Fractalkine as an Important Target of Aspirin in the Prevention of Atherogenesis

Editorial to: “Aspirin Inhibits Fractalkine Expression in Atherosclerotic Plaques and Reduces Atherosclerosis in ApoE Gene Knockout Mice” by H. Liu et al.

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Since its discovery in the 1850s, aspirin, or acetylsalicylic acid, has been widely used as an anti-inflammatory, analgesic and antipyretic drug. In addition, aspirin therapy reduces the incidence of myocardial infarction in patients with a high risk on occlusive vascular events [1], which has been attributed to aspirin's anti-platelet properties. By inhibiting COX activity, low-dose aspirin mainly prevents prothrombotic thromboxane A₂ production in platelets due to their inability to regenerate COX1. This shifts the haemostatic thromboxane-prostacyclin balance and favours the prostacyclin-mediated inhibition of platelet aggregation, thereby reducing the risk of thrombus formation in high-risk patients [2]. Interestingly, aspirin administration has also been reported to reduce the development and progression of atherosclerosis in hyperlipidaemic mice [3–6]. This property goes beyond aspirin's ability to block COX1 activity, as COX1 inhibition can only reduce atherosclerotic plaque formation in early stages, without affecting the progression of established lesions [7]. This emphasizes the importance of COX-independent anti-inflammatory actions of aspirin in its therapeutic value.

Atherosclerosis development and progression are indeed closely linked to inflammatory processes in the vessel wall [8]. The denudation and activation of the arterial endothelial cell (EC) layer by shear stress, reactive oxygen species (ROS) and oxidized low density lipoproteins (oxLDL) results in the production of soluble chemokines that recruit

leukocytes to the site of inflammation. Initial selectin-mediated rolling of monocytes over the vessel wall is followed by their firm adhesion and transmigration into the vessel, where they transform into macrophages involved in the uptake of accumulated modified lipids. Crucial for this firm monocyte-EC adhesion are the integrins on the monocyte cell surface that interact with their corresponding ligands on ECs. Also EC-mobilized chemokines play an important role in this adhesion step, as they increase the integrin avidity by stimulating their respective G-protein coupled chemokine receptors on monocytes. Recently, it was shown that inflammatory Ly-6C^{high} monocytes require the chemokine receptors CCR2, CCR5 and CX3CR1 to enter into atherosclerotic lesions, while the migration of Ly-6C^{low} monocytes did not rely on CX3CR1, but was partially dependent on CCR5 [9]. The importance of these chemokine receptors and their chemokine ligands in atherosclerosis is further demonstrated by their respective knock-out or antagonist treatment in hyperlipidaemic mice, displaying a reduction in atherosclerotic plaque formation and lesional macrophage accumulation, e.g. when targeting RANTES(CCL5)/CCR5 [10, 11], platelet-derived CCL5/CXCL4 heteromers [12], MCP1(CCL2)/CCR2 [11] and fractalkine (CX3CL1)/CX3CR1 [13–15]. These findings underline the important contribution of chemokine-mediated monocyte/macrophage accumulation to atherosclerotic lesion formation and open a new direction of therapeutic drug design and application.

Interestingly, aspirin has been shown to affect different steps in the inflammatory events associated with atherogenesis. As such, aspirin protects ECs from oxidative stress by interfering with the production of ROS and by reducing the ROS- and oxLDL-induced dysfunction of ECs [16]. In

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addition, aspirin reduces leukocyte chemotaxis and adhesion on ECs [17, 18], which can explain the lower foam cell content in atherosclerotic plaques of hypercholesterolaemic mice treated with aspirin [4, 6]. This reduction of leukocyte recruitment and adhesion can (at least partly) be ascribed to the ability of aspirin to inhibit the activity of IKK β [19] and the proteasome [20], which together underlie the aspirin-induced defect in the phosphorylation and degradation of I κ B- α , the inhibitor protein of NF- κ B [21, 22]. In this way, aspirin interferes with NF- κ B activation [21] and blocks the expression of NF- κ B target genes involved in monocyte chemotaxis, as CCL2 [23], and monocyte-EC adhesion, as E-selectin, VCAM1 and ICAM1 [18, 22].

In this issue, Liu and colleagues [24] observed that the reduction in atherosclerotic plaque size in hyperlipidaemic mice upon aspirin administration was associated with a reduced lesional expression of fractalkine, another NF- κ B target gene important in atherosclerosis [13]. Although the macrophage accumulation in the lesions was not measured in this study, previous data link reduced fractalkine expression to a lower foam cell content in the plaques [13]. In this context, fractalkine is unique in that it can both function as a soluble chemoattractant and as a membrane-bound leukocyte adhesion molecule, independent of selectins or integrins. Whereas this intrinsic adhesion function mediates initial, weak leukocyte interactions, the binding of fractalkine to its chemokine receptor CX3CR1 increases leukocytic integrin avidity, inducing firm leukocyte adhesion [25]. This unique adhesive function of fractalkine might explain why CX3CR1-expressing cells bound more rapidly and stronger to immobilized fractalkine than to VCAM1 [26]. Interestingly, it was shown that fractalkine and CCR2 contribute independently to monocyte/macrophage recruitment into atherosclerotic lesions [27]. Although fractalkine deletion had a considerable lower atheroprotective effect compared to the deletion of CCR2, fractalkine deficiency further reduced macrophage accumulation and atherosclerotic lesion formation in CCR2^{-/-} mice [27]. This interesting finding underlines the synergistic effect of chemokines and chemokine receptors in monocyte recruitment during atherogenesis, and indicates that a simultaneous blocking of two or more chemokine receptors might be beneficial in the treatment of atherosclerosis [27].

And this is precisely what underlies the property of aspirin to efficiently reduce leukocyte chemotaxis and adhesion on ECs: by interfering with NF- κ B activation, aspirin reduces the expression of synergistically acting chemokines as CCL2 and fractalkine, and in addition downregulates monocyte adhesion molecules, as VCAM1 and—again—fractalkine. This property can be linked to the ability of aspirin to attenuate atherosclerosis in mice [3–6], although it should be mentioned that this beneficial outcome was not observed by all groups studying the

effects of aspirin administration on atherogenesis [28, 29]. These discrepancies could be due to different animal models used, to differences in the high-fat diet and aspirin administration, and to the different time points at which atherosclerosis was examined. Furthermore, it remains unclear in how far aspirin and fractalkine exert true protective, anti-atherosclerotic functions in humans. Although CX3CR1 gene polymorphisms have been associated with a reduced risk of coronary artery disease (CAD) and therapeutics interfering with fractalkine activity might thus be beneficial in the treatment of CAD [30], the effectiveness of routine aspirin therapy for the primary prevention of CAD in healthy individuals remains high uncertain and requires further research [31].

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