MMP-2 gene silencing attenuates age-dependent carotid stiffness via reduction of elastin degradation and increased eNOS activation

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Funding Acknowledgement: Type of funding sources: Foundation. Main funding source(s): Swiss National Science Foundation,Foundation for Cardiovascular Research–Zurich Heart House

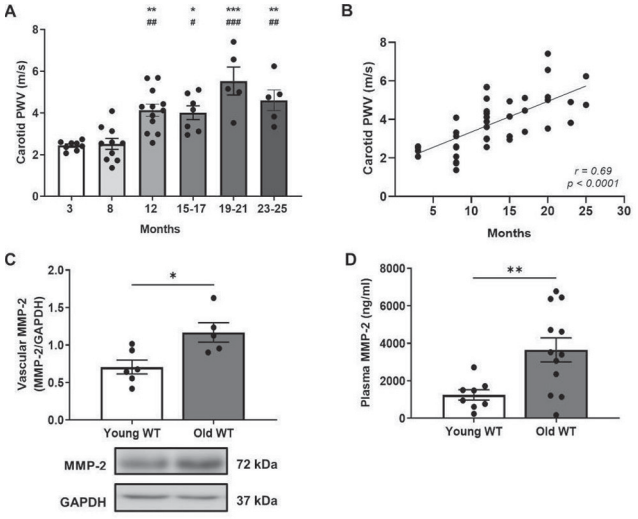
Background and aims: Arterial stiffness is a hallmark of vascular aging. Being characterized by a loss of elasticity of large arterial walls, arterial stiffness is associated with an increased risk of cardiovascular disease (CVD). The age-dependent arterial stiffness is primarily attributed to alterations in the elastic and collagen deposition that is regulated by a number of enzymes, including matrix metalloproteinase-2 (MMP-2). Nevertheless, the mechanistic link between age-dependent arterial stiffness and MMP-2 remains unclear.

In this study, we investigated the effect and efficacy of therapeutic MMP-2 knockdown using small interfering RNA (siRNA) on age-dependent arterial stiffness.

Methods: Pulse wave velocity (PWV) was assessed in the right carotid artery of wild-type (WT) mice of different age groups. MMP-2 levels and activity in the carotid artery and plasma of young (3 months) and aged (20–25 months) WT mice were determined. Old WT mice (18–21 months) were treated for 4 weeks with either MMP-2 or scrambled siRNA, in which carotid PWV was assessed at baseline, 2 and 4 weeks after the start of the treatment. Elastin to collagen ratio, desmosin (DES) level, and endothelial nitric oxide synthase (eNOS) pathways were also evaluated and compared. Lastly, levels of circulating MMP-2 and DES, the breakdown product of elastin, were measured in a human cohort (23–86 years old), in whom carotid-femoral PWV was assessed.

Results: Carotid PWV, as well as both vascular and circulating MMP-2 levels, were elevated with increasing age in WT mice (Figure 1). Therapeutic MMP-2 knockdown in aged WT mice reduced the vascular MMP-2 expression and attenuated age-dependent carotid stiffness. Increased elastin to collagen ratio and a lower plasma DES level were observed on MMP-2 silenced treated animals (Figure 2). Moreover, siMMP-2 treated mice showed enhanced eNOS phosphorylation on Ser1177. A direct interaction between MMP-2 and eNOS was also observed, which, interestingly, is augmented with age. Finally, collected human data showed a higher level of circulating MMP-2 levels on the elderly subjects. In addition, plasma DES level is positively correlated with age and aortic PWV, indicating the involvement of vascular elastin catabolism on arterial stiffness.

Conclusions: Therapeutic MMP-2 gene silencing, specifically targeting vascular MMP-2, attenuates age-dependent carotid stiffness. This effect is mediated by augmenting eNOS activation and reducing elastin degradation. Thus, our findings indicate MMP-2 as a potential therapeutic target to mitigate age-dependent arterial stiffness and CVD.



Carotid PWV and MMP-2 levels increase with age in WT mice. Carotid pulse wave velocity (PWV) measurement on different age groups of wild type (WT) mice showed a gradual increased with age (**A**) A positive correlation between carotid PWV and age in WT mice was also observed. Moreover, MMP-2 levels were significantly increased in the vascular and plasma old animals compared to the young ones (**C-D**) Results are presented as mean ± SEM, **P<0.05* ***P*<0.01 ****P*<0.0001 vs 3 months, # *P<0.05* ## *P*<0.01 ###*P*<0.0001 vs 8 months. MMP-2: matrix metalloproteinase 2; PWV: pulse wave velocity; RCCA: right common carotid artery; WT: wild-type.

Figure 1