HYDROGEN SULFIDE AND PRO-RESOLVING PATHWAY: A POSSIBLE INTERPLAY IN ENDOTHELIAL CELLS

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Introduction:n The management of inflammatory process is crucial in keeping the right balance between pro- and anti-inflammatory mediators to avoid chronic response and tissue damage. In this view, pro-resolving modulators such as formyl-peptide receptor 2 (FPR2) and annexin A1 (AnxA1) play a key role in dampening inflammation through the reduction of leukocyte migration and the facilitation of immune cells efferocytosis. The interest in assessing mechanisms of the pro-resolution pathways have mainly been focused on immune system and leukocytes actions at the interface with endothelium. In particular, activation of FPR2, together with production and release of AnxA1, lipoxin A_4 (LXA_4) and other specialized proresolving mediators (SPMs), reduces leukocytes emigration in inflamed tissues, ensuring a "controlled" inflammation. Interestingly, activation of AnxA1/FPR2 loop is also mediated by hydrogen sulfide (H_2S), an endogenous gaseous vasodilator with anti-inflammatory properties and synthesized by different enzymes, including cystathionine-γ-lyase (CSE). Although there is an increasing body of evidence with respect to pro-resolution in immune cells, still little is known about the role of AnxA1/FPR2 axis in endothelial cells. Indeed, we here aimed to investigate the role of endothelial AnxA1/FPR2 in inflammation and the possible cross-talk with H_2S pathway.

Materials and methods: Bovine aortic endothelial cells (BAEC) were cultured in DMEM medium supplemented with 10% FBS. Before the experimental procedure, cells have been starved overnight by replacing the medium with serum-free DMEM. BAEC were treated with TNFα (10ng/ml) for 6h alone or in combination with Ac2-26 (FPRs pan agonist, 0.1-1μM) or AP123 (H_2S donor, 1-10μM). Following the treatments, cell pellets were collected and used for western blot analysis to determine expression levels of CSE, eNOS, iNOS, COX2, FPR2 and AnxA1.

Results: Administration of TNFα to BAEC reduced expression of CSE, eNOS, and FPR2, while COX2 and AnxA1 showed a little reduction following TNFα treatment. Conversely, iNOS is increased by TNFα. The treatment with Ac2-26 substantially restored CSE, eNOS and FPR2 expression, with little or no changes in iNOS and AnxA1 levels. Conversely, COX2 was highly increased upon Ac2-26 treatment respect to TNFα alone. When AP123 was used, a consistent effect was again observed for CSE, eNOS and FPR2; however, AP123 was also able to restore levels of AnxA1 and to downregulate the increase in expression of iNOS induced by TNFα. Similarly to what observed for Ac2-26, AP123 also increased expression of COX2 in BAEC treated with TNFα.

Discussion and conclusion: The effect of Ac2-26 is evident on CSE and eNOS and indicates a possible role for FPR2 in the control of H_2S and NO biosynthesis, affected by TNFα; similarly, AP123 restored the expression of FPR2 as well as AnxA1, suggesting that H_2S may take part in the activation of AnxA1/FPR2 axis. In addition, the increase in COX2 expression induced by both Ac2-26 and AP123, despite their anti-inflammatory properties, maybe be relevant in the view of the crucial role of COX2-derived prostacyclin in endothelial cells, where it contributes to modulate vasodilation. Overall, these preliminary suggest a possible cross-talk between H_2S and AnxA1/FPR2 axis, cooperating to control vascular relaxation, besides the inflammatory response, thus providing a different approach to manage inflammatory-based vascular disease.