul, USA) was placed in each animal. ECG recording and experiments took place after a recovery period of 7 d to avoid possible effects of residual anesthetics and surgery. Serial (daily) acquisition of ECG data was obtained several times a day during the light cycle at a sample rate of 5 kHz for 30 min.

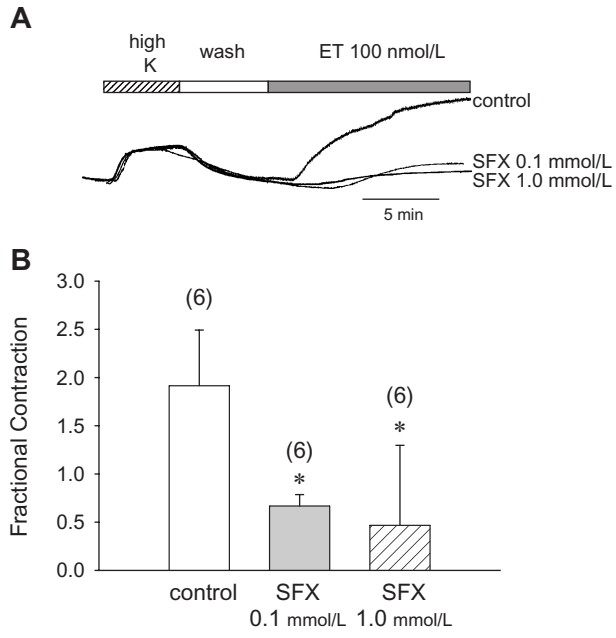

Fig. 1. *Effects of SFX on ET-induced contraction of the pulmonary artery. A: Representative traces of pulmonary artery ring contraction. Pulmonary artery rings were exposed to high K^+ solution followed by K-H solution (wash) and 100 nmol/L ET with or without SFX. B: Fractional contraction developed by 100 nmol/L ET was assessed with or without SFX. Data were expressed as a ratio to the maximal contraction induced by high K^+ solution. *$p < 0.05$ vs. control.*

From the ECG signals, a continuous series of RR intervals (*i.e.*, a tachogram) was initially obtained with the peak of the R wave as the fiducial point. The RR-interval data were used to generate power spectral density curves by means of a fast-Fourier transform (FFT), plotting power in ms^2/Hz *vs*. Hz for frequencies up to 2.0 Hz (*13*). Based on our previous investigations (*12*), a low frequency range (LF: 0.04–0.73 Hz) and a high frequency range (HF: 0.73–2.0 Hz) were chosen. The power and frequency of each spectral component are presented in absolute and normalized units. Normalized units were computed by dividing the absolute power of a given LF or HF component by total power (TP) or power of the other component, with or without multiplying this ratio by 100.

Ultrastructural Studies

For ultrastructural examination of ANP granules, small tissue blocks containing the right ventricular myocytes were fixed in 2.5% glutaraldehyde and 2% paraformaldehyde/0.1 mol/L cacodylate buffer (pH 7.4) for 2 h, and post-fixed in 2% osmium tetroxid for 2 h. These tissue blocks were dehydrated in a graded alcohol series and embedded in epoxy resin. Thin sections were stained with uranyl acetate and lead citrate, and examined with a JEOL electron microscope (Model JEM-100CX; Japan Electron Optical Laboratory, Ltd., Tokyo, Japan).

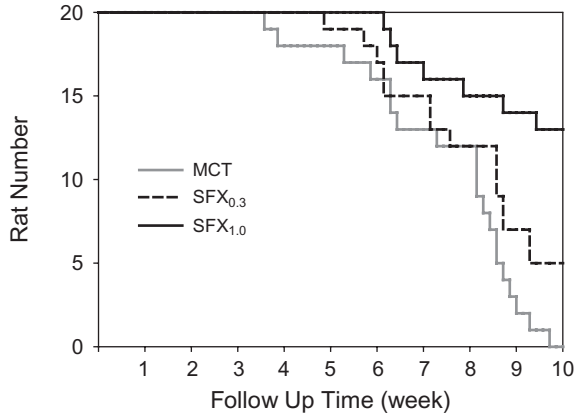

Fig. 2. *Survival curves for MCT-treated rats with or without SFX administration. Daily application of SFX (0.3 g/kg/d and 1.0 g/kg/d) extended survival in MCT-treated PH rats. $p < 0.001$, MCT group vs. $SFX_{1.0}$ group. $n = 20$ in each group.*

Plasma ANP Concentration

Blood samples for plasma ANP were taken from a systemic artery with a chilled syringe. Polypropylene tubes for plasma collection contained ethylenediaminetetraacetic acid (EDTA) and aprotinin. Plasma ANP concentrations were determined by using an RIA Kit (Peninsula Laboratories, San Carlos, USA) modified by the method of Radin *et al*. (*14*).

Drugs and Chemicals

MCT, SFX, and ET as well as all other chemicals, were purchased from Sigma Chemical Co.

Statistical Analysis

Statistical comparisons in PA contraction and PABP were performed by one-way ANOVA followed by Bonferroni/Dunn test for multiple-group comparisons. For ECG parameters, paired or unpaired Student's *t*-tests were used. The survival rate of each group was analyzed by Kaplan-Meier curves and the log-rank test. Each value was expressed as the mean±SEM. A p value <0.05 was considered statistically significant.

Results

SFX Prevented the Pulmonary Artery Contraction Induced by Endothelin

We first examined whether SFX could prevent ET-induced contraction of the pulmonary artery. As shown in Fig. 1A, 0.1

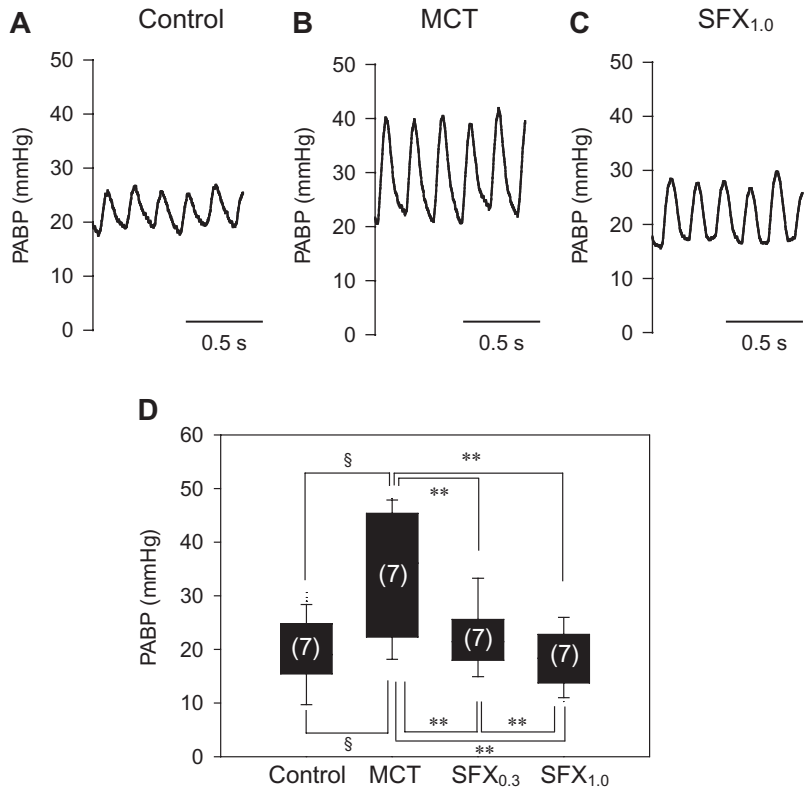

Fig. 3. *PABP measured from a control rat (A), an MCT group rat (B), and $SFX_{1.0}$ group rat at week 5 (C). D: Group data of mean systolic and diastolic PABP in control rats, MCT group rats, $SFX_{0.3}$ group rats, and $SFX_{1.0}$ group rats at week 5. n = 7 in each group. $^{\S}p < 0.01$ vs. control, $^{**}p < 0.01$ vs. MCT group.*

mmol/L SFX partially prevented the ring contraction induced by 100 nmol/L ET, while 1.0 mmol/L SFX highly attenuated the contraction. Figure 1B shows fractional contraction in each group as a ratio for high K^+–induced contraction. ET-induced contraction was significantly attenuated by SFX, although no statistical difference was detected between 0.1 mmol/L and 1.0 mmol/L SFX groups.

SFX Improved Survival Rate of MCT-Treated Rats

In response to the advancement of PH and resulting right heart failure, all MCT group rats eventually died. Figure 2 shows a dramatic difference in survival rate between the MCT group and $SFX_{1.0}$ group ($p < 0.001$). MCT group rats began to die at approximately 3 weeks, and all were dead within 10 weeks after MCT injection. In contrast, only seven of 20 rats treated with 1.0 g/kg/d SFX died during the observation period (survival rate, 65%). Administration of 0.3 g/kg/d SFX non-significantly improved the survival rate at week 10.

Attenuation of PH Development by SFX

Pulmonary artery blood pressure (PABP) of MCT group rats at week 5 was highly elevated (Fig. 3B). Systolic PABP (23.9±1.2 mmHg in control rats *vs*. 44.9±0.6 mmHg in MCT group rats) and diastolic PABP (14.7±0.7 mmHg in control rats *vs*. 24.1±1.1 mmHg in MCT group rats) were markedly elevated by MCT injection, however, the systolic and diastolic PABP were maintained at significantly low levels by oral treatment with SFX (27.0±1.2, 18.0±0.7 mmHg in $SFX_{0.3}$ group and 22.9±0.5, 13.7±0.4 mmHg in $SFX_{1.0}$ group, respectively), as shown in Fig. 3D. Meanwhile SFX did not modify systemic BP (98.6±1.3/69.6±2.9 mmHg, control *vs*. 95.0±1.4/64.3±1.0 mmHg, $SFX_{1.0}$ [$p = 0.11$]). As shown in Table 1, MCT treatment caused a significant increase in the ratio of RV/(LV+*S*), reflecting right ventricular hypertrophy (0.31±0.02 in control group *vs*. 0.63±0.07 in MCT group). In contrast, SFX treatment attenuated the development of PH and right ventricular hypertrophy in both the $SFX_{0.3}$ group (0.41±0.05, $p = 0.01$) and $SFX_{1.0}$ group (0.45±0.08, $p = 0.03$).

ECG Analyses in Control and RV Failing Rats

Representative ECG records from control rats, MCT-treated rats, and rats treated with SFX (1 g/kg/d) are shown in Fig. 4A, B and C, respectively. In the ECGs of PH rats (Fig. 4B), the RR intervals were shortened, the P-wave amplitude was

Table 1. Body Weight and Heart Weight in Rats at 5 Weeks after MCT Injection

	Control (n=7)	MCT		
		MCT (n=7)	$SFX_{0.3}$ (n=7)	$SFX_{1.0}$ (n=7)
BW, g	453±11	435±8	396±9*,†	394±8*,†
RV, mg	255±22	524±53*	308±35†	342±47†
LV+*S*, mg	816±28	842±30	755±17†	788±36†
RV/(LV+*S*)	0.31±0.02	0.63±0.07*	0.41±0.05†	0.45±0.08†

*p<0.05 *vs*. control, †p<0.05 *vs*. MCT. MCT, monocrotaline; $SFX_{0.3\ (1.0)}$, 0.3 (1.0) g/kg/d sulfisoxazole treatment; BW, body weight; RV, right ventricular free wall weight; LV, left ventricular free wall weight; *S*, sum of the septum.

enlarged, the R-wave amplitude was decreased, the QT interval was prolonged, and the QRS time was prolonged in comparison with control animals (Fig. 4A). On the other hand, ECG parameters of SFX-treated rats (Fig. 4C) were almost identical to those in control rats (Fig. 4A). Trendgrams of RR intervals indicated that the heart rate variability (HRV) was obviously decreased in the PH rats (Fig. 4E) in comparison with control rats (Fig. 4D). Beat-to-beat variability was maintained by SFX treatment (Fig. 4F). Power spectral analysis of HRV has been shown to be a quantitative and non-invasive method for assessing the effects of the autonomic nervous activity on the cardiovascular system (*15*). *Via* treatment with SFX, both LF and HF peaks were well preserved (Fig. 4I). On the other hand, both peaks disappeared in the PH rats not treated with SFX, indicating derangement of cardiac autonomic nerve function in the MCT-treated rats (Fig. 4H).

Serial Recording of Heart Rate and Spectral Parameters (TP, HF, Low- to High-Frequency Power Ratio) of HRV

MCT group rats showed an increased heart rate at 4 weeks after MCT injection, and this increase was maintained throughout the course of the study (Fig. 5A). From the data presented in the histogram shown in Fig. 5E, MCT group rats demonstrated a significantly faster heart rate at week 5 or later (316.9±5.1 bpm for the MCT group rats, compared to 280.2±3.3 bpm for the $SFX_{1.0}$ group rats, p<0.01). The TP and HF power in rats in the MCT group were markedly lower at 4 weeks or later after MCT injection compared to rats in the $SFX_{1.0}$ group throughout the observation period (Fig. 5B, C, F, G). As shown in Fig. 5D, the low- to high-frequency power ratio (L/H power ratio; an index of sympathetic nervous activity) of MCT group rats and $SFX_{1.0}$ group rats were almost identical. Histogram data for both groups showed a slight but insignificant decrease in L/H power ratio in weeks 7 and 8 (Fig. 5H).

Tissue ANP Granules and its Plasma Concentration

Histological study of right atrial and ventricular myocytes examined by transmission electron microscopy (Fig. 6) indicated that the number of ANP granules at the Golgi complex was markedly increased in number not only in right atrial myocytes (data not shown), but also in right ventricular myocytes in rats in the MCT group in comparison with control animals (Fig. 6A, B). However, the number of ANP granules in myocytes in rats treated with SFX was nearly identical (Fig. 6C) to that in the control rats (Fig. 6A). Consistent with the histological findings, plasma ANP concentrations in MCT group rat at week 5 were significantly increased in comparison with control rats, while administration of SFX to MCT-treated rats significantly prevented the increase in plasma ANP concentrations, which was almost identical to those in control rats (Fig. 6D).

Discussion

The present study demonstrated that an orally active sulfonamide derivative, SFX, improved the long-term survival rate, suppressed the elevation of pulmonary arterial blood pressure, prevented cardiac autonomic nerve derangement, and reduced the overexpression of ANP in cardiac myocytes in experimental PH induced by MCT in rats.

ET Antagonists Rescue PH

PH is often a progressive condition, characterized by a relentless increase in pulmonary vascular resistance that ultimately leads to right-sided heart failure and death. ET-1 is the most potent vasoconstrictor known (*1*, *2*) and ET levels are increased in humans who have primary and secondary PH (*3*, *4*). In PH patients, abnormal hemodynamic forces such as high pressure or increased blood flow in pulmonary arterial walls may stimulate ET production in pulmonary artery endothelial cells, and may lead to progression of the pathological condition. ET-1 exerts its major vascular effects through the activation of ET_A and ET_B receptors (*16*). Previous studies reported that LU 135252, an ET_A antagonist, reduced MCT-induced PH, rescued RVH, and restored vascular resistive properties (*17*). Another ET_A receptor antagonist, BQ-123, was also shown to significantly inhibit the development of PH and RVH (*18*). Bosentan, a nonselective $ET_{A/B}$ antagonist, which was approved by the U.S. Food and Drug Administration (FDA) in 2001 for oral treatment of PH, has been shown

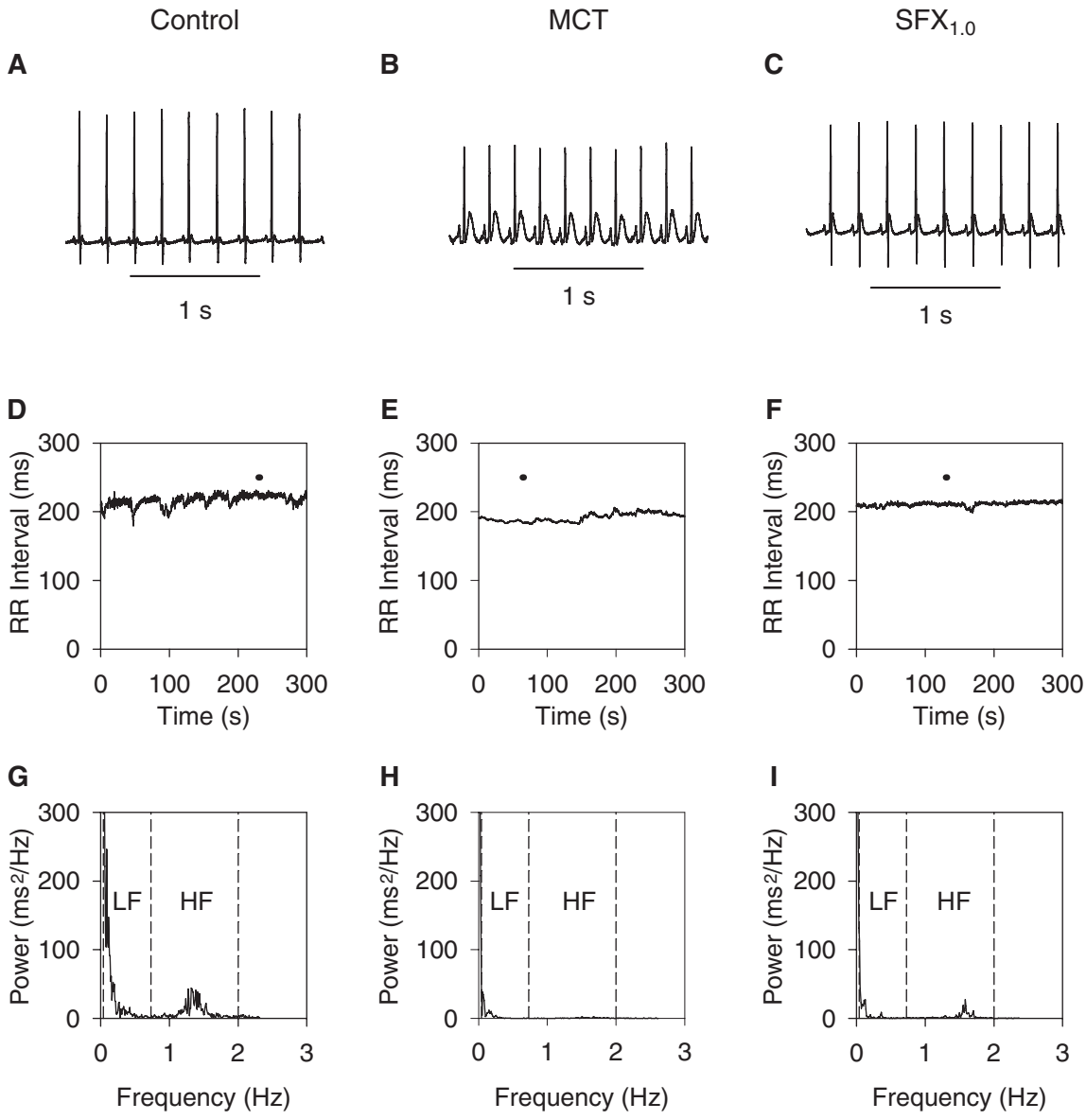

Fig. 4. *ECG, trendgram of RR intervals, and power spectrograms in a control Wistar rat, an MCT rat at 5 weeks and a SFX rat at 5 weeks treated for 4 weeks. A representative ECG record for 2 s in a control rat (A), an MCT rat (B) and an $SFX_{1.0}$ rat (C). Trendgrams of RR intervals for 300 s obtained from a control rat (D), an MCT rat (E) and an SFX rat (F). Short, thick bars in panels D, E, and F indicate the time during ECG records (A, B, and C) were obtained. Power spectra of heart rate (RR interval) fluctuations (G, H, I) were deduced from the trendgrams in D, E, and F, respectively.*

to effectively reduce PABP induced by hypoxia in animal studies (*6*), and are widely used in humans in many countries. Moreover, several previous studies reported that bosentan improved pulmonary hemodynamics, exercise capacity, functional status, and clinical outcome, as well as echocardiographic and Doppler parameters in patients with PH (*19*, *20*). In this context, we decided to examine an orally active selective ET_A receptor antagonist, SFX, for the treatment of PH in rats. SFX, an inhibitor of ET-induced pulmonary artery contraction (Fig. 1), dramatically improved PABP, and led to the long-term survival in MCT-treated rats (Figs. 2 and 3). These results suggest that excessive endogenous ET contributes to the development of PH and cardiovascular remodeling, and that SFX is beneficial for the break-up of the vicious cycle of pathological conditions in PH by antagonizing the ET_A receptor. In comparison with two other orally active non-peptide ET antagonists, YM598 and bosentan (*21*), SFX has an equal or even greater advantage in reducing PABP or right ventricular systolic pressure induced by MCT in rats, indicating that SFX is one of the most promising orally active agents

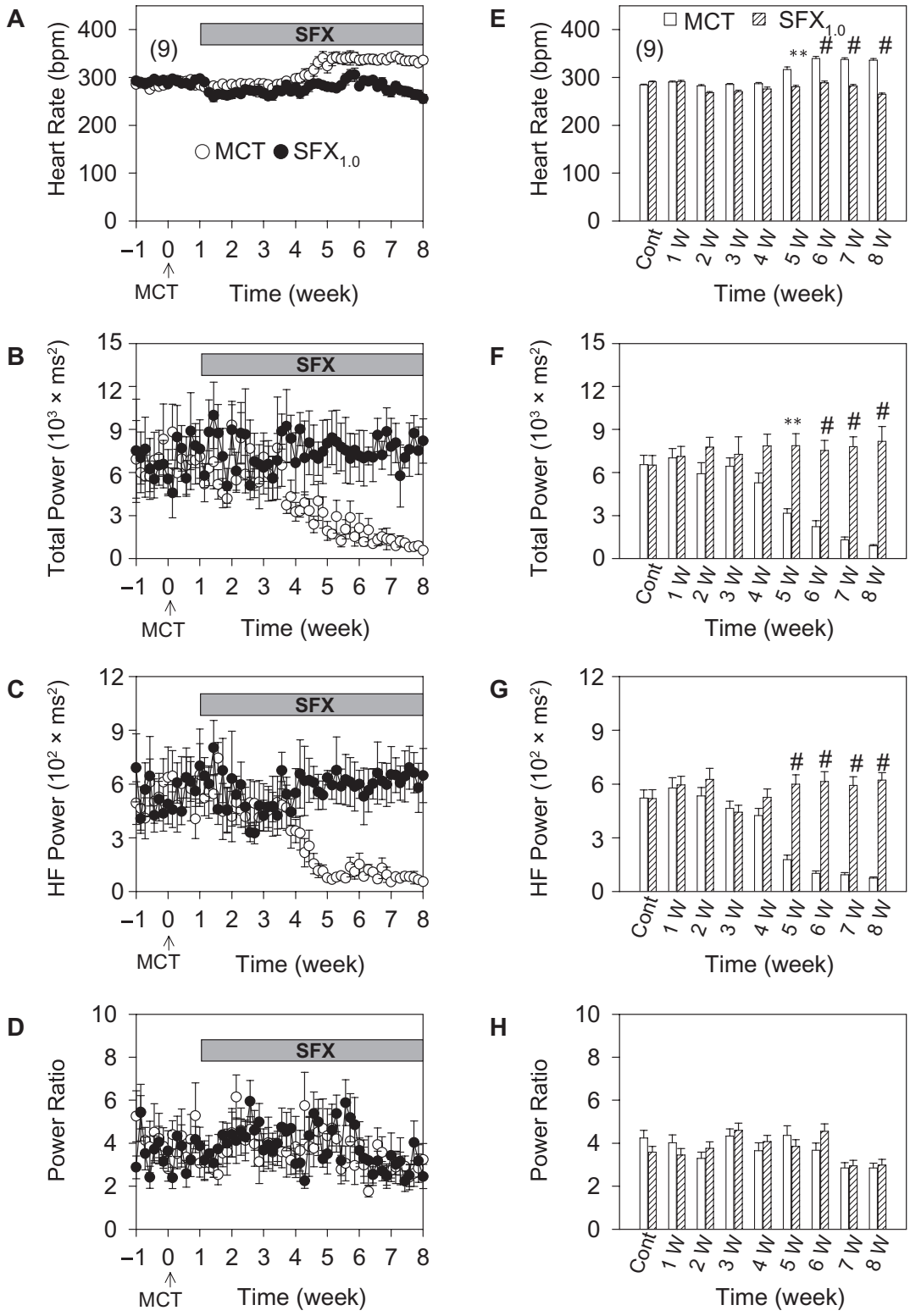

Fig. 5. *The serial (daily) recording of heart rate (HR), and histograms of weekly averaged HR obtained from MCT group rats and $SFX_{1.0}$ group rats (A, E). MCT was applied at week 0, and 1.0 g/kg/d SFX was applied daily for 7 d after MCT injection throughout the entire observation periods. The daily recording of total power (B), HF power (C), L/H power ratio (D), and histograms of weekly averages of these parameters (F, G and H) were obtained from MCT group and $SFX_{1.0}$ group rats. Times of MCT injection are indicated by arrows and SFX application period are marked. $^{**}p < 0.01$, $^{\#}p < 0.001$ vs. MCT group. $n = 9$ in each group.*

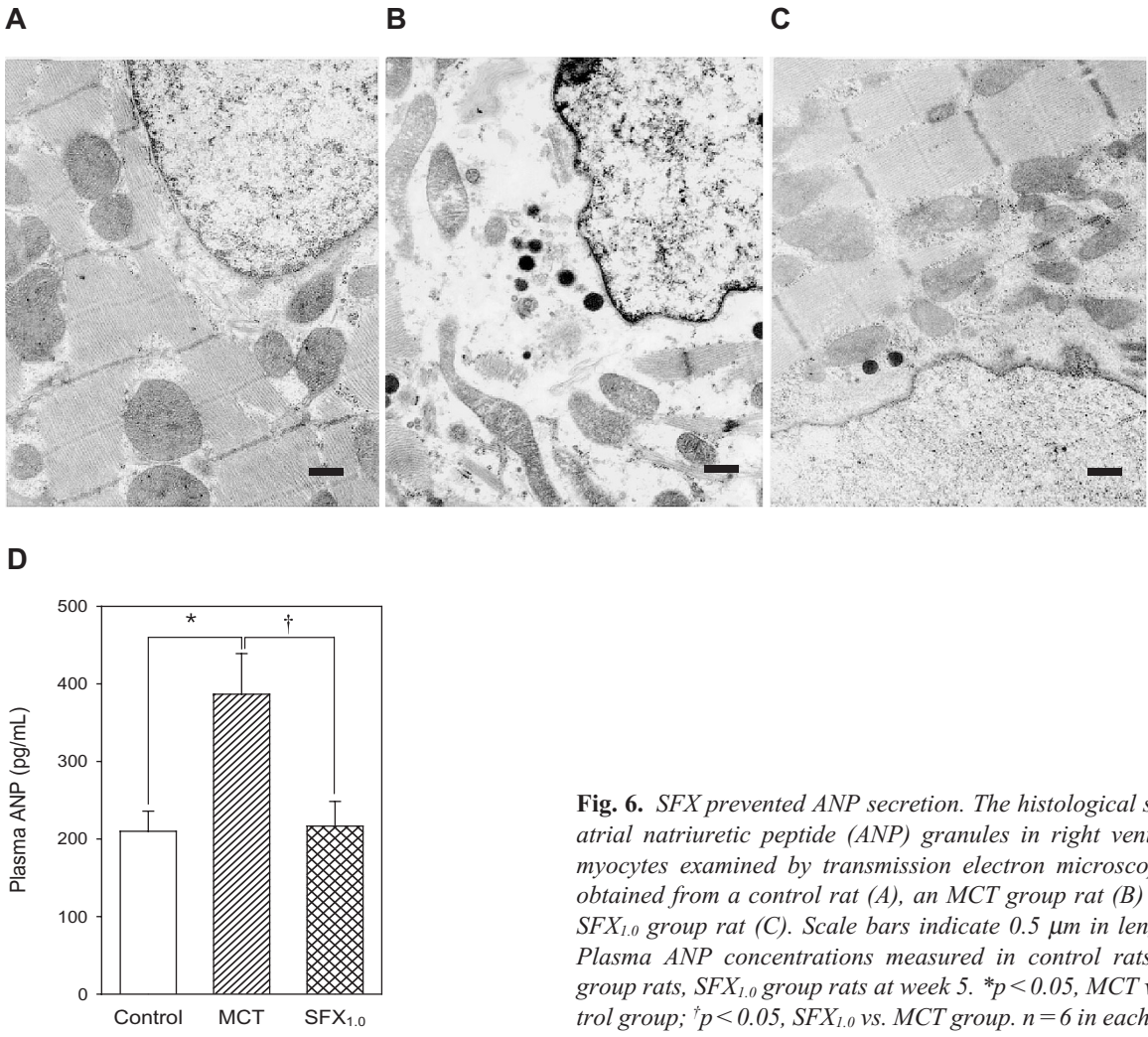

Fig. 6. *SFX prevented ANP secretion. The histological study of atrial natriuretic peptide (ANP) granules in right ventricular myocytes examined by transmission electron microscopy was obtained from a control rat (A), an MCT group rat (B) and an $SFX_{1.0}$ group rat (C). Scale bars indicate 0.5 μm in length. D: Plasma ANP concentrations measured in control rats, MCT group rats, $SFX_{1.0}$ group rats at week 5. *$p < 0.05$, MCT vs. control group; †$p < 0.05$, $SFX_{1.0}$ vs. MCT group. $n = 6$ in each group.*

clinically applicable to patients with PH. Actually, the mechanisms for PH are multifactorial, depending on the underlying etiology. Therefore, it is postulated that genotype- and phenotype-dependent therapeutic approach for PH patients would be desirable. Although it remains controversial whether nonselective $ET_{A/B}$ receptor antagonists or selective ET_A receptor antagonists should be used (*22*, *23*), the efficacy of orally active ET_A receptor blockade with this agent, in addition to conventional $ET_{A/B}$ receptor blockade, offers a therapeutic option and a rationale for the current treatment of patients with PH.

ET Antagonist Benefits in Heart Failure

It has been proposed by others that chronic ET_A inhibition improves the survival rate and ameliorates both LV and RV dysfunction in cardiomyopathic hamsters (*24*). It has also been reported that an intravenously administered nonselective $ET_{A/B}$ receptor antagonist, tezosentan, rapidly and effectively improved hemodynamics in patients with moderate to severe heart failure (*25*). As shown in Fig. 4, derangement of ECG parameters including QT prolongation was rescued by SFX treatment. Although we are unable to reveal the mechanism of QT prolongation in this study, we speculated that derangement of action potential repolarization caused long QT intervals. It is suggested that two distinct ion channels are mainly responsible for QT prolongation in this model based on the literature (*26*, *27*): voltage-dependent inward Na^+ current (I_{Na}) and transient outward current (I_{to1}). Administration of SFX presumably reversed the ion channel remodeling postulated in this study. Fauchier *et al*. reported that HRV was significantly decreased in patients with right heart failure due to PH, as well as in patients with chronic right and left heart failure (*28*). Based on the current consensus, HF power reflects parasympathetic tones whereas L/H ratio reflects sympathetic tones in the heart (*12*, *15*). We have previously proven that derangement of autonomic nerve control is correlated with the amelioration of right ventricular failure in MCT-induced

PH rats (*12*). The present HRV study demonstrated that HF power was highly maintained (Fig. 5C, G) by SFX, thus providing evidence that an orally active ET_A receptor antagonist sufficiently prevented early parasympathetic derangement in MCT-treated rats. As far as we know, this is the first report describing the effect of ET-1 receptor antagonist on the rescue of cardiac autonomic function. However, the effects of SFX on heart rate and cardiac autonomic functions are apparent as late as 5 weeks after continuous oral application of SFX. Therefore, it is speculated that SFX has no direct effect on autonomic functions.

ANP Secretion as a Marker of Heart Failure

Endogenous ANP plays a physiological role in modulating PABP, cardiac hypertrophy, and pulmonary vascular remodeling in both normoxic and hypoxic conditions (*29*). It has been reported that both plasma brain natriuretic peptide (BNP) and ANP levels were elevated in RV overload, correlating positively with mean PABP (*30*). By reducing PABP, indirectly or by the cellular signals *via* natriuretic peptide receptors, a reduction of ANP concentration in plasma by oral SFX consequently rescued the MCT-treated rats. From our knowledge, this is the first report demonstrating ANP granules in right ventricular myocytes in right-side heart failure, and rescue by an ET_A antagonist.

New Therapeutic Implications of SFX

SFX, a sulfonamide derivative, has long been used as a nonpatented low-cost antibacterial agent for topical use in the eye, as an oral treatment of urinary tract infection, and also in children with otitis media (*10*, *31*). Chan *et al*. have previously reported that based on a receptor binding study, SFX was a selective non-peptide ET_A receptor antagonist (*9*). In this context, we have proved for the first time that SFX has pharmacologically beneficial effects for the rescue of PH in addition to its antibacterial actions. In oral doses of 2 to 4 g/d, SFX is bound extensively to plasma proteins, reaching plasma concentrations of 110 to 250 μg/mL in 2 to 4 h. Approximately 95% of a single dose of SFX is excreted by the kidneys in 24 h (*10*). In this study, we decided to adopt a dose 10 times greater than that in clinical use in animal studies. Meanwhile, per the *in vitro* experiment as shown in Fig. 1, inhibition of ET-induced PA contraction was observed with as little as 0.1 mmol/L SFX, which was about 1/10 of the effective plasma concentration. Taken together, we hypothesized that daily oral administration of SFX could be therapeutically beneficial in other pathological conditions, such as PH. Based on our study, we conclude that oral treatment with the sulfonamide derivative, SFX, in MCT-treated rats, who developed PH and CHF, significantly improved survival rate, suppressed elevation of PABP, preserved the derangement of cardiac autonomic nerve functions, and prevented the increase of ANP in plasma concentration and in myocytes.

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