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Research Paper

Midkine Controls Arteriogenesis by Regulating the Bioavailability of Vascular Endothelial Growth Factor A and the Expression of Nitric Oxide Synthase 1 and 3

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ABSTRACT

Midkine is a pleiotropic factor, which is involved in angiogenesis. However, its mode of action in this process is still ill defined. The function of midkine in arteriogenesis, the growth of natural bypasses from pre-existing collateral arteries, compensating for the loss of an occluded artery has never been investigated. Arteriogenesis is an inflammatory process, which relies on the proliferation of endothelial cells and smooth muscle cells. We show that midkine deficiency strikingly interferes with the proliferation of endothelial cells in arteriogenesis, thereby interfering with the process of collateral artery growth. We identified midkine to be responsible for increased plasma levels of vascular endothelial growth factor A (VEGFA), necessary and sufficient to promote endothelial cell proliferation in growing collaterals. Mechanistically, we demonstrate that leukocyte domiciled midkine mediates increased plasma levels of VEGFA relevant for upregulation of endothelial nitric oxide synthase 1 and 3, necessary for proper endothelial cell proliferation, and that non-leukocyte domiciled midkine additionally improves vasodilation.

The data provided on the role of midkine in endothelial proliferation are likely to be relevant for both, the process of arteriogenesis and angiogenesis. Moreover, our data might help to estimate the therapeutic effect of clinically applied VEGFA in patients with vascular occlusive diseases.

1. Introduction

Vascular occlusive disease such as myocardial infarction, stroke or peripheral artery diseases are still a major cause of morbidity and mortality worldwide. Searching for new, non-invasive options to treat affected patients, much effort is made to understand the molecular mechanisms of arteriogenesis. Arteriogenesis is a tissue- and even life-saving process and presents the growth of pre-existing collateral arterioles to natural biological bypasses. It is provoked by increased fluid shear stress and is mediated by sterile inflammation (Chillo et al., 2016).

Midkine (MK) is a retinoic acid inducible cytokine, which is highly expressed during embryogenesis (Kadomatsu et al., 1990). In the adulthood its expression is restricted to certain tissues but strongly induced during inflammatory processes (Badila et al., 2015) such as rheumatoid arthritis, inflammatory bowel disease, and multiple sclerosis (for an overview see (Weckbach et al., 2011)), which are associated with angiogenesis. MK has also been implicated in angiogenesis and hence endothelial cell (EC) proliferation under conditions of tissue ischemia (Weckbach et al., 2012) and tumor growth (Choudhuri et al., 1997; Muramaki et al., 2003) as well as in neointima formation (Hayashi et al., 2005; Horiba et al., 2000). The functional role of MK in angiogenesis is still not well defined, however, it was shown that MK induces chemotaxis of neutrophils and supports neutrophil adhesion during inflammation (Takada et al., 1997; Weckbach et al., 2014). Neutrophils themselves express high levels of MK, but do not release it (Weckbach et al., 2012; Weckbach et al., 2014; Narita et al., 2008). However, after stimulation with the chemokine (C-X-C) ligand 1 (CXCL1) neutrophils release angiogenic growth factor such as vascular endothelial growth factor A (VEGFA) (Scapini et al., 2004).

The functional role of MK in arteriogenesis has never been investigated. Moreover, the functional role of VEGFA in arteriogenesis is controversially discussed (Jazwa et al., 2016). In vitro data from Tzima et al., who identified the VEGF receptor 2 (VEGFR-2), to be part of a mechanosensory complex and to be relevant for phosphoinositide 3-kinase (PI3K) activation, even suggested that VEGFR-2 is ligand-independent activated in response to shear stress (Tzima et al., 2005).

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Here, we demonstrate that leukocyte domiciled midkine controls the plasma bioavailability of VEGFA, which in turn is relevant for the expression of neuronal nitric oxide synthase (*Nos1*) and endothelial *Nos* (*Nos3*) (but not inducible *Nos* (*Nos2*)) in endothelial cells of collaterals. For arteriogenesis all three isoform of NOS, namely NOS1, NOS2 and NOS3 are described to be involved, although the function of the individual NOS is not well defined (Troidl et al., 2010; Pagel et al., 2011). Exclusively for NOS3 it has been shown that it mediates vasodilation in growing collaterals (Troidl et al., 2010). Here we show that *Nos1* or *Nos3* expression, respectively, is essential for collateral endothelial cell proliferation, whereby both isoforms are likely to be able to substitute for each other.

Collectively, our data provide mechanistic insights into MK-mediated endothelial cell proliferation, which are likely to be relevant not only for the process of arteriogenesis, but also for other inflammatory processes as well as tumor growth being associated with angiogenesis and hence endothelial cell proliferation.

2. Materials and Methods

2.1. Animals and Treatments

All experiments were carried out according to the German animal legislation guidelines and were approved by the Bavarian Animal Care and Use Committee. Mice were kept under a 12-hour (h) day and night cycle with chow and water provided ad libitum.

Mice were treated with either 100 μl DETA NONOate (Cayman Chemical, Ann Arbor, MI) 0.12 mg/ml intraperitoneal once daily in 0.9% NaCl (saline) (Braun, Melsungen, Germany), with 50 μl recombinant murine midkine (rmMK) (PeproTech, Rocky Hill, NJ) 0.1 mg/ml subcutaneously twice daily in saline or with 100 μl recombinant murine VEGFA164 (rmVEGFA) (Sigma-Aldrich, St. Louis, MO) 0.01 mg/ml intraperitoneal once daily in saline. Control groups received saline only. Sodium nitrite (Sigma-Aldrich) was applied via drinking water at a concentration of 1 g/l (Kumar et al., 2008; Hannas et al., 2010). Control groups received normal drinking water. Treatment was started 3 days (d) before the surgical procedure and continued until the end of the experiments.

2.2. Femoral Artery Ligation, Laser Doppler Imaging and Bone Marrow Transplantation

Collateral artery growth was investigated in a murine hindlimb model (Chillo et al., 2016) using 8- to 10-week-old male $Mdk^{-/-}$ (carrying the Mdk^{tm1Tmu} allele, RRID: MGI:3579532) (Nakamura et al., 1998), $Nos1^{-/-}$ (B6.129S4-$Nos1^{tm1Plh}$/J, RRID: IMSR_JAX:002986) (Huang et al., 1993) mice and appropriate WT controls (C57BL/6J mice, RRID: IMSR_JAX:000664; Charles River, Sulzfeld, Germany). Mice underwent ligation of the right femoral artery distally to the origin of the profunda femoris branch using a 7-0 silk braided suture (Peasalls Sutures, Somerset, Great Britain). During the surgical procedures mice were under general anesthesia with a combination of fentanyl (0.05 mg/kg body weight) (Janssen-Cilag, Neuss, Germany), midazolam (5.0 mg/kg body weight) (Ratiopharm, Ulm, Germany) and medetomidine (0.5 mg/kg body weight) (Pfizer, New York, NY), which was administered subcutaneously. The left leg underwent sham operation and was used as control. Perfusion of the paws was assessed using a LDI technique (Moor LDI 5061 and Moor Software Version 3.01, Moor Instruments, Remagen, Germany) under temperature-controlled conditions. The measurements were conducted before FAL (basal), immediately after FAL (aFAL), 3d and 7d after FAL. Color-coded images of the paws representing the flux value were used to calculate the ratio of the tissue perfusion of the occluded (Occ) to the Sham-operated (Sham) paw. For bone marrow transplantation see the Supplemental Experimental Procedures.

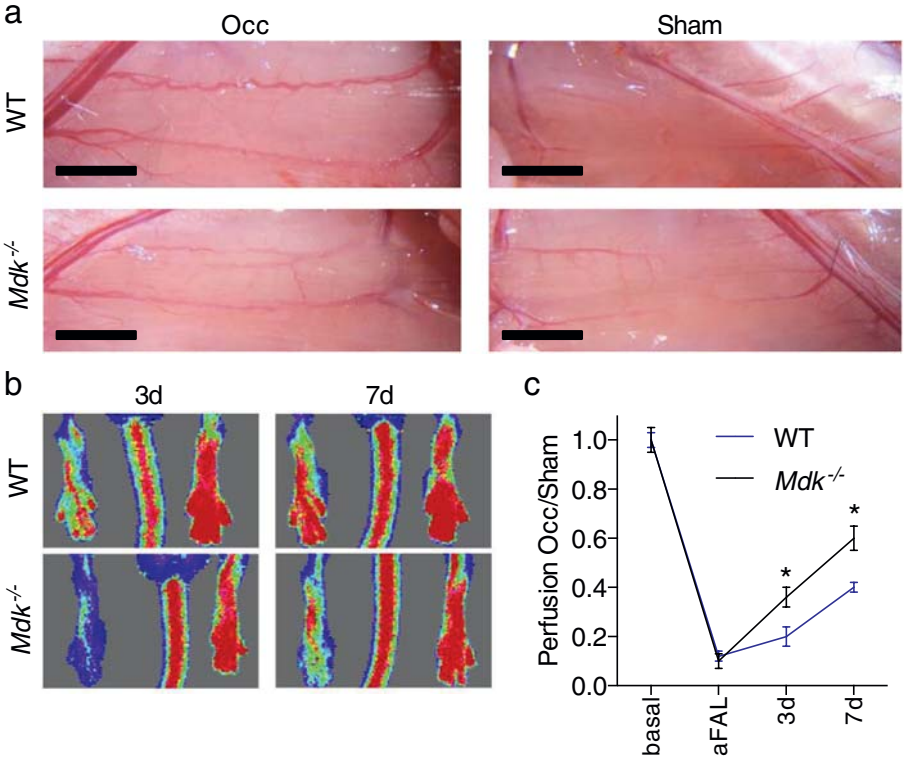

Fig. 1. $Mdk^{-/-}$ mice show reduced perfusion recovery after FAL. (a) Representative images of superficial collateral arteries in the adductor muscles of WT (upper panels) and $Mdk^{-/-}$ (lower panels) mice 7d after FAL (Occ) or sham operation (Sham). Scale bars: 3 mm. (b) Representative LDI images of WT (upper panel) and $Mdk^{-/-}$ (lower panel) mice 3d and 7d after FAL. (c) Line graph displaying the perfusion ratio (Occ/Sham) of WT and $Mdk^{-/-}$ mice before FAL (basal), immediately after FAL (aFAL), 3d and 7d after FAL. $n = 5$. *P < 0.05 WT compared with $Mdk^{-/-}$, two-way ANOVA with Bonferroni's multiple comparison test. Data shown are means ± SD.

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