Immunoadsorption Therapy for Dilated Cardiomyopathy Using Tryptophan Column—A Prospective, Multicenter, Randomized, Within-Patient and Parallel-Group Comparative Study to Evaluate Efficacy and Safety

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Over the past few decades, several cardiac autoantibodies have been reported in sera from patients with dilated cardiomyopathy (DCM). Immunoadsorption (IA) therapy is one of the therapeutic tools to remove such autoantibodies. The objective of this study was to investigate functional effects of IA therapy using a tryptophan column in severe DCM patients. Of 49 patients enrolled, 44 were randomized from 10 sites in Japan. IA therapy was conducted in 40 patients with DCM (refractory to standard therapy for heart failure, New York Heart Association [NYHA] class III/IV, left ventricular ejection fraction [LVEF] <30%). Mean echocardiographic LVEF was significantly improved ($23.8 \pm 1.3\%$ to $25.9 \pm 1.3\%$, $P = 0.0015$). However, mean radionuclide LVEF over 3 months of IA therapy was not significantly improved ($20.8 \pm 1.1\%$ to $21.9 \pm 1\%$, $P = 0.0605$). The cardiothoracic ratio was also significantly decreased ($P = 0.0010$). NYHA functional class ($P < 0.0001$), subjective symptoms assessed by a quality of life questionnaire ($P = 0.0022$), maximum oxygen consumption ($P = 0.0074$), and 6-minute walk distance ($P = 0.0050$) were improved after IA therapy. Subgroup analysis revealed improvement of echocardiographic LVEF in patients with higher baseline autoantibody scores but not in those with lower scores. IA therapy improved subjective symptoms and exercise capacity in patients with refractory heart failure resulting from DCM. Favorable effect on cardiac function was noted in patients with higher autoantibody scores. J. Clin. Apheresis 31:535–544, 2016. © 2016 The Authors. Journal of Clinical Apheresis Published by Wiley Periodicals, Inc.

Key words: dilated cardiomyopathy; antibodies; heart failure; immune system

INTRODUCTION

Autoimmunity is one of the pivotal mechanisms, along with viral infection and genetic predisposition, underlying the pathophysiology of dilated cardiomyopathy (DCM). Several autoantibodies found in sera from patients with DCM [1–5] play a role in mediating cardiac damage [6] and provide arrhythmic substrates [7], resulting in sudden cardiac death [8]. Immunoapheresis removing these autoantibodies has been developed to treat DCM patients with refractory heart failure [9–12]. In these preliminary trials except for Müller, et al. [9], intravenous immunoglobulin was replaced after each session of apheresis, because the columns used in these trials nonspecifically removed immunoglobulin. Historical studies have shown that clinically relevant autoantibodies are found in various IgG subclasses in sera from patients with DCM, but those belonging to IgG class 3 play a pivotal role in mediating efficacy of IA therapy [13,14]. Although protein A column under the modification may remove IgG3 subclass [15], tryptophan columns treating as low as 1.5 L plasma had a relatively high specificity for IgG3 than other IgG subclasses, and safely removed pathophysiologically relevant autoantibodies without immunoglobulin substitution in our proof-of-concept study [16]. We therefore

conducted a multicenter trial on immunoapheresis using tryptophan columns in patients with advanced heart failure resulting from DCM.

METHODS

The trial was carried out in accordance with the principles of good clinical practice and the Declaration of Helsinki; all patients provided written informed consent before any study-related procedure. The protocol was approved by the institutional review boards of all participating centers. The study was registered with the University Hospital Medical Information Network Clinical Trials Registry (trial number UMIN000003106).

Study Participants

Eligible patients had severe idiopathic DCM [17] resistant to standard therapy including angiotensin-converting enzyme inhibitor (ACE-I)/angiotensin-receptor blocker (ARB), β-blocker, and aldosterone antagonist for heart failure for at least 3 months. Other inclusion criteria were age over 18 years, New York Heart Association (NYHA) functional class III/IV, left ventricular (LV) ejection fraction (LVEF) measured by radionuclide ventriculography <30% or LVEF measured by echocardiography <35% in the past 3 months. Patients were excluded if they had a secondary DCM resulting from other diseases, were implanted with an LV assist device, received cardiac resynchronization therapy in the past 6 months, or underwent heart transplantation. Coronary arteriography was performed in all patients, but endomyocardial biopsy was not necessarily performed in all patients.

Study Design

This was a prospective, multicenter, randomized, within-patient and parallel-group comparative study. Eligible patients were randomized in a 1:1 ratio to the immunoadsorption (IA) and delay groups. In the IA group, after the baseline test was completed, five sessions of IA therapy over the course of 2 weeks (first treatment period) were performed using the IMMUSORBA TR-350(L) (Asahi Kasei Medical, Tokyo, Japan), followed, at 3 months after the start of the trial, by five more sessions of IA therapy, again administered over the course of 2 weeks (second treatment period). In total, 10 sessions were performed for patients in the IA group. In the delay group, after the baseline test was completed, patients were hospitalized for 2 weeks without IA therapy (nontreatment period), followed by five sessions of IA therapy over the course of 2 weeks (first treatment period) at 3 months after the start of the trial. In total, five sessions were performed for patients in the delay group. Endpoints were assessed at 3 months after IA therapy in the IA group (A period), 3 months after observation in the delay group (C period), and 3 months after IA therapy in the delay group (D period). Figure 1 shows an overview of the study design. We noted $+3.1 \pm 5.9\%$ increase of LVEF in the preliminary study [16], and we calculated sample size as 31, assuming $\alpha = 0.05$ and $\beta = 0.20$, according to the following formula:

$$N = \left(z_{1-2/\alpha} + z_{1-\beta}\right)^2 / \Delta^2 + z^2_{1-2/\alpha}/2$$

where Δ is the assumed difference/standard deviation. We decided to collect 40 cases, considering 20% will be dropped out.

IA Procedure

ACE-I was discontinued or switched to ARB at least 2 weeks before the first IA therapy, because use with the IMMUSORBA TR column is contraindicated in the presence of ACE-I with potential bradykinin production [18]. The patients were admitted to the hospital for the treatment period to avoid the risk of infection and bleeding. IA therapy was achieved with IMMUSORBA TR and Plasmaflo OP-05W(L) (Asahi Kasei Medical), as described previously [16]. The patients were treated with a plasma throughput of 1.5 L in each session of IA therapy, according to our preliminary study, in which 1.5 L treatment was sufficient to eliminate IgG3 antibodies without losing immunoglobulin specificity [16]. None received intravenous immunoglobulin treatment.

Efficacy and Safety Assessment

The primary endpoint was radionuclide LVEF change before and 3 months after IA therapy (A and D periods). Secondary endpoints included change of echocardiographic LVEF, 6-minute walk test, cardiothoracic ratio on chest radiograph, maximal oxygen consumption (VO$_2$ max) measured by cardiopulmonary exercise testing, brain natriuretic peptide (BNP) and atrial natriuretic peptide (ANP) levels, NYHA functional class, specific activity scale (SAS) determined by a quality of life questionnaire [19], and hospitalization for heart failure during the 12 months of the study. Cardiac events were defined as all-cause death; hospitalization for heart failure or arrhythmias; new addition or uptitration of cardiovascular medications because of worsening symptoms; and worsening by at least 1 grade of SAS score or NYHA class.

Assessment of Cardiac Function and Exercise Tolerance

After intravenous injection of technetium-99m-labeled human serum albumin, multigated radionuclide ventriculography was performed. Radionuclide LVEF was calculated by dividing the background-corrected
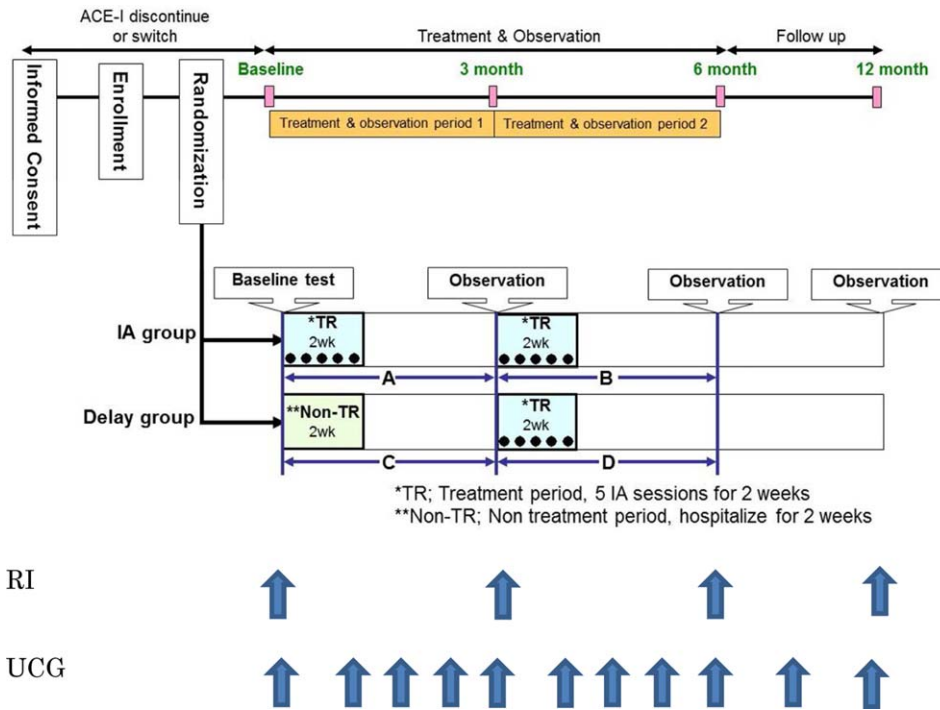
Fig. 1.   Study protocol. RI, radionuclide ventriculography; UCG, ultrasonic echocardiography

difference between end-systolic (minimum) and end-diastolic (maximum) counts by the end-diastolic counts. Two-dimensional echocardiography was done by experienced sonographers. Echocardiographic LVEF was evaluated by a modified Simpson's method with manual planimetry of the endocardial border at end-diastole and end-systole. To assess the trend of LVEF change during the 3 months after observation began, a regression line was created using five LVEF values during the 3 months. LVEF was judged as improvement when the slope of the regression line was more than $+5\%$ for 12 weeks and as aggravation when the slope was less than -5% [20]. Independent experienced physicians who were blinded to patient allocation judged the overall change of echocardiographic findings. Symptom-limited ergometer exercise test was performed to determine maximal oxygen consumption according to the previously reported method [21].

Autoantibody Measurement

Autoantibodies (total IgG and IgG3 subclass) directed against each antigen (β1-adrenergic receptor, muscarinic M2-receptor, Na-K-ATPase, troponin I, and myosin) were measured using enzyme-linked immunosorbent assay (ELISA) according to previously described methods [14]. The concentration of the target antibody was calculated from the standard curve using a known standard antibody. Total antibody score was calculated as $\log_{10} X_{0(1)} + \log_{10} X_{0(2)} + \log_{10} X_{0(3)} + \log_{10} X_{0(4)} + \log_{10} X_{0(5)}$, where $X_{0(1)}$ through $X_{0(5)}$ represent each autoanti-body. Using the total antibody score provided by five different IgG3 subclass autoantibodies, we divided patients into two groups according to the median value.

Statistical Analysis

All values are expressed as the mean ± standard deviation. The baseline characteristics of the two groups were compared with the use of Student's t-tests, Fisher's exact test, or the Wilcoxon rank-sum test. Statistical significance of baseline was defined as $P < 0.05$. The change of parameters before and after IA therapy was analyzed using paired t test (except for NYHA class, which was assessed by Wilcoxon signed-rank test). The relationship between response to IA therapy and baseline autoantibody profiles was evaluated using Spearman's rank correlation coefficient. The difference between groups was analyzed by Student's t test and Wilcoxon rank-sum test. Statistical significance was defined as $P < 0.05$. Analyses were conducted with the use of SAS software version 9.1.3 (SAS Institute, Cary, NC, USA).

RESULTS

Patient Disposition and Baseline Characteristics

Patient enrollment was started in March 2010 and completed in November 2011. Of 49 patients enrolled from 10 sites in Japan, 44 patients were randomized (IA group, $n = 22$; delay group, $n = 22$). Patient disposition is summarized in Figure 2. Overall, 49 patients registered were included in the safety analysis, and 43

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┌─────────────────────────────┐
│ Patients provided Informed  │
│          consent            │
│         N=  49              │
└─────────────────────────────┘
              │
┌─────────────────────────────┐
│     Patients registered     │
│         N=  49              │
└─────────────────────────────┘          ┌──────────────────────────────────────┐
              │                           │ Discontinue prior to randomization¹⁾  │
              ├───────────────────────────│            N=  5                      │
              │                           └──────────────────────────────────────┘
┌─────────────────────────────┐
│    Patients randomized      │
│         N=  44             │
│   IA  group:    22          │
│   Delay  group:  22         │          ┌──────────────────────────────────────┐
└─────────────────────────────┘          │  Discontinue within 3 months²⁾       │
              │                           │            N=  7                      │
              ├───────────────────────────│       IA  group:   4                 │
              │                           │       Delay  group:  3               │
┌─────────────────────────────┐          └──────────────────────────────────────┘
│  Patients finished 3 months │
│ (Treatment and observation 1)│
│         N=  37             │
│   IA  group:    18          │
│   Delay  group:   19        │          ┌──────────────────────────────────────┐
└─────────────────────────────┘          │  Discontinue within 6 months³⁾       │
              │                           │            N=  4                      │
              ├───────────────────────────│       IA  group:   1                 │
              │                           │       Delay  group:  3               │
┌─────────────────────────────┐          └──────────────────────────────────────┘
│  Patients finished treatment│
│  and observation period (6  │
│          months)            │
│         N=  33             │
│   IA  group:    17          │
│   Delay  group:   16        │
└─────────────────────────────┘
              │
┌─────────────────────────────┐
│  Patients finished follow-up│
│     period (12 months)      │
│         N=  33             │
│   IA  group:    17          │
│   Delay  group:   16        │
└─────────────────────────────┘
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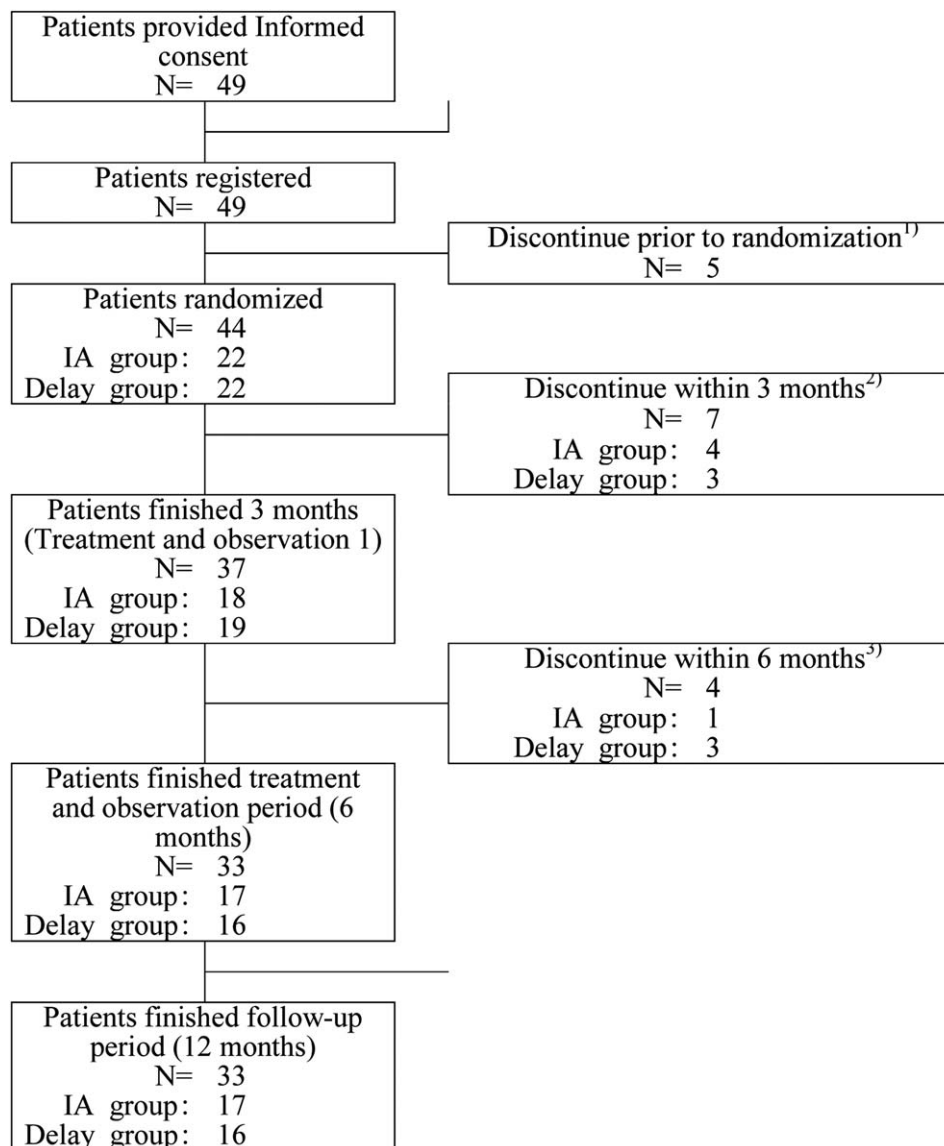
Fig. 2.   Randomization and allocation of the study subjects. 1) Patients withdrew consent, $n = 1$; physicians judged to exclude, $n = 4$. 2) Physicians discontinued because of adverse events, $n = 3$; patients withdrew consent, $n = 2$; physicians judged to discontinue IA, $n = 1$; withdrew because of cardiac event, $n = 1$. 3) Patients discontinued IA, $n = 2$; Patients withdrew consent, $n = 2$.

patients who completed baseline testing were eligible for efficacy analysis.

Demographic and baseline characteristics are summarized in Table I. Of the 43 patients, 37 were men. Mean patient age was 56 years old, and mean duration of heart failure was 93 months. The LVEF of all patients was severely reduced (mean radionuclide LVEF value, 19.1%). Almost all patients were in NYHA class III except for two patients who were in class IV. The baseline clinical characteristics of the patients were similar in the two groups. There were five patients in the IA group and eight patients in the delay group, who withdrew ACE-I and switched to ARB.

Echocardiographic data in each group over the 12 months were shown in Table II. LVEF as well as end-diastolic volumes decreased 4 months after IA in the IA group ($P < 0.05$). End-systolic volume decreased 3 months and just after 2nd treatment in addition to 4 months after IA. In the delay group these parameters improved only after IA. We did not see additional increase in LVEF after the 2nd treatment in the IA group. There was no significant change in the stroke volume.

The combined analysis of A and D periods is shown in Table III. Echocardiographic LVEF was significantly increased from $23.8 \pm 1.3\%$ to $25.9 \pm 1.3\%$ ($P = 0.0015$). In the trend analysis of LVEF using multiple data during the IA therapy, eight patients exhibited improvement (22%). The cardiothoracic ratio was also significantly decreased. There was a significant

TABLE I.   Demographic and Baseline Characteristics

	Total (N = 43)	IA group (N = 21)	Delay group (N = 22)
Age (yrs)	56 ± 13	56 ± 12	56 ± 13
Weight (kg)	60.0 ± 10.8	61.6 ± 11.9	58.5 ± 9.6
Duration of heart failure (months)	93 ± 57	115 ± 61	73 ± 47
Gender (Male/Female)	37/6	19/2	18/4
Atrial fibrillation (%)	16/43 (37)	10 (47)	6 (27)
Echocardiography LVEF (%)	24.5 ± 6.7[a]	23.1 ± 6.3[c]	25.7 ± 7.0
Radionuclide LVEF (%)	19.1 ± 6.7[a]	18.6 ± 6.7[c]	19.5 ± 6.7
NYHA class (III/IV)	41/2	19/2	22/0
SAS (METs)	3.3 ± 1.3	3.5 ± 1.3	3.1 ± 1.4
Cardiothoracic ratio (%)	57.8 ± 6.8	59.9 ± 6.1	55.9 ± 7.1
Six-minute walk distance (m)	347 ± 127[a]	353 ± 128	340 ± 128[c]
VO$_2$ max (mL/kg/min)	12.1 ± 4.8[b]	11.2 ± 5.6[d]	12.7 ± 4.6[d]
BNP (pg/mL)	471 ± 452	509 ± 523	435 ± 382
ANP (pg/mL)	187 ± 158	187 ± 153	187 ± 166

[a]Data for one patient were excluded.
[b]For VO$_2$ max, only patients who were able to undergo cardiopulmonary exercise testing (total, $n = 16$; IA group, $n = 6$; delay group, $n = 10$).
[c]Two-tailed Student's t test.
[d]Fisher's exact test.
ANP, atrial natriuretic peptide; BNP, brain natriuretic peptide; LVEF, left ventricular ejection fraction; METs, metabolic equivalents; NYHA, New York Heart Association; SAS, specific activity scale; VO$_2$ max, maximal oxygen consumption.

TABLE II.   Echocardiographic Data Over the 12 Months

IA group		LVEF (%)	LVEDV (ml)	LVESV (ml)	SV (ml)
	Before	23.1 ± 6.3	245 ± 105	192 ± 93	53 ± 18
	1st TR 2 weeks	23.2 ± 6.0	237 ± 97	185 ± 86	51 ± 17
	1 month	23.3 ± 6.7	247 ± 114	193 ± 102	53 ± 18
	2 months	23.6 ± 5.6	240 ± 120	186 ± 102	53 ± 22
	3 months	24.4 ± 5.7	237 ± 96	182 ± 85*	54 ± 14
	2nd TR 2 weeks	25.3 ± 6.2	230 ± 97	175 ± 87*	54 ± 16
	4 months	25.8 ± 7.4*	233 ± 97*	176 ± 86*	56 ± 18
	5 months	25.7 ± 7.3	241 ± 102	183 ± 94	57 ± 17
	6 months	24.1 ± 7.1	237 ± 108	183 ± 92	53 ± 21
	9 months	24.4 ± 5.0	250 ± 110	191 ± 93	58 ± 19
	12 months	25.6 ± 5.7	249 ± 106	188 ± 91	60 ± 18
Delay group	Before	25.7 ± 7.0	213 ± 56	159 ± 49	53 ± 15
	Non-TR 2 weeks	25.4 ± 9.8	204 ± 50	154 ± 49	49 ± 18
	1 months	24.4 ± 10.1	201 ± 51	156 ± 53	44 ± 24*
	2 months	25.3 ± 9.3	204 ± 54	154 ± 49	49 ± 18
	3 months	25.3 ± 8.5	206 ± 50	156 ± 48	50 ± 17
	TR 2 weeks	25.8 ± 9.0	193 ± 53*	145 ± 50*	47 ± 15
	4 month	26.5 ± 9.5	193 ± 51*	144 ± 50*	48 ± 15
	5 months	26.9 ± 9.3	193 ± 58*	144 ± 53*	49 ± 19
	6 months	27.5 ± 9.6	201 ± 61	148 ± 54	52 ± 20
	9 months	27.1 ± 9.2	206 ± 66	153 ± 57	53 ± 22
	12 months	26.9 ± 10.1	201 ± 57	149 ± 53	51 ± 19

LVEDV, left ventricular end-diastolic volume; LVESV, left ventricular end-systolic volume; SV, stroke volume.
*$P < 0.05$ vs. before.

increase in VO$_2$ max determined by cardiopulmonary testing. NYHA class was improved by at least 1 grade in 21 patients (52%). However, there was no improvement in the remaining 19 patients (48%), of whom one patient exhibited worsening of heart failure. The SAS score was increased from 3.4 ± 0.2 to 3.9 ± 0.2 metabolic equivalents (METs), and the 6-minute walk distance was increased from 359 ± 20 to 390 ± 19 m. Plasma BNP and ANP levels tended to be decreased, but the difference was not statistically significant.

Mean radionuclide LVEF 3 months after IA therapy improved nonsignificantly in the IA group (from

TABLE III.   Primary and Secondary Endpoints Changes Between Before and 3 Months After IA Therapy

Parameters	Number of patients	Combined A+D — Change for 3 months after IA								P values
Echocardiographic LVEF (%)[a]	39	23.8 ± 1.3 to 25.9 ± 1.3								0.0015
Trend analysis of Echocardiographic LVEF[b]	36	Improvement: eight patients (22%), Unchanged:27 patients (75%), Aggravation:one patient (3%)								–
Radionuclide LVEF (%)[c]	39	20.8 ± 1.1 to 21.9 ± 1.3								0.0605
Cardiothoracic ratio (%)	40	57.3 ± 1.2 to 55.9 ± 1.2								0.001
VO_2max (mL/min/kg)[d]	15	12.3 ± 1.2 → 16.0 ± 1.8								0.0064
NYHA class N (%)	40	IV	:	2	→	1	−3%			<0.0001
		III	:	33	→	18	−45%	Improvement:	21 −52%	
		II	:	4	→	15	−37%	unchanged:	18 −45%	
		II	:	1	→	5	−12%	Aggravation:	1 −3%	
		I	:	0	→	1	−3%			
SAS (METs)	40	3.4 ± 0.2 → 3.9 ± 0.2								0.0022
6-minute walk distance (m)	40	359 ± 20 to 390 ± 19								0.005
BNP (pg/mL)	40	426 ± 84 to 393 ± 82								0.4759
ANP (pg/mL)	40	172 ± 24 to 163 ± 27								0.5261

The data for changes in the 3 months after IA therapy in the IA group (A period) and the delay group (D period) were combined.

[a]Data were not obtained in one patient.

[b]The regression line was constructed using five LVEF values during the 3 months, and the slope of the regression line more than +5% or less than −5% for the period was defined as improvement or aggravation, respectively. Data missing in three patients.

[c]Data missing in one patient.

[d]Only patients who were able to undergo cardiopulmonary exercise testing.

ANP, atrial natriuretic peptide; BNP, brain natriuretic peptide; LVEF, left ventricular ejection fraction; METs, metabolic equivalents; NYHA, New York Heart Association; SAS, specific activity scale; VO_2 max, maximal oxygen consumption.

18.6% to 19.5%) and in the delay group (from 22.3% to 23.5%). However, there was a statistically significant increase in radionuclide LVEF during the 3-month observation period in the delay group without IA (from 19.5% to 22.3%, $P = 0.0343$). LVEF tended to be increased in the combined data from the A and D periods when IA therapy was performed.

Cardiac Events

Comparisons between experimental periods with and without IA therapy are shown in Supporting Information Tables S1 and S2. In the IA group, there were three cardiac events (two hospitalizations and one death) during the 3 months with IA therapy (A period), whereas in the delay group, there were six cardiac events (four hospitalizations, one death, and one treatment modification) during the 3 months without IA therapy (C period) ($P = 0.4566$). Furthermore, in the delay group, compared with the six cardiac events during the C period, there was only one event (hospitalization for heart failure) during the 3 months with IA therapy (D period) ($P = 0.0994$). From 6 months to 12 months, there were additional three cardiac events in the IA group and two cardiac events in the delay group.

Adverse Events

Adverse events were observed in 42 patients and severe adverse events in 19 patients, but most of the adverse events appeared to be attributed to the underlying heart failure. Hypotension occurred in 14 patients during the IA procedure, but symptoms resolved without any treatment or immediately after intravenous fluid administration. Most of the adverse events that occurred during the IA procedure, including hypotension, were mild in nature, easily managed, and similar to those observed in other extracorporeal circulation procedures.

Autoantibody Profiles

Total IgG was decreased from 1348 ± 449 mg/dL to 821 ± 269 mg/dL over the 1st set of five sessions of IA, and from 1397 ± 344 mg/dL to 925 ± 288 mg/dL during the 2nd set of IA in the IA group. IgG3 concentration decreased from 32 ± 22 mg/dL to 25 ± 17 mg/dL after single session of IA. Elimination rate of each IgG subclass and each autoantibody during the each single session of IA was presented in Table IV, showing IgG3 as well as five putative autoantibodies directed against specific epitopes were efficiently removed by 1.5 L plasma treatment. Full autoantibody profile is shown in Figure 3. Unfortunately, we were

TABLE IV.   Elimination Rate of IgG Subclasses and Each Autoantibody During the Single Session of IA

	0.5L treatment (n)	1.0L treatment (n)	1.5L treatment (n)
IgG1	99.8 ± 0.7 (39)	23.8 ± 15.0 (40)	1.5 ± 11.8 (39)
IgG2	99.1 ± 2.3 (39)	−13.8 ± 11.7 (40)	−11.4 ± 9.1 (39)
IgG3	98.0 ± 9.1 (38)	80.6 ± 22.4 (38)	62.1 ± 29.8 (38)
IgG4	98.9 ± 3.2 (36)	−26.9 ± 23.5 (37)	−9.2 ± 11.1 (36)
β1-antibody	92.9 ± 15.7 (27)	68.6 ± 40.1 (23)	61.9 ± 43.1 (22)
M2 antibody	100.0 ± 0.0 (5)	62.5 ± 51.3 (5)	54.1 ± 51.3 (6)
Na-K-ATPase antibody	93.6 ± 22.1 (12)	80.4 ± 40.9 (14)	75.9 ± 43.8 (12)
Troponin I antibody	100.0 ± 0.0 (17)	56.6 ± 44.0 (14)	51.8 ± 45.7 (14)
Myosin antibody	100.0 ± 0.0 (8)	100.0 ± 0.0 (10)	85.7 ± 40.4 (8)

Elimination rate (%) = (precolumn value − postcolumn value)/precolumn value × 100.
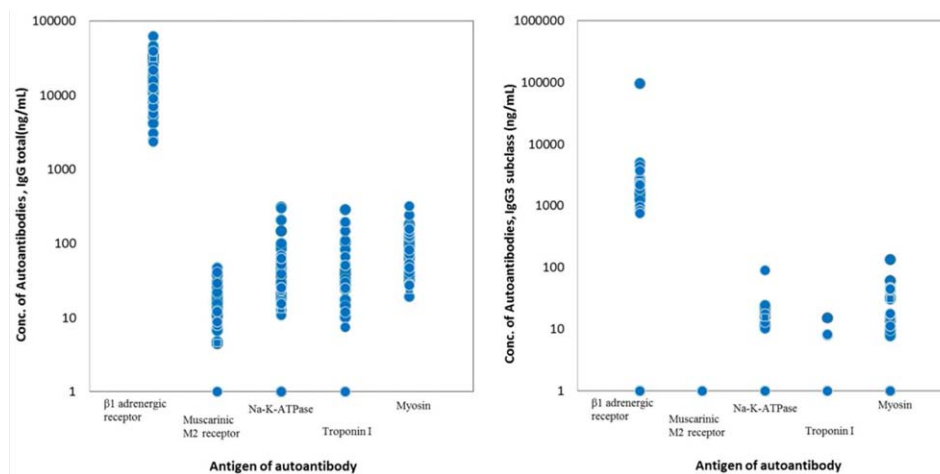
Fig. 3.   Baseline autoantibody concentrations. Left panel, total IgG antibodies; right panel, IgG3 subclass antibodies.

unable to find significant correlation between individual autoantibodies and LVEF response to IA therapy (data not shown). However, echocardiographic LVEF was significantly increased in patients with a higher autoantibody score but not in those with a lower score. Similarly, LV end-systolic volume (LVESV) was decreased in patients with a higher baseline autoantibody score but not in those with a lower score (Fig. 4).

DISCUSSION

We demonstrated that IA therapy using a tryptophan column could be safely conducted in patients with refractory heart failure resulting from DCM. Although we were not able to detect significant improvement of LVEF measured by radionuclide ventriculography, echocardiographic LVEF, NYHA functional class, cardiothoracic ratio, VO$_2$ max, SAS score, and 6-minute walk distance were significantly improved. Echocardiographic LVEF and LVESV were improved in patients with higher baseline autoantibody scores compared with those with lower scores.

Primary Endpoint

We defined a change in LVEF measured by radionuclide ventriculography 3 months after IA therapy by combining the A and D periods and failed to detect a significant change. We adopted this endpoint because radionuclide ventriculography is a highly objective tool to assess cardiac function. However, it is sometimes difficult to select the region of interest [22]. In addition, measurement is also affected by arrhythmias such as atrial fibrillation, commonly observed in patients with DCM [23]. We cannot completely exclude the possibility that LVEF spontaneously recovered during the 3 months without IA in the delay group.

We decided to enroll 40 patients in total assuming radionuclide LVEF was increased by 3.1% according to our preliminary finding [16]. Although previous report defined 5% or more increase in LVEF as a responder [12], we did not perform responder/nonresponder analysis.

Echocardiographic Assessment

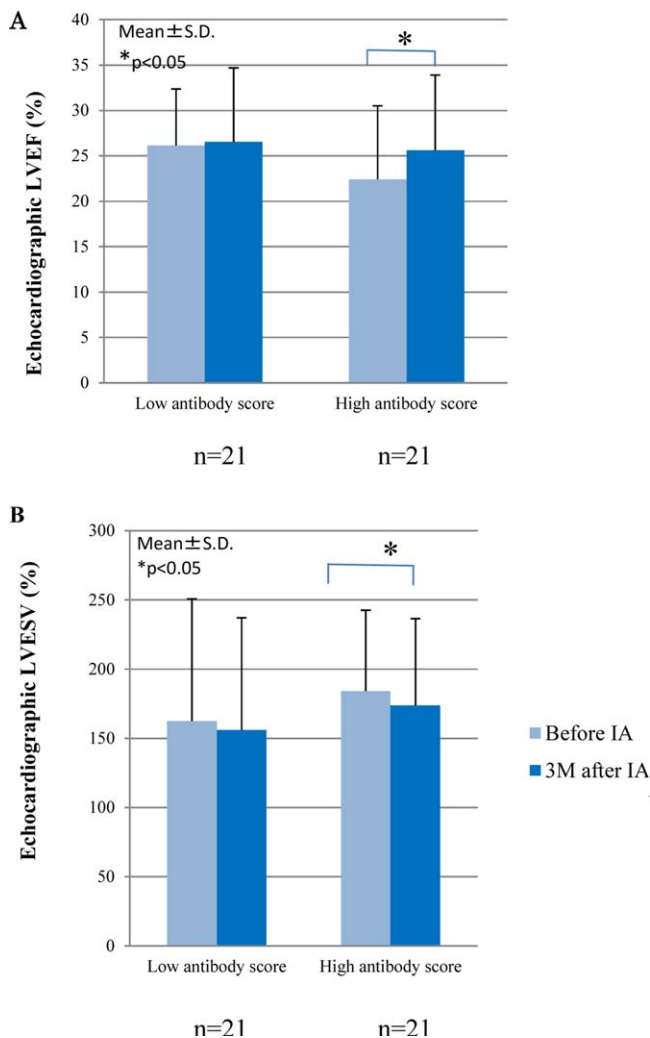Echocardiography has limitations in terms of objectivity but is superior to radionuclide ventriculography

Fig. 4. Changes in cardiac function before and 3 months (3M) after immunoadsorption (IA) therapy according to the baseline autoantibody score. *P < 0.05. (A) Left ventricular ejection fraction (LVEF); (B) left ventricular end-systolic volume (LVESV).

in terms of repeatability. Echocardiographic assessment is also able to avoid potential errors caused by atrial fibrillation by averaging multiple beats. We have shown that echocardiographic LVEF was significantly increased 3 months after IA. For comparison between A and C periods, the change in LVEF tended to be greater in A than C, although this difference was statistically insignificant. For comparison between C and D periods, LVEF was increased during IA therapy but not during the observation period without IA therapy. Trend analysis of echocardiography also showed that improvement was more common during IA therapy than during the observation period.

Other Secondary Endpoints

VO_2 max, NYHA functional class, SAS score, and 6-minute walk distance were improved 3 months after

IA therapy in the combined data of A and D periods. For comparison between A and C periods, the change in VO_2 max was significantly greater in A than C. The change in NYHA functional class also tended to be better in A than C. The change in VO_2 max was also better in D than C for the delay group. These findings suggest IA therapy improved maximal and submaximal exercise capacity. Plasma concentrations of BNP and ANP did not change significantly after IA. The tryptophan column may absorb natriuretic peptides [24], confounding the levels after IA. It may be difficult to estimate the severity of heart failure from the levels of natriuretic peptides, which are accepted biomarkers to estimate cardiac function.

Cardiac Events

Hospitalizations for heart failure tended to be less common during the 3 months after IA therapy than during the observation period. IA therapy may be useful to prevent such major cardiac events, thus ameliorating the quality of life in patients with heart failure.

In addition, our preliminary study showed that 1.5 L plasma treatment was sufficient to remove IgG3 subclass specifically [16]. A tryptophan column with high specificity for IgG3 may be superior to previously available columns such as anti-IgG and protein A columns, because removal of IgG is minimized to avoid substitution of high-dose immunoglobulin after the procedure.

Significance of Baseline IgG3 Autoantibody Score

We measured five different kinds of autoantibodies that are well known to play a major role in the pathophysiology of DCM [1,3–5,25] and failed to demonstrate that individual autoantibodies predict the LVEF response to IA therapy. Previous findings regarding the presence of autoantibodies in predicting efficacy of IA therapy have been inconsistent [9,26]. We previously showed that IA therapy was efficacious in patients who were positive for either autoantibody directed against β1-adrenergic receptor or muscarinic M2-receptor [16]. In contrast, some reports have shown that autoantibodies belonging to IgG subclass 3 have a pivotal role in mediating the pathophysiology of various autoimmune disorders [27,28]. As expected, removal of autoantibodies belonging to IgG3 was associated with clinical efficacy in patients who received IA therapy [13,14]. Dandel, et al. reported that heart transplantation or left ventricular assist device-free survival was better in patients with positive autoantibody for β1-receptors than those without autoantibody, who received IA therapy [10]. In the present study, we demonstrated that the total score for the five putative autoantibodies belonging to IgG3 subclass was associated with a

clinical response as defined by echocardiographically estimated LVEF and LVESV. Therapeutic efficacy appears to be more prominent in the study by Dandel, et al. as well as our preliminary experience than the present study. Inclusion criteria for autoantibody, study design (randomized or observational study), and plasma treatment are all different in these studies, accounting for the differential results.

Limitations

There was a discrepancy between radionuclide LVEF and echocardiographic LVEF. Unfortunately, we failed to show significant improvement for radionuclide LVEF. Instead, we attempted to minimize the time-to-time variation by multiple measurements during the 3 months of each observation using echocardiography. We added trend analysis by correlating the obtained data, and successfully validated the working hypothesis that IA therapy could improve cardiac function in patients with severe heart failure because of DCM. LVEF response was smaller than expected in this study. This may be because of the fact that the presence of β1-autoantibody was not a pre-requisite in this study. Cardiac events including heart failure hospitalization did not reach statistical significance. A larger sample size would successfully demonstrate clinical efficacy in addition to cardiac function.

CONCLUSIONS

We have demonstrated that IA therapy using a novel tryptophan column ameliorated subjective symptoms and exercise capacity in patients with refractory heart failure resulting from DCM. Assessment of a baseline autoantibody profile showed that this type of therapy was especially useful in patients who had a high auto-antibody score. This type of therapy may be also useful to reduce medical expenditure who are awaiting cardiac transplantation.

APPENDIX

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