«Η απομακρυσμένη ισχαιμική προπόνηση βελτιώνει την αρτηριακή λειτουργία, ενεργοποιεί την έκφραση καρδιοπροστατευτικών miRNAs και ελαττώνει το οξειδωτικό στρες σε ασθενείς με STEMI»

Δημήτριος Βλαστός, Ιγνάτιος Οικονομίδης, Μαρία Γαζούλη, Ιωάννα Ανδρεάδου, Παναγιώτης Εφεντάκης, Μαρία Βαρούδη, Στέφανος Βλάχος, Δημήτριος Μπενάς, Γεώργιος Παυλίδης, Ελένη Τριανταφυλλίδη, Ιωάννης Λεκάκης, Διονύσιος Κόκκινος, Ευστάθιος Κ. Ηλιοδρομίτης

Β' Πανεπιστημιακή Καρδιολογική Κλινική, ΑΤΤΙΚΟ Νοσοκομείο
BACKGROUND

- Remote ischemic conditioning attenuates ischemia-reperfusion injury in patients with STEMI.

- However, its effects on vascular function and biochemical mechanisms are not fully defined.
PULSATILE HEMODYNAMICS

- **Aortic elasticity** plays a major role in the regulation of *blood pressure* and *peripheral blood flow*.
- It allows the damping of the rhythmic left ventricular output and the translation of the pulsatile cardiac pump function into a nearly continuous peripheral blood flow, an effect that is well known as the aortic “**Windkessel function**”.
- This systolic-diastolic interplay can also affect the heart, **reducing the left ventricular afterload** and **improving the coronary blood flow** and ventricular relaxation.
PULSATILE HEMODYNAMICS

• On the contrary, **impaired aortic elasticity** would lead to an **increase in systolic blood pressure and a decrease in diastolic blood pressure** at any given mean pressure, an increase in systolic blood velocity, **an increase in left ventricular afterload**, and a **decrease in subendocardial blood supply during diastole**.

• **Aortic PWV (pulse wave velocity)** measurement is a simple, noninvasive, and reproducible method of **aortic stiffness estimation**.

• It essentially describes the speed of propagation of the shock wave that each stroke volume creates on the aortic wall, which is proportional to the aortic stiffness.
GLYOCALYX INTEGRITY

• **Endothelial glycocalyx** plays a vital role in vascular permeability and inflammation.

• Endothelial glycocalyx damage is associated with **lipoprotein influx**, macromolecule leakage, adhesion of circulating cells to the endothelium, and imbalance in enzymatic systems such as coagulation and antioxidant defence, as well as an impaired NO release.

• **Increased PBR** is an accurate index of impaired glycocalyx integrity, and consequently of **endothelial dysfunction**.
SERUM MDA LEVELS

- Excessive reactive oxygen species (ROS) production plays a significant role in the reperfusion injury mechanism.
- The abruptly reintroduced molecular oxygen into ischemic myocardium undergoes sequential reduction, which leads to oxygen free radicals formation.
- These free radicals react with polyunsaturated fatty acids, forming lipid peroxides and hydroperoxides that cause damage to the sarcollema and membrane-bound enzymes impairment.
- MDA is one of the products of the aforementioned lipid peroxidation, serving as a reliable index of free-radical induced injury.
It has been shown \textit{in vivo} that \textbf{nitrate and nitrite anions} serve as an \textit{independent of the L-arginine-NO-synthase source of NO}, particularly in the setting of hypoxia.

- Nitrite promotes vasodilation and blood pressure reduction, while by way of \textit{mitochondrial electron transfer modulation} it attenuates ischemia-reperfusion injury.

- Nitrate is converted to \textbf{nitrite} in the oral cavity by nitrate reductase containing bacteria, and therefore causes similar effects.
MicroRNAs (miRs)

- **MicroRNAs** are small, highly conserved, **non-coding RNA** molecules involved in the **regulation of gene expression**.
- The regulatory functions of microRNAs are accomplished through the RNA-induced silencing complex (**RISC**).
- The level of **complementarity between mRNA target and the respective RISC** determines **which silencing mechanism** will be employed; cleavage of target messenger RNA (mRNA) with subsequent **degradation or translation inhibition**.
- **miR-144** has been recognised as a **key mediator of RIC**.
- **miRs-150 and -499** exert **cardio-protective effects**, while **miRs-21 and -208** promote **adverse myocardial remodelling**.
METHODS

• We examined 180 patients with STEMI and 30 healthy controls.
• Patients were randomised in 2 remote ischemic conditioning protocols after a baseline assessment of vascular function (T0):
  a) one with two ischemic conditioning stimuli by brachial cuff inflation of both arms at 200mmHg for 5 min, separated by 15min, and each cuff deflation followed by vascular assessment (T1,T2), and a final vascular assessment (T3) 25 min after 2nd cuff deflation (double cuff inflation: n=90) or
  b) a second with omission of the second cuff inflation (single cuff inflation: n=90).
  c) 20 of the patients underwent a sham conditioning procedure.
METHODS
METHODS

In both protocols we measured:

• a) the carotid-femoral pulse wave velocity (PWV);
• b) the perfusion boundary region (PBR-micrometers) of the sublingual arterial microvessels as a marker of endothelial glycocalyx thickness;
• c) plasma malondialdehyde (MDA), NOx, and IL-6 levels as biomarkers of oxidative stress and inflammation; and
• d) miRs-144, -150, -499, -21, and -208 plasma levels (by PCR).
# RESULTS

<table>
<thead>
<tr>
<th>Patient characteristics</th>
<th></th>
<th>AMI characteristics</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>53±16</td>
<td>1 vessel disease</td>
<td>42% (n=76)</td>
</tr>
<tr>
<td>Sex</td>
<td>M:80% (n=144)</td>
<td>2 vessel disease</td>
<td>46% (n=83)</td>
</tr>
<tr>
<td>BMI</td>
<td>27±4</td>
<td>3 vessel disease</td>
<td>12% (n=22)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>27% (n=49)</td>
<td>Anterior</td>
<td>47% (n=85)</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>17% (n=30)</td>
<td>hs-Troponin</td>
<td>3843 [991-9338] ng/mL</td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>24% (n=43)</td>
<td>CPK</td>
<td>1210 [461-2923] U/L</td>
</tr>
<tr>
<td>Smoking</td>
<td>53% (n=05)</td>
<td>WBC</td>
<td>8.790±2.577/mcL</td>
</tr>
<tr>
<td>CAD family history</td>
<td>12% (n=22)</td>
<td>CRP</td>
<td>191±3 mg/L</td>
</tr>
</tbody>
</table>
RESULTS

• Compared to the double cuff inflation, the single cuff inflation protocol resulted in a more uniform aortic elasticity improvement (PWV T0: **12.09** m/s, T1: **11.54** m/s, T2: **11.39** m/s, T3: **11.71** m/s).

• Patients with baseline PWV>11 m/s benefited from an enhanced arterial elasticity improvement (mean ΔPWV=-3.5 m/s at T1, p<0.002); omission of the second ischemic stimulus in the same patient group resulted in a retained aortic stiffness alleviation at T3 (PWV=11.3 vs 13.8 m/s, p<0.05).

• A greater glycocalyx integrity restoration was observed in patients with baseline PBR> 2.1 (n=50) in both protocols (mean difference in PBR improvement=0.5 at T1, p<0.001).
RESULTS

PWV-single cuff

PWV-double cuff
RESULTS

• Compared to the double cuff inflation, the single cuff inflation protocol resulted in a more uniform aortic elasticity improvement (PWV T0: **12.09** m/s, T1: **11.54** m/s, T2: **11.39** m/s, T3: **11.71** m/s).

• Patients with baseline PWV>**11** m/s benefited from an enhanced arterial elasticity improvement (mean ΔPWV=**-3.5** m/s at T1, p<0.002); omission of the second ischemic stimulus in the same patient group resulted in a retained aortic stiffness alleviation at T3 (PWV=**11.3** vs **13.8** m/s, p<0.05).

• A greater glycocalyx integrity restoration was observed in patients with baseline PBR> 2.1 (n=80) in both protocols (mean difference in PBR improvement=**0.5** at T1, p<0.001).
RESULTS

• **MDA** was significantly reduced at T3 compared to baseline in STEMI (2.1 ± 0.15 at T3 vs 2.59 ± 0.15 at T0, p< 0.001) in **both protocols** while it remained unchanged in healthy controls.

• The **single cuff inflation protocol** promoted a greater increase in **NOx** levels compared to the double inflation protocol (T0: 8.25 ± 1.18 to T3: 11.1 ± 2 μM vs T0: 8.91 ± 2 to T3: 10.79 ± 1.18 μM, p< 0.05).

• **IL-6** levels were **not affected** by our conditioning protocols.
RESULTS

MDA

T0  T3

p > 0.05

NOx

T0  T3

p < 0.05
RESULTS

• miR-expression was significantly increased by both protocols; however, single cuff inflation protocol conferred a significantly greater miR-144 expression by 60% (p<0.001).

• miR-144 upregulation was positively and linearly correlated with PWV decrease at T1 (r=0.763, p<0.001).
RESULTS

<table>
<thead>
<tr>
<th></th>
<th>baseline</th>
<th>post</th>
</tr>
</thead>
<tbody>
<tr>
<td>sham</td>
<td>3.8</td>
<td>4.6</td>
</tr>
<tr>
<td>intervention</td>
<td>7.4</td>
<td>55.9</td>
</tr>
</tbody>
</table>
### miR-150

<table>
<thead>
<tr>
<th></th>
<th>baseline</th>
<th>post</th>
</tr>
</thead>
<tbody>
<tr>
<td>sham</td>
<td>1.8</td>
<td>2.3</td>
</tr>
<tr>
<td>RIPC</td>
<td>1.8</td>
<td>3.5</td>
</tr>
</tbody>
</table>

### miR-499

<table>
<thead>
<tr>
<th></th>
<th>baseline</th>
<th>post</th>
</tr>
</thead>
<tbody>
<tr>
<td>sham</td>
<td>1.39</td>
<td>1.8</td>
</tr>
<tr>
<td>intervention</td>
<td>1.6</td>
<td>3.5</td>
</tr>
</tbody>
</table>
CONCLUSION

• Remote ischemic conditioning by a single 5-min cuff inflation confers acute short-term improvement of vascular function, particularly in patients with greater aortic stiffness and glycocalyx integrity impairment, likely through oxidative stress reduction, nitrate-nitrite-NO pathway activation, and cardio-protective miR upregulation.