Successful Immunoglobulin Treatment for Fulminant Myocarditis and Serial Analysis of Serum Thioredoxin
—— A Case Report ——

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A 31-year-old woman suspected to have acute myocarditis was admitted to hospital and was managed with intra-aortic balloon pumping and a percutaneous cardiopulmonary support system because of sustained ventricular tachycardia. After immunoglobulin treatment, cardiac function and systemic inflammation were improved. The left ventricular endomyocardial biopsy revealed massive necrosis and degeneration of myocardial cells, and extensive infiltration of inflammatory cells. The clinicopathology of this patient was thought to be fulminant myocarditis. Serial serum thioredoxin (TRX) analysis showed that the serum level was high during the acute phase, and decreased during the chronic phase. Immunohistochemistry for TRX in the biopsy samples showed that inflammatory cells and cardiomyocytes were positively stained. (Circ J 2002; 66: 977–980)

Key Words: Fulminant myocarditis; Immunoglobulin treatment; Thioredoxin

Case Report

A 31-year-old woman presented with nausea, vomiting and diarrhea for the past 5 days, and was transferred to the coronary care unit because of ventricular tachycardia (VT) and elevation of creatine kinase (CK). She had a history of acute pancreatitis 6 months before, but not of Kawasaki disease. On physical examination, she looked pale and her blood pressure was 95/74 mmHg. There were no detectable cardiac murmurs. There were moist rales in both lower lung fields during end-inspiration. Abdominal findings were physiological. Pretibial edema was not detectable. An electrocardiogram showed sinus tachycardia at the rate of 112 beats/min with poor R wave progression in leads V1–3, and 0.1 mV-junctional ST depressions in leads V4–6 (Fig 1). The chest roentgenogram showed slight cardiomegaly and protrusion of the right atrium. The leukocyte count was elevated at 14,600/mm3 with 94.8% granulocytes, 2.6% lymphocytes and 2.6% monocytes. The serum levels of aspartate aminotransferase (109 IU/L), lactic dehydrogenase (336 IU/L), CK (1,243 IU/L) and CK-MB (164 IU/L) were also elevated, and the levels of electrolytes were

Fig 1. The ECG on admission.
normal. Ultrasonic cardiogram (UCG) showed normal valves, normal left ventricular chamber size without pericardial effusion, and diffuse reduced wall motion. She was stabilized with an intra-aortic balloon pump (IABP) and a percutaneous cardiopulmonary support (PCPS) system, and coronary angiography (CAG) and left ventriculography (LVG) was then performed (Fig 2). No significant stenosis in the coronary arteries was observed. LVG detected diffuse, reduced wall motion except in the anterobasal and posterobasal areas, and the left ventricular ejection fraction (LVEF) was 18%. These findings suggested fulminant myocarditis, and we initiated immunoglobulin (Venoglobulin-IH, Welfide Corporation, Japan) treatment (1 g·kg\(^{-1}\)·day\(^{-1}\)) on the 2nd hospital day for 2 days. After the treatment, the leukocyte count was decreased, and UCG showed recovery of wall motion on the 3rd hospital day (Fig 3). The PCPS was removed on the 4th hospital day, and the IABP was removed on the 5th hospital day. On the 19th hospital day, cardiac catheterization with a left ventricular endomyocardial biopsy was performed. LVG showed improvement of global wall motion (LVEF 49%). The left ventricular endomyocardial biopsy revealed massive and multiple necrosis and degeneration of myocardial cells, and extensive infiltration of inflammatory cells (Fig 4A, B). The clinicopathology of this patient was thought to be fulminant myocarditis according to the criteria of Lieberman et al.\(^\text{10}\) Measurement of neutralizing antibody titers in the paired samples for coxsackievirus and influenza virus were performed, but no diagnostic elevation was observed. The patient's clinical course was satisfactory and she was discharged on the 29th hospital day.

Fig 2. LVG and CAG on the 1st hospital day. Diastolic phase (A) and systolic phase (B) on the right anterior oblique view revealed diffuse reduced wall motion except in the anterobasal and posterobasal areas. LVEF was 18%. CAG showed that left coronary artery on the right anterior oblique view (C) and right coronary artery on the left anterior oblique view (D) were intact.

Methods

Analysis for Serum TRX

The serum TRX was measured using a sandwich enzyme-linked immunosorbent assay (ELISA) kit (MBL) according to the procedure described previously.\(^\text{11}\) After sample and blocking buffer containing 1% bovine serum albumin were incubated in ADF 21-antibody precoated 96-well microwell plates, horseradish peroxidase-labeled anti-ADF 21 antibody was added. After substrate solution containing 2,2-azino-di-3-ethyl-benzthiazoline sulfonic acid was incubated, stopping solution (1% oxalic acid) was added. The absorption at 405 nm was measured with an ELISA reader (Molecular Devices, Menlo Park, CA, USA). Data were analyzed by a software SOFTmax Version 2.31 (Molecular Devices). In this protocol, the normal TRX levels were 14.0±4.6 ng/ml.\(^\text{11}\)

Immunohistochemistry for TRX in the Endomyocardial Biopsy Samples

Immunohistochemistry for TRX was performed as previously described.\(^\text{8}\) Briefly, after paraffin sections of the heart were deparaffinized, endogenous peroxidase activity was inactivated with 3% H\(_2\)O\(_2\), and normal goat serum was added for the inhibition of nonspecific binding of second antibody. The sections were incubated with the primary antibody (mouse anti-human ADF 21 antibody) and then with peroxidase-conjugated goat anti-mouse secondary antibody, and the color was developed with the substrate 0.1% 3',3'-diaminobenzidine, followed by hematoxylin nuclear counterstaining. For negative controls, sections were processed through all steps of the immunohistochemical staining without primary antibody.

Results

Table 1 shows the serial changes of TRX. The serum TRX level on the 2nd hospital day was already elevated and was unchanged by the immunoglobulin treatment; it was still elevated on the 20th hospital day. On the 116th hospital day, the serum TRX level decreased, but did not
and autoimmune diseases, and has been reported as effective for acute myocarditis. We previously reported that immunoglobulin therapy suppressed experimental giant cell myocarditis in rats associated with the suppression of the expression of dendritic cell via inhibitory Fc receptor. Because the leukocyte count was immediately decreased by the treatment, anti-inflammatory effects of immunoglobulin were shown in this study and, moreover, it may be because of the immunomodulatory effects of immunoglobulin that the patient’s condition rapidly recovered, as spontaneous recovery of cardiac functions is in general slow in patients with acute myocarditis.

Increasing evidence supports the role of local and systemic oxidative stresses in the development and severity of myocarditis and cardiomyopathy. Excessive production of reactive oxygen species (ROS) at the inflammatory site contributes to the inflammatory process by inducing the expression of adhesion molecules, proinflammatory cytokines, and chemokines. ROS scavengers, such as superoxide dismutase, have therapeutic potential for myocarditis. TRX is a multifunctional protein that has a reductive antioxidant effect, an anti-inflammatory effect and immunosuppressive activity. Immunoglobulin treatment increased the serum TRX levels. Why did the discrepancy between the serum TRX levels and the clinical course occur? One of reasons is that the response to oxidative stresses may be delayed, as previously described in patients with burn injury. TRX was positively stained in the inflammatory cells and cardiomyocytes in the biopsied myocardium and, therefore, one of the sources of serum TRX might be damaged cardiomyocytes during the acute phase. In addition, systemic release of TRX may continue even during the chronic phase, because the levels of TRX were constantly high, which indicates a higher rate of release than elimination by cellular uptake, degradation, or clearance in the urine.

In conclusion, we report a patient with fulminant myocarditis who was successfully treated with immunoglobulin and in whom we analyzed the serial serum levels of TRX and its myocardial immunohistochemistry. Further studies on a large number of patients using endomyocardial biopsy samples and quantitative parameters, such as troponin T and brain natriuretic peptide, are required to determine the association between TRX levels and clinicopathology in acute myocarditis. However, these findings may yield important insights into the redox regulating mechanism in the pathogenesis of acute myocarditis.

### Discussion

Myocarditis is an important cause of heart failure among adolescents and young adults and frequently precedes the development of dilated cardiomyopathy (DCM). Immune or autoimmune mechanisms may be involved in the myocarditis and subsequent cardiomyopathy, but immunosuppressive therapy has not been shown to be beneficial in most patients with biopsy-proven myocarditis and there is as yet no general agreement on effective treatment for acute myocarditis. Immunomodulatory therapy with high-dose immunoglobulin has been reported for inflammatory and autoimmune diseases and has been reported as effective for acute myocarditis. We previously reported that immunoglobulin therapy suppressed acute viral myocarditis by an anti-viral effect, an anti-inflammatory effect and improvement in extracellular matrix changes. Moreover, immunoglobulin therapy suppressed experimental giant cell myocarditis in rats associated with the suppression of the expression of dendritic cell via inhibitory Fc receptor. Because the leukocyte count was immediately decreased by the treatment, anti-inflammatory effects of immunoglobulin were shown in this study and, moreover, it may be because of the immunomodulatory effects of immunoglobulin that the patient’s condition rapidly recovered, as spontaneous recovery of cardiac functions is in general slow in patients with acute myocarditis.

### Table 1 Serum TRX Levels During Clinical Course

<table>
<thead>
<tr>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
<th>Day 20</th>
<th>Day 116</th>
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<tbody>
<tr>
<td>TRX (ng/ml)</td>
<td>14.0±4.6 ng/ml (n=7, mean±SD)</td>
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Day 1 indicate the first hospital day. Immunoglobulin was administered on the 2nd and 3rd hospital day. The datum on Day 2 was checked before immunoglobulin treatment. TRX, thioredoxin. In this method, the normal TRX level was 14.0±4.6 ng/ml (n=7, mean±SD).


