Possible Participation of Endothelial Cell Apoptosis of Coronary Microvessels in the Genesis of Takotsubo Cardiomyopathy

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ABSTRACT

Background: Takotsubo cardiomyopathy (TCM) is characterized by systolic ballooning of the left ventricular apex. It is triggered by emotional or physical stress, but the exact mechanism through which stress leads to TCM is not known.

Hypothesis: Coronary microvessel apoptosis is the missing link between stress and TCM.

Methods: In 8 female patients with TCM, plasma catecholamines, Thrombolysis in Myocardial Infarction (TIMI) coronary flow grade and myocardial perfusion grade, and apoptosis of the coronary microvessels in the biopsied myocardial specimen by terminal deoxynucleotidyl transferase-mediated nick end-labeling (TUNEL) were examined.

Results: Plasma epinephrine and norepinephrine were increased to 663 ± 445 and 875 ± 812 pg/mL (mean $\pm$ SD), respectively. Acetylcholine-induced delayed myocardial perfusion through the ballooning apical segment without flow disturbance in the epicardial coronary arteries (indicating microvessel spasm) and focal myocardial necrosis were observed in all subjects. Apical ballooning disappeared and myocardial perfusion delay was not inducible 1 month later. The number of vessels having apoptotic endothelial cells/10 vessels in arterioles, venules, and capillaries at initial biopsy and repeat biopsy 1 month later were 8.3 ± 1.4 vs 0.4 ± 1.1 , $P < 0.0001$; 6.8 ± 1.8 vs 0.3 ± 0.7 , $P < 0.0001$; and 7.9 ± 1.0 vs 0.5 ± 0.9 , $P < 0.0001$, respectively.

Conclusions: Left ventricular apical ballooning in TCM was considered to be caused by coronary microvessel spasm due to catecholamine-induced endothelial cell apoptosis and myocardial stunning after release of microvessel spasm. Endothelial cell apoptosis of coronary microvessel is therefore considered to be the missing link between stress and TCM.

Introduction

Takotsubo cardiomyopathy (TCM) is a recently detected type of cardiac disorder in which symptoms and signs mimic acute myocardial infarction, but in which coronary lesions are not observed. It manifests as ballooning of the apical segment and augmented contraction of the middle-to-basal segments of the left ventricle during systole that recovers rapidly.¹ From its configuration by ventriculography, this phenomenon was named “Takotsubo cardiomyopathy” (Takotsubo is the Japanese word for “octopus pot,” a pot used to catch octopuses).^{1,2}

Since it was first recognized in 1991, TCM has gained increasing attention worldwide. TCM is a well-recognized cause of acute heart failure,³ lethal arrhythmias,⁴ and ventricular rupture.⁵ TCM tends to occur in postmenopausal women^{1,2} and is triggered by preceding emotional²

or physical stress.^{6,7} Coronary spasm,¹ catecholamine cardiotoxicity,⁸ and neurogenic stunned myocardium^{9,10} have been proposed as causative mechanism(s).

The link that connects stress and TCM, and the optimal management of this disease, remain unknown. The aim of the present study was to investigate the link that connects stress and TCM.

Methods

All patients provided informed consent with regard to the procedures. This prospective study protocol was approved by the ethical committees of Toho University Medical Center Sakura Hospital (Sakura, Japan) and Funabashi-Futawa Hospital (Funabashi, Japan).

Plasma Catecholamine Measurement

Eight female patients with TCM (mean $\pm$ SD age, 65.0 ± 10 y, Table 1A) were admitted to Toho University Sakura Hospital and Funabashi-Futawa Hospital. On admission,

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plasma epinephrine (normal range, <100 pg/mL) and norepinephrine (normal range, 100 to 410 pg/mL) were measured immediately after apical ballooning of the left ventricle was suspected by ultrasonography. Subsequently, left ventriculography, endomyocardial biopsy, coronary angiography, and a coronary spasm provocation test were performed.

Left Ventriculography, Endomyocardial Biopsy and Coronary Angiography

Patients were pretreated with oral diazepam (10 mg) before being transferred to the catheterization laboratory. The procedures mentioned above were carried out after IV administration of 50 mg xylocaine and 5000 IU heparin.

Coronary Angiography and Left Ventriculography

Coronary angiography was performed by introducing a 6F or 8F Judkins catheter through the right femoral artery. A 6F or 8F pigtail catheter was then introduced into the left ventricle for ventriculography.

Endomyocardial Biopsy

After left ventriculography, a 6F biptome was introduced into the apical segment of the left ventricle, where systolic ballooning was observed. Three myocardial specimens were obtained for each TCM patient. These specimens were immersed in 10% formaldehyde solution for fixation. Details of the endomyocardial biptomes and techniques are described elsewhere.¹¹

Table 1. Backgrounds of Patients With Takotsubo Cardiomyopathy

A. Triggers and Symptoms					
Case	Gender	Age (y)	Time From Symptom Onset	Trigger	Symptom
1	F	76	1 h	Death of sister	Chest pain
2	F	45	3 h	Gastroscopy	Dyspnea
3	F	66	12 h	Death of spouse	Chest pain
4	F	64	15 h	Financial	Chest pain
5	F	71	1 d	None	Epigastralgia
6	F	66	1 d	Relationship problems	Dyspnea
7	F	79	1 d	None	Epigastralgia
8	F	58	2 d	Illness of spouse	Chest pain
B. Electrocardiography and Plasma Catecholamines					
				Epinephrine/Norepinephrine (pg/mL) ^a	
				Initial	1 Mo
Case	Electrocardiography on Admission				
1	ST elevation in II, III, aVF, V ₁ through V ₆			1200/2453	230/171
2	ST elevation in II, III, aVF, V ₁ through V ₅			970/103	110/120
3	ST elevation in II, III, aVF, aVL, V ₁ through V ₆ ; abnormal Q in II, III, aVF, V ₁ through V ₃			1105/1710	200/110
4	ST elevation and negative T in II, III, aVF, V ₁ through V ₆			819/900	130/132
5	Abnormal Q and negative, T in II, III, aVF, V ₁ through V ₄			670/777	53/204
6	Abnormal Q and negative T in II, III, aVF, V ₁ through V ₅			402/370	101/116
7	Negative T in II, III, aVF, V ₁ through V ₅			34/400	89/210
8	Abnormal Q and negative T in II, III, aVF, V ₁ through V ₅			100/290	104/180
Abbreviations: F, female; Q, Q wave; T, T wave.					
^a Normal levels of plasma epinephrine and norepinephrine are <100 pg/mL and 100 to 450 pg/mL, respectively.					

Coronary Spasm Provocation

Acetylcholine (up to 100 µg into the right coronary artery and up to 200 µg into the left coronary artery) was selectively injected into the coronary artery to provoke spasm.

The Thrombolysis in Myocardial Infarction (TIMI) myocardial perfusion grade was examined using angiograms obtained before and after acetylcholine administration. The grading was as follows: grade 0, minimal or no myocardial blush by intracoronary contrast material; grade 1, contrast material stains the myocardium, and this stain persists on the next injection; grade 2, contrast material enters the myocardium but washes out slowly; and grade 3, normal entrance and exit of contrast material¹² in the ballooning apical segment.

Also, the TIMI coronary flow grade was examined before and after acetylcholine administration. The grading was: grade 0, no perfusion; grade 1, penetration without perfusion; grade 2, partial perfusion; and grade 3: complete perfusion.¹³

The coronary angiography, left ventriculography, endomyocardial biopsy, and spasm provocation test were repeated 1 month later. All patients were thereafter treated with oral aspirin (81 mg/d), nicorandil (a hybrid of nitrate and potassium channel opener; 15 mg/d), and a diuretic when necessary.

Histological Examinations

Biopsy specimens were cut into successive slices (thickness, 5 µm). One slice was stained with hematoxylin and eosin (H&E). The neighboring slice was used for staining of apoptotic cells by terminal deoxynucleotidyl transferase-mediated nick end-labeling (TUNEL), in which cell nuclei having fragmented DNA were stained in brown-black and normal nuclei were stained in green.^{14,15}

Five to 10 slices from 3 specimens per patient were used for counting the number of vessels having apoptotic cells and those without apoptotic cells. The number of arterioles, venules, or capillaries having apoptotic cells per 10 vessels and the number of apoptotic cardiomyocytes (CMs) per 10 CMs were counted.

Statistical Analysis

Data are presented as the mean ± SD. The paired Student *t* test was used for comparison between initial and follow-up endomyocardial biopsies. A *P* value <0.05 was considered significant.

Results

Catecholamines

Plasma epinephrine and norepinephrine levels on admission were 740 ± 137 and 992 ± 265 pg/mL, respectively (Table 1B).

Left Ventriculography

Figure 1 is a representative example of the appearance of left ventriculography. Left ventriculography showed systolic ballooning of the apical segment and augmented contraction of the mid-to-basal segments, presenting a typical “octopus pot” (Takotsubo) configuration. All 8 patients showed similar left ventricular dyssynergy. This dyssynergy was not observed in any patients 1 month later.

Coronary Angiography

Significant coronary stenosis (≥75% diameter) was not observed in any TCM patients. Intracoronary administration of acetylcholine provoked spasm of the left anterior descending coronary artery in 3 subjects, in the first diagonal branch in 2 subjects, and in the second diagonal branch in 1. Spasm of the distal segment of the left anterior descending artery that irrigated the ballooning left ventricular apical segment was not induced.

Before acetylcholine administration, TIMI myocardial perfusion through the ballooning apical segment was grade 1 in 1 patient and grade 2 in 2 patients; however, coronary flow was grade 3, indicating microcirculation disturbance without coronary flow disturbance in these patients.

Following acetylcholine administration in the other 5 patients, the myocardial perfusion grade changed from grade 3 to grade 2, whereas coronary flow remained at grade 3, also indicating microvessel spasm. One month later, acetylcholine administration failed to provoke spasm of the epicardial coronary artery, and myocardial perfusion was grade 3 before and after acetylcholine injection in all patients (Table 2).

Histology

Focal necrosis of the myocardium and infiltration of inflammatory cells at the initial biopsy, and focal myocardial fibrosis at biopsy 1 month later (characteristic of focal ischemia due to microvessel obstruction^{6,10}) were demonstrated by H&E staining in all patients (Figure 1). Figure 2 shows the histological changes obtained by H&E staining and by TUNEL. In this patient (case 1 in Table 1A), apoptotic ECs were observed not only in the arterioles, but also in venules and capillaries. Apoptotic CMs were also observed. Apoptotic arteriolar and venular smooth muscle cells were not seen. Extensive apoptosis of coronary microvessels was also observed in the other 7 patients at initial biopsy, but not at repeat biopsy 1 month later. Namely, the number of vessels having apoptotic ECs/10 vessels in arterioles, venules, and capillaries and the number of CMs/10 CMs at initial biopsy and at repeat biopsy 1 month later were 8.3 ± 1.4 vs 0.4 ± 1.1, *P* < 0.0001; 6.8 ± 1.8 vs 0.3 ± 0.7, *P* < 0.0001; 7.9 ± 1.0 vs 0.5 ± 0.9,

Figure 1. Ventriculographic and histological changes in a 78-year-old female with Takotsubo cardiomyopathy. A and B: Left ventriculograms at end diastole (ed) and end systole (es), respectively, taken 6 h after attack onset. Arrow in B: Typical ballooning of the apical wall segment. Arrowhead: Augmented contraction of the middle wall segment. C and D: Left ventriculograms at ed and es, respectively, taken 1 month later. E and F: Histological changes of the apical segment of the left ventricle in the same patient at initial biopsy and repeat biopsy 1 month later, respectively. Arrow in E: Focal myocardial necrosis and inflammatory cell infiltration. Arrow in F: Focal myocardial fibrosis. Horizontal bars in E and F: 20 μ m.

$P < 0.0001$, and 1.3 ± 0.5 vs 0.2 ± 0.5 , $P < 0.01$, respectively (Figure 3).

Discussion

Epicardial coronary artery spasm has been proposed as the causative mechanism of TCM,¹ but epinephrine and norepinephrine do not induce coronary spasm, while acetylcholine does clinically. The missing link between the stress, coronary spasm, and TCM thus remains unknown. We therefore investigated this missing link between stress and TCM, and observed extensive apoptosis of ECs of coronary microvessels and spontaneous or acetylcholine-induced myocardial perfusion disturbance localized to the ballooning apical segment of the left ventricle in TCM patients.

Spasm was induced by acetylcholine in epicardial coronary arteries in 3 patients in this study. However, the arteries did not irrigate the ballooning left ventricular apical segment. Therefore, it is unlikely that epicardial coronary spasm participated in the apical ballooning.

Significant coronary stenosis is not observed in approximately 15 percent of patients with acute coronary syndromes.^{16,17} Microvessel dysfunction is often observed in this category of patients. Microvessel obstruction with microemboli or endothelial dysfunction was considered to be a possible mechanism; however, this was without definite evidence.¹⁸ In the present study, microvessel emboli were not detected by endomyocardial biopsy. It is therefore unlikely that microvessel embolism participated in TCM.

Figure 2. Apoptotic endothelial cells (ECs) of coronary microvessels. The same patient depicted in Figure 1. A, B, C, and D: An arteriole, a venule, capillaries, and cardiomyocytes, respectively, stained by H&E. EC has a spindle-like nucleus, whereas smooth muscle cell has a round nucleus. a, b, c, and d: Apoptotic body stained black in the slices neighboring A, B, C, and D, respectively. Arrows: Apoptotic ECs. Numerals 1, 2, and 3 in B and in b: The same apoptotic ECs of a venule. Numerals 1 and 2 in C and in c: The same ECs of capillaries. Arrows in D and in d: The same apoptotic cardiomyocyte. Horizontal bars: 20 μ m. Abbreviations: H&E, hematoxylin and eosin; TUNEL, terminal deoxynucleotidyl transferase-mediated nick end-labeling.

Figure 3. Incidence of apoptotic endothelial cells (ECs) of coronary microvessels and apoptotic cardiomyocytes (CMs). ** $P < 0.01$ vs initial biopsy. **** $P < 0.0001$ vs initial biopsy.

Catecholamines and EC Apoptosis

Three different studies reported increased plasma epinephrine and/or norepinephrine levels in this category of patients^{10,19,20} as in the present study, whereas Madhavan et al observed no elevation of catecholamines.²¹ Uchida reported that plasma catecholamine levels were

dependent on the time from initiation of stress to blood collection.¹⁰

In this study, catecholamine levels were not elevated in 2 patients in whom the blood was collected 1 to 2 days after symptom onset, most likely due to delayed blood collection. It is well known that catecholamines induce

Table 2. Left Ventriculographic and Coronary Angiographic Changes

Case	Ventriculography		Coronary Angiography	
	LVEF (Initial/1 mo)	Spasm	TIMI Coronary Flow Grade (Initial/1 mo)	TIMI Myocardial Perfusion Grade (Initial/1 mo)
1	0.41/0.51	NI	3/3	2/3
2	0.33/0.49	I	3/3 ^a	1/3 ^a
3	0.35/0.55	I	3/3 ^a	1/3 ^a
4	0.28/0.47	I	3/3 ^a	2/3 ^a
5	0.39/0.50	NI	3/3	2/3
6	0.29/0.49	NI	3/3	2/3
7	0.43/0.57	NI	3/3	2/3
8	0.40/0.55	NI	3/3	2/3

Abbreviations: I, induced; LVEF, left ventricular ejection fraction; NI, not induced; TIMI, Thrombolysis in Myocardial Infarction. Case numbers are the same as those in Table 1.
^a Data were obtained before acetylcholine administration in cases 2, 3, and 4. Data were obtained after administration of acetylcholine in other cases.

apoptosis of vascular ECs via the catecholamine/ β -receptor/caspase-3 and catecholamine/Bcl-2/caspase cascades.^{22,23} Increased epinephrine and/or norepinephrine levels may have elicited apoptosis of ECs through these cascades in this study.

It is known that human vascular EC apoptosis occurs within 2 hours after application of the substances that cause apoptosis *in vitro*.²⁴ In this study, EC apoptosis was observed even in patients who underwent endomyocardial apoptosis a few hours after the symptom onset. It is therefore natural to assume that EC apoptosis occurred soon after the initiation of stress. CM apoptosis was not observed in a previous study in the acute phase of TCM,²⁵ whereas CM apoptosis was observed in this study. The reason for this difference is not known.

Mechanisms of Coronary Microvessel Spasm

Myocardial perfusion delay through the ballooning apical segment was observed without epicardial coronary flow disturbance in several patients before acetylcholine administration. Furthermore, myocardial perfusion delay, but not epicardial coronary flow disturbance, was induced by acetylcholine administration in other patients. All these findings indicate spontaneous or acetylcholine-induced microvessel spasm. This finding is in accordance with coronary microcirculation disturbance observed by Abdelmoneim using contrast echocardiography.²⁶ Wani et al observed reduced coronary flow reserve and suggested

participation of endothelial dysfunction in the reduced flow reserve; however, without definite evidence.²⁷

Coronary ECs produce nitric oxide and hyperpolarizing factor, which cause relaxation of smooth muscle and consequent coronary dilatation. A lack of these vasodilating substances makes the vessels prone to suffer from spasm.²⁸ In subarachnoid hemorrhage, it is known that persistent spasm develops in cerebral arteries having apoptotic ECs.²⁹ Lack of production of these vasodilating substances due to EC apoptosis may therefore have elicited persistent spasm of coronary microvessels (particularly arterioles) in patients in this study.

Mechanisms of Left Ventricular Apical Ballooning

Recovery from relatively long-lasting (≥ 20 min) myocardial ischemia results in calcium overload due to oxidative stress and consequent myocardial stunning ("ischemia-reperfusion stunning").^{30–32} The ventricular dyssynergy caused by this mechanism is reversible, as in the case of TCM. The microvessel relaxation caused by vasodilating substances (eg, H^+ , K^+ , and adenosine) released from the ischemic myocardium³³ may have eliminated spasm, resulting in myocardial stunning. This would have led to severe (but transient) left ventricular dyssynergy and pump dysfunction in TCM patients.

The apical segment of the left ventricle is thin and located in the most downstream part of the coronary blood supply. It is therefore very susceptible to ischemia. The degree of myocardial stunning may therefore have been the most severe in this segment, leading to systolic ballooning of this apical segment, showing a Takotsubo-like configuration. TCM with ballooning of other left ventricular wall segments does exist.³⁴ EC apoptosis probably occurred more severely in the irrigating microvessels. Thus, the results in this study suggest that targeting therapy of EC apoptosis is 1 choice for prevention and treatment of TCM.

Conclusion

The missing link between stress and TCM was investigated. Extensive apoptosis of ECs of coronary microvessels, focal myocardial necrosis, myocardial perfusion delay localized to the ballooning apical segment of the left ventricle, and elevated plasma catecholamines were observed in the acute phase of TCM. It was considered that: (1) the stress-induced increase in catecholamines caused apoptosis of ECs of coronary microvessels and the resultant microvessel spasm, and (2) release from spasm resulted in myocardial stunning by oxidative stress due to the ischemia-reperfusion mechanism, leading to left ventricular apical ballooning, showing a Takotsubo-like configuration of the left ventricle. Thus, EC apoptosis of coronary microvessels was hypothesized to be the missing link between stress and TCM.

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