NATURAL HISTORY AND PATHOGENESIS OF TAKOTSUBO CARDIOMYOPATHY

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Thesis submitted for the degree of
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In
Medicine
At
The University of Adelaide
(Faculty of health Sciences)

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Declaration

This thesis is the result of my own investigation, except where otherwise stated. It contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in future, be used in a submission for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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Kuljit Singh (9.2.2015)
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Finally, I would like to thank my wife Upinder Kaur Bhalla and my mother Amarjit Kaur for their support, patience and understanding.
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


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<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>AMI</td>
<td>Acute myocardial infarction</td>
</tr>
<tr>
<td>ACS</td>
<td>Acute coronary syndrome</td>
</tr>
<tr>
<td>ACE</td>
<td>Angiotensin converting enzyme</td>
</tr>
<tr>
<td>ADMA</td>
<td>Asymmetric dimethyl arginine</td>
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<tr>
<td>ARB</td>
<td>Angiotensin receptor blocker</td>
</tr>
<tr>
<td>BNP; NT-proBNP</td>
<td>B-type natriuretic peptide; amino-terminal prohormone of BNP</td>
</tr>
<tr>
<td>cGMP</td>
<td>Cyclic guanosine monophosphate</td>
</tr>
<tr>
<td>CA</td>
<td>Coronary angiogram</td>
</tr>
<tr>
<td>CMR</td>
<td>Cardiac magnetic resonance imaging</td>
</tr>
<tr>
<td>CVA</td>
<td>Cerebrovascular accident</td>
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<tr>
<td>ECG</td>
<td>Electrocardiogram</td>
</tr>
<tr>
<td>TOE</td>
<td>Transesophageal echocardiography</td>
</tr>
<tr>
<td>TTE</td>
<td>Transthoracic echocardiography</td>
</tr>
<tr>
<td>TTC</td>
<td>Takotsubo cardiomyopathy</td>
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<tr>
<td>LAD</td>
<td>Left anterior descending artery</td>
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<tr>
<td>LGE</td>
<td>Late gadolinium enhancement</td>
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<tr>
<td>LOS</td>
<td>Length of stay</td>
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<tr>
<td>LVEF</td>
<td>Left ventricular ejection fraction</td>
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<tr>
<td>LVSV</td>
<td>Left ventricular stroke volume</td>
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<tr>
<td>LVOT</td>
<td>Left ventricular outflow tract</td>
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<tr>
<td>LV</td>
<td>Left ventricle</td>
</tr>
<tr>
<td>MR</td>
<td>Mitral regurgitation</td>
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<tr>
<td>MRS</td>
<td>Magnetic resonance spectroscopy</td>
</tr>
<tr>
<td>NOS</td>
<td>Nitric oxide synthase</td>
</tr>
<tr>
<td>NSTEMI</td>
<td>Non-ST elevation myocardial infarction</td>
</tr>
<tr>
<td>PASP</td>
<td>Pulmonary artery systolic pressure</td>
</tr>
<tr>
<td>PAR</td>
<td>Poly (ADP) ribose</td>
</tr>
<tr>
<td>PARP</td>
<td>Poly (ADP) ribose polymerase</td>
</tr>
<tr>
<td>PCWP</td>
<td>Pulmonary capillary wedge pressure</td>
</tr>
<tr>
<td>PHS</td>
<td>Prolonged hospital stay</td>
</tr>
<tr>
<td>RV</td>
<td>Right ventricle</td>
</tr>
<tr>
<td>SAH</td>
<td>Subarachnoid hemorrhage</td>
</tr>
<tr>
<td>STEMI</td>
<td>ST elevation myocardial infarction</td>
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<tr>
<td>TXNIP</td>
<td>Thioredoxin interacting protein</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
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<tr>
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</tr>
<tr>
<td>VT</td>
<td>Ventricular tachycardia</td>
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<tr>
<td>VF</td>
<td>Ventricular fibrillation</td>
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<tr>
<td>WMSI</td>
<td>Wall motion score index</td>
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<tr>
<td>3NT</td>
<td>3-Nitrotyrosine</td>
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<tr>
<td>3AB</td>
<td>3-Aminobenzamide</td>
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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 footprint 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.