

Abstract

Background

Thrombosis is viewed as a multi-step, multi-cellular process driven by inflammatory stimuli and leukocyte (e.g. neutrophil) activation, and is a major pathophysiological contributor of stroke. Accumulating data supports the hypothesis that anti-inflammatory therapies may limit thrombosis and anti-thrombotic therapies may reduce vascular inflammation. One such target is the anti-inflammatory and pro-resolving endogenous mediator Annexin A1 (AnxA1). The development of therapies that temper inflammation and enhance resolution offer potential therapeutic strategies for the treatment and management of stroke and other thromboinflammatory diseases. Here we sought to comprehensively elucidate the functional significance of targeting the AnxA1/Fpr2/ALX pathway in thrombosis.

Material and Methods

We used a well-characterized light/dye thrombosis model, coupled with pharmacological and genetic approaches. Male and female adult wild type (WT, C57/BL6) or AnxA1 knock-out (AnxA1^{-/-}) mice were used. Fluorescence intravital microscopy (IVM) was used to monitor cerebral thrombosis, along with flow cytometry. Mice were treated with AnxA1 (1 µg/mouse) or saline vehicle 20 minutes before the onset of cerebral thrombus formation. Thrombogenesis and blood flow cessation times were quantified. (All animal experiments were approved by relevant institutional animal welfare panels and were conducted in accordance with the American Association for Accreditation of Laboratory Animal Care guidelines and complied with ARRIVE [Animal Research: Reporting In Vivo Experiments]).

Results

IVM revealed that AnxA1 treatment was able to prolong blood flow cessation times in both cerebral arterioles and venules, via regulation of the key inflammation resolution receptor: FPR2/ALX, as evidenced by the co-administration of the FPR2/ALX antagonist WRW4. Using AnxA1^{-/-} mice, we found that a deletion of *AnxA1* impacts thrombosis as shown by the accelerated cerebral microvascular thrombus formation (decrease in blood flow cessation time) observed in both arterioles and venules of these mice compared to their WT counterparts (arterioles: 17.9 ± 2.3 vs 33.2 ± 1.9 min and venules: 13.2 ± 2.4 vs 20.9 ± 2.2 min. $p < 0.05$). Furthermore, we found that in an inflammatory

backdrop (induced by the administration of lipopolysaccharide [LPS]), AnxA1 treatment was able to suppress both platelet $\alpha\text{IIb}\beta\text{3}$ and P-selectin expression.

Conclusion

Herein, we found a previously unknown phenomenon that the anti-inflammatory protein AnxA1 can modify a thromboinflammatory environment to promote resolution. These findings have far-reaching applicability for drug discovery programs focused on resolution biologics targeting thromboinflammatory diseases such as cardiovascular disease and stroke.