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 a sudden emotional stress or in the context of a severe and stressful concomitant illness.(2) Although initially their medical presentation is similar to an acute myocardial infarction with chest pain, ECG changes and troponin elevation, in modern healthcare rapid diagnostic coronary angiography excludes culprit coronary artery disease and the diagnosis is usually made following left ventriculography. The left ventricular function is characterised by the striking appearance with hypo- or akinetic apical and mid ventricular 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 recognised mortality of 4-5% and up to 50% of TTS patients have acute complications including pulmonary oedema and cardiogenic shock.(2) Most patients with TTS who survive make a good recovery, but 10-15% have chronic cardiac problems, including recurrent episodes of Takotsubo syndrome where 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 phaeochromocytomas suggest catecholamines play a central role. Different hypotheses proposed include direct catecholaminergic myocardial stunning, multivessel coronary vasospasm, microvascular endothelial dysfunction and abnormal ventriculo-arterial coupling. Preclinical models reproduce the anatomical pattern of acute apical dysfunction with administration of high doses of epinephrine or isoproterenol.(4,5) One hypothesis is based on the observation that epinephrine is a positive inotrope at normal physiological levels, but induces a negative inotropic response at higher concentrations via the cardiac β-adrenoceptors (βAR), such as the concentrations to which the heart is exposed during extreme stress.(6) This is due to a negative feedback mechanism whereby β1AR-dependent phosphorylation of the β2AR via protein kinase A (PKA) or G protein receptor kinases (GRKs) uncouples the receptor from stimulatory Gs signalling 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, as β2AR-Gi activates an antiapoptotic signalling cascade, and thus may minimise the 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 β1ARs and β2ARs, explaining the apical hypokinesia in most cases. (6) In experimental models inhibiting the Gi pathway blocked the development of the TTS phenotype, but at the cost of increased mortality consistent with β2AR-Gi signalling imparting a cardioprotective effect.(4,5) How this mechanism contributes to the development of TTS in humans remains to be determined, but one interesting observation was the increased frequency of the GRK5 L41Q polymorphism in TTS patients in one cohort.(8) This polymorphism increases GRK5 kinase activity, resulting in increased β2AR-Gi coupling, and imparts a survival advantage in human heart failure populations. (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 suggests age, loss of estrogen with its sympatholytic properties, and the environmental stressor, are all potentially necessary factors for TTS, although it can occur in men, younger women, and

spontaneously without a stressful trigger. Several groups have explored a genetic basis, or predisposition to TTS, reporting variable results in targeted sequencing of the main candidate genes including α1AR, β1AR, β2AR, GRK5, estrogen receptors and various microRNAs. However these have been limited by relatively small cohorts, a priori hypotheses and sequencing technologies applied, and no consistent result has emerged.

Inducible pluripotent stem cells (iPSC) are a new scientific platform to study 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 have been able to successfully generate 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 further characterise 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 JACC Borchert and colleagues developed iPSC-CMs and iPSC-CM-EHT from four unrelated TTS patients and four female controls, and studied the cardiac physiology and catecholamine responses at the cellular and tissue level in this unique experimental preparation.(*JACC REF*) The authors performed a detailed characterisation of the pharmacological responses to epinephrine and isoproterenol, and identified exaggerated catecholamine responses at the highest doses in the TTS iPSC-CMs compared to controls, with larger acute cAMP transients following catecholamine application, particularly at the highest concentrations. This was matched by higher phosphorylation levels of PKA targets including the ryanodine receptor, phospholamban, and caveolin 1.2, leading to increased Ca^{2+} transients and accelerated Ca^{2+} transient decay in the TTS iPSC-CMs. In the acute cellular iPSC-CM studies the authors report that the increased response was predominantly β1AR-mediated, although they present evidence of both β1AR and β2AR-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 half the TTS iPSC-CMs studied, but rarely in control iPSC-CMs. Following epinephrine treatment there was a dose-dependent increase in lipid droplet accumulation in the TTS iPSC-CMs but not in controls, which is relevant as lipid droplets were observed in endomyocardial biopsy specimens from TTS patients, and also in preclinical models.(16) There was also a reduction in mitochondrial area observed using confocal microscopy suggesting a major metabolic disruption resulting from exposure to high catecholamine levels.

Finally authors also generated iPSC-CM-EHT constructs from both TTS patients and controls. Interestingly they found the TTS iPSC-CM-EHTs had reduced basal absolute contractile force generation compared to controls, but higher sensitivity to catecholamines acutely with a leftwards shift in the isoproterenol dose-contraction relationship, which was both β1AR and β2AR dependent. Chronic catecholamine exposure for 24 hours induced desensitisation with a rightward shift in the

dose-contraction relationship, with the degree of desensitisation less in TTS iPSC-CM-EHTs compared to controls.

Using TTS iPSC-CMs it was possible to create the first human 'Takotsubo-in-a-dish' model which recapitulates some of the pathophysiology observed in patients during the acute phase of TTS, and this will allow further opportunity to explore the underlying mechanisms.

This study raises a number of scientific questions. It is interesting that skin fibroblasts and circulating blood mononuclear cells sampled following an acute episode can retain individual characteristics after dedifferentiation and redifferentiation during the process required to generate iPSC-CMs. These cells will have been exposed to the high circulating catecholamine levels during the acute episode, and it is possible that epigenetic changes associated with severe stress may contribute to the phenotype observed rather than it solely reflecting the DNA sequence, and different cell sources may lead to differential epigenetic expression.(17) The results suggest that individuals with TTS have a higher sensitivity to catecholamines with higher cAMP levels and larger Ca^{2+} transients, but this paradoxically leads to reduced contractility during chronic exposure, with electrical silence, lipid droplet accumulation and reduced mitochondrial area. One important factor in studies of TTS is the timing, as the cellular, molecular and organ pathophysiology evolve with different features when studied at different time points.(18) This appears to also be the case with TTS iPSC-CMs and TTS iPSC-CM EHTs, and therefore serial assessments are helpful to clarify how the initial heightened β1AR and β2AR responses lead to a secondary reduction in inotropy and electrical quiescence. These would correlate with the hypokinetic apical myocardium during the acute phase as lack of electrical activity in the paralysed apical myocardium would be one potential mechanism leading to the deep T wave inversion observed.

A number of caveats and limitations should also be considered. Firstly in TTS the whole body is exposed to the sudden stress and catecholamine surge, and understanding the effects requires assessment of integrated systems physiology not possible with an *in vitro* iPSC-CM model. The effects on the brain and peripheral vasculature may be particularly important in the development of TTS, and factors including left ventricular outflow tract obstruction and increased afterload may also contribute to the development or severity of the clinical phenotype. (19)

Secondly the total number of cases and controls was low, reflecting the complexity of developing TTS iPSC-CMs, but this introduces the potential for type 1 errors and larger numbers of different TTS iPSC-CMs would be important to confirm these findings from 4 cases. Thirdly the choice of controls is suboptimal. Ideally all control iPSC-CMs should be from postmenopausal women, and perfection would be to study control samples from both age-matched postmenopausal women exposed to similar stressful events but who did not develop TTS and from a cohort of postmenopausal women with acute heart failure from other causes e.g. acute MI. In reality this would be extremely challenging, but it is possible that some of the results observed in this study may reflect either the postmenopausal state, or epigenetic effects of acute heart failure, rather than specifically TTS. Fourthly the influence of medication was not controlled for or discussed as potentially betablockers and ACE inhibitors could have epigenetic effects on the source cells. Fifthly several of the studies assessing the β1AR and β2AR responses measure amplitude of contraction or calcium transients, without separating the effects of Gs from the antagonistic effects of Gi signalling, which may be enhanced by receptor activation and stimulus trafficking, but are compounded in the measured

assay. Finally a challenge for all myocardial iPSC-CM studies is that the cells generated are morphologically and pharmacologically immature and very different to the mature adult CM phenotype. Even different catecholamine responses have been reported.(20) Whilst these TTS iPSC-CMs exhibit many characteristics of cardiac cells, to what extent the results can be directly translatable to the human adult heart remains to be determined. In some cases, such as electrophysiological screening of antiarrhythmic drug strategies they have been helpful, but for more complex diseases including TTS this remains a limitation.

What is emerging from this study and others is that TTS is a complex condition with potential genetic and epigenetic predispositions, interacting with environmental factors including age, postmenopausal hormonal status and emotional or physical stresses. Catecholaminergic hyperstimulation of the left ventricular myocardium initiates a cascade of molecular and cellular changes leading to altered β1AR and β2AR signalling, lipid accumulation, mitochondrial dysfunction and electrical quiescence during the acute phase which all contribute to the acute negatively inotropic response observed in the affected LV segments. These are all potentially reversible phenomena, although some of these effects last for many months following the acute episode including acute oedema and inflammation. Hopefully these and future studies using TTS iPSC-CMs, TTS iPSC-CM EHTs and other models will identify new treatment targets to rescue patients with TTS complicated by cardiogenic shock where mortality and morbidity is high, and current treatment options are limited. TTS should now be considered a catecholaminergic and metabolic acute heart failure syndrome, and Borchert and colleagues should be congratulated on completing this range of complex experiments which shed further light on the mechanisms underpinning this interesting cardiac syndrome.

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