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EDITORIAL COMMENT

Stress in a Dish

Exploring the Mechanisms of Takotsubo Syndrome*

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Takotsubo syndrome (TTS), also known as stress cardiomyopathy, is an acute heart failure syndrome first described by Hikaru Sato and colleagues in Hiroshima, Japan, 27 years ago (1). TTS is most commonly seen in postmenopausal women who present with chest pain and breathlessness after sudden emotional stress or in the context of a severe and stressful concomitant illness (2). Although initially the medical presentation of these patients is similar to that of patients with an acute myocardial infarction with chest pain, electrocardiographic changes, and troponin elevation, in modern health care rapid diagnostic coronary angiography excludes culprit coronary artery disease, and the diagnosis is usually made with left ventriculography. Left ventricular function in TTS is characterized by the striking appearance, with hypokinetic or akinetic apical and midventricular myocardium and preserved or hyperkinetic basal myocardium, leading to the end-systolic appearance likened to the "takotsubo" or Japanese fisherman's octopus pot. The acute course can be severe, with a recognized mortality rate of 4% to 5%, and up to 50% of patients with TTS have acute complications including pulmonary edema and cardiogenic shock (2). Most patients with TTS who survive make a good recovery, but 10% to 15% have chronic cardiac problems, including recurrent episodes of TTS, in which they enter the high-risk acute phase (3). Currently there is a lack of evidence-based treatments for these patients, both during the acute phase to rescue from cardiogenic shock and at follow-up to prevent recurrent episodes (2).

The pathophysiology of TTS is incompletely understood. The stressful trigger and TTS cases caused by epinephrine or dobutamine administration or pheochromocytomas suggest that catecholamines play a central role. Different hypotheses proposed include direct catecholaminergic myocardial stunning, multivessel coronary vasospasm, microvascular endothelial dysfunction, and abnormal ventriculoarterial coupling. Preclinical models reproduce the anatomic pattern of acute apical dysfunction with administration of high doses of epinephrine or isoproterenol (4,5). The basis of 1 hypothesis is the observation that although epinephrine is a positive inotrope at normal physiological levels, it induces a negative inotropic response at higher concentrations through the cardiac β-adrenoceptors (β-ARs), such as the concentrations to which the heart is exposed during extreme stress (6). This property results from a negative feedback mechanism whereby β_1-AR-dependent phosphorylation of the β_2-AR through protein kinase A (PKA) or G-protein receptor kinases (GRKs) uncouples the receptor from stimulatory Gs signaling and increases coupling to the inhibitory Gi protein. This molecular switch, initiated by only the highest epinephrine doses, is known as stimulus trafficking or biased agonism. Although it is negatively inotropic, it is also cardioprotective because β_2-AR-Gi activates an antiapoptotic signaling cascade and thus may minimize cardiac toxicity during acute severe stress. Indeed, activation of antiapoptotic pathways is reported in endomyocardial biopsy specimens from TTS cases during the acute phase (7). The apical myocardium has a higher density of β_1-ARs and β_2-ARs, a finding that explains the apical hypokinesia in most cases (6). In experimental models, inhibiting the Gi pathway blocked the development of

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the TTS phenotype, but at the cost of increased mortality consistent with β_2-AR-Gi signaling imparting a cardioprotective effect (4,5). How this mechanism contributes to the development of TTS in humans remains to be determined, but an interesting observation was the increased frequency of the GRK5 L41Q polymorphism in patients with TTS in 1 cohort (8). This polymorphism increases GRK5 kinase activity, results in increased β_2-AR-Gi coupling, and imparts a survival advantage in human patients with heart failure (9). However, the higher frequency of GRK5 L41Q was not reproduced in a second TTS cohort (10).

TTS is most commonly seen in postmenopausal women following a stressful episode. This setting suggests that age, loss of estrogen with its sympatholytic properties, and the environmental stressor are all potentially necessary factors for TTS, although the syndrome can occur in men, younger women, and spontaneously without a stressful trigger. Several groups of investigators have explored a genetic basis, or predisposition to TTS, and have reported variable results in targeted sequencing of the main candidate genes including α_1-AR, β_1-AR, β_2-AR, GRK5, estrogen receptors, and various microRNAs. However, these studies have been limited by relatively small cohorts, a priori hypotheses, and sequencing technologies applied, and no consistent result has emerged.

Inducible pluripotent stem cells (iPSCs) are a more recent scientific platform for studying the cellular pathophysiology of human disease. Since the first report by Shinya Yamanaka (11), who with John Gurdon was awarded the 2012 Nobel Prize for medicine for this discovery, cardiovascular research groups have combined iPSC reprogramming technology with the required factors and conditions to usher stem cells to differentiate along a cardiac lineage, and these investigators have succeeded in generating patient-specific iPSC-derived cardiomyocytes (iPSC-CMs). This method has been successfully applied to create iPSC-CMs from patients with known inherited cardiac diseases including long QT syndrome and hypertrophic cardiomyopathy (12,13). Coupling iPSC-CMs with tissue engineering science allows the creation of multicellular tissue preparations from patient-specific iPSCs to characterize further the disease phenotype at the tissue level, sometimes termed engineered heart tissue (EHT) (14). In addition to retaining the genetic information from the donor patient, epigenetic information relating to environmental events may also be retained in these cell models despite the extensive genetic reprogramming and multiple cell passages to achieve tissue-specific cell types (15).

In this issue of the *Journal,* Borchert et al. (16) report that they developed iPSC-CMs and iPSC-CM EHT from 4 unrelated patients with TTS and 4 female control subjects, and these investigators studied the cardiac physiology and catecholamine responses at the cellular and tissue level in this unique experimental preparation. They performed detailed characterization of the pharmacological responses to epinephrine and isoproterenol and identified exaggerated catecholamine responses at the highest doses in the TTS iPSC-CMs compared with controls, with larger acute cyclic adenosine monophosphate (cAMP) transients following catecholamine application, particularly at the highest concentrations. This finding was matched by higher phosphorylation levels of PKA targets, including the ryanodine receptor, phospholamban, and caveolin 1.2, thus leading to increased calcium (Ca^{2+}) transients and accelerated Ca^{2+} transient decay in the TTS iPSC-CMs. In the acute cellular iPSC-CM studies, Borchert et al. (16) report that the increased response was predominantly β_1-AR mediated, although they present evidence of both β_1-AR- and β_2-AR-dependent cAMP responses and PKA-phosphorylated targets. Electrophysiology studies were performed using the multielectrode array preparation, and isoproterenol application resulted in electrical silence in more than one-half the TTS iPSC-CMs studied, but rarely in control iPSC-CMs.

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Following epinephrine treatment there was a dose-dependent increase in lipid droplet accumulation in the TTS iPSC-CMs but not in controls, a finding that is relevant because lipid droplets were observed in endomyocardial biopsy specimens from patients with TTS, as well as in preclinical models (17). There was also a reduction in mitochondrial area observed using confocal microscopy that suggested a major metabolic disruption resulting from exposure to high catecholamine levels.

Finally, Borchert et al. (16) also generated iPSC-CM EHT constructs from both patients with TTS and control subjects. Interestingly, these investigators found that the TTS iPSC-CM EHTs had reduced basal absolute contractile force generation compared with controls, but higher sensitivity to catecholamines acutely with a leftward shift in the isoproterenol dose-contraction relationship, which was both β_1-AR and β_2-AR dependent. Long-term catecholamine exposure for 24 h induced desensitization with a rightward shift in the dose-contraction relationship, with the degree of desensitization less in TTS iPSC-CM EHTs compared with controls.