A Case of Influenza Subtype A Virus-Induced Fulminant Myocarditis: An Experience of Percutaneous Cardio-Pulmonary Support (PCPS) Treatment and Immunohistochemical Analysis

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Miura, M., Asaumi, Y., Wada, Y., Ogata, K., Sato, T., Sugawara, T., Yano, M., Mitsuoka, M., Takai, O., Ota, K., Namiki, K., Sato, D., Sato, E., Nagura, H. and Kimura, T. A Case of Influenza Subtype A Virus-Induced Fulminant Myocarditis: An Experience of Percutaneous Cardio-Pulmonary Support (PCPS) Treatment and Immunohistochemical Analysis. Tohoku J. Exp. Med., 2001, 195(1), 11–19 —— A 64-year-old man was admitted to the emergency center of Furukawa City Hospital because of common cold-like symptoms and hypotension. He was diagnosed as fulminant myocarditis with cardiogenic shock and arrhythmia elicited by influenza virus subtype A. Cardiac angiography, echocardiography and biopsy also showed myocarditis, and serum antibody titer to influenza virus subtype A was increased to 4-fold in paired sera. Treatments of both percutaneous cardio-pulmonary support (PCPS) and intra-aortic balloon pumping (IABP) were carried out to sustain the general circulation. PCPS treatment was discontinued on the 25th day of the admission, but IABP was continued. Finally, he died of multiple organ failure. The autopsy revealed myocardial necrosis with a slight fibrosis and a small amount of lymphocytic infiltration into the ventricular wall, which were compatible with restrictive myocarditis. Moreover, immunohistochemical analysis also showed the presence of viral antigens in cardiac myocytes. This case clearly showed that PCPS and IABP can be beneficial to sustain the general circulation in fulminant myocarditis, but cardiac pumping function failed completely to recover from myocardial damage.

Received May 24, 2001; revision accepted for publication September 14, 2001.
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It is well known that acute myocarditis can be caused not only by infection such as virus, bacteria, rickettsia, and spirochaeta, but also by toxins, drugs and immunological reaction.

Especially, in elderly persons and infants infection of coxackiviruses (A, B) and other viruses such as echo, polio and influenza sometimes give rise to myocarditis (Lerner and Wilson 1973; Woodruff 1980). Viral infection is considered to induce myocardial necrosis due to direct cytotoxicity, to auto-immune-mediated lysis of infected cells and/or to the alteration of intracellular metabolisms (Woodruff 1980; Herzum et al. 1995). Also, influenza viruses have been reported to complicate pericarditis, restrictive myocarditis and endomyocarditis as well as pulmonary diseases and encephalitis. Moreover, three antigenic subtypes A, B and C in influenza viruses are known, and former two types, in particular, are outbreak in winter and produce clinically indistinguishable symptoms such as fever, chills, malaise, cough and myalgia (Woodruff 1980).

To our knowledge, so far, there have been only a few reports which assess clinical, hemodynamic, histological, therapeutic and prognostic profiles of acute myocarditis induced by influenza subtype A. Herein, we reported a case of acute fulminant myocarditis with influenza subtype A infection sustained with percutaneous cardio-pulmonary support (PCPS) for relatively a long period. Its precise pathological findings were also described.

**CASE REPORT**

A 64-year-old man, suffering from fever, chill, general malaise, cough, cold sweating, palpitation, and precordial distress was admitted to the emergency center of Furukawa City Hospital in February 1999, and died on 41 days after. He was referred to our hospital suspicious of common cold from a practitioner. He was healthy until this episode. On admission, his consciousness was alert. Body weight was 64 kg, body length 165 cm, and nutrition intermediate. Cold sweating and cyanosis were noticed, but anemia and jaundice were not identified. Blood pressure was 90/40 mmHg, pulse rate 54 bpm and irregular, and body temperature 35.4°C. The third heart sound without murmur and pulmonary massive rales were audible. From these findings, he was diagnosed as cardiogenic shock. Abdominal wall was flat and soft, and liver, spleen and kidneys were not palpable. No edema was in pretibial regions, no enlargement of lymph nodes was noticed, and neurological examinations were not remarkable.

As shown in Table 1, both hematological findings and coagulation tests were not remarkable. Liver function tests were within normal values, except for elevated glutamic-oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), creatine phosphokinase (CPK), and lactate dehydrogenase (LDH). Serum protein, lipids, fasting blood glucose also were not remarkable. Serum electrolytes were normal, but blood urea nitrogen (BUN) and serum creatinine were elevated. Myosin light-chain and myocardial troponin T were much increased in the circulating blood. Arterial blood gas analysis showed PH7.436, PaO₂ 110, PaCO₂ 20.8, HCO₃ 13.7, BE-8.1 and SaO₂ 98.3% (O₂ 5 liter/min.). Electrocardiogram (ECG) showed that the frequent and alternative occurrences of complete AV block and ventricular tachycardia (150/min.), then, clear judgement of abnormal QS segment and T wave were impossible. Ultrasonic cardiography (UCG) showed an impairment of left
ventricular movement with 24% left ventricular ejection fraction (LVEF, Fig. 1), and left ventricular wall motion was diffusely impaired and its cardiac chamber was enlarged with the hypertrophied myocardium (12 mm). Chest x-ray showed the enlargement of the heart and cardiothoracic ratio (CTR) was 66%. Lung field also showed severe pulmonary congestion.

Antibody titers to various viruses in paired sera were shown in Table 2. Influenza virus subtype A titer was apparently elevated compared with other viral titers and showed about 4 times increase in the paired sera.

Examination by cardiac catheterization and biopsy findings: Left ventricular angiography showed an impairment of left ventricular movement, but there was neither obstruction nor stenosis on the coronary angiography (Fig. 2). These findings suggested the presence of myocarditis. Right ventricular biopsy specimens also revealed myocardial edema with mononuclear cell infiltration and cardiac muscle degeneration.

Clinical course and treatments using PCPS and intra-aortic balloon pumping (IABP): Since cardiogenic shock with ventricular tachycardia was not effectively improved by catecholamine treatments, implantation of IABP followed by PCPS was performed. Blood flow rate at the onset was 3 liter/minute to maintain more than 80 mmHg in systolic blood pressure. To prevent blood coagulation, gabexate mesilate (2 g/day) was administered into the PCPS system and whole body to maintain more than 200 seconds of activated coagulation time (ACT).

In addition, dopamine (5 mg • kg⁻¹ • min⁻¹)
Fig. 1. Echocardiogram illustrates impaired left ventricular movement (LVEF 24%).
Right: M-mode from the parasternal position.
Left: parasternal long axis view.

<table>
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<th>February 9*</th>
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<tr>
<td>(H3N2)</td>
<td>512</td>
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<td>4</td>
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<tr>
<td>HSV</td>
<td>16</td>
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HSV, herpes simplex virus (*At the admission).

Fig. 2. Coronary angiography shows neither obstruction nor stenosis. (left picture: right coronary artery, right picture: left coronary artery)
Fig. 3. Clinical course and treatments of the patient. The period of percutaneous cardiopulmonary support-PCPS, intra-aortic balloon pumping-IABP, hemodialysis, and medications are illustrated along with laboratory data. Black arrows indicate blood transfusion. Antibiotics consists of Cefoxopran-CZOP, Minocycline-MINO, Clavulanic acid/Ticarillin-CVA/TIPC, Imipenem/Cilastatin-IPM/CS, Clindamycin-CLDM, ○, Cardiac Index-C.I.; +, Saturation of mixed venous oxygen-SVO₂; ■, Creatinine Phosphokinase-CPK; △, C-reactive protein-CRP, Clinical admission days-Days.

and dobutamine (5 mg·kg⁻¹·min⁻¹) were also used to maintain the circulation. Diuretics, when necessary, were used, and antibiotics and γ-globulin were administered. Steroid treatment was not carried out.

Since LVEF and systolic blood pressure were gradually improved, and on day 25, PCPS was excluded from the general circulation under more than 100 mmHg of systolic blood pressure (SBP) after weaning blood flow rate in PCPS. General circulation was maintained with epinephrine and norepinephrine, and blood pressure was stable. On day 27, bleeding occurred at the site of femoral artery, which was used for PCPS, but circulatory states were recovered by blood transfusion and IABP. On day-32, multiple organ failure occurred following endotoxin shock and fungal infection, and he finally died on day 41, despite continuous hemodialysis (Fig. 3).

Pathological findings

Autopsy revealed left ventricular dilatation and calcification of the aortic valve. Cardiac weight was 400 g. Microscopic findings showed a diffuse elimination or disappearance of myocardial cells and their necrosis in both right and left ventricular walls. Focal myocardial fibrosis was noticed in both ventricular walls, and lots of lymphocytes were infiltrated in the right ventricular wall (Fig. 4). These findings consisted with angiographic profile and confirmed the clinical diagnosis of fulminant myocarditis. The intensity of lymphocytes infiltration was almost equivalent to biopsy specimens, however, Hematoxylin-
Eosin staining and Elastica-Masson staining revealed cardiac myocytes degeneration, and fibrosis was more advanced than biopsy specimens. Particular findings were not noticed in other organs.

Immunohistochemical analysis

Methods. Formalin-fixed and paraffin-embedded cardiac muscle specimens obtained from the ventriculum septum (biopsy specimen) and left posterior wall (autopsy specimen) were sectioned at 2.5 μm and were immunohistochemically stained according to the method
described by Tsutsumi et al. (1991). In this method, patient’s immunoglobulins that react to antigens including viral-produced proteins in infected cells can be detected. Briefly, the specimens were deparaffinized in xylene, hydrated through graded series of ethanol, then placed in 0.01 M phosphate buffered saline (PBS), and sections were incubated in 10% bovine serum albumin (BSA) for 20 minutes to reduce nonspecific bindings. Then, they were reacted with patient’s serum, which is corrected at autopsy and stored at –70°C until using, diluted with PBS at 1:1000 for 16 hours at 4°C. After washing with PBS, they were incubated in 0.3% hydrogen peroxidase in methanol for 12 minutes at room temperature to block endogenous peroxidase activity. After being washed again with PBS, sections were incubated with the second antibody, horseradish peroxidase labeled anti-human IgG (Biosource International, Camarillo, CA, USA) at a dilution of 1:800 in room temperature for 2 hours. Finally, they were washed again with PBS, and reacted with 3,3’-diaminobenzidine tetrahydrochloride (DAB) and hydrogen peroxide in Tris-HCl buffer, pH 7.6, with 0.04% CoCl₂, then washed in distilled water and dehydrated and mounted.

RESULTS

Positive stainings were confirmed in cytoplasm of degenerated cardiac myocytes (Fig. 5a: cardiac biopsy, Fig. 5b: autopsy, arrow). No apparent positive stainings were detected in non-degenerated myocytes.

DISCUSSION

The present case is compatible with acute fulminant myocarditis induced by influenza virus subtype A infection from hemodynamic, laboratory and histologic findings as well as immunohistochemical analysis. In addition, common cold-like symptoms related to influenza virus were prevalent in the northern part of Japan in the winter of 1999, and also a specifically increased antibody titer in influenza virus type A was noticed in paired sera in the present case. Moreover, immunohistochemical analysis, using patient’s serum as the primary antibody, was performed to determine the presence of viral antigens in the cardiac myocytes as well as to detect the existence of viral infection in these cells. Presumably, the patient’s serum contains immunoglobulin which reacts to such viral antigens. Positive stainings were confirmed in the cytoplasm of degenerated cardiac myocytes, but not in non-degenerated myocytes. This suggests that there was the viral infection in these degenerated cells, although the presence of the virus could not directly be confirmed. The staining pattern of the cardiac biopsy was the same as that of autopsy specimens. Cardiac myocytes degeneration and fibrosis were more apparent in autopsy specimen, while the intensity of lymphocytes infiltration was almost equivalent in both specimens. These findings suggest that cardiac myocytes damages were gradually developed during the course of the disease with continuous viral infection.

Acute myocarditis shows variable symptoms such as arrhythmia, heart failure, and cardiogenic shock, and sometimes absence for symptoms. Especially myocarditis, which abruptly occurs and combines heart failure with ventricular arrhythmia and cardiogenic shock with conduction block, is called a fulminant type, and ventricular and/or supra ventricular arrhythmia are common in acute myocarditis. Mortality rate in fulminant myocarditis induced by influenza virus is higher in elderly patients with related basal diseases such as severe cardiac diseases (Sprenger et al. 1993). ECG shows several abnormal findings, echocardiography ventricular hypertrophy, and cardiac catheterization increases in Left Ventricular Endiastolic Pressure, as well as endomyocardial biopsy reveals fibrosis, cellular hypertrophy and nuclear alterations (Ortolani et al. 1989).

Myocarditis in mild and moderate degrees
can be treated with drugs such as digitalis and catecholamines in order to maintain the systemic circulation. For fulminant myocarditis with cardiogenic shock, however, implantation of IABP and PCPS are recommended to maintain the general circulation (Rockman et al. 1991; Morishima et al. 1994; Reiss et al. 1996).

In the present case, the treatment with PCPS and IABP was chosen because the patient was diagnosed as fulminant myocarditis, and PCPS was continued for 25 days, and IABP was necessary to maintain blood pressure thereafter. The presence of massive myocardial damage required this treatment. Success of catheterization in relatively large blood vessels and the absence of severe atherosclerosis in
the present patient could make PCPS treatment possible for a long period. However, the long-term application of PCPS may cause systemic infection and circulatory impairments in the distal regions maintained by PCPS, whereby leading to the multiple organ failure with high mortality rate.

It has been recognized that treatments for acute myocarditis prevent the myocardial cell necrosis and enhance recovery of the early cardiac function. For these purposes, steroid pulse, γ-globulin, and immunosuppressive or immunoactivative treatments have been tried, but their effectiveness has not yet been established (Woodruff 1980). On the other hand, rather steroid and immunosuppressive treatments were reported to be harmful to myocarditis (Mason 1995).

Moreover, recently, several anti-viral drugs have been developed, but, to our knowledge, whether or not these drugs have effects on the entire clinical course of viral myocarditis have been elusive. Either cardiac organ transplantation or left ventricular assist system (LVAS) as well as bi-ventricular assist system (BiVAS) is another possibility for patients with severe myocarditis, but it is almost impossible to find a suitable donor in Japan for a limited time and to clinically use LVAS and BiVAS for patients in a hospital.

From our experience, for the treatment of fulminant myocarditis at present, the development of PCPS being possible for a long term application is necessary. It is more important to establish methods for the prevention and treatment of viral myocarditis including effective vaccination.

Acknowledgments

We are deeply indebted Ms. Emiko Sakai and Ms. Rutsuko Sasagawa for expert secretarial assistance. This work was supported by a Grant-in-Aid for severe hypothalamo-hypophysial diseases and in part by the Health Science Research Grants for Research on Specific Diseases from the Japanese Ministry of Health, Labour and Welfare.

References


