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Elevated Levels of Oxidative DNA Damage in Serum and Myocardium of Patients With Heart Failure

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Background Oxidative stress has been implicated in the pathogenesis of chronic heart failure. The present study investigated whether the levels of 8-hydroxy-2-deoxyguanosine (8-OHdG), a marker of oxidative DNA damage, were elevated in the serum and myocardium of patients with dilated cardiomyopathy (DCM), and furthermore whether carvedilol, a vasodilating β-blocker with antioxidant activity, could reduce the levels.
Methods and Results Serum levels of 8-OHdG were measured by enzyme immunoassay in 56 patients with DCM and in 20 control subjects. DCM patients had significantly elevated serum levels of 8-OHdG compared with control subjects. Endomyocardial biopsy samples obtained from 12 DCM patients and 5 control subjects with normal cardiac function were studied immunohistochemically for the expression of 8-OHdG. Positive 8-OHdG staining was found in the nuclei of cardiomyocytes from DCM patients but not in those from control subjects. After treatment with carvedilol, the serum levels of 8-OHdG in DCM patients significantly decreased by 19%, together with amelioration of heart failure.
Conclusions Levels of 8-OHdG are elevated in the serum and myocardium of patients with heart failure. Treatment with carvedilol might be effective for decreasing the oxidative DNA damage. (*Circ J* 2006; **70:** 1001 – 1005)

Key Words: DNA; Heart failure; Oxidative stress

The mortality rate of patients with heart failure remains high despite recent advances in medical therapies such as the use of angiotensin-converting enzyme inhibitors, β-blockers and angiotensin-receptor blockers[1–3] Furthermore, the number of patients with heart failure is expected to increase with aging of the population[4] so understanding the fundamental mechanisms responsible for heart failure is important.

Oxidative stress due to reactive oxygen species (ROS) is thought to be a factor exacerbating heart failure[5–13] because ROS directly damage the cellular membrane[14,15] myofibrillar proteins[16] and subcellular organelles such as mitochondria[17] or sarcoplasmic reticulum[18] in cardiac myocytes and thus impair cardiac function. Furthermore, ROS activate intracellular signaling cascades and consequently induce apoptosis or hypertrophy in cardiac myocytes[7,19]

DNA in the nucleus is one of the major targets of ROS and oxidative DNA damage has been implicated in the pathogenesis of cancer, neurodegenerative diseases and in aging[20–23] Hydroxyl radical or singlet oxygen is responsible for hydroxylation of the C-8 position of 2'-deoxyguanosine to produce 8-hydroxy-2'deoxyguanosine (8-OHdG), which is used as a reliable maker of oxidative DNA damage[20–23] In a recent study, 8-OHdG was detected in cardiomyocytes of patients with severe dilated cardiomyopathy (DCM)[24] so we hypothesized that oxidative DNA damage would be elevated in patients with heart failure. To test this hypothesis, we investigated whether the levels of 8-OHdG were elevated in the serum and myocardium of patients with DCM. We also investigated whether carvedilol, a vasodilating β-blocker with antioxidant activity, could reduce the levels.

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Methods

Subjects

The patient population studied comprised 56 consecutive patients not taking β-blockers (42 men, 14 women; mean age, 53±12 years) admitted to Okayama (Japan) University Hospital between 1998 and 2003 (Table 1). After admission to hospital, DCM was diagnosed according to the criteria of the 1995 World Health Organization/International Society and Federation of Cardiology Task Force. None of the patients showed any evidence of coronary artery disease, valvular heart disease, or pericardial heart disease. We excluded patients with systemic hypertension, hyperlipidemia or smoking habit, because those risk factors increase oxidative stress[25]

Echocardiographic studies were performed with commercially available Aloka ProSound SSD-5500. A cardiac catheterization study, including coronary angiography, left ventriculography and pressure analysis, was performed by the percutaneous Seldinger technique in all patients. Endomyocardial biopsy samples (3 or 4 per patient) were obtained from the right ventricular side of the septum of all

Table 1 Clinical Characteristics of 56 Patients With Dilated Cardiomyopathy

Age (years)	*53±12*
Sex (M/F)	*42/14*
NYHA functional class	*2.3±0.7*
Cardiothoracic ratio (%)	*54±4*
Laboratory data	
WBC (/μL)	*6,400±1,600*
Creatinine (mg/dl)	*0.84±0.37*
CK (IU/L)	*148±209*
BNP (pg/mL)	*146±184*
Echocardiographic data	
LVDd (mm)	*62±7*
LVDs (mm)	*51±8*
FS (%)	*17±6*
Hemodynamic data	
LVEF (%)	*38±11*
LVEDP (mmHg)	*6±4*
CI ($L \cdot min^{-1} \cdot m^{-2}$)	*2.5±0.6*
PCWP (mmHg)	*7±5*
Medical treatment (n)	
ACEI	*40*
Angiotensin-receptor blocker	*12*
Ca-channel blocker	*13*
Diuretics	*31*

Data are mean±SD.
NYHA, New York Heart Association; WBC, white blood cells; CK, creatine kinase; BNP, B-type natriuretic peptide; LVDd, left ventricular end-diastolic diameter; LVDs, left ventricular end-systolic diameter; FS, fractional shortening; LVEF, left ventricular ejection fraction; LVEDP, left ventricular end-diastolic pressure; CI, cardiac index; PCWP, pulmonary capillary wedge pressure; ACEI, angiotensin converting-enzyme inhibitor.

patients by the internal jugular approach. Left ventricular ejection fraction (LVEF) was calculated by the area–length method and was less than 50% in patients with DCM.

The control subjects were 20 gender- and age-matched healthy blood donors (14 men, 6 women; mean age, 50±12 years) without any history of cardiac disease. Endomyocardial biopsy samples obtained from 5 patients with primary arrhythmia (lone atrial fibrillation, n=4; atrial tachycardia, n=1) and with LVEF >60% were used as control samples in the immunohistochemical examination. Arrhythmia of unknown etiology is sometimes related to myocarditis and early cardiomyopathy, so to obtain a definite diagnosis, we performed biopsies in patients with arrhythmia. In this study, we used biopsy samples without histological evidence of myocardial inflammation, hypertrophy or fibrosis as the control samples. We explained to the arrhythmia patients that we would perform biopsy for diagnosis and for this study. We explained the risk of endomyocardial biopsy to all patients and obtained their consent. There were no significant complications and no prolonged hospitalizations. The study protocol was approved by the local medical ethical committee and written informed consent was given by all subjects before each investigation.

Serum 8-OHdG Levels

Blood was collected from an antecubital vein into a plain glass tube and allowed to clot for 1 h before centrifugation at 3,000 rpm for 10 min. After centrifugation, the samples were immediately stored at –80°C until analysis. Serum 8-OHdG levels were measured using a commercially available enzyme-linked immunosorbent assay kit (Japan Institute for the Control of Aging, Fukuroi, Japan).

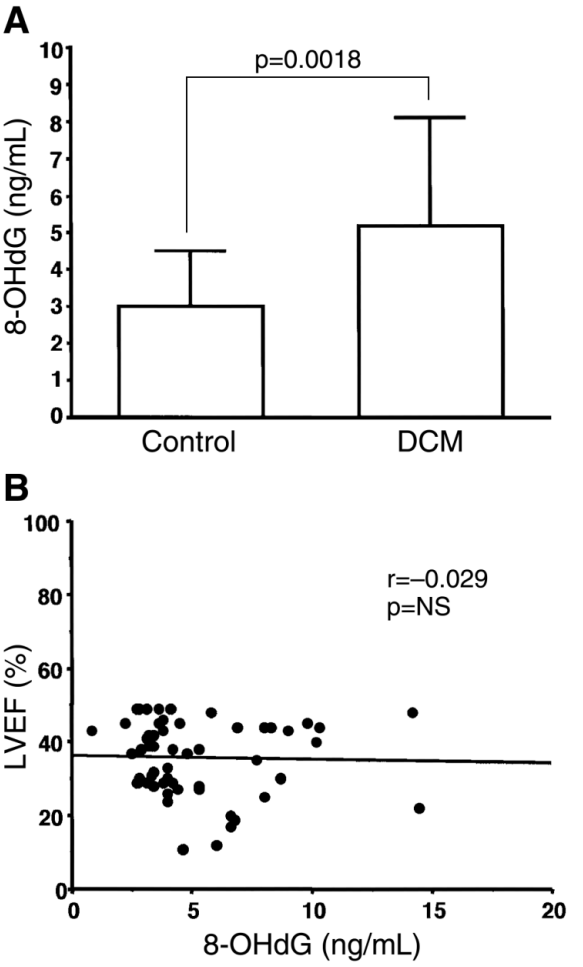

Fig 1. Serum levels of 8-hydroxy-2-deoxyguanosine (8-OHdG) in patients with dilated cardiomyopathy (DCM). (A) DCM patients had significantly elevated serum levels of 8-OHdG compared with control subjects. Data are mean±SD. (B) Relationship between serum 8-OHdG level and left ventricular ejection fraction (LVEF).

Immunohistochemistry

Endomyocardial biopsy samples were fixed in 10% formalin and embedded in paraffin. Each tissue sample was serially cut into 5-μm-thick sections and immunoenzymatic staining was performed using a DAKO LSAB System (DakoCytomation, Kyoto, Japan) according to the manufacturer's instructions. Briefly, the heart sections embedded in paraffin were preincubated with 1.5% hydrogen peroxide and normal bovine serum albumin to block nonspecific reactions. Mouse monoclonal anti-8-OHdG antibody (1:100 dilution, NOF Corporation, Tokyo, Japan) was added, and the sections were incubated at 4°C overnight. As a negative control study, mouse monoclonal IgG1 antibody (1:50 dilution, DakoCytomation, Kyoto, Japan) was added. The sections were then incubated with biotinylated anti-mouse immunogloblin for 20 min and subsequently with horseradish peroxidase-labeled streptavidin solution for 20 min. The slides were rinsed in cold tris-buffered saline after each step of incubation. Peroxidase activity was visualized with diaminobenzidine tetrahydrochloride solution.

Protocol for Carvedilol Treatment

After cardiac catheterization, treatment with carvedilol was started at a dosage of 1 mg/day in all patients with

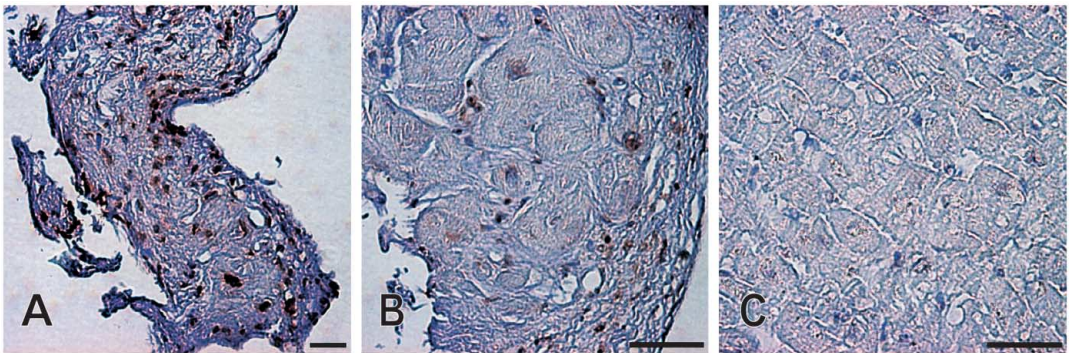

Fig 2. Immunohistochemical examination of 8-hydroxy-2-deoxyguanosine (8-OHdG) in representative myocardial biopsy samples. Patient with dilated cardiomyopathy (DCM): (A) Low-power field: positive staining (brown) for 8-OHdG is distinct in the nuclei of cardiac myocytes. (B) High-power field. (C) Control subject. Bar = 50 μm.

Table 2 Amerioration of Cardiac Function by Carvedilol

	Before	*After*	*p value*
Systolic BP (mmHg)	*127±20*	*127±17*	*NS*
Diastolic BP (mmHg)	*78±11*	*73±9*	*NS*
Heart rate (beats/min)	*83±13*	*68±11*	*p=0.0001*
NYHA	*2.3±0.7*	*1.7±0.6*	*p=0.0004*
LVDd (mm)	*64±9*	*60±9*	*p<0.005*
LVDs (mm)	*53±10*	*48±11*	*p<0.005*
LVEF (%)	*34±14*	*40±12*	*p<0.05*
PCWP (mmHg)	*7±4*	*7±4*	*NS*

Data are mean ± SD.
BP, blood pressure. Other abbreviations see in Table 1.

DCM. The dosage was gradually increased to a maximum of 5–30 mg/day. In 11 patients from whom we obtained consent, cardiac catheterization and measurement of serum 8-OHdG levels were performed during carvedilol treatment. The mean carvedilol treatment period was 12±9 months and the mean carvedilol dosage was 20±8 mg/day.

Statistical Analysis

All data are expressed as mean ± SD. Statistical significance for comparison between 2 measurements was determined using Student's t-test. Correlation coefficients (r) were calculated by linear regression analysis. Statistical significance for comparison between before and after treatment with carvedilol was determined using Student's t-test. Values of $p<0.05$ were considered to be significant.

Results

Serum Levels of 8-OHdG in Patients With DCM

DCM patients had significantly elevated serum levels of 8-OHdG compared with control subjects (DCM patients: 5.2±2.9 ng/mL vs control subjects: 3.0±1.5 ng/mL, p=0.0018) (Fig 1A).

It is reported that oxidative stress is enhanced in healthy young men compared with age-matched women;[26] so we investigated the difference between the serum levels of 8-OHdG in male and female patients with DCM and found no significant difference (men: 6.1±4.3 ng/mL vs women: 4.9±2.3 ng/mL, p=NS).

The serum 8-OHdG levels in patients with DCM were not correlated with left ventricular end-diastolic diameter (LVDd) (r=0.049, p=NS) or LVEF (r=–0.029, p=NS) (Fig 1B). Furthermore, the serum 8-OHdG levels were not correlated with either parameter in men or women with DCM (LVDd: men, r=0.015, p=NS; women, r=0.179, p=NS; LVEF: men, r=–0.007, p=NS; women, r=–0.029, p=NS).

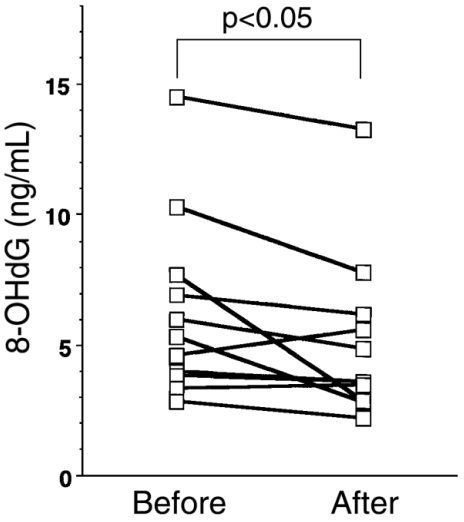

Fig 3. Decrease in serum 8-hydroxy-2-deoxyguanosine (8-OHdG) levels after treatment with carvedilol (6.3±3.5 ng/mL before and 5.1±3.2 ng/mL after carvedilol administration (19%), p<0.05).

Myocardial Levels of 8-OHdG in Patients With DCM

Positive 8-OHdG staining was found in the nuclei of cardiac myocytes of samples from DCM patients but not in those from control subjects (Fig 2).

Effect of Treatment With Carvedilol

Treatment with carvedilol reduced heart rate (p=0.0001), New York Heart Association class (p=0.0004) and LVDd (p<0.005), and ameliorated LVEF (p<0.05) (Table 2). Serum 8-OHdG levels were decreased by 19% during treatment (6.3±3.5 ng/mL before and 5.1±3.2 ng/mL after carvedilol administration, p<0.05) (Fig 3). These findings indicate that carvedilol reduced the level of oxidative DNA damage, together with amelioration of heart failure.

Discussion

Three major new findings were obtained in the present study. First, the level of a marker of oxidative DNA dam-

age was elevated in both the serum and myocardium of patients with DCM. Second, carvedilol reduced those levels and ameliorated the heart failure. Our hypothesis that oxidative DNA damage is elevated in patients with heart failure is supported by these findings.

DNA damage is increased in human hearts under various conditions, such as myocardial ischemia and myocarditis[24,27] In the present study, we found increased oxidative DNA damage in the serum and myocardium of patients with DCM, so oxidative stress is also a condition under which DNA damage occurs. Progression of heart failure because of oxidative stress has been implicated in damage to the cellular membrane,[14,15] myofibrillar proteins,[16] mitochondria[17] and sarcoplasmic reticulum[18] in cardiac myocytes. We found that oxidative DNA damage in the nucleus is also elevated in heart failure, so repair of DNA damage may be an efficient medical treatment for progression of heart failure caused by oxidative stress.

Oxidative DNA damage has been implicated in the pathogenesis of cancer, neurodegenerative diseases and in aging[20–23] Oxidative DNA damage has mutagenic effects. In particular, 8-OHdG has biological significance, including G:C to T:A transversions at DNA replication[23,28,29] DNA mutations in the nucleus result in neuronal death and carcinogenesis, so oxidative DNA damage in cardiac myocytes may also have mutagenic effects and result in myocardial dysfunction or death. Furthermore, oxidative DNA damage triggers p53 autoproteolytic activity, leading to the generation of p50 fragments lacking either the carboxyl (p50 (ΔC)) or amino terminal (p50 (ΔN)).[30] p50 (ΔC) promotes DNA repair, whereas p50 (ΔN) leads to apoptosis.[31] Therefore, oxidative DNA damage may cause apoptotic cell death in the failing myocardium. In fact, increased oxidative DNA damage has been shown at 2–4 weeks after myocardial infarction, together with apoptotic cardiomyocytes in the peri-infarct areas.[32]

The serum 8-OHdG levels were elevated in patients with DCM, but did not correlate with LVDd or LVEF in this study. The serum 8-OHdG levels are affected by oxidative stress not only in heart but systemically. Elevation of circulating 8-OHdG levels may reflect other organ damage, such as endothelial dysfunction.[33] Further studies are needed.

Carvedilol reduced the oxidative DNA damage in patients with DCM by several possible mechanisms. The β-blocking effects of carvedilol may be important, because catecholamines such as isoproterenol or norepinephrine induce oxidative stress in the myocardium[8,34,35] Other effects, such as anti-ischemic effects, including negative chronotropic effects via β-receptors, and the direct antioxidative effect of carvedilol, may contribute to the reduction of oxidative stress[8] Carvedilol reduced oxidative DNA damage with amelioration of heart failure, but only decreased the oxidative stress level by 19%. More potent and effective antioxidants might be needed to greatly reduce oxidative DNA damage.

Study Limitation

The present study was limited by the absence of a placebo group. Therefore, it is not clear that the changes of the 8-OHdG were solely attributable to carvedilol.

In conclusion, the levels of oxidative DNA damage were elevated in the serum and myocardium of patients with DCM. Carvedilol reduced the levels of oxidative DNA damage, together with amelioration of heart failure.

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