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Mice lacking the gene encoding for MMP-9 and resistance artery reactivity

Jun Su, Desiree I. Palen, Pamela A. Lucchesi, Khalid Matrougui *

Department of Pharmacology, LSU Health Sciences Center, 1901 Perdido Street, New Orleans, LA 70112, USA

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Abstract

Objectives: To define the link between the deletion of gene encoding for metalloproteinase 9 and resistance artery reactivity, we studied in vitro smooth muscle and endothelial cell function in response to pressure, shear stress, and pharmacological agents.

Background: Matrix metalloproteinases play a crucial role in the regulation of extracellular matrix turnover and structural artery wall remodeling.

Methods: Resistance arteries were isolated from mice lacking gene encoding for MMP-9 (KO) and their control (WT). Hemodynamic, pharmacology approaches, and Western blot analysis were used in this study.

Results: The measurement of blood pressure in vivo was similar in KO and WT mice. Pressure-induced myogenic tone, contractions to angiotensin-II and phenylephrine were similar in both groups. The inhibition of MMP2/9 ((2*R*)-2-[(4-biphenylylsulfonyl) amino]-3-phenylpropionic acid) significantly decreased myogenic tone in WT and had no effect in KO mice. Relaxation endothelium-dependent (flow-induced- dilation 41.3 ± 0.6 vs. 21 ± 1.6 at 10 μl/min in KO and WT mice, respectively, $P < 0.05$) and eNOS expression were increased in KO compared to WT mice. The inhibition of eNOS with L-NAME significantly decreased endothelium response to shear stress, which was more pronounced in KO mice resistance arteries (-26.83 ± 2.5 vs. -15.84 ± 2.3 at 10 μl/min in KO and WT, respectively, $P < 0.05$). However, the relaxation to exogenous nitric oxide-donor was similar in both groups.

Conclusion: Our study provides evidence of a selective effect of MMP-9 on endothelium function. Thus, MMP-9 gene deletion specifically increased resistance artery dilation endothelium-dependent and eNOS expression. Based on our results, MMP-9 could be a potential therapeutic target in cardiovascular disease associated with resistance arteries dysfunction.

Keywords: Metalloproteinases; Microcirculation; Pressure; Myogenic tone; Shear stress; eNOS

Resistance arteries, defined as pre-capillary vessels, contribute actively to resistance arterial tone and to the blood flow control depending on tissue metabolic demands [1]. Resistance arterial tone is mainly regulated by mechanical factors, such as transmural pressure and intraluminal shear stress. Thus, the control of resistance arterial tone is dependent upon a complex interplay between endothelial cells and VSMC. In general, flow (shear stress)-induced endothelium-dependent vasodilation occurs via release of endothelium-dependent relaxing factors such as nitric oxide [2]. On the other hand, pressure-induced contraction (myogenic tone, MT) is generally endothelium-independent and is mediated by direct stimulation of VSMC [3]. Mechanisms involved in flow-induced dilation and MT are not yet resolved. In previous studies, we showed that the disturbance of gene encoding for vimentin or dystrophin selectively decreased flow-induced dilation of resistance arteries [4–7] indicating a selective role of cytoskeleton elements in the response of endothelial cells to shear stress increases.

The role of MMPs has been relatively well characterized in artery structural remodeling in cardiovascular diseases, but their contributions in acute response to shear stress-induced dilation and MT are not known especially

* Corresponding author. Fax: +1 504 568 2361.
E-mail address: kmatro@lsuhsc.edu (K. Matrougui).

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in resistance artery. Thus, extracellular matrix (ECM) degradation by MMPs, specifically MMP-9, is involved in the pathogenesis of a wide spectrum of cardiovascular disorders, including atherosclerosis, restenosis, cardiomyopathy, congestive heart failure, myocardial infarction, and aortic aneurysm [8,9]. A strict regulation of MMPs expression and activity is crucial in the control of ECM homeostasis. Several studies have documented the importance of MMP-mediated ECM destruction for tumor initiation, growth, migration, angiogenesis, invasion, and metastasis. Recently we showed that MMP-2 and -9 are involved in myogenic tone development through Heparin binding-EGF shedding from SMC surface and subsequent EGF receptor transactivation [10]. In the current study we investigated the role of the absence of MMP-9 on mice mesenteric resistance arteries response to shear stress and pressure changes.

Methods

Animals. The Animal Protocol Review committee at LSU Health Sciences Center approved the experimental protocol. Adult male mice lacking the gene encoding for MMP-9 (MMP-$9^{-/-}$, KO, 3–4 months old, $n = 25$) and age-matched wild type male mice (MMP-$9^{+/+}$, WT, $n = 25$) were obtained from Jackson Laboratory (Bar Harbor, ME). Mice were anesthetized by intraperitoneal injection of a mixture of 100-mg/kg ketamine and 20-mg/kg xylazine.

In vivo blood pressure measurement. Mice were anesthetized with ketamine/xylazine and a catheter, connected to a pressure sensor (Living System Instrumentation, Burlington, Vermont), was inserted into left carotid. After surgery, the mice were subjected to a 20-min equilibration period before mean arterial pressure was measured.

Resistance arterial tone. KO and WT mesenteric resistance arteries (100–120 μm) were dissected, isolated, and mounted onto two glass cannulas in a vessel chamber and then pressurized to 100 mmHg by using a pressure-servo-control perfusion (Living System Instrumentation) in order to stretch the artery and set a constant arterial length. Vessel diameter was continuously monitored via a video image analyzer as previously described [10]. The cannulated arterial segments were submerged in 2 ml of physiological salt solution (pH 7.4), oxygenated with (10% O_2–5% CO_2 and 85% N_2). Functional integrity of endothelium was assessed by test of the endothelium-dependent vasodilation effect on acetylcholine (1 μM) after precontraction with phenylephrine (1 μM). Vessels not responding by contraction and relaxation more than 75% to phenylephrine and acetylcholine, respectively, were discarded. Next, after a 45-min equilibration period, diameter changes were measured when intraluminal pressure was increased from 25 to 125 mmHg (active diameter) by 25 mmHg for each step. The time difference between each step was set at 3–5 min. Then pressure–diameter relationship was repeated in the presence of different drugs: (MMP2/9 and EGFR inhibitors, (2R)-2-[(4-biphenylylsulfonyl) amino]-3-phenylpropionic acid and AG1478 "Calbiochem", respectively). Each vessel will be submitted to one drug. At the end of each experiment, the passive diameter was determined in the presence of EGTA (2 mM) and sodium nitroprusside (SNP, 100 μM).

Flow (shear stress)-induced dilation. Resistance arteries were pressurized at 75 mmHg of intraluminal pressure and subjected to incremental levels from 3 to 25 μl/min under control and eNOS inhibition conditions. Shear stress (dyn/cm^2) was calculated based on the equation: shear stress $= 4 \times$ viscosity $\times$ the flow rate/$\pi \times$ vessel radius^3.

Pharmacology approach. Acetylcholine, phenylephrine, nitric oxide donor (SNP) (10^{-10}–0^{-5} M), and angiotensin-II (10^{-10}–10^{-7} M), in a dose–response manner, were performed in pressurized mesenteric resistance arteries isolated from KO and WT mice.

Immunoprecipitation and Western blot analysis. In vivo mesenteric resistance arteries were isolated from KO and WT mice, and immediately frozen with liquid nitrogen and stored at −80 °C. Frozen vessel segments were pulverized and re-suspended in ice-cold lysis buffer [11]. Each sample was immunoprecipitated with a specific anti-total eNOS antibody and then subjected to immunoblotting with a specific antibody anti-total eNOS (1:1000, Cell Signaling Technology).

Statistical analysis. Results are expressed as means ± SEM. Significance of the differences between groups was determined by repeated 1- or 2-factor ANOVA, where appropriate, followed by Bonferroni *post hoc* analysis (InStat). The two-tailed Student's *t*-test was also used to compare the variables "mean arterial pressure and body weight" between the two groups. Differences were considered significant at $P < 0.05$.

Results

Animals

Body weight and mean arterial pressure (MAP) were similar in WT and KO mice.

Pressure- induced myogenic tone

In isolated mesenteric resistance arteries, stepwise increases in intraluminal pressure induced myogenic tone (MT) development. MT was similar in mesenteric resistance arteries from both groups (Fig. 1). The inhibition of MMP-2 and -9 significantly decreased MT in WT mice resistance arteries (Fig. 2A). However, the inhibition of MMP2 had no effect of MT in KO mice resistance arteries (Fig. 2B), suggesting that the deletion of gene encoding for MMP-9 affected the involvement of MMPs-2 in MT development. EGF receptor inhibition (AG1478, 5 μM) significantly blocked MT in both animals (Fig. 2A and B), indicating that EGF receptor transactivation involvement is preserved in KO mice resistance arteries.

DMSO was used as vehicle for MMP 2/9 and EGF receptor inhibitors. The application of DMSO alone had no effect on MT (data not shown).

Flow-induced dilation

At 75 mm Hg of intraluminal pressure, stepwise increases in intraluminal flow induced a progressive vascular dilation in both animals (Fig. 3A) but the dilation was significantly enhanced in KO mice compared to WT (41.3 ± 0.6 vs. 21 ± 1.6, at 10 μl/min, in KO and WT, respectively, $P < 0.05$). Blockade of nitric oxide synthesis by L-NAME (*N*(G)-nitro-L-arginine methylester, 100 μM) significantly reduced flow-induced dilation in both groups (Fig. 3A and B), but was more pronounced in KO resistance arteries (-26.83 ± 2.5 vs. -15.84 ± 2.3, at 10μl/min, in KO and WT, respectively, $P < 0.05$). In addition, endothelial nitric oxide synthesis (eNOS) expression was significantly high by 3-fold in KO mice mesenteric resistance arteries compared to WT (Fig. 3D).