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Clinical and Immunologic Predictors of Scleroderma Renal Crisis in Japanese Systemic Sclerosis Patients With Anti–RNA Polymerase III Autoantibodies

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Objective. **To identify predictive factors for scleroderma renal crisis (SRC) in patients with anti–RNA polymerase III (anti–RNAP III) antibodies.**

Methods. **A total of 583 adult Japanese patients with systemic sclerosis (SSc) were screened for anti–RNAP III using a commercially available enzyme-linked immunosorbent assay (ELISA) kit. RNAP subsets were further identified by immunoprecipitation (IP) assays. The association of clinical and immunologic factors with SRC was examined by logistic analyses.**

Results. **In this cohort, 37 patients (6%) were positive for anti–RNAP III, as determined by anti–RNAP III–specific ELISA. Further IP assays revealed that 19 patients were positive for anti–RNAP I/III, 17 for anti–RNAP I/II/III, and 1 for anti-RNAP III. SRC occurred in a total of 17 (2.9%) of 583 patients, with a significantly higher frequency in anti–RNAP III–positive SSc patients (9 of 37 [24%]) than those without anti–RNAP III (8 of 546 [1%]) (odds ratio [OR] 21.6 [95% confidence interval (95% CI) 7.8–60.3], $P < 0.00001$). Our multivariate analyses using the Cox proportional hazards regression model revealed that anti–RNAP I/II/III positivity (OR 11.0 [95% CI 1.6–222.8], $P = 0.0118$) and an ELISA index for anti–RNAP III of ≥157 (OR 2.4 × 10^9 [95% CI 2.1–uncalculated], $P = 0.0093$) were independent factors associated with the development of SRC.**

Conclusion. **Our findings indicate that anti–RNAP III is associated with SRC, as reported previously. In addition, the presence of anti–RNAP II in combination with anti–RNAP I/III (anti–RNAP I/II/III) and a higher ELISA index for anti–RNAP III may be associated with the development of SRC in SSc patients with anti–RNAP III.**

Systemic sclerosis (SSc) is a multisystem connective tissue disorder characterized by excessive fibrosis of skin and internal organs and microvascular damage. While the etiology of SSc remains unclear, autoimmunity is considered to be involved in the pathophysiology. Serum antinuclear antibodies (ANAs) are detected in >90% of patients with SSc and are a hallmark of the disease (1). Many ANAs are specific for SSc, including antibodies against topoisomerase I (topo I), centromeres, RNA polymerase III (RNAP III), Th/To, and U3 RNP. Autoantibodies are present at diagnosis of the disease. These antibodies do not coexist in most patients, and patients' autoantibody profiles usually do not change during the disease course (2). Importantly, ANAs are closely associated with distinct clinical subsets, and thus detection of SSc-related antibodies is useful not only in the diagnosis of the disease, but also for predicting organ involvement and prognosis.

SSc is a heterogeneous disease with variable clinical presentations. Scleroderma renal crisis (SRC) is a major complication in patients with SSc (3). SRC occurs in 5% of patients with SSc, particularly in the first years of disease evolution, and typically in the diffuse

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form (4). It is characterized by accelerated hypertension and acute progressive renal impairment. Although the prognosis of SRC has been dramatically improved by the use of angiotensin-converting enzyme (ACE) inhibitors, long-term survival after SRC remains poor (5,6). Furthermore, SRC is one of the most rapidly progressive conditions among SSc complications. Therefore, identification of high-risk groups and early detection of SRC are critical for improving outcomes. While several indicators for SRC have been reported, anti–RNAP III antibodies are present in one-third of the patients who develop SRC. Nonetheless, the indicators for SRC within the anti–RNAP III–positive group have not been identified. Thus, in this study we examined factors that were associated with SRC in anti–RNAP III–positive SSc patients.

PATIENTS AND METHODS

Patients. We analyzed 583 consecutive Japanese patients with SSc (490 women and 93 men) who were evaluated between 1995 and 2012 at Kanazawa University Hospital or Social Insurance Chukyo Hospital. All patients met one of the following criteria (7): the American College of Rheumatology preliminary criteria for the classification of SSc (8); the presence of at least 3 of the 5 features of CREST syndrome (calcinosis, Raynaud's phenomenon, esophageal dysmotility, sclerodactyly, telangiectasias); or the presence of definite Raynaud's phenomenon, abnormal nailfold capillaries, and scleroderma-specific autoantibodies (9). SSc patients were classified as having limited cutaneous SSc (lcSSc) or diffuse cutaneous SSc (dcSSc) according to the criteria proposed by LeRoy et al (10). All serum samples were obtained prior to the onset of SRC. Fresh venous blood samples were centrifuged shortly after clot formation. Samples were stored at −70°C until used. Written informed consent was obtained from all subjects, and the protocol was approved by Kanazawa University Hospital.

Clinical assessments. The modified Rodnan skin thickness score (MRSS) was used to semiquantitatively assess the degree of skin sclerosis (11), and the maximum score during the disease course was recorded. The skin thickness progression rate was defined as the MRSS on the first visit divided by the duration, in years, from when the patient reported or a physician judged or recorded the onset of skin thickening, in MRSS units per year (12). Organ system involvement was defined principally as previously described (13–17), and definitions are listed in Supplementary Table 1, available on the *Arthritis & Rheumatology* web site at http://onlinelibrary.wiley.com/doi/10.1002/art.38994/abstract.

Enzyme-linked immunosorbent assays (ELISAs). Samples were screened for anti–RNAP III by ELISA kits according to the recommendations of the manufacturer (MBL) (18,19).

Immunoprecipitation (IP) assays. IP assays were performed using extracts of the leukemia cell line K562, as previously described (20). Briefly, 10 μl of patient sera was mixed with protein A–Sepharose CL-4B (Pharmacia Biotech) in IP buffer (10 m*M* Tris HCl, pH 8.0, 500 m*M* NaCl, and 0.1% Nonidet P40) and incubated for 2 hours at 4°C, and then washed 5 times with IP buffer. For protein analysis, the antibody-coated Sepharose beads were incubated with ^{35}S-methionine–labeled K562 cell extracts at 4°C for 2 hours. After 9 washes, the immunoprecipitated materials were subjected to 8% sodium dodecyl sulfate–polyacrylamide gel electrophoresis. Radiolabeled polypeptide components were analyzed by autoradiography.

Statistical analysis. Comparisons between anti–RNAP III–positive SSc patients with SRC and those without SRC were made using a chi-square test with Yates' correction or Fisher's exact test, when appropriate. Continuous variables were divided into 2 groups based on a cutoff value that was determined by the receiver operating characteristic curve in a univariate analysis. For instance, the cutoff value for the anti–RNAP III ELISA index was 157, which yielded a maximum value of "sensitivity − (1 − specificity)" (see Supplementary Figure 1, available on the *Arthritis & Rheumatology* web site at http://onlinelibrary.wiley.com/doi/10.1002/art.38994/abstract). Variables selected by the univariate analyses were further subjected to multivariate analyses using a Cox proportional hazards regression model to determine independent factors associated with SRC development. We used the Kaplan-Meier method to estimate SRC development between the 2 patient groups. The results are presented as the odds ratio (OR) and 95% confidence interval (95% CI). Statistical analyses were performed using JMP statistical software version 10.

RESULTS

Assessment of anti–RNAP III in SSc patients. Of the 583 SSc patients included in this study, 60% had lcSSc and 40% had dcSSc (352 and 231 patients, respectively). In this cohort, 37 patients (6%) (13 men and 24 women) were positive for anti–RNAP III, as determined by an anti–RNAP III–specific ELISA. Six of these patients had lcSSc, and 31 had dcSSc. Their mean ± SD maximum MRSS was 17.9 ± 10.8.

We examined the immunoprecipitation pattern of RNAP in the 37 anti–RNAP III–positive patients identified by ELISA. Sera from the 37 anti–RNAP III–positive patients were divided into 3 groups based on the immunoprecipitation of RNAP. Seventeen patients had both anti–RNAP I/III and anti–RNAP II (anti–RNAP I/II/III), 19 had anti–RNAP I/III, and 1 had anti–RNAP III alone. All 17 serum samples that were positive for anti–RNA I/III and II recognized both the phosphorylated and the nonphosphorylated forms of RNAP II (Figure 1). All 37 anti–RNAP III–positive patients were then classified into 2 groups based on the presence or absence of anti–RNAP II. Therefore, the one patient with anti-RNAP III alone was included in the anti–RNAP I/III patient group.

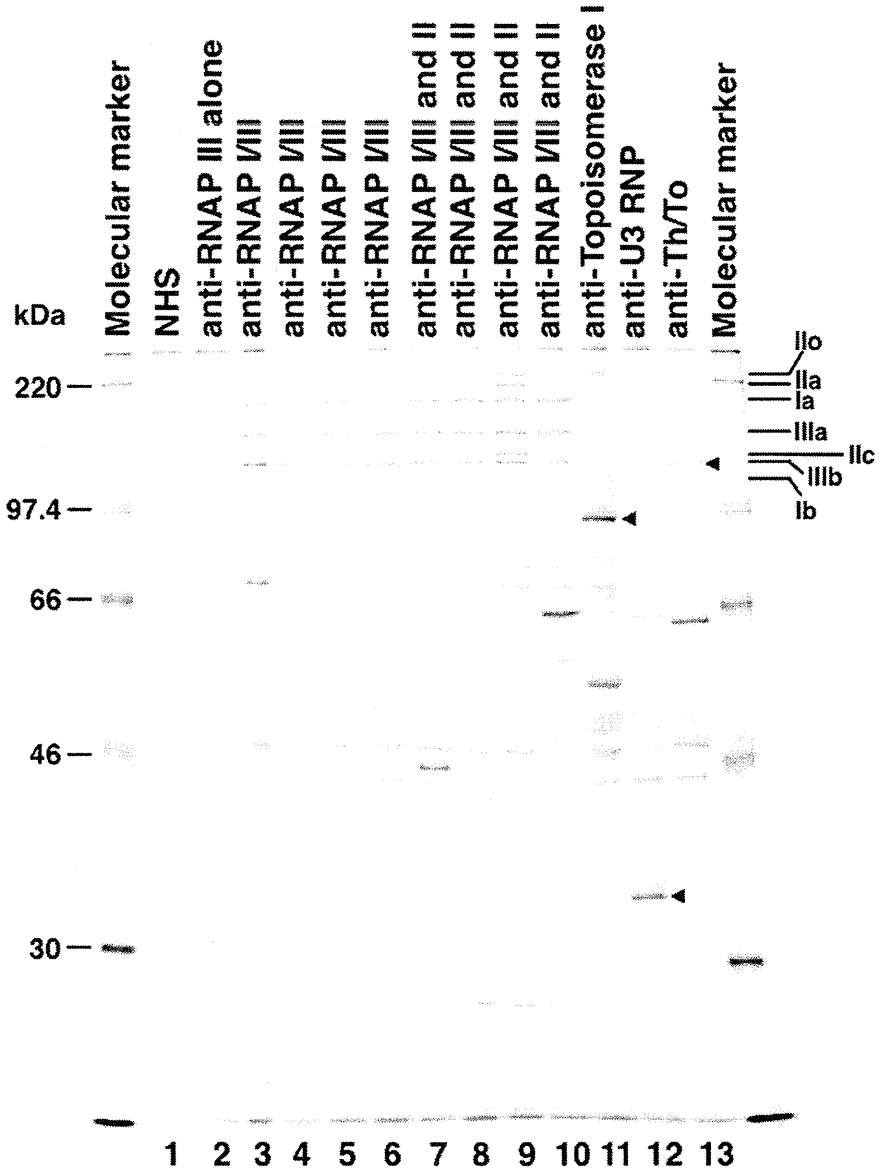

Figure 1. Immunoprecipitation assay of autoantibodies related to systemic sclerosis (SSc). Immunoprecipitation of ^{35}S-methionine–labeled K562 cell extracts was performed on sera from patients with SSc (lanes 2–13) and normal human serum (NHS; lane 1), separated by 7.5% sodium dodecyl sulfate–polyacrylamide gel electrophoresis, and analyzed by autoradiography. RNA polymerase III (RNAP III) alone (lane 2), RNAP I/III (lanes 3–6), and RNAP I/III and II (lanes 7–10) proteins are shown by the indicated lines. Arrowheads indicate topoisomerase I (100 kd; lane 11), U3 RNP (34 kd; lane 12), and 7-2 RNA (120 kd; lane 13). All 17 serum samples that were positive for anti–RNA I/III and II recognized both the phosphorylated (IIo) and the nonphosphorylated (IIa) forms of RNAP II.

SRC frequency in Japanese SSc patients. SRC occurred in 17 (2.9%) of the 583 SSc patients (4 men and 13 women). All 17 patients had dcSSc. Consistent with the findings of previous studies (5), SRC occurred at a significantly higher frequency in anti–RNAP III–positive SSc patients (9 of 37 [24%]) than in those without anti–RNAP III (8 of 546 [1%]) (OR 21.6 [95% CI 7.8–60.3], $P < 0.00001$). Thus, we confirmed that anti–

Table 1. Clinical outcomes in the 37 anti–RNAP III–positive patients with SSc with or without SRC*

	Patients with SRC (n = 9)	Patients without SRC (n = 28)
Age at onset, years	54 ± 16	55 ± 13
Duration, years	1.8 ± 1.1	2.0 ± 1.8
Followup period, years	4.4 ± 2.8	7.8 ± 6.2
SSc subtype, no. with dcSSc/no. with lcSSc	9/0	22/6
MRSS	21 ± 11	17 ± 11
Skin thickness progression rate, MRSS units/year	19 ± 17	16 ± 18
Maximum oral steroid, mg/day	26 ± 15	19 ± 11
ELISA index for anti–RNAP III	250.4 ± 71.2	141.6 ± 84.2†

* Except where indicated otherwise, values are the mean ± SD. Anti–RNAP III = anti–RNA polymerase III; SSc = systemic sclerosis; dcSSc = diffuse cutaneous SSc; lcSSc = limited cutaneous SSc; MRSS = modified Rodnan skin thickness score; ELISA = enzyme-linked immunosorbent assay.
† $P = 0.0013$ versus patients with scleroderma renal crisis (SRC).

RNAP III positivity is characteristic of Japanese SSc patients with SRC. All 9 anti–RNAP–positive and 8 anti–RNAP–negative (7 with anti–topo I and 1 with anti–U3 RNP) patients with SRC had the diffuse form and typical SRC. No anti–RNAP III–positive patients with SRC died of SRC, but 2 of the 8 anti–RNAP III–negative patients with SRC died of SRC despite administration of ACE inhibitors. Of 9 anti–RNAP III–positive patients with SRC, 8 (89%) received corticosteroids, and the corticosteroid dosage was >15 mg/day in 7 (88%) of those 8 patients. Those 8 patients received the maximum dose of corticosteroids in the period preceding SRC. Of 28 anti–RNAP III–positive patients without SRC, 23 patients (82%) received corticosteroids, and 20 (87%) of 23 patients received corticosteroids >15 mg/day.

Clinical characteristics of SSc patients with anti–RNAP III antibodies. We compared 9 patients with anti–RNAP III who developed SRC and 28 patients with anti–RNAP III who did not. As shown in Table 1, there was no significant difference in age at onset, disease duration, or followup period between the 2 groups. Although the differences were not significant, the percentage of patients with dcSSc was higher in the group with SRC than in the group without SRC (100% versus 79%; $P = 0.3025$), and the MRSS was higher in those with SRC than in those without SRC (mean ± SD 21 ± 11 versus 17 ± 11, $P = 0.2778$). Skin thickness progression rate and maximum oral steroid dosage were not statistically significantly different between the 2 groups. However, the ELISA indexes for anti–RNAP III were significantly higher in patients with SRC compared to those without SRC (mean ± SD 250.4 ± 71.2 versus 141.6 ± 84.2; $P = 0.0013$). Additionally, we examined the association of an ELISA index for anti–RNAP III with the maximum skin score. ELISA indexes for anti–RNAP III were positively associated with the maximum skin score, although the correlation was mild ($P = 0.0413$, $R^2 = 0.11365$) (see Supplementary Figure 2, available on the *Arthritis & Rheumatology* web site at http://onlinelibrary.wiley.com/doi/10.1002/art.38994/abstract).

Predictive factors for SRC and clinical course in anti–RNAP III–positive SSc patients. We examined the baseline clinical and immunologic factors associated with the development of SRC as indicated by logistic analysis. The steroid dosage used for analysis was the maximum dosage used during the patient's entire clinical course. We found that the factors associated with the development of SRC in the univariate analysis were anti–RNAP I/II/III positivity (OR 16.9 [95% CI 1.8–156.3], $P = 0.0055$) and an ELISA index for anti–RNAP III of ≥157 (OR 33.5 [95% CI 4.2–268.2], $P < 0.0001$) (Table 2).

For our multivariate analysis, in addition to anti–RNAP I/II/III positivity and an ELISA index for anti–RNAP III of >157, we added the factors of MRSS and oral steroid dosage, since previous studies indicated that these 2 factors were associated with the development of

Table 2. Univariate logistic regression analysis to predict SRC development*

Variable baseline factor	OR of SRC development (95% CI)
Age at onset >59 years	0.923 (0.204–4.179)
Male	0.900 (0.184–4.400)
MRSS >12	6.000 (0.659–54.667)
Skin thickness progression rate >7.3	3.033 (0.533–17.250)
Digital pitting scars	3.000 (0.525–17.159)
Digital ulcers	7.714 (0.608–97.846)
Interstitial lung disease	1.500 (0.310–7.247)
Gastrointestinal tract involvement	1.400 (0.238–8.240)
Heart involvement	3.000 (0.525–17.159)
Joint involvement	0.375 (0.040–3.551)
Skeletal muscle involvement	7.714 (0.608–97.846)
Malignancy	2.300 (0.424–12.465)
Oral steroids	1.739 (0.176–17.222)
Maximum oral steroid dosage >30 mg/day	2.639 (0.568–12.254)
Anti–RNAP I/II/III positivity	16.889 (1.825–156.282)†
ELISA index for anti–RNAP III ≥157	33.476 (4.178–268.216)‡

* SRC = scleroderma renal crisis; OR = odds ratio; 95% CI = 95% confidence interval; MRSS = modified Rodnan skin thickness score; anti–RNAP I/II/III = anti–RNA polymerase I/II/III; ELISA = enzyme-linked immunosorbent assay.
† $P = 0.0055$.
‡ $P < 0.0001$.

Table 3. Selected variables from the Cox proportional hazards regression model to predict SRC development*

Variable baseline factors	OR of SRC development (95% CI)
MRSS >12	4.743 (0.601–96.331)
Maximum oral steroid dosage >30 mg/day	1.790 (0.386–9.698)
Anti–RNAP I/II/III positivity	10.967 (1.617–222.846)†
ELISA index for anti–RNAP III ≥157	2.367×10^9 (2.122–uncalculated)‡

* SRC = scleroderma renal crisis; OR = odds ratio; 95% CI = 95% confidence interval; MRSS = modified Rodnan skin thickness score; anti–RNAP I/II/III = anti–RNA polymerase I/II/III; ELISA = enzyme-linked immunosorbent assay.
† $P = 0.0118$.
‡ $P = 0.0093$.

SRC (4,6). Our multivariate analyses using a Cox proportional hazards regression model revealed that anti–RNAP I/II/III positivity (OR 11.0 [95% CI 1.6–222.8], $P = 0.0118$) and an ELISA index for anti–RNAP III of ≥157 (OR 2.4×10^9 [95% CI 2.1–uncalculated], $P = 0.0093$) were the independent factors associated with the development of SRC (Table 3).

Based on our multivariate analysis, the development of SRC was further evaluated by Kaplan-Meier analyses of anti–RNAP I/II/III and anti–RNAP I/III (Figure 2A) and of an ELISA index of ≥157 and of <157 (Figure 2B). Eight (47%) of 17 patients with anti–RNAP I/II/III developed SRC, whereas only 1 patient had SRC in the anti–RNAP I/III–positive group ($P = 0.0023$ by log rank test). While 9 (47%) of 19 patients whose ELISA indexes for anti–RNAP III were ≥157 had SRC, none of the patients with an ELISA index of <157 developed SRC ($P = 0.0005$ by log rank test). Figure 3 summarizes the association between the development of SRC, anti-RNAP subtype, and ELISA index for anti–RNAP III. The difference in ELISA indexes for anti–RNAP III between patients with anti–RNAP I/II/III and those with anti–RNAP I/III was not significant ($P = 0.231$).

DISCUSSION

In this study, we demonstrated that coexistence of anti–RNAP II with anti–RNAP I/III (anti–RNAP I/II/III) and a higher ELISA index for anti–RNAP III were immunologic predictors of developing SRC in anti–RNAP III–positive patients with SSc. Moreover, we did not detect any contribution of skin thickness or oral prednisolone dosage to the risk of developing SRC.

RNAPs I, II, and III are major targets of autoantibody responses in SSc patients. Most SSc sera that react with RNAPs recognize more than one class of RNAP, with a pattern of RNAP I/III or RNAP I/II/III. Anti–RNAP II alone is detected at a low frequency in sera from SSc patients, often in a combination with anti–topo I, and also in sera from some patients with systemic lupus erythematosus or overlap syndrome (21,22). Thus, anti–RNAP I and anti–RNAP III (anti–RNAP I/III) are highly specific for SSc and almost always coexist in most anti–RNAP III–positive SSc sera, while some of these sera contain anti–RNAP II as well (anti–RNAP I/II/III) (23,24).

The frequency of anti–RNAP III positivity varies in different ethnicities. In a US cohort, 61 (25%) of 247 patients with SSc were positive for anti–RNAP III, whereas in a French cohort 5 (4%) of 127 patients were

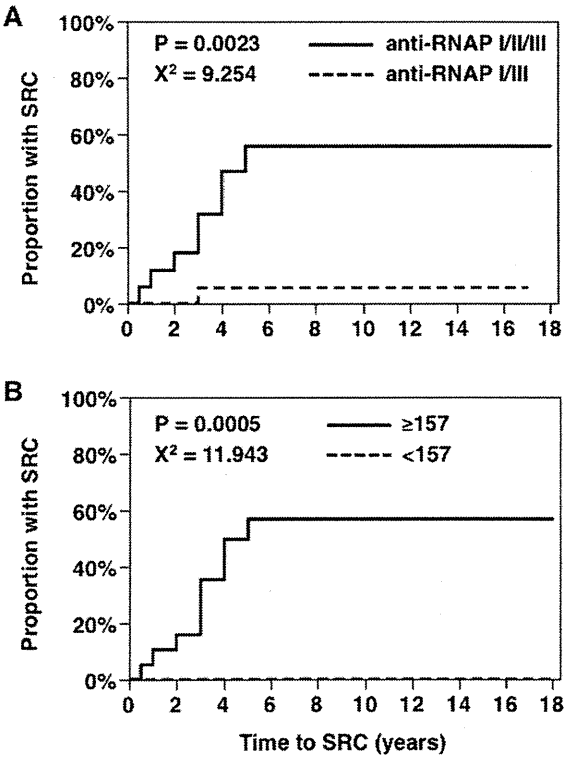

Figure 2. Kaplan-Meier plots of time to the development of scleroderma renal crisis (SRC). **A**, Significantly increased incidence of SRC in patients with anti–RNA polymerase I/II/III (anti–RNAP I/II/III) versus those with anti–RNAP I/III ($P = 0.0023$ by log rank test). **B**, Significantly increased incidence of SRC in patients with an enzyme-linked immunosorbent assay (ELISA) index for anti–RNAP III of ≥157 versus those with an ELISA index for anti–RNAP III of <157 ($P = 0.0005$ by log rank test).

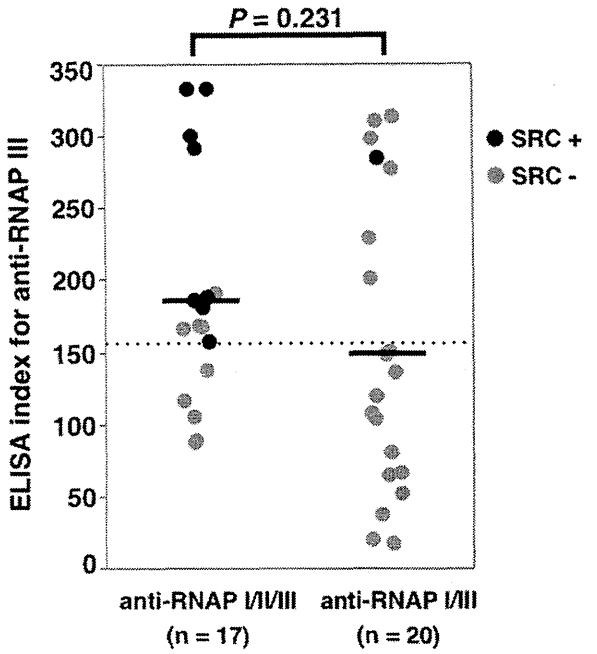

Figure 3. Enzyme-linked immunosorbent assay (ELISA) index for anti–RNA polymerase III (anti–RNAP III) in patients with anti–RNAP I/II/III with or without scleroderma renal crisis (SRC) and patients with anti–RNAP I/III with or without SRC. Circles represent individual patients; horizontal lines show the mean. The broken line indicates the cutoff value of 157.

positive (25). Nikpour et al reported that 69 (15.3%) of 451 SSc patients had anti–RNAP III in an Australian cohort (26). In a Japanese population, anti–RNAP III was detected in 38 (10.7%) of 354 patients with SSc (19). In the present study, the frequency of anti–RNAP III was 6% (37 of 583 SSc patients), confirming the previous finding that anti–RNAP III is more prevalent in North American than in Japanese SSc patients (14,15).

Several factors associated with SRC have been identified, including a disease duration of <4 years, diffuse and rapidly progressive skin thickening, new cardiac events, and the presence of anti–RNAP III (4,6,27). Steen (28) also demonstrated that reduction of renal blood flow caused by sepsis, dehydration, and cardiac dysfunction is a potential trigger for SRC. Regarding genetic factors, HLA–DRB1*0407 and *1304 are independent risk factors for the development of SRC (29).

Our study revealed that the presence of anti–RNAP II in combination with anti–RNAP I/III is a predictive factor for SRC. Harvey et al reported a detailed consideration of clinical associations with a subset of anti-RNAP (30). The incidence of renal involvement was associated with anti-RNAP. The highest rate of SRC was observed in patients with anti–RNAP I/II/III (40%), while the anti–RNAP I/III group had a lower frequency of SRC (14.3%). There was no statistically significant difference between these 2 groups, possibly due to the small numbers involved. Our results confirmed the findings of that previous study. Therefore, it is likely that coexistence of anti–RNAP II and anti–RNAP I/III (anti–RNAP I/II/III) is associated with an increased incidence of SRC.

In this study, all 9 patients with SRC had an ELISA index for anti–RNAP III of ≥157. There are several studies that describe the association of an ELISA index for anti–RNAP III with clinical features that include internal organ involvement. Kuwana et al (18) reported the association of anti–RNAP III levels with clinical findings and found that patients with a high level of anti–RNAP III had dcSSc more frequently than those with a lower level. The maximum total skin score and frequency of tendon friction rubs were also significantly increased in the high-level group compared with the low-level group. However, there were no significant differences in the frequencies of internal organ involvement, including SRC (18). Nihtyanova et al (31) also reported no correlation between peak anti–RNAP III ELISA index levels and the onset of SRC.

In contrast, our multivariate analyses revealed that a higher ELISA index for anti–RNAP III was another predictive factor for SRC. Several possibilities could account for this discrepancy. The study by Kuwana et al (18) analyzed a total of 90 patients (17 from Tokyo and 73 from Pittsburgh), a larger number of patients than our study, although with fewer Japanese patients. The study by Nihtyanova et al (31) was limited to Caucasian patients. In contrast, our study included all Japanese patients. Therefore, it cannot be ruled out that ethnicity can affect the results. Alternatively, the small number of patients in this study may explain this difference, since this study included only 9 anti–RNAP III–positive patients who developed SRC.

Exposure to corticosteroids has been considered a risk factor for SRC. In a case–control study, the use of corticosteroid therapy at a prednisone dosage of >15 mg/day was prescribed significantly more frequently in SRC patients (36%) than in controls (12%) (32). In addition, Helfrich et al observed an association of high-dose (>30 mg/day) corticosteroid therapy with normotensive SRC (33). In this study, we did not detect any influence of corticosteroid treatment in the development

of SRC. This may be explained in part by the fact that most patients with anti–RNAP III were treated with corticosteroids despite the presence or absence of SRC. However, the possibility cannot be ruled out that the use of corticosteroids increases the overall incidence of SRC. Whether there is an association of the use of corticosteroids with the incidence of SRC in Japanese SSc patients remains unclear. Further studies are needed to clarify the influence of corticosteroids as a trigger for developing SRC.

In conclusion, this study demonstrated the clinical importance of the presence of anti–RNAP I/II/III and/or an elevated ELISA index for anti–RNAP III as predictive factors for SRC. However, clinicians should watch all anti–RNAP III–positive patients for the development of SRC, since the identification of anti–RNAP II is limited to certain facilities. Establishing a system that could routinely screen for anti–RNAP II, such as ELISA, is needed. Our findings should be confirmed in a large-scale study of patients of different ethnicities in future studies.

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AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. Fujimoto had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study conception and design. Hamaguchi, Kodera, Matsushita, Hasegawa, Inaba, Usuda, Kuwana, Takehara, Fujimoto.

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RESEARCH ARTICLE

Rapid Initiation of Intravenous Epoprostenol Infusion Is the Favored Option in Patients with Advanced Pulmonary Arterial Hypertension

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Abstract

Background

Intravenous infusion (IVI) of epoprostenol is an effective treatment for patients with advanced pulmonary arterial hypertension (PAH). However, there is no widely accepted standard method for initiating the IVI therapy. This study evaluated the hemodynamic improvements achieved with IVI epoprostenol to determine the optimal protocol for treatment initiation.

Methods and Results

We retrospectively analyzed 42 consecutive PAH patients who underwent IVI epoprostenol in Keio University Hospital from 2001 to 2013. The study group comprised 30 women with a mean age of 34.3 ± 1.9 years. The etiology of PAH was idiopathic or heritable PAH (I/HPAH) in 38 cases, PAH associated with connective tissue disease in 3, and Eissenmenger's syndrome in the remaining case. We divided the patients into rapid- and slow-initiation therapy groups according to the cumulative epoprostenol dose administered during the first 180 days, and compared the hemodynamic changes between the groups. The median cumulative doses were 6142 ± 165 μg/kg and 3998 ± 132 μg/kg epoprostenol, respectively. While there were no significant differences in mean pulmonary artery pressure (mPAP), pulmonary vascular resistance (PVR), or cardiac index (CI) between the groups before the IVI epoprostenol therapy, the rapid-initiation therapy group achieved significant improvements in these hemodynamic data compared with the slow-initiation therapy group ($P < 0.005$) at the follow-up right-heart catheterization (RHC).

manuscript, this does not alter their adherence to PLOS ONE Editorial policies and criteria.

Conclusion

Rapid initiation of IVI epoprostenol therapy achieved the optimal hemodynamic improvements in patients with severe PAH.

Background

Pulmonary arterial hypertension (PAH) is a rare, progressive, and fatal disease characterized by raised pulmonary vascular resistance (PVR), and resulting in right ventricular dysfunction due to increased right-heart afterload. Raised PVR is caused by pulmonary vasoconstriction, vascular remodeling of the small pulmonary arteries, and thrombosis [1]. In the absence of treatment, the median reported survival after diagnosis for idiopathic PAH (IPAH) is only 2.8 years [2].

Epoprostenol is a prostaglandin analogue that was first approved for patients with advanced PAH as a continuous intravenous infusion (IVI) in 1995 [3]. A 12-week, open, randomized, prospective study showed a positive effect of epoprostenol on survival in patients with New York Heart Association (NYHA) functional class III or IV IPAH [4]. Since then, many trials of epoprostenol have shown clinical and hemodynamic improvement with increased survival [5,6].

The dose of IVI epoprostenol is adjusted upward depending on the severity of PAH and side effects of the drug such as thrombocytopenia, hypotension, or high cardiac output. However, despite the many promising trial results with this drug, the optimal dose of IVI epoprostenol remains controversial. Some reports described the appropriate dose as 25 to 40 ng/kg/min [7–11], while others such as Akagi et al. [12] showed the efficacy of high-dose IVI epoprostenol. Moreover, there is no widely accepted standard method for initiating the IVI epoprostenol treatment, or any conclusive findings as to whether the increase in epoprostenol delivery should be rapid or slow with respect to eliciting improvements in PAH status. To address these important knowledge gaps, we evaluated the hemodynamic changes in PAH patients treated with IVI epoprostenol at our hospital, according to the original dose-up protocol, and we investigated the optimal protocol for initiating IVI epoprostenol.

Methods

Patients

This is a single-center, retrospective study. All PAH patients who received IVI epoprostenol at Keio University Hospital (Tokyo, Japan) from January 2001 to April 2013 were identified using a clinical database. This study was approved by the local ethical committee (KEIO UNIVERSITY SCHOOL OF MEDICINE AN ETHICAL COMMITTEE, Tokyo, Japan, approval number: 2010008). And written informed consent for their clinical records to be used in this study was given by all of the participants. In each patient, the IVI epoprostenol treatment was initiated according to the different protocols considered to be optimal at the time. Continuous infusion of IVI epoprostenol was delivered via a tunneled central venous catheter (Hickmann's catheter), inserted under fluoroscopic guidance. We enrolled all patients for whom we could obtain protocols for the initiation of IVI epoprostenol and who received follow up right-heart catheterization (RHC) in Keio University Hospital within several months after the initiation of IVI epoprostenol. Patient files and the clinical database were reviewed and data were collected on NYHA functional classification (FC) at the initial visit to our hospital, protocol of

the IVI epoprostenol initiation, medication for pulmonary hypertension (phosphodiesterase type 5 inhibitors (PDE5i), endothelin receptor antagonist (ERA), prostanoids), hemodynamic data (mean pulmonary artery pressure (mPAP), pulmonary vascular resistance (PVR), and cardiac index (CI)) assessed by RHC, before and after the initiation of IVI epoprostenol. The NYHA FC of patients was allocated by the treatment physician, according to the WHO Functional Classification of PAH (whereby an FC of 1–4 is derived from patient symptoms in relation to exercise capacity).

Group classification

The dose-up protocols of IVI epoprostenol were extensively reviewed, and the cumulative IVI epoprostenol dose for 180 days after initiation was determined. The patients were then divided into two groups according to the cumulative epoprostenol dose, with patients who had received 4700 μg/body weight [kg] or more during the initial 180 days classified into a rapid-initiation therapy group, and those who had received less than 4700 μg/body weight [kg] classified into a slow-initiation therapy group. Hemodynamic data assessed by RHC before and after the initiation of IVI epoprostenol were compared between the groups.

Statistical analysis

We analyzed differences in the patient hemodynamic data (mPAP, PVR, CI) between the groups (rapid initiation therapy group and slow initiation therapy group). Data are presented as mean ± SEM. Mean PAP before and after the initiation of IVI epoprostenol exhibited normal distributions, so Student's t-test was performed to analyze this data. Mann-Whitney's U test was performed to analyze PVR and CI, both before and after the initiation of the therapy, since PVR and CI after the initiation of IVI epoprostenol did not exhibit normal distributions.

Two-sided *P*-values of less than 0.05 were considered to be statistically significant. Statistical analyses were performed with use of SPSS version 21.

Results

Patient enrollment

From January 2001 to June 2013, a total of 58 PAH patients received IVI epoprostenol treatment in Keio University Hospital. Four patients died soon after the initiation of IVI epoprostenol, three were loss to follow-up, three underwent lung transplantation in the United States, and another six were excluded due to missing protocols for the initiation of IVI epoprostenol.

Finally, we enrolled 42 patients, whose protocol for the initiation of IVI epoprostenol could be obtained and who received follow-up RHC within several months (Fig 1). The enrolled cohort comprised 30 women, with a mean age of 34.3 ± 1.9 years. The etiology of PAH was idiopathic PAH (IPAH) for 33 patients, heritable PAH (HPAH) for 5 patients, associated with connective tissue disease (CTD-PAH) for 3 patents, and Eissenmenger's syndrome (ES) for 1 patient. Seven patients were classified as NYHA FC 2 at the first visit to our hospital, but their symptoms subsequently deteriorated, such that they became indicated for IVI epoprostenol treatment.

Dosing of IVI epoprostenol

The initial dose of epoprostenol was 1 ng/kg/min for all patients, and then increased by 1 ng/kg/min every day, every several days, or weeks, according to each protocol. The typical protocols of both groups are shown in Fig 2. And the precise protocol of each patient is also shown

Figure 1.

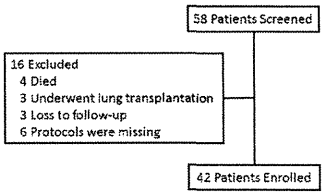

Fig 1. Patient inclusion. Flow chart describing patient inclusion protocol. Within the study period (2001–1013) 58 patients with PAH received IVI epoprostenol in Keio University hospital. Among the group, 16 patients were excluded from this study because they were lost to follow up, had missing protocols, died, or underwent a lung transplantation soon after the initiation of IVI epoprostenol.

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in S1 Fig To compare the protocols, the cumulative IVI epoprostenol doses for 180 days after the initiation were calculated (Fig 3). The mean cumulative epoprostenol dose (± SE) during the initial 180 days was 4968 ± 136 mg/body weight [kg] (range 2673–6575 mg/body weight [kg]), and we classified them into two groups by the median value with 23 classified into the slow-initiation therapy group and 19 into the rapid-initiation therapy group. We used inotropic agents as appropriate at the beginning for severe patients with right heart failure, and no patients failed the intended protocol. The years when the patients initiated epoprostenol in each group are shown in S1 Table. Baseline characteristics of the two groups are shown in Table 1. More patients in the rapid-initiation therapy group were taking PDE5i and ERA than in the slow-initiation therapy group ($p = 0.007$, $p = 0.042$, respectively).

Figure 2.

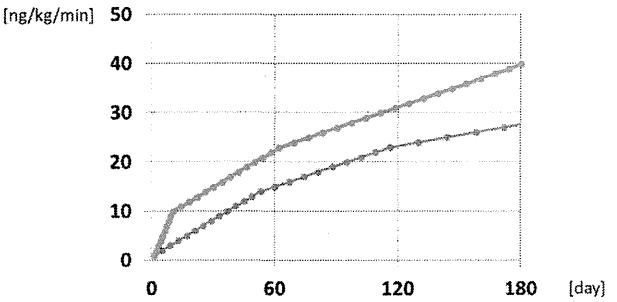

Fig 2. Typical protocols for rapid and slow initiation of therapy. The blue and red lines indicate the standard dosing schedules for the slow- and rapid-initiation IVI epoprostenol therapy, respectively.

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Figure 3.

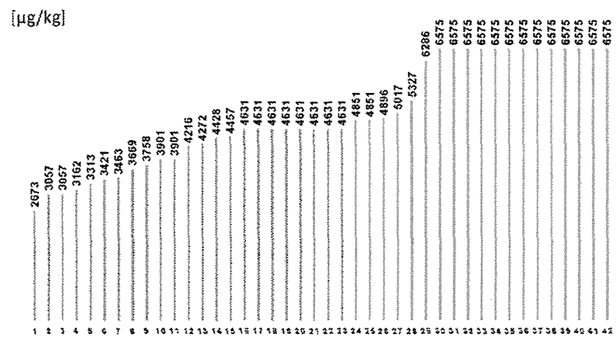

Fig 3. Cumulative epoprostenol dose for each patient. Bars show the cumulative dose of epoprostenol per body weight within the initial 180 days. The blue and red bars describe each patient's cumulative dose in the slow- and rapid-initiation groups, respectively.

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Table 1. Baseline clinical characteristics.

	Slow initiation therapy group (n = 23)	Rapid initiation therapy group (n = 19)	Total (n = 42)	*P* value*
Age, yr	35.2 ± 2.1	33.2 ± 3.5	34.3 ± 1.9	0.612
Female sex, n (%)	18 (78.2)	12 (63.1)	30 (71)	0.300
Asian race, n (%)	23(100)	19 (100)	42 (100)	-
Body mass index, kg/m^2	21.0 ± 0.8	20.2 ± 0.6	20.6 ± 0.5	0.356
Etiology of PAH, n (%)				
IPAH	17 (73.9)	16 (84.2)	33 (78.6)	0.431
HPAH	5 (21.7)	0 (0)	5 (11.9)	0.022
CTD-PAH	1 (4.3)	2 (10.5)	3 (7.1)	0.451
ES	0 (0)	1 (5.3)	1 (2.3)	0.331
NYHA functional class at the first visit, n (%)				
II	3 (13.0)	3 (15.8)	6 (14.3)	0.814
III	19 (82.6)	14 (73.7)	33 (78.6)	
IV	1 (4.3)	2 (10.5)	3 (7.1)	
Medication				
PDE5i	5 (21.7)	12 (63.1)	17 (40.4)	0.007
ERA	3 (13.0)	8 (42.1)	11 (26.1)	0.042
Prostanoid	6 (26.1)	5 (26.3)	11 (26.1)	0.987
Warfarin	7 (30.4)	5 (26.3)	12 (28.6)	0.775
Duration of IVI epoprostenol, days	240 ± 20	194 ± 12	219 ± 12	0.052
Cumulative epoprostenol dose, μg/body weight [kg]	3998 ± 132	6142 ± 165	4968 ± 136	< 0.001

Plus–minus values are means ± SE.

* *P* values indicate the statistical difference between slow and rapid initiation of therapy.

PAH, pulmonary arterial hypertension; IPAH, idiopathic pulmonary arterial hypertension; HPAH, heritable pulmonary arterial hypertension; CTD-PAH, PAH associated with connective tissue disease, ES, Eissenmenger's syndrome; NYHA, New York Heart Association; PDE5i, phosphodiesterase type 5 inhibitor; ERA, endothelin receptor antagonist.

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Figure 4.

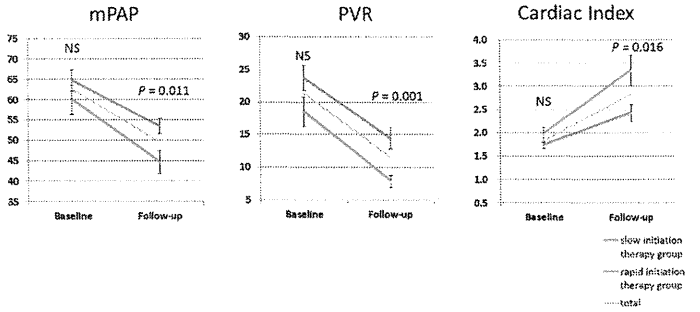

Fig 4. Improvements in hemodynamic data following IVI epoprostenol therapy. At follow up, the rapid-initiation group achieved significant improvements in mPAP, PVR and CI compared with the slow-initiation group, while there were no significant differences at baseline. mPAP: mean pulmonary artery pressure, PVR: pulmonary vascular resistance, CI: cardiac index, NS: not significant

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Hemodynamic measurements

Follow up RHC was performed 219 ± 12 days (240 ±20 days for slow-initiation therapy group, and 194 ± 12 days for rapid-initiation therapy group) after the initiation of IVI epoprostenol. In the slow- and rapid-initiation groups at baseline, mPAPs were 64.7 ± 2.7 mmHg and 59.9 ± 3.6 mmHg, PVR were 23.7 ± 1.9 Wood's Units and 18.5 ± 2.3 Wood's Units, and CI were 1.73 ± 0.07 L/min/m^2 and 1.97 ± 0.14 L/min/m^2, respectively, whereas at the follow-up RHC, the comparative values were 53.4 ± 1.9 mmHg and 44.6 ± 2.8 mmHg, 14.4 ± 1.6 Wood's Units and 7.9 ± 0.9 Wood's Units, and 2.43 ± 0.18 L/min/m^2 and 3.34 ± 0.33 L/min/m^2, respectively.

As shown in Fig 4, mPAP, PVR, and CI were not significantly different between the groups before the initiation of IVI epoprostenol, whereas at the follow-up RHC, both mPAP and PVR were significantly decreased, and CI was significantly improved, in the rapid-initiation therapy group compared with the slow-initiation therapy group, suggesting a significant improvement in hemodynamic parameters with rapid initiation of IVI epoprostenol.

In order to make sure that the hemodynamic improvement was not achieved just by high dose of epoprostenol, but by rapid initiation, we compared the hemodynamic data of the groups one year after the initiation, when most of the patients reached to the final dose. One year follow up RHC was performed 381 ± 19 days (412 ± 124 days for slow-initiation therapy group, and 344 ± 29 days for rapid-initiation therapy group, p = 0.081). The mean values of mPAPs were 48.3 ± 2.4 mmHg and 40.9 ± 2.7 mmHg, PVR were 12.2 ± 1.3 Wood's Units and 7.4 ± 0.8 Wood's Units, and CI were 2.51 ± 0.16 L/min/m^2 and 3.16 ± 0.23 L/min/m^2 in the slow- and rapid-initiation groups, respectively. And as shown in S2 Fig, hemodynamic data in the rapid-initiation therapy group showed the significant improvement compared with the slow-initiation therapy group. On the other hand, the dosages of epoprostenol were not significantly different between the groups (35.1 ± 1.7 ng/kg/min for slow-initiation therapy group, and 39.5 ± 1.2 ng/kg/min for rapid-initiation therapy group, p = 0.063). Therefore, we concluded that the hemodynamic improvement of the rapid initiation therapy group was achieved by the initiation protocol, not by the dose of epoprostenol, and which brought long term improvements in hemodynamics.

Subgroup analyses

A significantly larger number of patients in the rapid-initiation therapy group was taking PDE5i or ERA before the initiation of IVI epoprostenol compared to the slow-initiation therapy group, thus a subgroup comparison was conducted in the rapid-initiation therapy patients between those who taking none or one vasodilatory agent (PDE5i, ERA, or prostanoid) and those taking two or more agents at baseline.

As shown in Table 2, although both mPAP and PVR were significantly low, and CI was significantly high in those who took two or more agents at baseline, there were no significant differences in mPAP, PVR, or CI between the two groups at the follow-up RHC.

We also compared the hemodynamic data between the groups particularly in patients with IPAH and HPAH. As shown in S3 Fig, mPAP, PVR, and CI were not significantly different between the slow- and rapid-initiation groups before the initiation of IVI epoprostenol (mPAP were 64.8 ± 2.8 mmHg and 61.1 ± 4.0 mmHg, PVR were 23.5 ± 1.9 Wood's Units and 19.3 ± 2.7 Wood's Units, and CI were 1.73 ± 0.07 L/min/m^2 and 1.97 ± 0.14 L/min/m^2, respectively), whereas at the follow-up RHC, both mPAP and PVR were significantly decreased, and CI was significantly increased in the rapid-initiation therapy group compared with the slow-initiation therapy group (180 day follow up RHC; mPAP were 53.2 ± 2.0 mmHg and 44.2 ± 3.2 mmHg, PVR were 14.0 ± 1.6 Wood's Units and 7.7 ± 1.0 Wood's Units, and CI were 1.73 ± 0.07 L/min/m^2 and 1.97 ± 0.14 L/min/m^2, one year follow up RHC; mPAP were 48.2 ± 2.5 mmHg and 39.8 ± 2.9 mmHg, PVR were 12.2 ± 1.3 Wood's Units and 7.4 ± 0.8 Wood's Units, and CI were 2.51 ± 0.16 L/min/m^2 and 3.16 ± 0.23 L/min/m^2, respectively). These findings suggested that there was also the significant improvement in hemodynamic parameters with rapid initiation of IVI epoprostenol in the patients with IPAH/ HPAH.

Discussion

This study showed the efficacy of rapid initiation of epoprostenol IVI therapy. Compared with the patients in the slow-initiation group (cumulative dose of epoprostenol less than 4700 μg/body weight [kg] in initial 180 days), patients receiving a rapid initiation of epoprostenol IVI enjoyed superior hemodynamic improvements in mPAP, CI, and PVR.

Although many oral agents for PAH have been approved, IVI epoprostenol is still the so-called "last line of defense for PAH treatment". In some clinical trials for IPAH or PAH

Table 2. Changes in hemodynamic data from baseline to follow-up right heart catheterization with rapid initiation therapy.

	Oral vasodilator agents		Total	*P* value*
	none or one (n = 11)	two or three (n = 8)		
Baseline				
mPAP [mmHg]	61.5 ± 5.0	57.8 ± 5.4	59.1 ± 3.5	0.616
PVR [Wood's Units]	19.4 ± 3.3	17.3 ± 3.3	18.3 ± 2.2	0.717
CI [L/min/m^2]	1.82 ± 0.19	2.17 ± 0.19	1.97 ± 0.13	0.238
Follow-up				
mPAP [mmHg]	44.7 ± 4.1	44.5 ± 3.8	44.2 ± 2.7	0.969
PVR [Wood's Units]	8.1 ± 1.3	7.6 ± 1.2	7.7 ± 0.9	0.778
CI [L/min/m^2]	3.48 ± 0.50	3.15 ± 0.37	3.41 ± 0.32	0.657

Plus–minus values are means ±SE.

* *P* values indicate the statistical difference between slow and rapid initiation of therapy.

mPAP, mean pulmonary arterial pressure; PVR, pulmonary vascular resistance; CI, cardiac index.

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associated with scleroderma, epoprostenol improves disease symptoms, exercise capacity, and hemodynamics [15,16]. Moreover epoprostenol is the only therapy that has achieved mortality reduction in a randomized study for IPAH [4].

A meta-analysis for total mortality of the three epoprostenol RCTs [4,13,14] showed a 70% relative risk reduction, and based on this the recent guidelines classify only epoprostenol as a Class I therapy in patients with severe (WHO-FC IV) PAH [15]. In addition, a recent report revealed a survival benefit of the upfront triple-combination therapy including IVI epoprostenol in patients with severe PAH [16].

The maximum dose-dependent epoprostenol efficacy in patients with PAH [12,17], and a dose-dependent reduction in PVR with epoprostenol IVI therapy [18] were previously reported in patients with PAH. However, there is no apparent evidence that the efficacy of epoprostenol also depends on the initiation schedule, despite anecdotal evidence from PAH specialists of the merits of rapid initiation. Accordingly, we aimed to design the best way to optimize the initiation of epoprostenol in order to achieve maximal hemodynamic improvement, and herein, confirmed the importance of rapid initiation of epoprostenol as well as high-dose usage in patients with severe PAH, which may also suggest further long-term survival benefit with such a protocol.

This study has some limitations. First, it was a single-center, retrospective study. As our institution is a center for PAH, some of the patients were diagnosed as IPAH in other hospitals and then referred to our center after initiations of various oral agents, possibly introducing a selection bias. Secondly, application of the initiation protocols differs from time to time. For instance, recent patients tend to undergo the rapid-initiation protocol and take oral combination therapies. Indeed, in this study, the number of patients who were taking PDE5i or ERA before the initiation of IVI epoprostenol was significantly larger in the rapid-initiation therapy group than in the slow-initiation therapy group. Therefore, we conducted subgroup analysis in the rapid-initiation group to compare between patients taking none or one vasodilatory agent and those taking two or more agents at baseline. However, there were no significant differences in any hemodynamic parameters (mPAP, PVR, or CI) between these groups at the follow-up RHC, suggesting that the improvements in hemodynamic data achieved by IVI epoprostenol exceeded the effect of concomitant medications. Finally, this study analyzed only a short period (180 days and one year) of data during the initiation of epoprostenol, and analysis of a possible association between long-term mortality and the cumulative dose of epoprostenol will be needed in the future.

In conclusion, compared with slow initiation, rapid initiation of IVI epoprostenol therapy significantly improved the hemodynamics in patients with PAH.

Supporting Information

S1 Fig. Precise protocols for all the enrolled patients. The blue and red lines indicate the dosing schedules for the patient classified into slow- and rapid-initiation therapy, respectively.
(TIF)

S2 Fig. Improvements in hemodynamic data one year after the initiation of IVI epoprostenol therapy. At the time, rapid-initiation group showed significant improvements in mPAP, PVR, and CI compared with the slow-initiation group, as well as those of 180 days follow up RHC. mPAP: mean pulmonary artery pressure, PVR: pulmonary vascular resistance, CI: cardiac index, NS: not significant
(TIF)

S3 Fig. Improvements in hemodynamic data 180 day and one year after the initiation of IVI epoprostenol therapy in patients of IPAH and HPAH (N = 38). The rapid-initiation group achieved significant improvements in mPAP, PVR and CI compared with the slow-initiation group in 180 days and one-year follow up RHC. mPAP: mean pulmonary artery pressure, PVR: pulmonary vascular resistance, CI: cardiac index, NS: not significant
(TIF)

S1 Table. The number of patients whom enrolled slow- or rapid-initiation therapy protocols in each year.
(DOC)

Author Contributions

Conceived and designed the experiments: YT. Performed the experiments: M. Kimura MT. Analyzed the data: TO. Contributed reagents/materials/analysis tools: MT TY. Wrote the paper: M. Kimura YT M. Kuwana TS KF.

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Oral vasopressin receptor antagonist tolvaptan in right heart failure due to pulmonary hypertension

To the Editor:

During the past decade, the prognosis of patients with pulmonary hypertension (PH) has improved due to the development of pulmonary arterial vasodilators [1]. Yet, treatment for right-sided heart failure (RHF) associated with PH has not improved, despite being the most important prognostic factor for chronic RHF [2, 3]. Existing treatment strategies for RHF associated with PH are restricted to administration of loop diuretics and inotropes [4]; however, administration of high doses of loop diuretics may worsen the outcome of chronic heart failure [5, 6]. In recent years, tolvaptan, an oral, nonpeptide, selective vasopressin V2-receptor antagonist, has been developed for the treatment of hyponatraemia [7], autosomal dominant polycystic kidney disease [8] and heart failure. Used in addition to standard therapy that includes diuretics, tolvaptan improves many signs and symptoms of heart failure without serious adverse events [9]. However, there is no report describing the effect of tolvaptan on RHF, independent of left-sided heart disease.

We evaluated both the short- and long-term effects of tolvaptan, and explored the possibility of loop diuretics dose reduction and improved fluid retention in patients with refractory and loop diuretics-dependent RHF associated with PH.

We enrolled 10 outpatients with chronic RHF associated with PH, who were refractory to loop diuretics, between September 2012 and March 2014 at Keio University Hospital (Tokyo, Japan). This study was an open-label, single-arm, prospective study to investigate the safety and efficacy of tolvaptan in patients with RHF due to PH. The study consisted of a 12-week open-label treatment period with tolvaptan (with visits at baseline, days 1–7, and weeks 2, 4, 6 and 12), and followed the ethical standards of the responsible committee on human experimentation (Keio University School of Medicine ethical committee, Tokyo, Japan) and the Helsinki Declaration. The trial was registered at UMIN before initiating recruitment (identifier number: UMIN000010196). Written informed consent was obtained from all patients in the study. All patients had been previously diagnosed as having idiopathic pulmonary arterial hypertension (IPAH) (n=5) or chronic thromboembolic pulmonary hypertension (CTEPH) (n=5), as confirmed by right heart catheterisation. The baseline dose of furosemide was 60 mg·day^{-1} for more than 4 weeks and patients were also taking specific therapies for PH such as oral phosphodiesterase type-5 inhibitors and/or endothelin receptor antagonists. All patients were free from defects such as a predominant left-to-right shunt or any other kind of cardiomyopathy (*e.g.* ischaemic cardiomyopathy or valvular heart disease). The mean±SD age of the patients was 47.8±15.6 years and the number of females was five (50%). Each patient had been receiving loop diuretics, with an average furosemide dose of 88±17 mg·day^{-1}. None of the patients had taken another type of diuretic such as spironolactone or hydrochlorothiazide.

The primary end-point was a changed furosemide dosage at week 12 relative to baseline. Tolvaptan was administered orally at a dose of 3.75 mg, 7.5 mg or 15 mg per day. For safety reasons, all participants commenced tolvaptan at a dose of 3.75 mg·day^{-1} for 3 days. Provided urine output did not increase during this initial 3-day period, the dosage of tolvaptan was increased to 7.5 mg·day^{-1} and 15 mg·day^{-1}. The dosage of furosemide was reduced in cases satisfying the following conditions: 1) urine output was greater than baseline levels, and more than 1000 mL per day; and 2) there was no evidence of increased body weight compared with the previous day. In cases free from increased fluid retention (leg oedema, hepatomegaly or jugular vein distention), a reduction of furosemide would be considered from 160 mg, 120 mg, 80 mg, 60 mg, 40 mg, 20 mg, to 10 mg per day.

The mean±SD dosage of tolvaptan at day 7 and week 12 were 6.38±1.8 mg·day^{-1} and 7.87±4.1 mg·day^{-1}, respectively. During the adjustment of tolvaptan dosing, neither overcorrection of serum sodium (>150 mEq·L^{-1}) or elevated liver enzymes were noted, and the main adverse event was a dry mouth (80%). There were no withdrawals from the study due to adverse events. There were no patients who developed hypotension or experienced worsening heart failure during treatment.

The comparisons of clinical variables at baseline and post-tolvaptan treatment are shown in figure 1. The mean dose of furosemide administered at day 7 and week 12 compared with baseline was significantly reduced by tolvaptan treatment (p=0.001). Tolvaptan was also associated with a significant decrease in average urine osmolality and brain natriuretic peptide levels at week 12 compared with baseline (p=0.001