**PS569 I BENCH**

Endothelial dysfunction in arteries from patients with induced hyperhomocysteinemia is associated with eNOS-mediated nitrooxidative stress

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**Aim:** Endothelial dysfunction, defined as a decreased level of bioavailable nitric oxide (NO) in the vessel wall, is underlying mechanism of endothelium-dependent vascular disorders. Oxidative stress seems to be critical mechanism of the impairment of endothelial function. Endothelial NO synthesis (eNOS) can be considered as a significant source of reactive oxygen and nitrogen species, that are the products of the reaction between NO and O2. Indirect observations indicate that hyperhomocysteinemia (HHcy) promotes oxidative and nitrooxidative stress in the blood vessel leading to the endothelial dysfunction. In previous studies, we examined the functional and structural state of eNOS to establish the role of eNOS in nitrooxidative stress in the endothelium of human arteries from patients with induced HHcy.

**Methods:** To investigate the role of eNOS in the pathogenesis of cardiomyopathy - emerging evidence for an inherited mechanism of Takotsubo cardiomyopathy.

**Results:** Reduced bioavailability of Nitric Oxide (NO) is a key factor contributing to myocardial ischemia and reperfusion injury. One mechanism behind reduction of NO deficiency is the NO Synthase (NOS) substrate L-arginine and the cofactor tetrahydrobiopterin (BH4) resulting in NOS uncoupling. The aim of the study was to investigate if the combination of L-arginine and BH4 given iv in a coronary artery reperfusion protects from reperfusion injury using two clinically relevant animal models.

**Methods:** Anesthetized rats and pigs were subjected to 30 min left coronary artery ligation and 2 h reperfusion. At 25 min of ischemia rats were given iv injections of 1) saline (n=8); 2) BH4 (10 mg/kg) followed by L-arginine (100 mg/kg, n=6); 3) BH4 (10 mg/kg) followed by saline (n=6); 4) saline followed by L-arginine (100 mg/kg, n=6); 5) the NOS inhibitor NG-monomethyl-L-arginine (L-NMMA, 10 mg/kg, n=6). BH4 was given iv (10 mg/kg) and L-arginine (100 mg/kg, n=6). Pigs were subjected to 40 min LAD ligation and 4 h reperfusion. They received infusions into the left main coronary artery of: 1) saline (n=6); 2) BH4 (0.03 mg/kg/min, n=6); 3) L-arginine (3.0 mg/kg/min, n=6) or 4) the combination of L-arginine and BH4 (n=6). All infusions started at 30 min of ischemia and continued during 20 min of reperfusion. Infarct size (IS) was determined using staining with 2,3,5-triphenyltetrazolium chloride and expressed as percentage of the area at risk.

**Conclusions:** Our data indicate that HHcy-induced nitrooxidative stress is the effect of increased production of ONOO- in the reaction between eNOS-derived NO and O2-. Elevated expression of eNOS enzyme in patients with HHcy may serve in the group with HHcy vs. control group. In contrast, total eNOS protein expression was elevated in the endothelium of patients with HHcy. The ratio of eNOS dimers to monomers was analyzed SDS-PAGE electrophoresis under both reducing and nonreducing conditions. The extent of zinc ions released from purified eNOS was measured spectrophotometrically.

**Results:** Significantly reduced release of bioactive NO with a parallel increase in the release of both O2- and ONOO- from a single endothelial cell was observed by the highly sensitive electrochemo NO/ O2- /ONOO-nanosensors. The expression of eNOS dimers and monomers was assessed using SDS-PAGE electrophoresis under both reducing and nonreducing conditions. The extent of zinc ions released from purified eNOS was measured spectrophotometrically.

**Conclusions:** We observed a higher prevalence of haplotypes T in patients with TKCM both in ESR1 –397 and ESR2 –1839 (Table 2). On logistic regression analysis the haplotype T of ESR1 –397 was significantly associated with TKCM, whereas the haplotypes G for ESR1 –351 and for ESR2 –1839, respectively, were associated with MI (Table 3).

**Table 1. Pearson's chi square – ESR1 -397 T>C**

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>Controls</th>
<th>Takotsubo</th>
<th>Myocardial Infarction</th>
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<tbody>
<tr>
<td>TT</td>
<td>55.9%</td>
<td>44.1%</td>
<td>57.1%</td>
</tr>
<tr>
<td>CT</td>
<td>43.1%</td>
<td>55.6%</td>
<td>42.1%</td>
</tr>
<tr>
<td>CC</td>
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**Table 2. Pearson's chi square – Polymorphisms**

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>ESR1 -397</th>
<th>ESR1 -351</th>
<th>ESR2 -1082</th>
<th>ESR2 -1839</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>55.9%</td>
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<td>57.1%</td>
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<tr>
<td>Takotsubo</td>
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<td>41.7%</td>
</tr>
<tr>
<td>Myocardial Infarction</td>
<td>0.9%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
</tbody>
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