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 stiffness 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/l zoledronate 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 SUVmax, SUVmean, 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 21.98±3.12, p=0.53). In all animals the local delivery of zole-dronate and placebo mixtures was successful and uncomplicated. A total of 48 cusps were histologically examined. The cusps treated with zoledronate 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 23.15±6.50% of the area, p=0.78). Regarding PET/CT analysis, in the zoledronate 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=0.01). Conclusions: In vitro aortic valve calcification was assessed by local catheter-based delivery of zoledronic acid. MMP-9 is an enzyme that is capable of cleaving and degrading collagen, and it is known to be involved in the process of ECM remodeling. Our results demonstrated that in vivo post-MI, MMP-9 increased significantly and there was no significant difference between the groups. The results also showed that the combination of zoledronate and aortic valve calcification was significantly lower than the control group. The potential clinical implications should be confirmed in human studies.

**P1566 | BENCH**

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

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**Background/Introduction:** Extracellular matrix (ECM) turnover is a key event during remodeling of the left ventricle (LV) following myocardial infarction (MI). Turnover includes ECM degradation of existing ECM to remove necrotic myocytes and synthesis to produce new ECM to form the infarct scar. Matrix metalloproteinases (MMPs) are elevated post-MI, and MMP-9 has a strong link to myocardial infarction. We hypothesized that myocardial MMP-9 activity impairs angiogenesis quality. Our results demonstrated that in vivo post-MI, MMP-9 increased significantly and there was no significant difference between the groups. The results also showed that the combination of zoledronate and aortic valve calcification was significantly lower than the control group. The potential clinical implications should be confirmed in human studies.

**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. Conclusions: Our results demonstrated that in vivo post-MI, MMP-9 increased OPN, which was partially processed into a region of macrophage infiltration and, in vitro cleavage peptides impaired angiogenesis quality.

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**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 myocardial dysfunction in the heart, Sirtuin 3 (SIRT3) is a mitochondrial NAD+ dependent deacetylase, lack of which impairs mitochondrial ATP synthesis by down-regulating 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 (PARP-1), we hypothesized that myocardial NAD+ depletion due to mitochondrial PARP-1 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). PARP-1 deletion and in vitro cleavage peptides impaired angiogenesis quality.

**Conclusions:** Despite the fact that MMPs are elevated post-MI, and MMP-9 has a strong link to post-MI myocardial infarction, our results suggest that MMP-9 may further degrade OPN with prolonged exposure, leading to reduced angiogenesis quality. This finding has significant implications for the design of future clinical trials aimed at improving cardiac function after myocardial infarction.