Macrophage-derived osteopontin is fragmented by MMP-9 to hinder angiogenesis in the post-myocardial infarction left ventricle

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sound to assess aortic stenosis and PET/computed tomography (CT) with 18F-NaF to assess calcification severity. Thereafter, 8 animals were subsequently treated with local delivery of a mixture containing 500 μg bolus zolendronate that was delivered on the cusps of the aortic valve, by a dedicated balloon catheter. A placebo mixture was administered on the rest 8 animals (control group). At 28 days all animals underwent a follow-up cardiac imaging with PET-CT 18F-NaF. The progression of calcification was assessed by calculating the difference in SU-Vmax, SU-Vmean, TBRmax and TBR mean at baseline and at follow up both for AV and ascending aorta (AA). After the second PET/CT examination all animals were sacrificed and all aortic valves were collected and analyzed by histology.

**Results:** At baseline, all animals developed aortic valve stenosis with severe calcification. No differences regarding AVA were recorded between both groups. (21.37±1.76 vs. 21.95±3.12, p<0.53). In all animals the local delivery of zolendronate and placebo mixtures was successful and uncomplicated. A total of 48 cusps were histologically examined. The cusps treated with zolendronate had significantly lower expression of calcium content compared to the cusps of the placebo group (16.40±0.90 vs 24.88±1.90% of the area, p<0.0001), whereas the ascending aortas of both groups showed similar expression of calcium content (23.58±4.43 vs 23.12±5.05% of the area, p=0.78). Regarding PET/CT analysis, in the zolendronate group, TBRmean and TBRmax at the level of AA showed a significant increase of calcification during follow up (1.31±0.11 versus 1.63±1.84, p<0.001 and 1.42±0.11 versus 1.64±0.20, p<0.001). In the same group TBRmean and TBRmax at the level of AV did not show any significant change in calcification during the same period (1.20±0.12 versus 1.17±0.78, p=0.29 and 1.30±0.33 versus 1.40±0.67, p>0.08). Interestingly TBRmean showed a regression of calcification at the level of AV compared to AA (0.34±0.07 versus 0.30±0.11, p<0.001).

OPN, which was proteolytically processed as a result of macrophage infiltration, was a strong linear post-LV dysfunction. The ECM protein osteopontin (OPN) increases post-MI, and we previously identified by mass spectrometry a novel MMP-9 cleavage site of OPN between amino acids 151 and 152. In vitro, peptides both upstream and downstream of the cleavage site increased cardiac fibroblast migration without affecting proliferation.

**Purpose:** The aim was to determine the biological function of the MMP-9 generated OPN fragments in vivo post-MI, using full length and cleavage-site specific OPN antibodies.

**Methods:** C57BL/6J wild type (WT) and MMP-9 null mice (3–6 months old) were used for coronary artery ligation and examined at days 0, 1, 3, 5, and 7 post-MI. All animal procedures were approved by the Institutional Animal Care and Use Committee at a University Medical Center in accordance with the Guide for the Care and Use of Laboratory Animals. Immunoblotting and immunohistochemistry were used to quantify full-length and OPN fragments. In vitro angiogenesis assay was performed using HUVECs to compare spanning OPN fragment peptide to fragment peptides upstream and downstream of the cleavage site.

**Results:** In vivo, both full length OPN and the cleaved OPN fragment increased in the LV infarct in WT from days 1 to 5, with a peak elevation at day 5 post-MI. Compared to WT, post-MI MMP-9 null LV showed a surprising increase in cleavage product, indicating that MMP-9 may further degrade OPN with prolonged exposure. This was confirmed by in vitro cleavage assay. By immunohistochemistry, we identified myocytes as the main cellular source of OPN in the absence of MI. Post-MI, macrophages were robustly positive for both full length OPN and the cleaved fragment. An angiogenesis assay was performed to further elucidate the biological function of MMP-9 generated OPN fragments. While total uptake of H2 was not altered, the OPN spanning peptide increased total branching and segment length, effects that were abolished when peptides upstream or downstream of the cleavage site were used.

**Conclusions:** Our results demonstrated that in vivo post-MI, MMP-9 increased OPN, which was processed and released in response to macrophage infiltration, and in vitro cleavage peptides impaired angiogenesis quality.

**Acknowledgement/Funding:** NIH HL075360, HL129823, HL051971, GM104357, GM114833, GM15428, GM103476, and GM103328 and VA 5101B000505

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**P1566 | BENCH**

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**P1567 | BENCH**

**Mitochondrial calpains mediate SIRT3-dependent cardiac dysfunction in LPS-induced endotoxemia**

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Sepsis may result in myocardial dysfunction, likely related to concomitant mitochondrial dysfunction in the heart. Sirtuin 3 (SIRT3) is a mitochondrial NAD+-dependent deacetylase, lack of which impairs mitochondrial ATP synthesis by downregulating deacetylation of proteins of fatty acid oxidation, the TCA cycle and electron transport chain. Since sepsis is characterized by hyperactivation of the NAD+-dependent DNA repair enzyme poly(ADP-ribose)polymerase-1 (PARP1), we hypothesized that myocardial NAD+-depletion due to mitochondrial PARP1 activation may impair SIRT3 activity and thereby contribute to mitochondrial and contractile dysfunction in sepsis. In isolated working hearts, 6 hours of LPS treatment resulted in a decrease in cardiac power (-22%), palmitate oxidation (-33%) and cardiac efficiency (-34%), accompanied by a 57% decrease of the myocardial NAD+/NADH ratio compared to non-treated mice (all p<0.05). PARP1 deletion increased cardiac power, mitochondrial ATP synthesis and palmitate oxidation, whereas the NAD+/NADH ratio was elevated (all p<0.05). In vivo, both full length OPN and the cleaved OPN fragment increased in the LV infarct in WT from days 1 to 5, with a peak elevation at day 5 post-MI. Compared to WT, post-MI MMP-9 null LV showed a surprising increase in cleavage product, indicating that MMP-9 may further degrade OPN with prolonged exposure. This was confirmed by in vitro cleavage assay. By immunohistochemistry, we identified myocytes as the main cellular source of OPN in the absence of MI. Post-MI, macrophages were robustly positive for both full length OPN and the cleaved fragment. An angiogenesis assay was performed to further elucidate the biological function of MMP-9 generated OPN fragments. While total uptake of H2 was not altered, the OPN spanning peptide increased total branching and segment length, effects that were abolished when peptides upstream or downstream of the cleavage site were used.

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