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Letter to the Editor

Aspirin-triggered 15-epi-lipoxin A_4 signals through FPR2/ALX in vascular smooth muscle cells and protects against intimal hyperplasia after carotid ligation

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The benefit of aspirin is well established in cardiovascular prevention, and involves the irreversible inhibition of platelet cyclooxygenase (COX)-1 and, as a consequence, decreased thromboxane levels [1]. In addition to inhibition of platelet aggregation, aspirin may also have anti-inflammatory properties, with potential additional benefit in atherosclerosis prevention. Acetylation of the COX-2 enzyme by aspirin directs the metabolism of polyunsaturated fatty acids (PUFAs) towards a biosynthetic pathway, which in addition involves lipoxygenase enzymes and leads to the formation of specific mediators, such as 15-epi-lipoxin A_4, also referred to as aspirin-triggered lipoxin (ATL) [2]. ATL has been shown to promote the resolution of inflammation [3].

ATL shares its receptor with several other ligands, for example antibacterial peptides such as the cathelicidin LL-37, and this receptor has therefore been denoted as FPR2/ALX (formyl peptide receptor 2 and A type lipoxin receptor) [4]. It was recently shown that FPR2/ALX is expressed in human carotid atherosclerotic lesions, and that higher expression levels are inversely associated with clinical signs of cerebral ischemia, suggesting that this receptor may transduce increased atherosclerotic plaque stability [5]. In addition to macrophages, the latter study also identified vascular smooth muscle cells (vSMCs) in human atherosclerotic lesions expressing the FPR2/ALX receptor [5]. Furthermore, murine vSMCs that lack this receptor exhibit a decreased collagen production and cross-linking, whereas collagenases are increased, accompanied by decreased collagen content in atherosclerotic lesions in hyperlipidemic mice lacking FPR2/ALX [5].

Importantly, lipoxins are produced during percutaneous coronary interventions (PCI), and their levels increase after aspirin treatment [6]. However, the role of ATL signaling through the FPR2/ALX receptor in vSMC and vascular injury has remained unexplored. Therefore, the aim of the present study was to explore the ATL signaling through FPR2/ALX in vSMCs in vitro and in a mouse model of carotid artery ligation in vivo.

Mice lacking the FPR2/ALX homologue ($Fpr2^{-/-}$; FPR2/ALX knockout [KO] mice) and wild type ($Fpr2^{+/+}$; WT) mice were generated as previously described [7]. Aortic vSMCs were isolated from WT and KO mice, and used for evaluation of proliferation and migration according to established protocols [1,5,8]. Finally, FPR2/ALX WT and KO mice were subjected to ligation of the left common carotid artery. Furthermore, based on observations in the present and previous [3] studies that ATL acts as an inhibitor of vSMC responses in vitro, either ATL (10 μg/kg) or vehicle (15% ethanol in PBS) was administrated by means of osmotic pumps following carotid ligation. Experimental details are given in the Supplementary material. All animal experiments were performed according to procedures approved by the local ethics committee.

Vascular SMC derived from FPR2/ALX KO mice exhibited 2.3 ± 0.2 fold higher proliferation compared with wild type vSMCs ($P < 0.05$; Fig. 1A). To further extend this observation, vSMC migration was assessed by a scratch assay, which revealed that ATL (100 nM) reduced WT vSMC migration by 26 ± 3% ($P < 0.05$). Fig. 1B depicts a representative experiment of the effects of ATL after 24 h in WT vSMCs. The critical role of FPR2/ALX in vSMC signaling was further reinforced by the observation that FPR2/ALX KO vSMCs exhibited an accelerated migration, closing 75 ± 9% of initial wound at 24 h, compared with WT 45 ± 6% closure in vSMCs ($P < 0.05$; Fig. 1C). Importantly, and in contrast to the results obtained in WT cells, FPR2/ALX KO cells remained unresponsive to ATL (Fig. 1E). Taken together, these results support a previous study demonstrating that ATL inhibited PDGF-induced migration of

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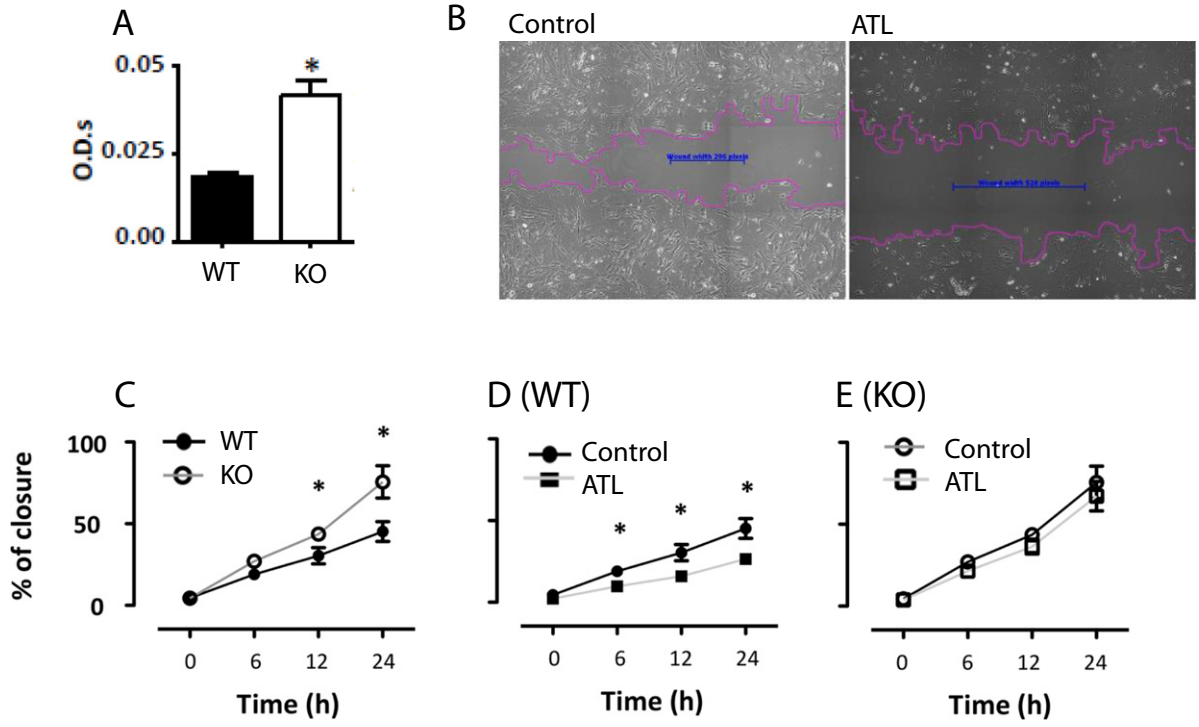

Fig. 1. (A) Comparison of vSMC proliferation in vascular smooth muscle cells (vSMCs) derived from either wild type (WT) or FPR2/ALX knock-out (KO) mice. Results (mean ± SEM) are expressed in ODs and each bar represents quadruplicates of cells derived from 3 different mice of each genotype. *$P < 0.05$ vs WT. (B) Representative micrographs of the scratch assay at 24 h, showing WT vSMCs in the absence (left panel) and presence (right panel) of aspirin-triggered lipoxin (ATL; 100 nM). (C–E) Time course of vSMC migration in the scratch assay. Results (mean ± SEM) are expressed as percent closure of initial scratch over a period of 24 h. Four groups were compared; WT in the absence (closed circles) and presence (closed squares) of ATL (100 nM), and FPR2/ALX KO in the absence (open circles) and presence (open squares) of ATL (100 nM), N = 4. *$P < 0.05$ compared with either WT (C) or ATL-treated (D).

venous SMCs [3] and extend the observation by providing a first support to the notion that ATL inhibits vSMC migration through FPR2/ALX receptor signaling.

Based on these in vitro findings, a carotid ligation model was used to assess the impact of FPR2/ALX signaling on intimal hyperplasia in vivo. Administration of ATL reduced the intima area and intima/media ratio 4 weeks after carotid ligation in WT mice (Fig. 2), supporting previous studies of D-series resolvins [9] and the murine homologue of LL-37 [10]. Interestingly, ATL failed to induce any significant effects when administered to KO mice (Fig. 2). The latter results for the first time point out a crucial role of ATL-signaling through FPR2/ALX in the response to vascular injury.

In summary, two major observations emerge from the present study. First, ATL induced effective inhibition of vSMC migration and conferred protection against intimal hyperplasia following carotid ligation. Second, these effects of ATL were transduced through the receptor FPR2/ALX, as shown by the lack of effect of this ligand in FPR2/ALX KO mice, both in vitro and in vivo. In addition, an increased proliferation and faster wound closure were observed in FPR2/ALX KO vSMCs compared with WT cells. In conclusion, these findings, together with the

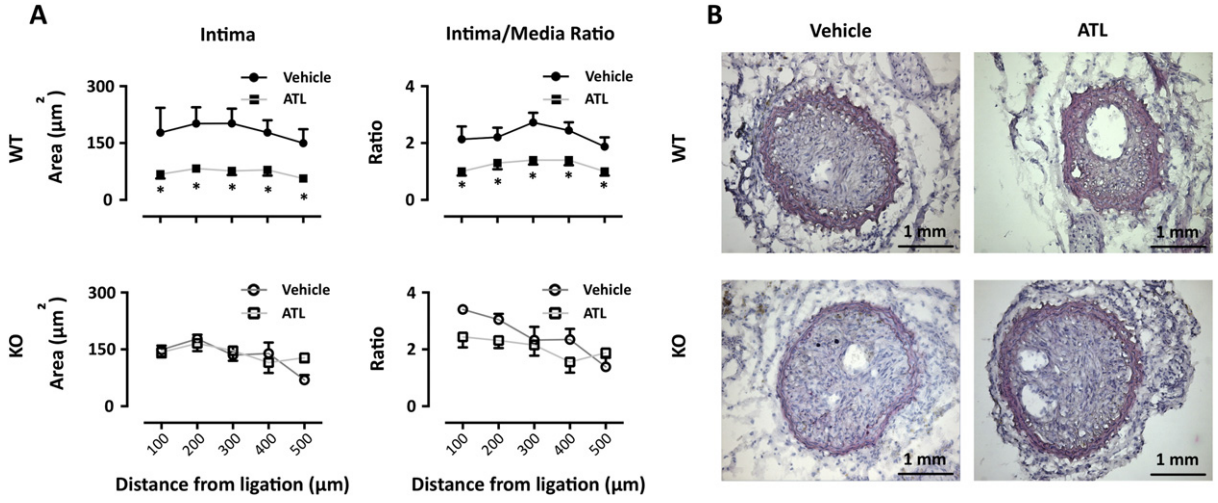

Fig. 2. (A) Effects of ATL (10 μg/kg) on the development of intima hyperplasia 28 days after carotid ligation in wilt type (WT; upper panels) and FPR2/ALX knock-out (KO; lower panels) mice in vivo. In each graph, the intimal hyperplasia was quantified every 100 μm from carotid bifurcation/ligation, and expressed as either intima area (left panels) or intima/media ratio (right panels). WT mice treated with either vehicle (N = 9, closed circles) or ATL (N = 7, closed squares), and KO mice treated with either vehicle (N = 9, open circles) or ATL (N = 8, open squares). Each point represents by mean ± SEM, *$P < 0.05$ vs. Vehicle. (B) Representative micrographs at the 300 μm level from the carotid ligation.

recently described effects on extracellular matrix deposition and maturation [5], indicate a potential therapeutic implication of ATL and FPR2/ALX vSMC signaling in vascular diseases. The pertinence of the observations for human pathology is further reinforced by the expression of FPR2/ALX in atherosclerotic lesions [5] and the increased ATL production during PCI [6], and provides support for additional benefit of aspirin in interventional cardiology, which goes beyond platelet inhibition.

Disclosures

The authors report no relationships that could be construed as a conflict of interest.

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Appendix A. Supplementary methods description

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.ijcard.2014.11.010.

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