NATURAL HISTORY AND PATHOGENESIS OF TAKOTSUBO CARDIOMYOPATHY

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In
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Declaration

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Kuljit Singh (9.2.2015)

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Statement of contribution to research

The studies were conceived and designed jointly by Professor John Horowitz and myself.

Execution

I performed all the recruitment and organization of patients into the studies, with the assistance of Ms. Jeanette Stansborough (research nurse). I collected all the clinical data with assistance from Ms. Stansborough. I performed all the data search and meta-analysis with the assistance of Ms. Kristen Carson. I analysed of the echocardiographic studies for humans and animals. Some of the immunohistochemistry studies, in particular detection of apoptosis (TUNEL staining), Poly ADP ribose (PAR Staining) were performed by me. I also performed platelet aggregometry on new patients diagnosed with TTC between 2012 and 2014. Dr. Giovanni Licari performed immunohistochemistry studies of measurement of 3-nitrotyrosine and TXNIP. I performed quantification of staining for 3-nitrotyrosine and TXNIP with the assistance of Dr. Giovanni Licari. I performed quantification of staining for apoptosis, and inter-observer analysis were performed with Dr. Betty Raman. Plasma 3-Nitrotyrosine levels were analyzed in Prof. Tsikas's laboratory in Hannover, Germany.

Analysis

All the data were collated and analyzed by myself.

List of published studies

1. Kuljit Singh, Kristin Carson, Zafar Usmani, Gagandeep Sawhney, John Horowitz: Systematic Review and Meta-Analysis of Incidence and Correlates of Recurrence of Takotsubo Cardiomyopathy. International Journal of Cardiology 04/2014; 174(3):696-701

2. Kuljit Singh, Kristin Carson, Ranjit Shah, Gagandeep Sawhney, Balwinder Singh, Ajay Parsaik, Harel Gilutz, Zafar Usmani, John Horowitz: Meta-Analysis of Clinical Correlates of Acute Mortality in Takotsubo Cardiomyopathy. The American journal of cardiology 04/2014; 113(8):1420-1428.

3. Kuljit Singh, Christopher Neil, Thanh Nguyen, Jeanette Stansborough, Cherin Chong, Dana Dawson, Micheal Frenneaux, John Horowitz. Dissociation of early shock in Takotsubo cardiomyopathy from either right or left ventricular systolic dysfunction. Heart Lung and Circulation. 12/2014; 23(12):1141-8

4. Betty Raman, Kuljit Singh, Christopher J Zeitz, John D Horowitz: Takotsubo cardiomyopathy presenting as S-T elevation myocardial infarction: Not gone but forgotten? International Journal of Cardiology. 01/2014; 172(1):e261-2

5. Kuljit Singh, Tina Marinelli, John D Horowitz: Takotsubo cardiomyopathy after anti-influenza vaccination: catecholaminergic effects of immune system. The American journal of emergency medicine 07/2013; 31(11): 1627.

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List of Abbreviations

AMI	Acute myocardial infarction
ACS	Acute coronary syndrome
ACE	Angiotensin converting enzyme
ADMA	Asymmetric dimethyl arginine
ARB	Angiotensin receptor blocker
BNP; NT-proBNP	B-type natriuretic peptide; amino-terminal prohormone of BNP
cGMP	Cyclic guanosine monophosphate
CA	Coronary angiogram
CMR	Cardiac magnetic resonance imaging
CVA	Cerebrovascular accident
ECG	Electrocardiogram
TOE	Transesophageal echocardiography
TTE	Transthoracic echocardiography
TTC	Takotsubo cardiomyopathy
LAD	Left anterior descending artery
LGE	Late gadolinium enhancement
LOS	Length of stay
LVEF	Left ventricular ejection fraction
LVSV	Left ventricular stroke volume
LVOT	Left ventricular outflow tract
LV	Left ventricle
MR	Mitral regurgitation
MRS	Magnetic resonance spectroscopy
NOS	Nitric oxide synthase
NSTEMI	Non-ST elevation myocardial infarction
PASP	Pulmonary artery systolic pressure
PAR	Poly (ADP) ribose
PARP	Poly (ADP) ribose polymerase
PCWP	Pulmonary capillary wedge pressure
PHS	Prolonged hospital stay
RV	Right ventricle
SAH	Subarachnoid hemmorhage
STEMI	ST elevation myocardial infarction
TXNIP	Thioredoxin interacting protein

VT	Ventricular tachycardia
VF	Ventricular fibrillation
WMSI	Wall motion score index
3NT	3-Nitrotyrosine
3AB	3-Aminobenzamide

Abstract

Introduction: Takotsubo cardiomyopathy (TTC) is a transient left ventricular (LV) systolic dysfunction of uncertain pathogenesis, which occurs predominantly in ageing women. Although there is considerable uncertainty about the pathogenesis of TTC, pronounced catecholamine release and an acute inflammatory process is implicated. Furthermore, natural history of TTC is unknown and correlates of acute complications and incomplete recovery have not been evaluated.

Methods: In the 5 experimental chapters, this thesis examines aspects of (a) pathogenesis and (b) natural history of TTC. As regard the pathogenesis, we hypothesized that increased release of nitric oxide (NO) in patients with TTC potentially induces the formation of peroxynitrite ($ONOO^-$) anion with associated redox stress, protein nitration and downstream activation of thioredoxin interacting protein (TXNIP). Evaluation of presence of nitrosative stress was performed in a rat model of TTC in parallel with human experiments looking at the local and systemic rise in the 3-nitrotyrosine (3-NT) as a footmark of $ONOO^-$ formation. As a part of clinical investigations, we evaluated the role of RV involvement in early hemodynamic derangement. We performed a meta-analysis to assess the impact of "secondary" TTC, male gender, advancing age and catecholamine use on mortality in TTC. We used a similar approach to assess the correlates of recurrence rate of TTC.

Results:

A. **Pathogenesis:** In rat model of TTC, there was substantially increased myocardial 3-NT (2.9 ± 0.6 % and 0.3 ± 0.1 %; $p< 0.01$) and TXNIP content (16.5 ± 5.2 vs 0.5± 0.2%; $p < 0.01$). Furthermore, use of poly (ADP) ribose polymerase (PARP)-1 inhibitor attenuated the isoprenaline induced LV systolic dysfunction. In human experiments, plasma concentrations of 3-NT did not differ significantly between TTC (2.26 ± 0.22 nmol/L) and control subjects (2.20 ± 0.25 nmol/L). However, myocardial 3-NT and TxNIP content

were increased 4-fold and 10-fold respectively. Furthermore, myocardial content for poly (ADP) ribose (PAR) activity was increased 4 folds.

B. **Clinical investigations:** RV involvement occurred in 1/3rd of TTC patients. Hypotension was noted in 21% of TTC patients, while shock occurred in 16%. RV involvement was a univariate but not a multivariate correlate of either hypotension or shock and did not result in prolonged hospital stay. RV involvement predicted more extensive LV hypokinesis and LV systolic dysfunction.

C. **Meta-Analysis:** In-hospital mortality among patients with TTC was 4.5% (95% CI, 3.1%-6.2%). Male gender was associated with higher mortality (OR 2.6, 95% CI, 1.5-4.6, p=0.0008) so was "secondary" TTC (RD-0.11, 95%CI; -0.18 to -0.04, p=0.003).

TTC had 1-2% annual recurrence rate, which was independent of clinic utilization of beta-blocker prescription, but inversely correlated (r =-0.45, p = 0.016) with ACEi/ARB prescription. Patients with severe TTC at index admission were noted to have more recurrences.

Conclusion:

A. TTC is associated with evidence of nitrosative stress within left ventricular myocardium.
B. RV involvement is not an independent predictor of hemodynamic derangement.
C. Male gender and "secondary" TTC are associated with higher mortality and use of ACEi might reduce recurrence rate.