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Relationship between the Myocardial Oxidative Stress and Cardiac Sympathetic Hyperactivity in Patients with Takotsubo Cardiomyopathy

Seiko Oda, Shigeki Kobayashi, Takuma Nanno, Hironori Ishiguchi, Takeki Myoren, Wakako Murakami, Mamoru Mochizuki, Tetsuro Oda, Shinichi Okuda, Jutaro Yamada, Takayuki Okamura, Masunori Matsuzaki and Masafumi Yano

Division of Cardiology, Department of Medicine and Clinical Science, Yamaguchi University Graduate School of Medicine, 1-1-1 Minamikogushi, Ube, Yamaguchi 755-8505, Japan
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Correspondence to Shigeki Kobayashi, M.D., Ph.D. Email: skoba@yamaguchi-u.ac.jp

Abstract We investigated the relationship between myocardial oxidative stress and cardiac sympathetic hyperactivity in patients with takotsubo cardiomyopathy (TC) compared with acute anteroseptal myocardial infarction (AMI). **Methods:** In 10 TC patients and 10 AMI patients, electrocardiogram, echocardiography, cardiac catheterization were conducted, and plasma catecholamines and urinary (U) 8-hydroxy-2'-deoxyguanosine (8-OHdG) as a marker of oxidative DNA damage were taken for one week from onset. **Results:** On admission, the coronary sinus (CS) had significantly higher norepinephrine (NE) and 8-OHdG levels than the aortic root (Ao) and peripheral blood vessels. Circulating catecholamines in TC patients tended to be higher than those in AMI patients; especially, peripheral plasma NE levels (day 1) in patients with TC were significantly higher than those in patients with AMI. TC patients had elevated U-8-OHdG in parallel with elevated NE, whereas, AMI patients had U-8-OHdG level that decreased to almost normal range within one week. On day 1, in patients with TC, serum 8-OHdG differences (CS vs Ao) and U-8-OHdG increased proportionally with the wall-motion score as an index of LV dysfunction, whereas serum NE (CS vs Ao) did not. **Conclusion:** Myocardial oxidative stress induced by cardiac sympathetic hyperactivity may play a critical role in transient LV dysfunction in TC.

Key words: takotsubo cardiomyopathy, oxidative stress, catecholamine

Introduction

Takotsubo cardiomyopathy (TC) is characterized by transient cardiac dysfunction, commonly triggered by physical or emotional stress.[1-3] Emotional stress activates the autonomic nervous system causing norepinephrine (NE) release from postsynaptic sympathetic neurons and epinephrine secretion from the adrenal medulla.[1,4,5] Based on evidence that catecholamine levels in TC were several times higher than those in acute myocardial infarction (AMI), Wittstein et al. reported that catecholamine toxicity might be central to TC pathophysiology.[1] However, little is known about the molecular mechanism by which catecholamine toxicity induces transient left ventricular (LV) dysfunction. Here, we hypothesized that a marked increase of catecholamines in cardiac tissue causes enhanced production of reactive oxygen species (ROS) in cardiomyocytes, leading to transient LV dysfunction. In the present study, we investigated how myocardial oxidative stress (OS) associated with sympathetic hyperactivity influenced myocardial dysfunction in

patients with TC in comparison to patients with anteroseptal AMI.

ROS attack nuclear and mitochondrial DNA, oxidizing the 2'-deoxyguanosine base molecule to 8-hydroxy-2'-deoxyguanosine (8-OHdG). This 8-OHdG is cleaved from the DNA and excreted via the blood and urine.[6-9] The concentration of 8-OHdG in blood and urine can be measured using an enzyme-linked immunosorbent assay with an anti-8-OHdG antibody.[6-8] In the present study, we clarified the relationship between sympathetic hyperactivity and myocardial OS in patients with TC.

Methods

Study patients

Ten patients with TC with chest pain or symptomatic heart failure precipitated by acute emotional stress were enrolled. The clinical features of these patients were compared with those in 10 patients with AMI. All patients were admitted to the coronary care unit of Yamaguchi University Hospital between April 2009 and March 2015. Ten patients with AMI had acute chest pain or an acute chest oppressive sensation, and immediately, percutaneous coronary intervention for the left anterior descending branch (No.6 or No.7) was performed. All patients were excluded if they had inflammatory disease, malignancy, or severe renal disease (>2.0 mg/mL creatinine), or if they were a smoker.

Informed consent was obtained from all patients, and the protocol was approved by the Institutional Review Board of Yamaguchi University School of Medicine. All procedures were conducted in line with The Code of Ethics of the World Medical Association (Declaration of Helsinki).

The TC patients were admitted to our hospital within 24 hours from the onset of chest pain or symptomatic heart failure precipitated by acute emotional stress. The diagnosis of TC was based on the Mayo Clinic guidelines (Table 1).[10] All patients underwent coronary angiography. Concomitantly, left ventriculography was performed in 10 patients with TC and right-heart catheterization in 6 TC patients, blood sampling from the coronary sinus (CS) and aortic root (Ao) to measure plasma catecholamines and serum 8-OHdG in 8 patients. All patients underwent two-dimensional transthoracic echocardiography within 24 hours after the onset of symptoms and again on days 7, and, if possible, 14 of their hospital stay. Wall-motion abnormalities on echocardiography were assessed using a standard 16-segment model with numerical scores of contractile function as follows: 1, normal contraction; 2, mild hypokinesis; 3, severe hypokinesis; 4, akinesis; and 5, dyskinesis.[11]

Measurements of levels of peripheral vein plasma catecholamines, brain natriuretic peptide, and creatine phosphokinase, and a urinary oxidative stress marker

In patients, plasma catecholamines and urinary (U) 8-OHdG were measured on days 1, 3, 7, and, if possible, 14 of the hospital stay. The AMI (New York Heart Association [NYHA] III or IV) patients were chosen as comparators because they had similar clinical

Table 1 Mayo Clinic criteria for TC

1. Transient hypokinesis, akinesis, or dyskinesis in the left ventricular mid segments with or without apical involvement; regional wall-motion abnormalities that extend beyond a single epicardial vascular distribution; and frequently, but not always, a stressful trigger.
2. The absence of obstructive coronary disease or angiographic evidence of acute plaque rupture.
3. New electrocardiogram abnormalities (ST-segment elevation and/or T-wave inversion) or modest elevation in cardiac troponin.
4. The absence of pheochromocytoma and myocarditis.

presentations and were expected to have high a sympathetic tone. Blood samples were placed on ice and immediately centrifuged, and the plasma was flash-frozen. Serial measurements of CK and CK-MB (the bound combination of the two isoenzymes of CK) were performed to obtain the maximum CK(CK-MB). Plasma catecholamines were measured using high-performance liquid chromatography, and brain natriuretic peptide (BNP) was measured using an enzyme immunoassay or radioimmunoassay.[6,7]

Statistical analyses

All results are expressed as the mean ± standard deviation (SD) or standard error (SE). Categorical variables are presented as frequency counts and percentages, and intergroup comparisons were analyzed using the χ^2 test. Because BNP, U-8-OHdG, the differences between the groups were detected using the Mann-Whitney U test with 2-tailed *P* values significant at <0.05. The Wilcoxon signed-rank test was used to compare left ventricular ejection fraction (LVEF) and echocardiographic scores in patients with TC at various times. A *P* value <0.05 was considered significant.

Results

Table 2 depicts the clinical characteristics of patients with TC. Coronary angiography showed that the coronary artery was intact without vasospastic findings or delayed flow in all patients with TC. All patients showed apical ballooning on left ventriculography. The LVEF profile in TC shows that the decreased LVEF (44.6 ± 7.9%) noted on day 1 of the hospital stay was restored to almost normal levels within 2 weeks (Fig. 1).

Table 3 showed the medication for TC patients after admission. A β-blocker was administrated after admission in 90% TC patients in order to protect the heart from excess catecholamines. Inotropes were not necessary for the treatment of congestive heart failure in all TC patients.

Table 4 shows the comparison of clinical parameters between patients with TC and AMI. We could not perform sex-matching between the groups, but there were no significant differences between them in terms of age, BMI, blood pressure, NYHA class, LVEF, BNP, or creatinine levels. Serum CK and CK-MB in the AMI group were significantly higher compared with those in the TC group (both P <0.05). On day 1, the peripheral blood plasma catecholamines and U-8-OHdG levels were higher in the TC group compared with those

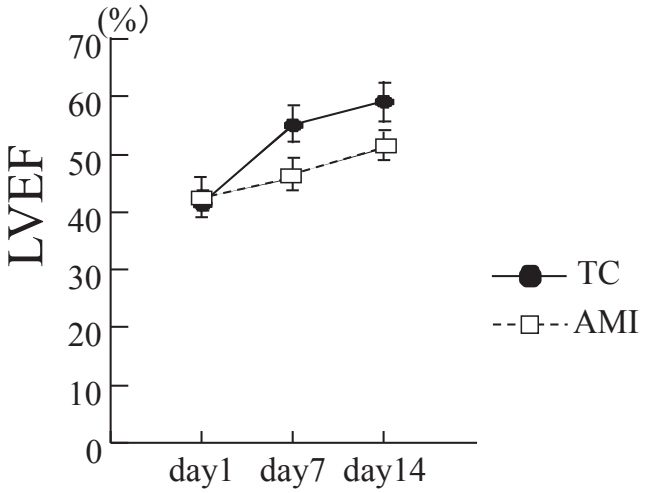

Fig. 1 Time course of LVEF in patients with TC and AMI
Echocardiography was performed on admission (day 1), and on days 7, and 14 (outpatients) after onset.
Graph represents the mean (SE).

Table 2 Clinical characteristics of TC

Patient No.	Age (Years)	Sex	Emotional Stressor	Clinical Presentation				Right-heart catheterization		Blood sampling from CS and Ao	ECG abnormality	Defect size on dual scintigraphy
				Time after onset (hours)	HR (bpm)	SBP (mmHg)	Symptoms	Pcw (mmHg)	CI (1/min/m^2)			
1	77	F	Family trouble	<12	76	152	Chest pain	15	4.1	+	GNTW	TI<MIGB
2	79	F	Family cancer	<24	128	200	Dyspnea	28	1.4	+	STE	TI<MIGB
3	77	F	Money	<24	82	160	Dyspnea	13	2.8	+	STE	TI<MIGB
4	76	F	Family trouble	<12	104	92	Chest discomfort	25	1.97	+	STE	TI<MIGB
5	74	F	Fear of illness	<24	88	120	Heart failure	ND	ND	+	NTW	TI<MIGB
6	76	F	Son's divorce	<3	90	80	Dyspnea	16	2.5	+	STE	TI<MIGB
7	73	F	Family trouble	<1	115	220	Dyspnea	ND	ND	+	STE	ND
8	72	F	Family death	<24	117	90	Heart failure	14	2.7	+	STE	TI<MIGB
9	54	F	Car accident	<24	83	154	Chest pain	ND	ND	ND	STE	TI<MIGB
10	70	F	Family altercation	<6	80	118	Chest pain	ND	ND	ND	STE	ND

HR, heart rate; SBP, systolic blood pressure; Pcw, pulmonary capillary wedge pressure; CI, cardiac index; CS, coronary sinus; Ao, aorta; ECG, electrocardiography; F, female; ND, not detected; GNTW, giant negative T wave; STE, ST elevation; NTW, negative T wave; Tl, Tl^{201} scintigraphy; MIBG, I^{123} meta-iodobenzylguanidine scintigraphy

Table 3 Medication for TC patients after admission

medication	TC (n=10)
β-blocker	9
ACEI/ARB	7
Ca2+blocker	2
diuretics	5
statine	5

ACEI, angiotensin-converting enzyme; ARB, angiotensin receptor blocker

Table 4 The comparison of baseline characteristics between TC and AMI

	TC (n = 10)	AMI (n = 10)	P	
Age (years)	72.5 ± 6.6	65 ± 10.4	0.129	ns
Sex (M/F)	0/10	8/2	0.000	<0.05
BMI (kg/m2)	22.1 ± 5.4	22.2 ± 3.3	0.821	ns
NYHA class	3.1 ± 0.32	3.3 ± 0.48	0.276	ns
HR (bpm)	96.3 ± 18.3	79.6 ± 13.1	0.028	<0.05
SBP (mmHg)	138.6 ± 47.2	136.9 ± 28.9	0.880	ns
DBP (mmHg)	83.9 ± 35.2	77.4 ± 16.9	0.677	ns
LVDd (mm)	43.2 ± 7.4	48.1 ± 3.1	0.063	ns
LVEF (%)	44.6 ± 7.9	42.2 ± 8.2	0.789	ns
Peak CK (IU/L)	238.8 ± 152.1	2898 ± 2244	0.001	<0.05
Peak CK-MB (IU/L)	28.9 ± 20.8	250.4 ± 218.7	0.001	<0.05
Hb (g/dL)	13.2 ± 1.2	13.6 ± 2.2	0.597	ns
BS (mg/dL)	190.7 ± 93.3	152.4 ± 57.7	0.427	ns
TC (mg/dL)	193 ± 32.6	169.5 ± 28.3	0.102	ns
Peak BNP (pg/mL)	443.2 ± 332.5	281.8 ± 277.8	0.112	ns
Cr (pg/mL)	0.65 ± 0.29	0.80 ± 0.32	0.289	ns
Na (mmol/L)	138.6 ± 2.9	138.3 ± 3.3	1.000	ns
K (mmol/L)	3.8 ± 0.45	3.85 ± 0.52	0.879	ns
NE (pg/mL)	1500 ± 800.7	728 ± 332.7	0.049	<0.05
Epinephrine(pg/mL)	158.1 ± 156.4	83.714 ± 126.1	0.105	ns
Dopamine (pg/mL)	42.3 ± 36.7	24 ± 12.7	0.450	ns
U-8-OHdG (ng/mg Cr)	61.0 ± 26.8	21.6 ± 13.4	0.002	<0.05
HT	8/10	7/10	0.500	ns
DM	1/10	2/10	0.500	ns
DL	6/10	5/10	0.500	ns

Numerical data are expressed as mean ± SD.
BMI, body mass index; NYHA, New York Heart Association; HR, heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; LVDd, left ventricular end-diastolic dimension; LVEF, left ventricle ejection fraction; CK, creatinine kinase; Hb, hemoglobin; BS, blood sugar; TC, total cholesterol; BNP, brain natriuretic peptide; Cr, creatinine; Na, sodium; K, potassium; NE, norepinephrine; U-8-OHdG, urinary 8-hydroxy-2'-deoxyguanosine; HT, hypertension; DM, diabetes mellitus; DL, dyslipidemia; ns, not significant,
Catecholamines were measured by high performance liquid chromatography (SRL, Inc, Tokyo, Japan); Normal range: NE 100-450 pg/mL, Epinephrine 0-100 pg/mL, Dopamine 0-20 pg/mL.
Normal range of U-8-OHdG is under 10 ng/mg Cr adapted from reference No.6.

in the AMI group. In addition, there were no differences in coronary risk factors such as hypertension, diabetes mellitus, or dyslipidemia between the groups.

Fig. 2A shows the blood sampling data from the CS and Ao in patients with TC. NE concentration in the CS was significantly higher than that in the Ao, whereas the epinephrine and dopamine concentrations were not different between the CS and Ao, suggesting that NE was released from cardiac tissues. Fig. 2B shows that the serum 8-OHdG levels in the CS were significantly higher than those in the Ao, indicating that 8-OHdG was released from the cardiac tissue.

Fig. 3 shows the profiles of peripheral plasma NE, epinephrine, and dopamine, and the U-8-OHdG within a week of onset. On day 1, peripheral NE concentrations were significantly higher in the TC group compared with the AMI group (Fig. 3A). By contrast, the maximum CK concentration was

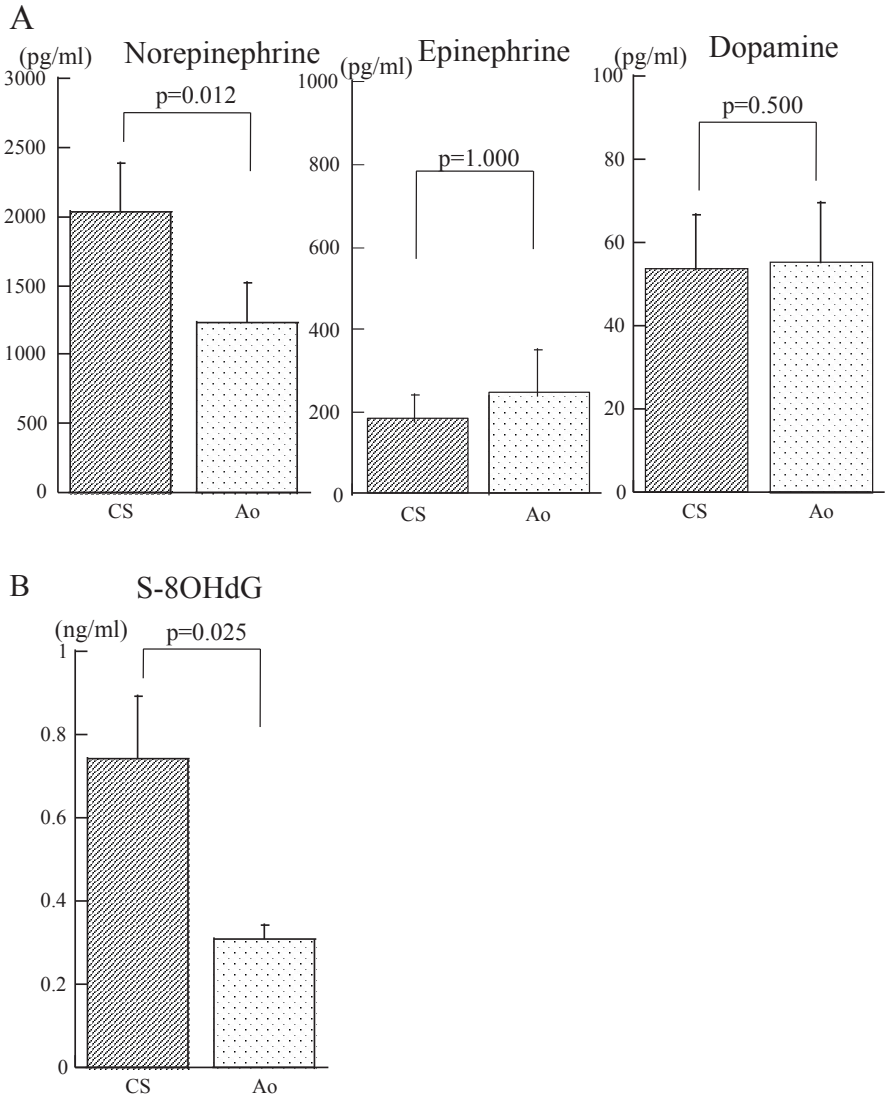

Fig. 2 Blood sampling data from the coronary sinus (CS) and aorta root (Ao) in TC patients

A: NE level in the CS was significantly higher than that in the Ao, epinephrine and dopamine levels were not significantly different between the CS and Ao.

B: Serum 8-OHdG level in the CS was significantly higher than that in Ao.

NE;norepinephrine

Each bar graph represents the mean (SE).

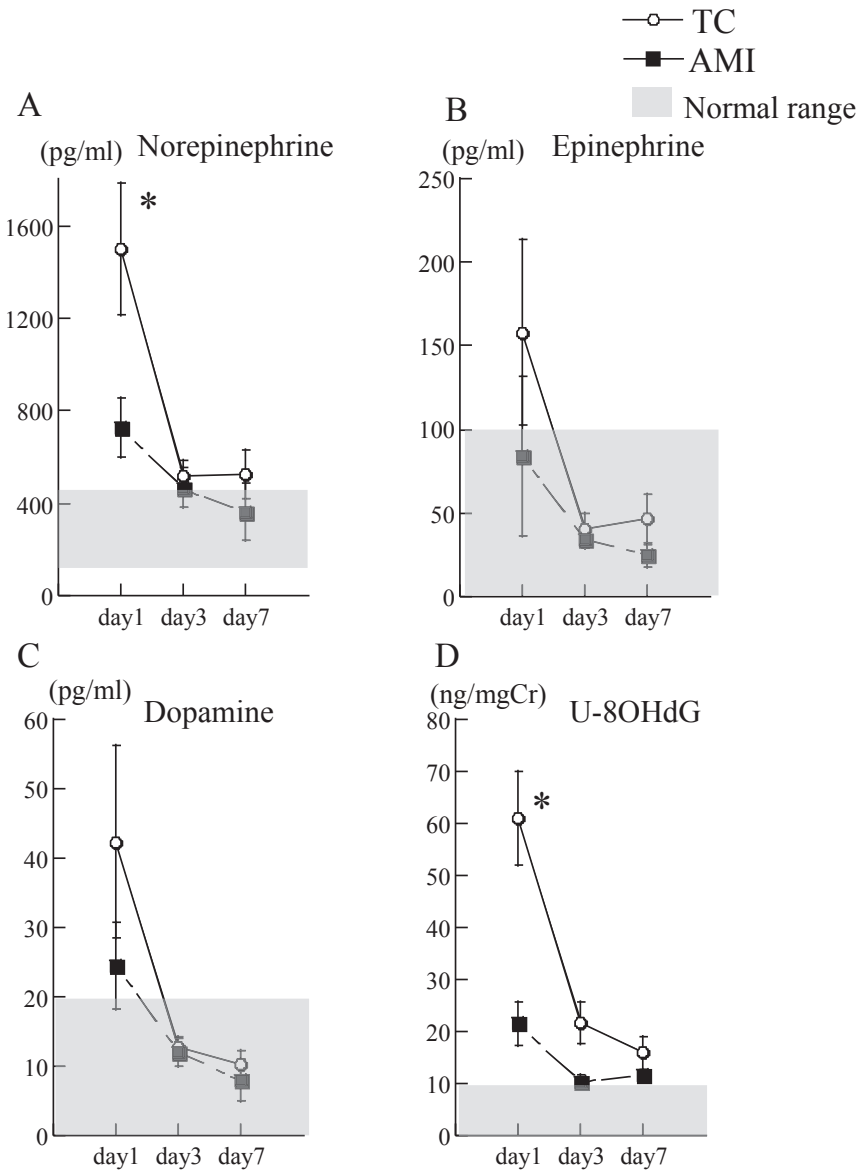

Fig. 3 Peripheral plasma catecholamines and U8-OHdG profiles within a week in patients with TC and AMI

A, B, and C. On day 1, plasma NE was significantly higher in the TC group compared with the AMI group (A). Although plasma epinephrine (B) and dopamine (C) in the TC group tended to be higher those in the AMI group, the differences were not significant at any stage. The elevated plasma NE, epinephrine, and dopamine levels on day 1 decreased gradually until day 7. * $P<0.05$ vs AMI.

NE;norepinephrine, TC;takotsubo cardiomyopathy, AMI;acute myocardial infarction

Each graph represents the mean (SE).

Catecholamines were measured by high performance liquid chromatography (SRL, Inc, Tokyo, Japan); Normal range: NE 100-450 pg/mL, Epinephrine 0-100 pg/mL, Dopamine 0-20 pg/mL

D. On day 1, U-8-OHdG was significantly higher in the TC group compared with that in the AMI group. The marked increase in U-8-OHdG level on day 1 decreased gradually in parallel with NE levels. * $P<0.05$ vs AMI.

Graph represents the mean (SE).

Normal range of U-8-OHdG is under 10 ng/mg Cr adapted from reference No.6.

significantly lower in the TC group compared with the AMI group. On days 3 and 7, there was no difference in the NE levels in the TC and the AMI groups. (Fig. 3A). The epinephrine and dopamine profiles were similar to that of NE but did not differ significantly between the groups (Fig. 3B-3C). U-8-OHdG in the TC group was elevated in parallel with the profile of plasma NE (Fig. 3D).

Echocardiography showed that the LVEF in most of the patients with TC improved within 2 weeks of onset (Fig. 1). Interestingly, in patients with TC on day 1, differences in serum 8-OHdG (CS vs. Ao) and U-8-OHdG increased proportionally with wall-motion scores as an index of LV dysfunction (R = 0.93, P <0.001), while differences in serum NE levels (CA vs. Ao) did not (Fig. 4).

Discussion

General overview

The most important aspect of this study is that a surge of catecholamines associated with hypersympathetic activity causes direct myocardial injury due to intracellular ROS production. This is the first clinical report to demonstrate the relationship between hypersympathetic activity and myocardial OS in patients with emotional stress-induced TC. This notion is supported by the following findings. 1) Circulating catecholamines in patients with TC tended to be higher than those in patients with AMI; especially, peripheral plasma NE levels in patients with TC were significantly higher than those in patients with AMI. 2) Serum NE and 8-OHdG levels in the CS were significantly higher than those

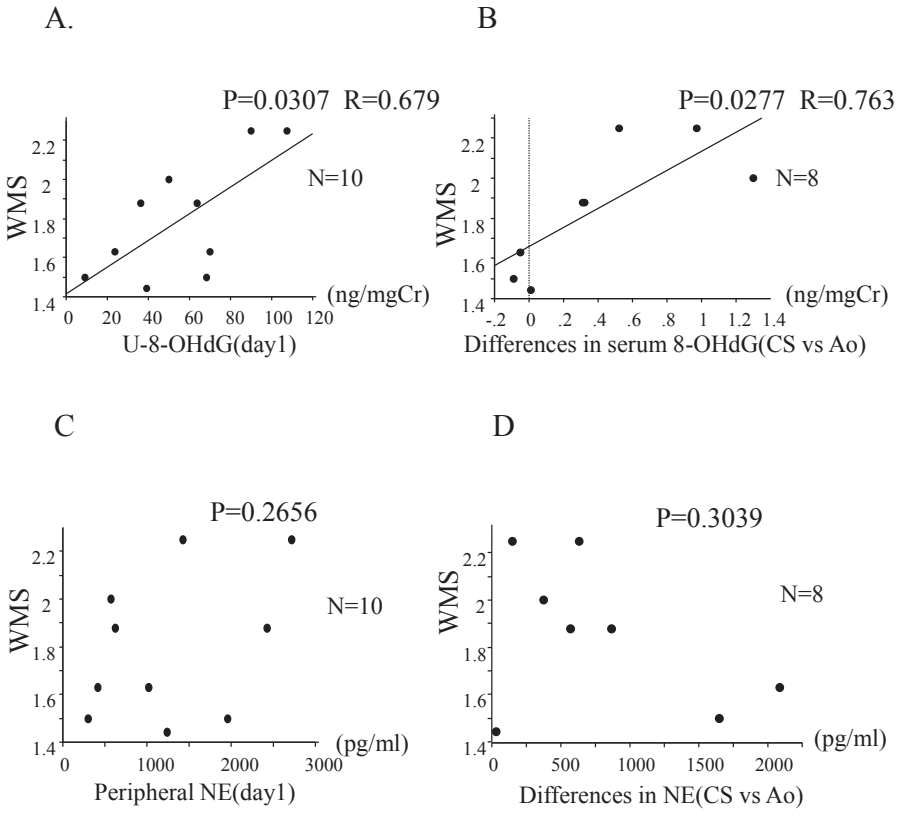

Fig. 4 Relationship between LV dysfunction and myocardial OS

A, B. In the TC group on day 1, the U-8-OHdG and the differences in serum 8-OHdG (CS vs. Ao) increased proportionally with the wall-motion score (R = 0.679, p <0.05 and R = 0.763, p <0.05, respectively).

C, D. Peripheral NE and the differences in NE (CS vs Ao) did not correlate with wall-motion scores.

OS;oxidative stress, TC;takotsubo cardiomyopathy, CS;coronary sinus, Ao; aortic root

in the Ao. 3) In patients with TC, U-8-OHdG was elevated in parallel with plasma catecholamines, whereas, in patients with AMI, it decreased to almost normal range within one week. 4) In patients with TC, U-8-OHdG levels as well as differences in serum 8-OHdG levels (CS vs. Ao) increased proportionally with wall-motion scores as an index of LV dysfunction, while those for serum NE did not.

Local catecholamine release from sympathetic nerve endings in cardiac tissue as well as circulating catecholamines may initiate transient myocardial injury

In the present study, in patients with TC, plasma NE level in the CS was higher than that in the Ao, whereas plasma epinephrine and dopamine were not. In addition, on the first admission day, peripheral vein plasma NE levels were significantly higher in patients with TC when compared with those in patients with AMI. Catecholamine profiles also showed that peripheral vein epinephrine and dopamine levels were higher in patients with TC compared with those in patients with AMI. These findings were congruent with those from a previous study.[1]

The mechanism by which excess catecholamines caused transient left ventricle dysfunction

Excess catecholamines cause intracellular Ca^{2+} overload, leading to cardiomyocyte injury through activation of cyclic adenosine monophosphate-mediated protein kinase A.[12] One mechanism of catecholamine-induced intracellular Ca^{2+} overload is attributed to diastolic Ca^{2+} leak via the ryanodine receptor channel on the sarcoplasmic reticulum.[12-18] Experimental studies have demonstrated how excessive intracellular Ca^{2+} leads to the mitochondrial production of intracellular ROS via the action of nicotinamide adenine dinucleotide phosphate-oxidase.[13] Excessive catecholamine release would, therefore, enhance ROS generation, interfering with Na^{+} and Ca^{2+} transporters, and possibly causing myocyte dysfunction through increased transsarcolemmal Ca^{2+} influx and overload.[12-18] Taken together, this would indicate that excessive catecholamine release is profoundly associated with intracellular Ca^{2+} overload and myocardial OS. Thus, catecholamine release would likely contribute to the ROS - Ca^{2+} overload interaction to cause LV dysfunction. Histologically, in diseases such as pheochromocytoma catecholamine excess has been associated with contraction band necrosis,[19] which might occur subsequent to the intracellular Ca^{2+} overload.[20, 21]

Do urinary 8-OHdG levels reflect myocardial oxidative stress?

U-8-OHdG is not a cardiac-specific marker. However, in the present study, blood sampling data from the CS and Ao strongly suggested that cardiac tissue was a major source of U-8-OHdG. In addition, we previously reported that 8-OHdG was produced in cardiac diseases such as dilated cardiomyopathy, old myocardial infarction, and cardiac sarcoidosis.[3,6,7] This suggested that U-8-OHdG levels reflected clinical myocardial OS. The reason being that cardiac cells are enriched with mitochondria, and, therefore, there is an abundance of mitochondrial DNA that can be oxidized easily, subsequently increasing the levels of 8-OHdG in the blood and urine.

Other molecular mechanisms in TC

Several factors underlying TC pathogenesis other than sympathetic hyperactivation have been proposed including coronary vasospasm, microcirculatory disorders, and a lack of estrogen.[22-25] In the present study, coronary angiography was performed within 24 hours of onset. However, there was no evidence of epicardial coronary spasm and no delay of coronary flow in any patients. Therefore, there was scarce possibility that epicardial coronary spasm and microvascular injury were involved in the TC pathophysiology in the present study. Because all of the patients in the TC group were postmenopausal women, there is a possibility that low estrogen levels might have been involved in TC pathophysiology. Recently, epinephrine stimulation of the β2-receptor has been advocated as a novel signal-switching mechanism,[26,27] therefore, dense distribution of the β2-receptor in the cardiac apex could play a key role in TC. High epinephrine levels could trigger signaling-switching of the β2-receptor from

stimulatory G-protein to inhibitory G-protein and thereby trigger typical apical systolic dysfunction. Such signal-switching might have contributed to transient LV apical dysfunction in the present study, although it is impossible to demonstrate signal-switching in the clinical setting.

Limitations

The present study has several limitations. First, the cohort size was small, and a larger study is warranted to confirm whether transient LV dysfunction is attributed to myocardial OS associated with sympathetic hyperactivity. Second, sex-matching between the groups could not be performed owing to the greater percentage of females in the TC group. However, this might not affect the findings of the present study because a previous large population study indicated that there was no significant sex-bias for U-8-OHdG levels.[28]

Conclusion

A marked increase in catecholamine-induced myocardial OS might be related to transient LV dysfunction in patients with TC. Monitoring U-8-OHdG levels in these patients might provide relevant functional and tissue information that could help to resolve the pathophysiology of TC.

Acknowledgements

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Conflict of Interest

The authors state no conflict of interest.

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