Marked Vascular Dysfunction in a Case of Peripartum Cardiomyopathy

Saereh Khanamiri^{a, b} June-Wha Rhee^{a, b} David T. Paik^{a, b} Ian Y. Chen^{a, b}
Chun Liu^{a, b} Nazish Sayed^{a, b}

^aStanford Cardiovascular Institute, Stanford, CA, USA; ^bDivision of Cardiovascular Medicine, Department of Medicine, Stanford University School of Medicine, Stanford, CA, USA

Keywords

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Abstract

Peripartum cardiomyopathy (PPCM) is a rare form of congestive heart failure characterized by left ventricular dysfunction that develops towards the end of pregnancy or during the early postpartum phase. Even though the majority of PPCM patients show partial or complete recovery of their heart functions, the mortality rate of PPCM remains high. Previous research has suggested that vascular dysfunction triggered by late-gestational hormones and potent anti-angiogenic factors play key roles in the pathogenesis of PPCM; however, the exact mechanisms remain elusive due to limited patient tissues for characterization. Here, we report a case of PPCM where the coronary vessels from the patient's explanted heart showed marked vascular dysfunction with impaired nitric oxide response. Importantly, these vessels exhibited deficient adenosine-mediated vasorelaxation when subjected to myograph studies, suggesting impaired Kv7 ion channels. Results from this work may lead to new therapeutic strategies for improving Kv7 function in PPCM patients.

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Introduction

Peripartum cardiomyopathy (PPCM) is a rare form of potentially fatal heart disease that develops toward the end of pregnancy or during the early post-partum phase. It is marked by significant left ventricular (LV) systolic dysfunction, and limited data indicate that up to 38% of the peripartum fatalities are attributable to sudden cardiac death, suggesting a high burden of arrhythmia [1]. In addition, endothelial dysfunction has been found to be of prognostic value in predicting adverse outcomes even in the absence of coronary artery disease [2]. Previous research has suggested that vascular dysfunction triggered by late-gestational hormones plays a key role in the pathogenesis of PPCM [3]. More recently, it has been suggested that PPCM may share genetic traits with dilated cardiomyopathy (DCM) [4]. However, the exact mechanism remains unknown due to the lack of adequate experimental models and available patient tissues.

Methods and Results

Here, we describe a case of PPCM with progressive systolic heart failure that eventually required a heart transplant. The patient, a 55-year-old female, was diagnosed

Fig. 1. Clinical features of the patients. **a** Medical history of the healthy controls (with or without CV risk factors), DCM control, and the PPCM patients. **b** Echocardiogram of the PPCM patient showing stably reduced LV systolic function without any significant changes. **c** Angiogram of the PPCM patient showing coronaries without any significant obstruction. LVEF, left ventricular ejection fraction; CV, cardiovascular; HDL, high-density lipoprotein.

	Healthy control (no CV risk factors)	Healthy control (with CV risk factors)	DCM	PPCM
Age, years	41	62	63	55
Sex	F	M	F	F
Ethnicity	Caucasian	Caucasian	Caucasian	Caucasian
LVEF, %	51	44	25	<25
CV risk factors	No	Yes (Hypertension, 1HDL)	No	No

with PPCM at age 29 when significant ventricular ectopic beats were observed at the time of emergent caesarean section. She was subsequently managed with optimal medical and device therapies until she became refractory to available maximal therapies, ultimately requiring an orthotopic heart transplant. Review of the chart showed no apparent epigenetic insults till the time of her transplant to negatively influence her coronary vascular function, and she was free of conventional cardiovascular risk factors including dyslipidemia, obesity, diabetes, hypertension, and substance abuse (Fig. 1a). Despite no pre-existing heart condition, her echocardiogram showed marked LV dilatation with severely reduced LV ejection fraction (<20%) (Fig. 1b). Importantly, her angiogram revealed coronaries without significant obstructive disease (Fig. 1c).

Knowing that systemic angiogenic imbalance could lead to PPCM [3], we hypothesized that PPCM patients may suffer from coronary vascular dysfunction. Using isometric tension recordings of vessel reactivity, we investigated the coronary vasculature *ex vivo* utilizing explanted hearts from the PPCM patient (Fig. 2a), an age/gender-matched DCM patient, and a gender-matched healthy donor patient. Of note, the healthy donor died of a noncardiac cause (massive hemorrhagic stroke) and had no pre-existing conventional cardiac risk factors.

Furthermore, gross examination of the donor heart revealed no palpable calcification to suggest any significant coronary artery disease. The DCM control heart was used, as PPCM is often considered a subtype of DCM. Wire myograph measurements found impaired endothelial cell function and nitric oxide production in PPCM and DCM as compared to the healthy control (Fig. 2b).

Next, we investigated the vascular smooth muscle cell compartment, which is known to play a critical role in regulating vascular tone [5]. Our initial screen for the possible involvement of ion channels found that only voltage-gated K^+ (Kv7) channels, which are activated by depolarization and are key for maintaining the coronary circulation [5], were impaired in the diseased PPCM coronary arteries. Pre-constricted PPCM left anterior descending arteries (LADs) showed significantly reduced relaxation compared to the controls in response to retigabine, a Kv7.2–7.5 channel activator, with this relaxation being completely abrogated in the presence of linopirdine, a specific Kv7 inhibitor (data not shown). In addition, responses to linopirdine failed to elicit vasoconstriction in PPCM LADs at basal tone as compared to both the healthy control and DCM LADs (Fig. 2c, left panel). This clearly suggests that Kv7 activity is impaired in coronary vasculature of the PPCM patient. Furthermore, we ascertained the

Fig. 2. Vascular reactivity in the PPCM heart. **a** Explanted PPCM heart, dissected coronary arteries, and wire myograph. **b** Endothelial function in the PPCM heart. Isometric tension recordings of relaxation to acetylcholine (10 μ M), carbachol (10 μ M), and NO donor SNP (3 μ M) upon pre-constriction with U46619. **c** Tension recordings of PPCM LAD segments showing less contraction at basal tone upon application of 10 μ M linopirdine when compared

to DCM and the healthy control (left panel); impaired adenosine response in PPCM or in the presence of 10 μ M linopirdine (right panel). Statistical analyses were done on three segments ($n = 3$) from one coronary vessel per patient ($n = 1$) using two-way ANOVA. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. ACh, acetylcholine; SNP, sodium nitroprusside; CV, cardiovascular.

role of Kv7 channels in response to adenosine, an intrinsic vasodilator. In healthy LADs, adenosine induced $\sim 30\%$ dilation, but this effect was absent in PPCM coronaries incubated with linopirdine (Fig. 2c, right panel).

Since our wire myograph measurements showed impairment of endothelial function in PPCM hearts, we next investigated the molecular characteristics of the endothelium in the PPCM patient to gain more insights into the vascular dysfunction. For this, we isolated primary endo-

thelial cells (ECs) from the control and PPCM hearts' coronary vasculature (Fig. 3a) and then subjected them to functional assays to assess their endothelial phenotype. Specifically, we tested the inherent ability of ECs to form three-dimensional vascular networks or uptake acetylated low-density lipoprotein (Ac-LDL) [6]. Consistent with our wire myography data, primary ECs isolated from the PPCM patient's LADs showed a decreased capacity to form networks of tubular structures when placed on ma-

Fig. 3. Functional characteristics of the primary endothelial cells (ECs) from the PPCM heart. **a** Representative images of the primary ECs isolated from the healthy and PPCM hearts. **b** Representative images of capillary-like networks formed by primary ECs, showing impaired tube formation by PPCM ECs compared to healthy controls. **c** Bar graph showing quantification of the number of tubes formed by the primary ECs. **d** Representative fluores-

cent images of Ac-LDL uptake by primary ECs, showing reduced capacity to incorporate Ac-LDL by PPCM ECs compared to healthy controls. **e** Bar graph showing quantification of Ac-LDL fluorescence intensity in primary ECs. All data are represented as mean $\pm$ SEM, $n = 3$, * $p < 0.05$. Statistical analyses were done using the standard Student *t* test and Mann-Whitney nonparametric test.

trigel compared to primary ECs isolated from healthy controls (Fig. 3b, c). Similarly, the PPCM patient's ECs incorporated significantly less Ac-LDL when compared to the control patient (Fig. 3d, e). Taken together, these results suggest that ECs isolated from the PPCM patient show abnormal EC function, further strengthening our observation of marked vascular dysfunction in this PPCM patient.

Discussion

In summary, our report shows that the PPCM patient exhibited marked coronary vascular dysfunction using direct reactivity assays on blood vessels of explanted human hearts. We observed endothelial dysfunction with a clear impairment in nitric oxide responses in PPCM, and ECs isolated from these PPCM coronaries showed functional impairment. Moreover, PPCM coronaries exhibited an apparent lack of adenosine-mediated vasorelax-

ation, related to impaired Kv7 channel activity. While previous animal studies have suggested vascular dysfunction related to PPCM [3, 7], this is the first human study to show a direct link between PPCM and impaired coronary vascular function. Whether this observed dysfunction is a bystander effect of PPCM or a significant contributor to the underlying pathology remains unknown. Preeclampsia is considered a risk factor for PPCM [8] and is often associated with vascular dysfunction; however, only a small percentage of women with preeclampsia eventually develops PPCM, suggesting other possible mechanisms. Indeed, our patient did not have preeclampsia prior to developing PPCM and moreover did not show any signs of conventional cardiovascular risk factors. We speculate that the observed coronary artery dysfunction resulted in myocardial underperfusion and compromised reactive hyperemia, especially during the peripartum phase of marked hormonal and metabolic changes, possibly leading to ischemic insult to the myocardium and

subsequent myocardial dysfunction. This might explain in part why early treatment with bromocriptine, a dopamine agonist known to vasodilate, resulted in an improved outcome of PPCM [9].

Even though our study brings forth a novel understanding of PPCM that is scientifically and clinically important, there are some limitations that need to be acknowledged. Our report is representative of 1 patient that was diagnosed based on the timing of LV dysfunction related to pregnancy and in the absence of other competing factors. Moreover, as PPCM can occur months before or after pregnancy, not all PPCM patients present with similar phenotypes, making it difficult to compare results from different studies [10]. Further studies are warranted to elucidate the role of coronary vascular dysfunction in the pathogenesis of PPCM.

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Statement of Ethics

Human tissues were obtained from and procured by the Human Biorepository Tissue Research Bank at Stanford University under the approved IRB protocol No. 32769. All personal information was de-identified in accordance to the HIPAA regulations, and tissues were collected with informed patient consent.

Disclosure Statement

The authors have nothing to disclose.

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