Biochemical and Biophysical Research Communications 436 (2013) 145–151

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Impact of dietary iron restriction on the development of monocrotaline-induced pulmonary vascular remodeling and right ventricular failure in rats

Yoshiro Naito [a,*], Manami Hosokawa [a], Hiroyuki Hao [b,*], Hisashi Sawada [a], Shinichi Hirotani [a], Toshihiro Iwasaku [a], Yoshitaka Okuhara [a], Akiyo Eguchi [a], Seiichi Hirota [b], Mitsumasa Ohyanagi [c], Takeshi Tsujino [d], Tohru Masuyama [a]

[a] Cardiovascular Division, Department of Internal Medicine, Hyogo College of Medicine, Nishinomiya, Japan
[b] Department of Surgical Pathology, Hyogo College of Medicine, Nishinomiya, Japan
[c] Division of Coronary Heart Disease, Department of Internal Medicine, Hyogo College of Medicine, Nishinomiya, Japan
[d] Department of Pharmacy, Hyogo University of Health Sciences, Kobe, Japan

ARTICLE INFO

Article history:
Received 10 May 2013
Available online 23 May 2013

Keywords:
Iron
Monocrotaline
Pulmonary hypertension
Right ventricular failure
Transferrin receptor 1

ABSTRACT

Pulmonary hypertension (PH) is characterized by pulmonary vascular remodeling leading to right ventricular (RV) failure. Recently, iron deficiency is reported to be prevalent in patients with PH. However, the mechanism by which iron deficiency occurs in patients with PH remains unknown. Here, we investigated the effects of dietary iron restriction on the development of monocrotaline-induced pulmonary vascular remodeling and the involved mechanisms. Male Sprague–Dawley rats were subcutaneously injected with monocrotaline (60 mg/kg). Afterwards, monocrotaline-injected rats were randomly divided into two groups and were given a normal diet ($n = 6$) or an iron-restricted diet ($n = 6$) for 4 weeks. Saline-injected rats given a normal diet were served as controls ($n = 6$). Monocrotaline-injected rats showed pulmonary vascular remodeling, increased RV pressure, RV hypertrophy, and decreased RV ejection fraction, followed by RV failure after 4 weeks. In contrast, iron restriction attenuated the development of pulmonary vascular remodeling and RV failure. Of interest, expression of cellular iron transport protein, transferrin receptor 1 was increased in the pulmonary remodeled artery and the failing right ventricle of monocrotaline-injected rats, as compared with the controls. Moreover, a key regulator of iron homeostasis, hepcidin gene expression was increased in the failing right ventricle of monocrotaline-injected rats. Iron restriction attenuated the development of monocrotaline-induced pulmonary vascular remodeling and RV failure. Cellular iron transport might be involved in the pathophysiology of PH and PH induced RV failure.

1. Introduction

Pulmonary hypertension (PH) is characterized by progressive pulmonary vascular remodeling resulting from significant proliferation of pulmonary artery smooth muscle cells and endothelial cells [1]. PH is a public health problem leading to right ventricular (RV) failure and sudden death. RV failure is the main cause of death in patients with PH [2]. Thus, understanding of the pathophysiology of PH and PH induced RV failure is important for the improvement of the morbidity and mortality.

Iron is a necessary element for maintaining physiological homeostasis in the body, whereas excess iron leads to toxicity and free radical damage by the Fenton reaction, thereby resulting in tissue damage. Hence, iron is considered to be implicated in the pathogenesis of several cardiovascular diseases [3–5]. Of interest, several reports suggest that iron availability affects acute pulmonary vascular responses to hypoxia [6,7]. Additionally, recent reports indicate that iron deficiency is prevalent without overt anemia in patients with idiopathic and heritable PH [8–10]. However, the mechanism by which iron deficiency occurs in patients with PH remains unknown.

Most cells regulate iron uptake by modulating the amount of transferrin receptor 1 (TfR1), which plays a key role in cellular iron transport. Low iron conditions normally lead to upregulate TfR1 expression. Conversely, high iron conditions induce to downregulate TfR1 expression [11]. We have recently shown that TfR1 is involved in the development of vascular remodeling in Dahl

* Corresponding authors. Address: Cardiovascular Division,Department of Internal Medicine, Hyogo College of Medicine, 1-1 Mukogawa-cho, Nishinomiya 663-8501, Japan. Fax: +81 798 45 6551 (Y. Naito). Address: Department of Surgical Pathology, Hyogo College of Medicine, 1-1 Mukogawa-cho, Nishinomiya 663-8501, Japan. Fax: +81 798 45 6671 (H. Hao).
E-mail addresses: ynaito@hyo-med.ac.jp (Y. Naito), haohiro@hyo-med.ac.jp (H. Hao).

0006-291X/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2013.05.059

salt-sensitive rats. In addition, dietary iron restriction has preventive effects on salt-induced cardiovascular remodeling in Dahl salt-sensitive rats. [12]. To the best of our knowledge, there is no report to investigate whether TfR1 is involved in the development of PH and PH associated RV failure. Besides, it is completely unknown whether dietary iron restriction impacts the development of pulmonary vascular remodeling. We therefore hypothesized that TfR1 participates in the development of pulmonary vascular remodeling and dietary iron restriction might affect the development of pulmonary vascular remodeling. In the present study, we investigated the effects of dietary iron restriction on monocrotaline (MCT)-induced pulmonary vascular remodeling and the involved mechanisms.

2. Materials and methods

2.1. Animals

9-Week-old male Sprague–Dawley rats (body weight; 350–400 g) were fed on a normal diet for 1 week. Afterwards, Male Sprague–Dawley rats were subcutaneously injected with MCT (60 mg/kg, Sigma). MCT was dissolved in 1 N HCl, the pH was adjusted to 7.4. After 1 day injection, MCT-treated rats were randomly divided into two groups and were given a normal diet ([PH] $n = 6$) or an iron-restricted diet ([PH + iron restriction (IR)] n = 6) for 4 weeks. Saline-injected rats given a normal diet were served as controls ([CTRL] $n = 6$). The nutrients of a normal diet consist of cornstarch 33%, casein 22%, cellulose 5%, sucrose 30%, corn oil 5%, mineral mixture 4%, and vitamin mix 1%. Mineral mixture contains dicalcium phosphate dihydrate 0.43%, potassium dihydrogen phosphate 34.31%, sodium chloride 25.06%, ferric citrate 0.623%, magnesium sulfate 4.8764%, zinc chloride 0.02%, manganese (II) sulfate pentahydrate 0.121%, copper (II) sulfate pentahydrate 0.156%, potassium iodide 0.0005%, calcium carbonate 29.29%, ammonium molybdate tetrahydrate 0.0025%, and microcrystalline cellulose 5.11%. An iron-restricted diet was based on a normal diet, but with a mineral mixture free of ferric citrate [13]. After 4 weeks diet, rats were sacrificed. The tissues were resected and quickly snap-frozen in liquid nitrogen and stored at −80 °C. In a separate study, 7-week-old male Sprague–Dawley rats (body weight; 250–300 g) were treated by a single subcutaneous injection of MCT (60 mg/kg) and were randomly given a normal diet (PH) or an iron-restricted diet (PH + IR) for 8 weeks. The survival rate was compared among the CTRL ($n = 6$), PH ($n = 15$), and PH + IR ($n = 14$) groups for 8 weeks diet. Rats were maintained on a 12 hr light/dark cycle and had free access to food and water. All of our experimental procedures were approved by the Animal Research Committee of Hyogo College of Medicine.

2.2. Cardiac and pulmonary hemodynamics

Rats were anesthetized with ketamine HCl (50 mg/kg) and xylazine HCl (10 mg/kg). B-mode, M-mode, and pulmonary pulsed-wave Doppler echocardiography were performed by transthoracic echocardiography (Aplio, Toshiba Medical Systems Co.). RV end-diastolic and end-systolic dimension were determined from epigastrium views. RV pressure was also measured directly by inserting a catheter connected to a pressure transducer into the RV before sacrifice (ADVantage, Scisense).

2.3. Assessments of hematologic parameters

Peripheral blood cell count, and serum iron and erythropoietin levels were determined as previously reported [14].

2.4. Histomorphometric analysis

The lung and RV tissues were fixed with buffered 4% paraformaldehyde, embedded in paraffin, and cut into 4-μm-thick sections. Elastica van Gieson stain (EVG) staining was performed using serial lung sections. Pulmonary arteriolar wall thickness was quantified using ImageJ software by measuring the maximum thickness of arteriolar walls. The values are normalized to CTRL (100%). There were at least 5–10 arterioles measured per slide and 2 slides per animal ($n = 3$–4 rats per group). Hematoxilin-eosin (HE) staining and Masson's trichrome staining were performed using serial RV sections. Cross-sectional area and fibrosis area of the RV were evaluated by semiquantitative score using the method as previously described [14].

2.5. Immunohistochemical analysis

The pulmonary and RV sections were immunohistochemically stained with a primary mouse anti-TfR1 antibody (Zymed Laboratories; dilution 1:200). Immunostains were visualized with the use of an avidin–biotin-peroxidase conjugate and 3,3′-diaminobenzidine substrate. Every section was counterstained with hematoxylin. Quantification of TfR1 positive area was performed by counting the number of TfR1 positive cells in 50 pulmonary arteries and 80 cardiomyocytes in 20 randamly selected fields.

2.6. Gene expression analysis

Total RNA was extracted from the tissues using TRIzol reagent (Invitrogen). DNase-treated RNA was reverse-transcribed into cDNA using random primers (Applied Biosystems). Real-time PCR reactions were performed using the ABI PRISM 7900 with TaqMan Universal PCR Master Mix and TaqMan Gene Expression Assays (Applied Biosystems) [12]. TaqMan Gene Expression Assays used as primers and probes for each gene were as follows: hepcidin (assay ID Rn00584987_m1), interleukin-6 (IL-6) (assay ID Rn01410330_m1), bone morphogenetic protein receptor type II (BMPR2) (assay ID Rn01437214_m1), and glyceraldehyde-3-phosphatedehydrogenase (GAPDH) (assay ID Rn99999916_s1). GAPDH was used as an internal control.

2.7. Statistical analysis

Values are reported as the means ± SEM. Statistical analysis was performed using one way analysis of variance. Analysis of variance (Kruskal–Wallis test, followed by Mann–Whitney U test) was used for statistical comparisons. The probability value <0.05 were considered to be significant. Survival rate was assessed by the Kaplan–Meier survival curves.

3. Results

3.1. Effects of iron restriction on the development of pulmonary vascular remodeling in MCT-injected rats

First, we evaluated the effects of iron restriction on the development of pulmonary vascular remodeling. EVG staining showed a single injection of MCT in rats induced massive pulmonary vascular remodeling; however, dietary iron restriction prevented this change (Fig. 1A and B). In addition, Doppler echocardiography demonstrated the appearance of midsystolic notching on pulmonary artery flow and the increase in pulmonary artery acceleration time in MCT-injected rats, whereas iron restriction suppressed these changes (Fig. 1C). Moreover, MCT-injected rats showed a significant increase in RV systolic pressure, while iron restriction

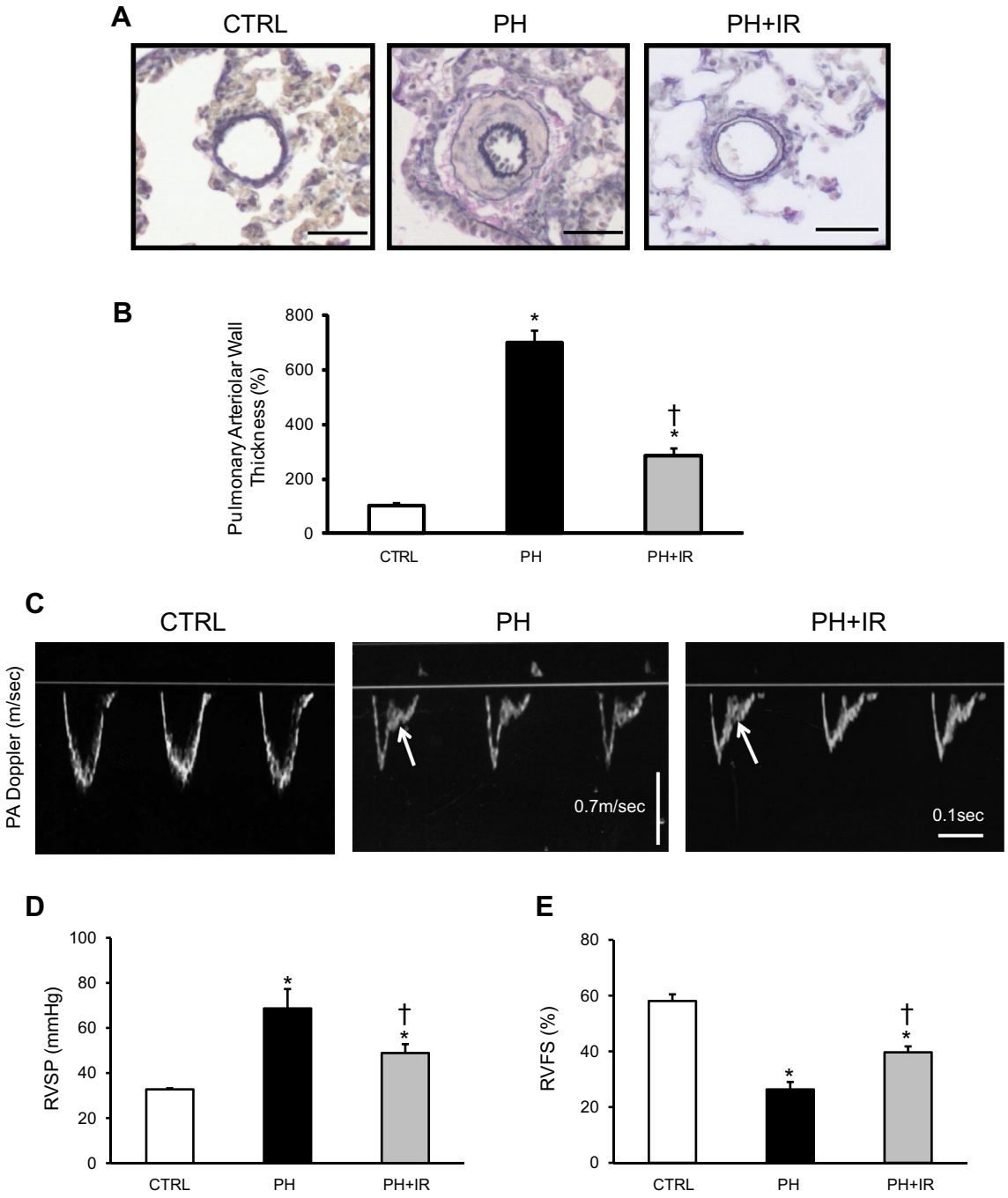

Fig. 1. Effects of dietary iron restriction on pulmonary arteriolar wall thickness and right ventricular systolic pressure in MCT-treated rats. (A) Representative images of Elastica van Gieson staining of the pulmonary artery sections. Scale bars: 50 μm. (B) Quantification of pulmonary arteriolar medial wall thickness in the CTRL (white bar), PH (black bar), and PH + IR (gray bar) groups ($n = 6$ in each group). (C) Representative pulse-waved Doppler in pulmonary artery flow. Arrows indicate midsystolic notch. (D) RV systolic pressure (RVSP) and (E) RV fractional shortening (RVFS) in the CTRL (white bar), PH (black bar), and PH + IR (gray bar) groups ($n = 6$ in each group). CTRL, Saline-treated rats fed a normal diet; PH, MCT-treated rats fed a normal diet; PH + IR, MCT-treated rats fed an iron-restricted diet. $^{*}p < 0.05$ versus the CTRL group, $^{\dagger}p < 0.05$ versus the PH group.

attenuated the increase in RV systolic pressure of MCT-injected rats (Fig. 1D). Interestingly, RV fractional shortening was decreased in MCT-injected rats. In contrast, iron restriction attenuated the decrease in RV fractional shortening of MCT-injected rats (Fig. 1E). These results suggest that dietary iron restriction prevents the development of pulmonary vascular remodeling in MCT-injected rats. On the other hand, as shown in Table 1, serum iron concentration was decreased in the PH + IR group, whereas anemia was not observed in all groups.

3.2. Effects of iron restriction on RV structure in MCT-injected rats

Since PH leads to pressure overload of the RV, with hypertrophy and dilation, followed by RV failure, we next evaluated the effect of iron restriction on the development of RV hypertrophy and dilatation. RV free wall thickness and dilatation were macroscopically observed in MCT-injected rats. As a result, the interventricular septum was shifted to LV in the PH group; however, these changes ware attenuated in the PH + IR group (Fig. 2A). In fact,

Table 1
Hematologic parameters in all groups at 4 weeks after MCT injection.

Parameter	CTRL	PH	PH + IR
n	6	6	6
Hemoglobin (g/dL)	16.3 ± 0.8	15.5 ± 0.5	14.2 ± 0.5
Serum iron concentration (μg/dL)	186.3 ± 10.3	181.9 ± 10.8	142.5 ± 10.4*†
Serum Epo concentration (mU/mL)	15.6 ± 0.9	14.6 ± 0.9	12.6 ± 1.4

MCT, monocrotaline; CTRL, Saline-treated rats fed a normal diet; PH, MCT-injected rats fed a normal diet; PH + IR, MCT-injected rats fed an iron-restricted diet; IR, iron-restricted diet; Epo, erythropoietin.
* $p < 0.05$ versus CTRL group.
† $p < 0.05$ versus PH group.

MCT-injected rats displayed a marked increase in the RV weight to left ventricular + interventricular septum weight ratio compared with the CTRL group, while this increase was attenuated in the PH + IR group (Fig. 2C). Histological analysis revealed that MCT-injected rats exhibited the increase in RV cardiomyocyte cross- sectional area by HE staining, whereas this increase tended to be reduced in the PH + IR group (Fig. 2B and D). In addition, Masson's trichrome staining showed RV interstitial fibrosis was increased in the PH group, while it was attenuated in the PH + IR group (Fig. 2B and E). Collectively, these data indicated that iron restriction suppressed the progression of RV failure in MCT-injected rats. Of interest, in the PH group, some rats died from 26 days after MCT injection and reached 50% survival by 8 weeks, while iron restriction resulted in 79% survival after 8 weeks diet (Fig. 2F).

3.3. Increased TfR1 expression in the pulmonary artery and RV of MCT-injected rats

In order to explore the possibility that TfR1, which plays a key role in intracellular iron transport, is associated with the mechanisms of PH and PH induced RV failure in MCT-injected rats, we next investigated TfR1 expression in the pulmonary artery (PA) and RV of these groups. TfR1 is required for the uptake of transferrin-bound iron into the cells [11]. Of interest, immunohistochemical analysis revealed that TfR1 was slightly expressed in the PA of CTRL rats, while TfR1 expression was increased in the remodeled PA of MCT-injected PH rats (Fig. 3A and C). Moreover, TfR1expression was increased in the failing RV of MCT-injected rats, as compared with CTRL rats (Fig. 3B and D). Meanwhile, TfR1 expression was increased in the PA and RV of PH + IR rats in response to iron deficiency (Fig. 3A–D). These results suggest that TfR1 may participate in the development of PA remodeling and RV failure of MCT-injected rats.

3.4. Increased hepcidin gene expression in the RV of MCT-injected rats

Recently, hepcidin, which is expressed in the liver, was identified to be a key regulator of iron homeostasis [15]. In order to evaluate the possibility that hepcidin is involved in the mechanisms of PH and RV failure in MCT-injected rats, we next examined hepcidin gene expression in these groups. Hepatic hepcidin gene expression was decreased in the PH + IR group in response to iron deficiency (Fig. 4A). On the other hand, pulmonary hepcidin gene expression was not different among three groups (Fig. 4B). Of interest, hepcidin gene expression was increased in the failing RV of MCT-injected rats compared with the other groups (Fig. 4C). These data suggest that hepcidin may be associated with the development of RV failure of MCT-injected rats.

3.5. Regulation of hepcidin gene expression in the RV of MCT-injected rats

Hepcidin expression is reported to be induced by IL-6 in HepG2/2.2.1 cells [16]. In order to assess whether hepcidin is induced by IL-6 in the RV of MCT-injected rats, we examined IL-6 gene expression in the RV of these groups. IL-6 gene expression was not increased in MCT-injected rats, while IL-6 gene expression was decreased in the PH + IR group, as compared with the other groups (Fig. 4D). These data suggest that IL-6 may not be the main cause of increased hepcidin gene expression in the RV of MCT-injected rats, while IL-6 may contribute to decreased hepcidin gene expression in the RV of PH + IR rats. On the other hand, BMPR2 is reported to be associated with hepcidin expression in HepG2 cells [8]; however, BMPR2 gene expression was not different among the RV of our experimental groups (Fig. 4E). Importantly, pulmonary vascular expression of BMPR2 is reported to be decreased in patients with primary PH [17] and MCT-injected rats [18]. Therefore, we examined whether iron restriction affects BMPR2 expression in the lung of MCT-injected rats. In consistent with the previous report [18], BMPR2 gene expression was decreased in the lung of MCT-injected rats. Meanwhile, BMPR2 gene expression was also decreased in the lung of PH + IR rats (Fig. 4F), suggesting that the beneficial effects of iron restriction against the development of PH might not be associated with BMPR2 signaling.

4. Discussion

To the best of our knowledge, this is the first study to show that dietary iron restriction attenuates the development of pulmonary vascular remodeling and RV failure in MCT-injected rats. Besides, TfR1 expression was increased in the PA and RV of MCT-injected rats. Moreover, hepcidin gene expression was increased in the RV of MCT-injected PH rats. These data indicate that dysregulation of cellular iron transport may be associated with the pathophysiology of PH and PH induced RV failure.

Iron is a vital element necessary for life. However, excess iron accumulation promotes increased oxidative stress, which actually leads to cell and tissue damage. Thus, it is extremely important to consider the participation of iron on the pathophysiology of various diseases. In fact, iron is reported to be associated with the control of pulmonary vascular tone in response to acute hypoxic condition [6,7]. In the current study, we elucidated the effects of dietary iron restriction on the development of pulmonary vascular remodeling in MCT-injected rats. Of note, MCT-injected rats showed pulmonary vascular remodeling and RV failure; however, dietary iron restriction attenuated these changes. This salutary effect of iron restriction on pulmonary vascular remodeling is consistent with the results previously reported that an iron chelator, deferoxamine inhibited the development of pulmonary vascular remodeling in a hypoxia induced PH model rat [19]. Since oxidative stress is associated with the mechanism of pulmonary vascular remodeling [20], iron restriction may prevent the development of pulmonary vascular remodeling through inhibition of oxidative stress.

Recently, iron deficiency is reported to be prevalent without overt anemia in patients with idiopathic and heritable PH [8–10]. However, the mechanism by which iron deficiency occurs in patients with PH remains unknown. In the current study, we found for the first time that TfR1 expression was increased in the PA and RV of MCT-injected rats. Low iron conditions normally lead to upregulate TfR1 expression, conversely high iron conditions induce to downregulate TfR1 expression [11]. In the present study, we observed increased expression of TfR1 in the PA and RV of PH + IR rats normally in response to low iron conditions.

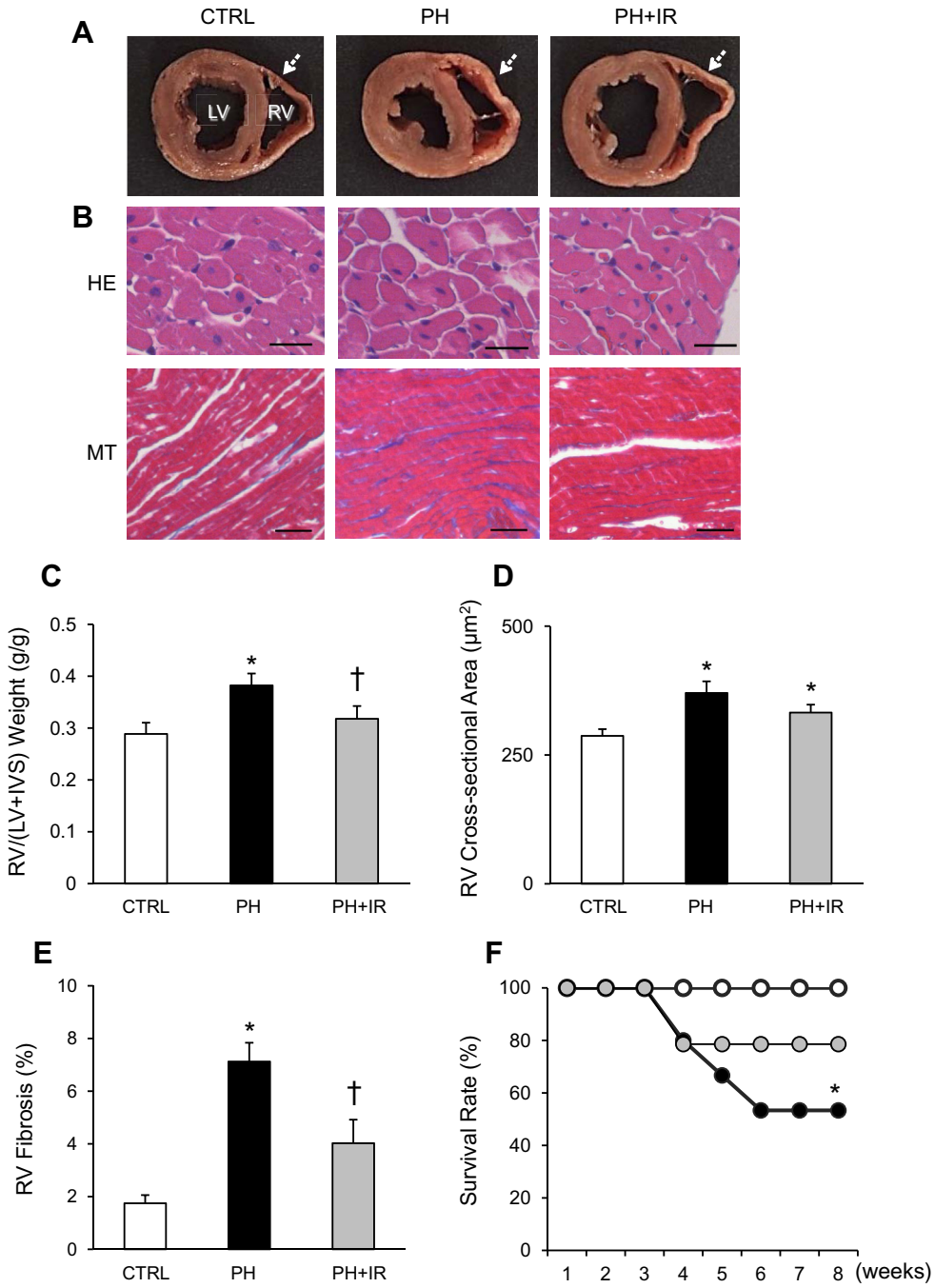

Fig. 2. Effects of dietary iron restriction on right ventricular failure and prognosis in MCT-treated rats. (A) Representative gross morphology of the heart cross-sections. Arrows indicate RV free wall. (B) Representative images of hematoxylin-eosin and Masson's trichrome staining of the RV sections. Scale bars: 50 μm. (C) RV/(LV + IVS) weight ratio and quantitative analysis of (D) RV myocyte cross-sectional area and (E) myocardial interstitial fibrosis in the CTRL (white bar), PH (black bar), and PH + IR (gray bar) groups ($n = 6$ in each group). (F) Survival rate after diet in the CTRL (white circle, $n = 6$), PH (black circle, $n = 15$), and PH + IR (gray circle, $n = 14$) groups. HE, hematoxylin-eosin staining; MT, Masson's trichrome staining. $^{*}p < 0.05$ versus the CTRL group, $^{\dagger}p < 0.05$ versus the PH group.

Meanwhile, we also detected increased TfR1 expression in the PA and RV of MCT-injected rats without artificial low iron conditions. These results suggest that dysregulation of cellular iron transport may be associated with the pathophysiology of pulmonary vascular remodeling and RV failure, and partially with the mechanism of iron deficiency in PH.

Another novel finding of this study is that hepcidin gene expression is increased in the RV of MCT-injected rats. Hepcidin is mainly expressed in the liver and a pivotal regulator of iron homeostasis. Hepatic hepcidin is predominantly decreased in response to iron deficiency. In contrast, hepatic hepcidin expression is increased by iron overload [21]. In this study, we observed decreased hepatic hepcidin gene expression in the PH + IR group in response to iron deficiency, similarly to the previous report [21]. Of interest, hepcidin gene expression was increased in the failing RV of MCT-injected rats. There is no report to assess hepcidin expression in the RV so far. To our best knowledge, this is the first report to show increased hepcidin gene expression in the failing RV of MCT-injected rats. However, it remains unknown how hepcidin contributes to the failing RV or how hepcidin gene expression in the

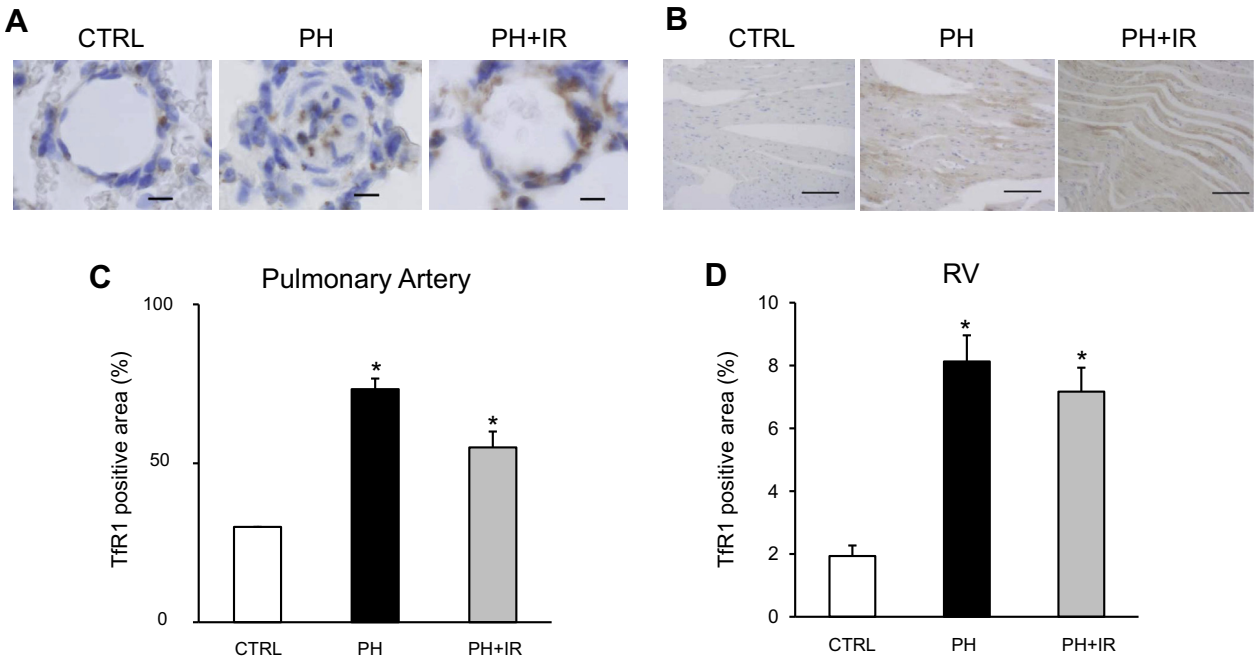

Fig. 3. TfR1 expression in the pulmonary artery and right ventricle of MCT-treated rats. Representative images of TfR1 staining of the (A) pulmonary artery and (B) RV sections. Scale bars: 10 μm for pulmonary artery and 100 μm for RV. Quantitative analysis of TfR1-positive area of the (C) pulmonary artery and (D) RV in the CTRL (white bar), PH (black bar), and PH + IR (gray bar) groups ($n = 6$ in each group). $^{*}p < 0.05$ versus the CTRL group.

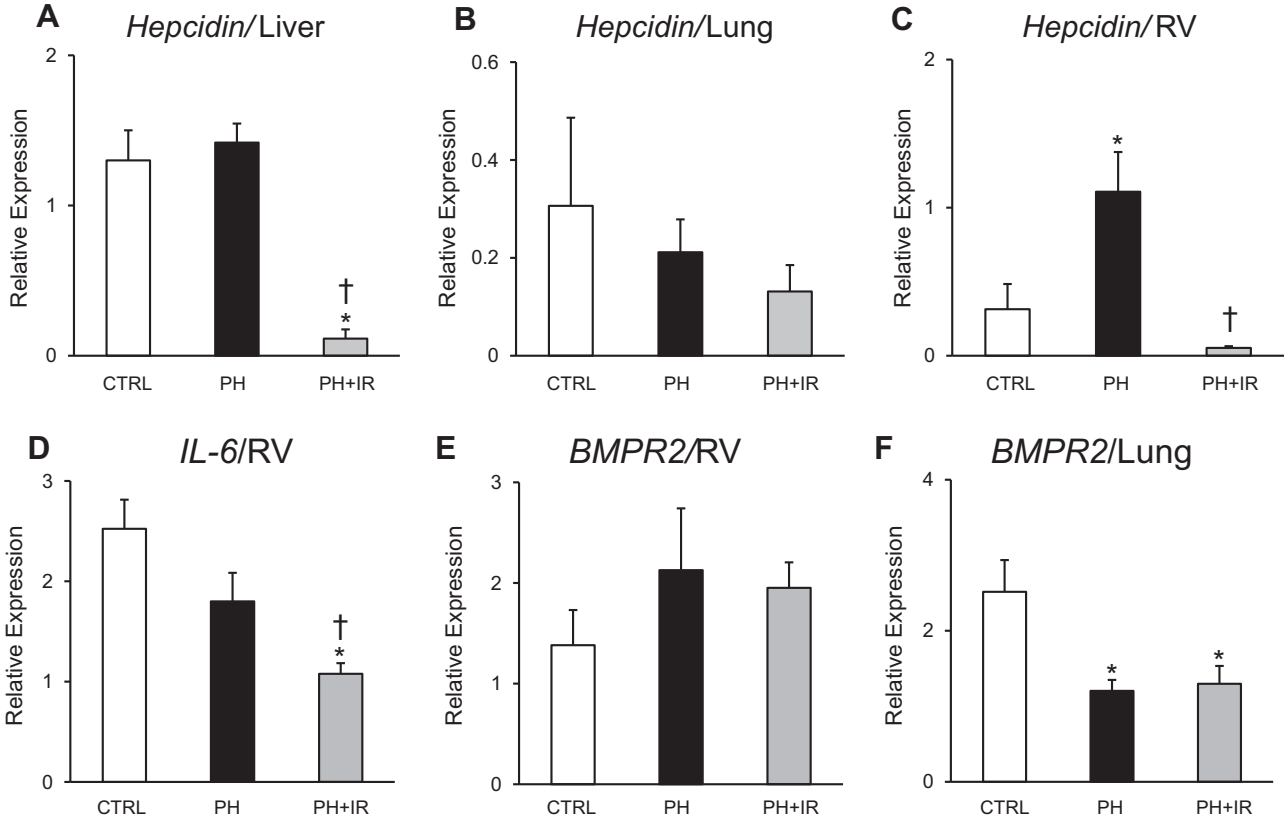

Fig. 4. Hepcidin gene expression in the liver, lung, and right ventricle of MCT-treated rats. *Hepcidin* gene expression in the (A) liver, (B) lung, and (C) RV of the CTRL (white bar), PH (black bar), and PH + IR (gray bar) groups ($n = 6$ in each group). (D) *IL-6* and (E) *BMPR2* gene expression in the RV, and (F) *BMPR2* gene expression in the lung of the CTRL (white bar), PH (black bar), and PH + IR (gray bar) groups ($n = 6$ in each group). Gene expression of *hepcidin, IL-6,* and *BMPR2* was normalized with *GAPDH* gene expression, and relative levels of gene expression are shown in the graph. $^{*}p < 0.05$ versus the CTRL group, $^{\dagger}p < 0.05$ versus the PH group.

failing RV is regulated in MCT-injected rats. IL-6 and BMPR2 are reported to be associated with hepcidin expression in HepG2 cells [8,16]. In the current study, IL-6 and BMPR2 gene expression was not increased in the RV of MCT-injected rats. Therefore, IL-6 and BMPR2 do not seem to be critical for regulating hepcidin expression in the RV of MCT-injected rats. In addition, erythropoietin is reported to mediate hepcidin expression in hepatocytes [22]. Because serum erythropoietin concentration did not differ among three groups, erythropoietin does not likely contribute to increased hepcidin gene expression in the RV of MCT-injected rats. Although many studies on heart failure have focused on the left ventricle, little is known about the mechanisms underlying RV failure. Besides, RV function is a critical determinant of survival in patients with PH [23]. Future efforts are certainly warranted to delineate the molecular mechanism by which hepcidin acts on the failing RV of MCT-injected rats.

Since PH is a chronic disease, pulmonary vascular remodeling has already been developed when patients with PH are diagnosed. Therefore, it is difficult to diagnose at the early phase and prevent the development of PH. Although progressive iron deficiency by venesection has been reported to promote acute pulmonary vasoconstriction in 11 high-altitude residents with chronic mountain sickness [7], we showed the salutary effects of iron restriction against the development of pulmonary vascular remodeling in MCT-injected rats. The differences seen in these studies may be due to the differences in the extent and duration of iron deficiency. Smith et al. provided acute effects of iron deficiency on pulmonary vasoconstriction [7], whereas we showed chronic effects of iron deficiency on pulmonary vascular remodeling. Iron is considered to be implicated in the pathogenesis of several cardiovascular diseases including PH [3–7]. From the view point of prevention, future studies are necessary to consider the role of iron on the development of PH.

In conclusions, we found for the first time that dietary iron restriction attenuates the development of pulmonary vascular remodeling and RV failure in MCT-injected rats. TfR1 expression was increased in the PA and RV of MCT-injected rats. Moreover, hepcidin gene expression was increased in the RV of MCT-injected rats. Dysregulation of cellular iron transport might be involved in the pathophysiology of pulmonary vascular remodeling and RV failure.

Acknowledgments

We gratefully acknowledge the technical assistance of Noriko Kumon and Sachi Ito. This study was supported by a Grant-in-Aid for Scientific Research (C) (JSPS KAKENHI Grant No. 25460919) and grants from The Salt Science Research Foundation (No. 1232), Takeda Science Foundation, and Hyogo Science and Technology Association (to Y. Naito).

References

[1] S.L. Archer, E.K. Weir, M.R. Wilkins, Basic science of pulmonary arterial hypertension for clinicians: new concepts and experimental therapies, Circulation 121 (2010) 2045–2066.
[2] N.F. Voelkel, R.A. Quaife, L.A. Leinwand, R.J. Barst, M.D. McGoon, D.R. Meldrum, J. Dupuis, C.S. Long, L.J. Rubin, F.W. Smart, Y.J. Suzuki, M. Gladwin, E.M. Denholm, D.B. Gail, Right ventricular function and failure: report of a National Heart, Lung, and Blood Institute Working Group on Cellular and Molecular Mechanisms of Right Heart Failure, Circulation 114 (2006) 1883–1891.
[3] A. Ascherio, W.C. Willett, E.B. Rimm, E.L. Giovannucci, M.J. Stampfer, Dietary iron intake and risk of coronary disease among men, Circulation 89 (1994) 969–974.
[4] K. Jomova, M. Valko, Advances in metal-induced oxidative stress and human disease, Toxicology 283 (2011) 65–87.
[5] J. Kaluza, A. Wolk, S.C. Larsson, Heme iron intake and risk of stroke: a prospective study of men, Stroke 44 (2013) 334–339.
[6] T.G. Smith, G.M. Balanos, Q.P. Croft, N.P. Talbot, K.L. Dorrington, P.J. Ratcliffe, P.A. Robbins, The increase in pulmonary arterial pressure caused by hypoxia depends on iron status, J. Physiol. 586 (2008) 5999–6005.
[7] T.G. Smith, N.P. Talbot, C. Privat, M. Rivera-Ch, A.H. Nickol, P.J. Ratcliffe, K.L. Dorrington, F. León-Velarde, P.A. Robbins, Effects of iron supplementation and depletion on hypoxic pulmonary hypertension: two randomized controlled trials, JAMA 302 (2009) 1444–1450.
[8] C.J. Rhodes, L.S. Howard, M. Busbridge, D. Ashby, E. Kondili, J.S. Gibbs, J. Wharton, M.R. Wilkins, Iron deficiency and raised hepcidin in idiopathic pulmonary arterial hypertension: clinical prevalence, outcomes, and mechanistic insights, J. Am. Coll. Cardiol. 58 (2011) 300–309.
[9] G. Ruiter, S. Lankhorst, A. Boonstra, P.E. Postmus, S. Zweegman, N. Westerhof, W.J. van der Laarse, A. Vonk-Noordegraaf, Iron deficiency is common in idiopathic pulmonary arterial hypertension, Eur. Respir. J. 37 (2011) 1386–1391.
[10] E. Soon, C.M. Treacy, M.R. Toshner, R. MacKenzie-Ross, V. Manglam, M. Busbridge, M. Sinclair-McGarvie, J. Arnold, K.K. Sheares, N.W. Morrell, J. Pepke-Zaba, Unexplained iron deficiency in idiopathic and heritable pulmonary arterial hypertension, Thorax 66 (2011) 326–332.
[11] T.R. Daniels, T. Delgado, J.A. Rodriguez, G. Helguera, M.L. Penichet, The transferrin receptor part I: Biology and targeting with cytotoxic antibodies for the treatment of cancer, Clin. Immunol. 121 (2006) 144–158.
[12] Y. Naito, S. Hirotani, H. Sawada, H. Akahori, T. Tsujino, T. Masuyama, Dietary iron restriction prevents hypertensive cardiovascular remodeling in Dahl salt-sensitive rats, Hypertension 57 (2011) 497–504.
[13] Y. Naito, A. Fujii, H. Sawada, S. Hirotani, T. Iwasaku, A. Eguchi, M. Ohyanagi, T. Tsujino, T. Masuyama, Effect of iron restrictio t n on renal damage and mineralocorticoid receptor signaling in a rat model of chronic kidney disease, J. Hypertens. 30 (2012) 2192–2201.
[14] Y. Naito, T. Tsujino, M. Matsumoto, T. Sakoda, M. Ohyanagi, T. Masuyama, Adaptive response of the heart to long term anemia induced by iron deficiency, Am. J. Physiol. Heart Circ. Physiol. 296 (2009) H585–H593.
[15] M.W. Hentze, M.U. Muckenthaler, B. Galy, C. Camaschella, Two to tango: regulation of Mammalian iron metabolism, Cell 142 (2010) 24–38.
[16] D.M. Wrighting, N.C. Andrews, Interleukin-6 induces hepcidin expression through STAT3, Blood 108 (2006) 3204–3209.
[17] C. Atkinson, S. Stewart, P.D. Upton, R. Machado, J.R. Thomson, R.C. Trembath, N.W. Morrell, Primary pulmonary hypertension is associated with reduced pulmonary vascular expression of type II bone morphogenetic protein receptor, Circulation 105 (2002) 1672–1678.
[18] R.E. Morty, B. Nejman, G. Kwapiszewska, M. Hecker, A. Zakrzewicz, F.M. Kouri, D.M. Peters, R. Dumitrascu, W. Seeger, P. Knaus, R.T. Schermuly, O. Eickelberg, Dysregulated bone morphogenetic protein signaling in monocrotaline-induced pulmonary arterial hypertension, Arterioscler., Thromb., Vasc. Biol. 27 (2007) 1072–1078.
[19] C.M. Wong, I.R. Preston, N.S. Hill, Y.J. Suzuki, Iron chelation inhibits the development of pulmonary vascular remodeling, Free Radical Biol. Med. 53 (2012) 1738–1747.
[20] S. Wedgwood, R.W. Dettman, S.M. Black, ET-1 stimulates pulmonary arterial smooth muscle cell proliferation via induction of reactive oxygen species, Am. J. Physiol. Lung Cell Mol. Physiol. 281 (2001) L1058–L1067.
[21] G. Nicolas, C. Chauvet, L. Viatte, J.L. Danan, X. Bigard, I. Devaux, C. Beaumont, A. Kahn, S. Vaulont, The gene encoding the iron regulatory peptide hepcidin is regulated by anemia, hypoxia, and inflammation, J. Clin. Invest. 110 (2002) 1037–1044.
[22] J.P. Pinto, S. Ribeiro, H. Pontes, S. Thowfeequ, D. Tosh, F. Carvalho, G. Porto, Erythropoietin mediates hepcidin expression in hepatocytes through EPOR signaling and regulation of C/EBPalpha, Blood 111 (2008) 5727–5733.
[23] M.R. Bristow, L.S. Zisman, B.D. Lowes, W.T. Abraham, D.B. Badesch, B.M. Groves, N.F. Voelkel, D.M. Lynch, R.A. Quaife, The pressure-overloaded right ventricle in pulmonary hypertension, Chest 114 (Suppl. 1) (1998) 101S–106S.