SOCIETÀ ITALIANA DI FARMACOLOGIA

MODELLO PER INVIO RELAZIONE DI METÀ E FINE PERIODO

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TIPOLOGIA DI BORSA RICEVUTA: BORSA DI STUDIO PER BREVI PERIODI ALL'ESTERO SIF

TIPOLOGIA DI RELAZIONE (es.: metà periodo o finale): FINALE

TITOLO DELLA RELAZIONE: H_2S AND INFLAMMATION: INTERPLAY WITH RESOLUTION PATHWAYS AND SPECIALIZED PRO-RESOLVING MEDIATORS

RELAZIONE:

Hydrogen sulfide (H_2S) is a gaseous mediator synthesized in mammalian tissues by three enzymes, cystationine-γ-lyase (CSE) and cystationine-β-synthase (CBS) and 3-mercaptopyruvate sulfurtransferase (3-MST) [1]. H_2S is identified as the youngest member of gaseous transmitter family, along with nitric oxide (NO) and carbon monoxide (CO), for its modulatory effects on several signalling molecules [2-3]. Furthermore, H_2S is involved in a variety of pathophysiological processes, such as vasodilation, monocyte and granulocytes adhesion, lipid metabolism, tissue repair and inflammation [2]. There is growing evidence of crosstalk between H_2S and NO. In particular, the interaction of these endogenous mediators has been shown to affect the endothelial function, where NO may increase the cellular uptake of cystine, indirectly increasing H_2S

production [4]. Conversely, H_2S affects NO production as shown in rat corpus cavernosum where NaHS, source of exogenous H_2S donor, increased eNOS mRNA and protein levels and enhanced NO production [5]. One of the first physiological effect identified for H_2S was its ability to relax vSMCs, resulting in vasodilation, a hallmark of inflammation. Indeed, the literature on the role of hydrogen sulfide in inflammation was initially contradictory as it was for NO, however, recent studies demonstrated that this mediator exerts potential anti-inflammatory effects, expected at high concentrations [6]. In another report, it was demonstrated that H_2S anti-inflammatory response involved the activation of AnxA1, a glucocorticoid-regulated inhibitor of inflammation acting through formylated-peptide receptor 2 (ALX) [7]. Therefore, hydrogen sulfide promotes resolution of inflammation. The resolution response is an active process that it is also operated by an important group of specialized pro-resolving mediators (SPMs), that include lipoxins (LXs), maresins (MaRs), protectins (PDs) and resolvins (RvDs), that derived from polyunsaturated fatty acids (PUFAs) and lead to regression of inflammatory processes [8]. The study of these lipid mediators provides new opportunities for the development of an effective strategy for treating chronic inflammatory diseases [9]. Their biosynthetic production is a transcellular process that occurs between inflammatory cells (i.e. eosinophils, macrophages, and dendritic cells) and resident cells in the inflamed tissue (i.e. endothelial cells, epithelial cells) [8]. Indeed, investigators have observed several effects of SPMs on ECs that reduce their inflammatory activation and promote a more quiescent state [10]. Moreover, recently, the regulation of macrophage function by H_2S has been actively recognized. For instance, in response to interferon-γ (IFN- γ) stimulation, the expression of CSE was elevated in a time-dependent pattern. Furthermore, CSE was similarly detected in bone marrow-derived macrophages (BMDMs) [11]. Although H_2S donors have a biological activity, their function can be inconsistent. Additionally, donors have different H_2S release properties, which could lead to different effects [12]. Finally, prostaglandins, whose formation is catalysed by COX-1 and COX-2, play a key role in the generation of the inflammatory response. Their biosynthesis is significantly increased in inflamed tissue and they contribute to the development of the cardinal signs of acute inflammation. Although the pro-inflammatory properties of individual prostaglandins during the acute inflammatory response are well established, their role in the resolution is more controversial. Nonetheless, several aspects have not been fully elucidated. For instance, the anti-inflammatory role of lipid mediators has not been sufficiently delineated respect to the overall modulation of vascular function. At a similar extent, the possible relationship between H_2S and lipid mediators has not been highlighted at vascular level, as well as in immunity. For this purpose, we wanted test whether the novel H_2S donor molecule, AP123, and a specific CSE inhibitor, PAG, could affect the changes to lipid mediators' activity, in an established model of *in vitro*

inflammation on macrophages. First of all, we evaluated the effect of H_2S donor on several prostaglandins such as LTB_4, PGE_2, PGD_2, $PGF_{2\alpha}$ and TBX_2. To obtain human monocyte-derived macrophages, blood was received from were procured from the National Health Service Blood and Transplant Bank. Human peripheral blood mononuclear cells (PBMCs) were prepared following density separation by layering Ficoll-Histopaque (1077 Sigma). These were then cultured for 7 days in RPMI 1640 (10% human serum). M1 macrophages were obtained by incubating isolated monocytes for 7 days with 20ng/mL GM-CSF, and therefore stimulating them for 24 hours with 1 ng/mL LPS. Then, M1 macrophages (1.0x106 cells/1.5mL) were incubated with RPMI 1640, without phenol red, and treated with PAG (5mM) and AP123 (1nM, 10nM, 100nM and 1μM) at two different time points (45min and 24h). After incubation, cells were collected to perform LC-MS/MS analysis. Preliminary data regarding the levels of LTB_4 showed that they seem not to be regulated by AP123. Interestingly, H_2S donor determined the changes in PGE_2 levels in time- and concentration-dependent manner (fig.1A-B).

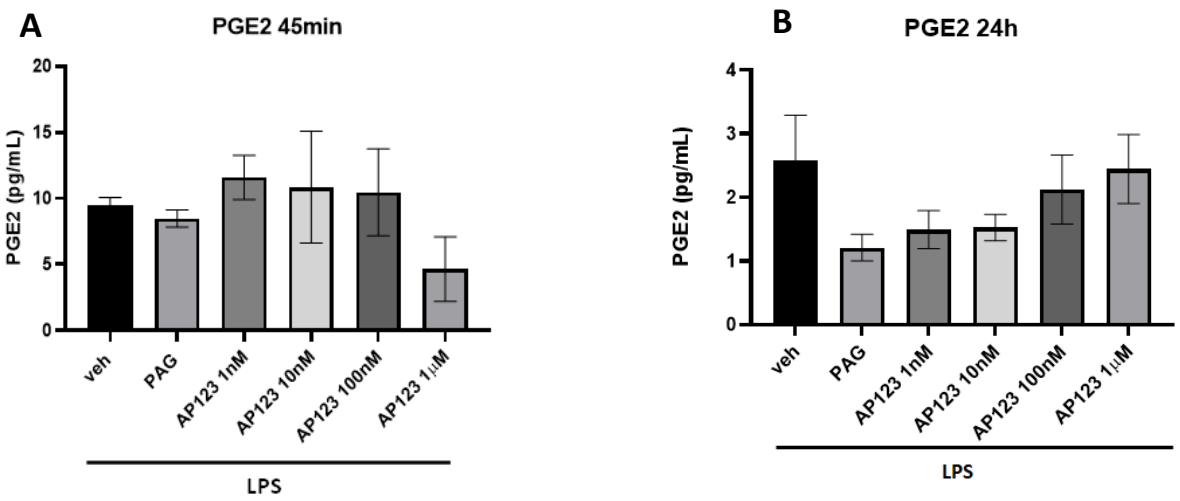

Fig.1. Levels of PGE_2 following treatment with PAG or AP123 at 45 min (A) and 24h (B)

Similarly, AP123 treatment showed a positive response on PGF_{2a} levels, leading to a concentration-dependent decrease (fig.2A-B).

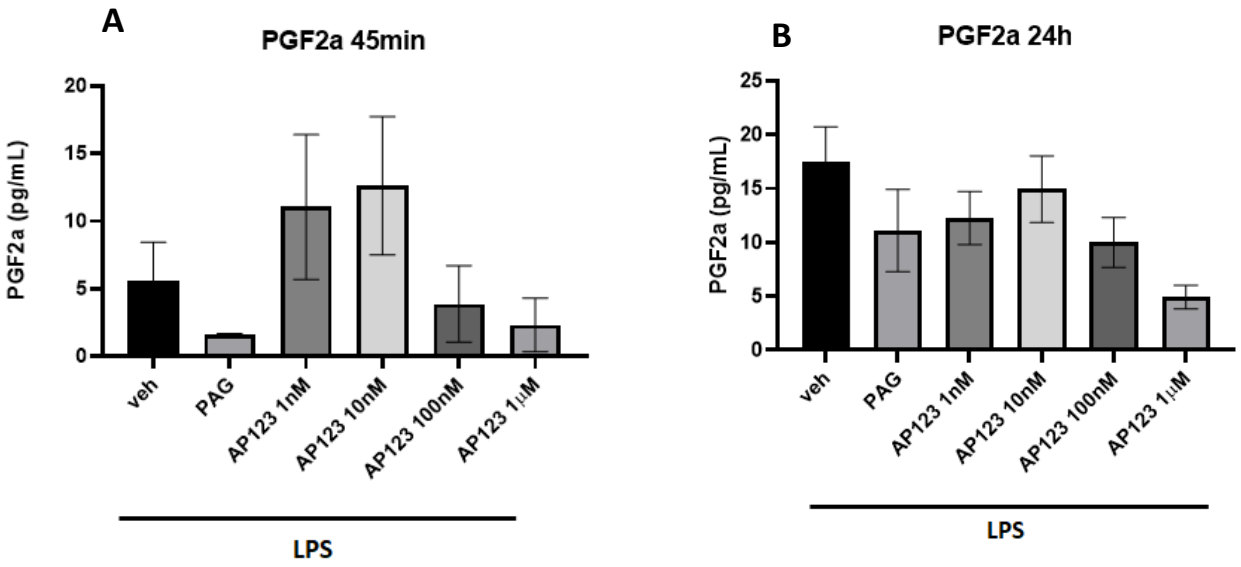

Fig.2. Levels of $PGF_{2\alpha}$ following treatment with PAG or AP123 at 45 min (A) and 24h (B)

Evaluation of PGD_2 (fig. 3A-B) and TBX_2 (fig. 4A-B) levels indicates that these mediators do not change upon treatment with PAG, while a slight increase is observed following addition of AP123.

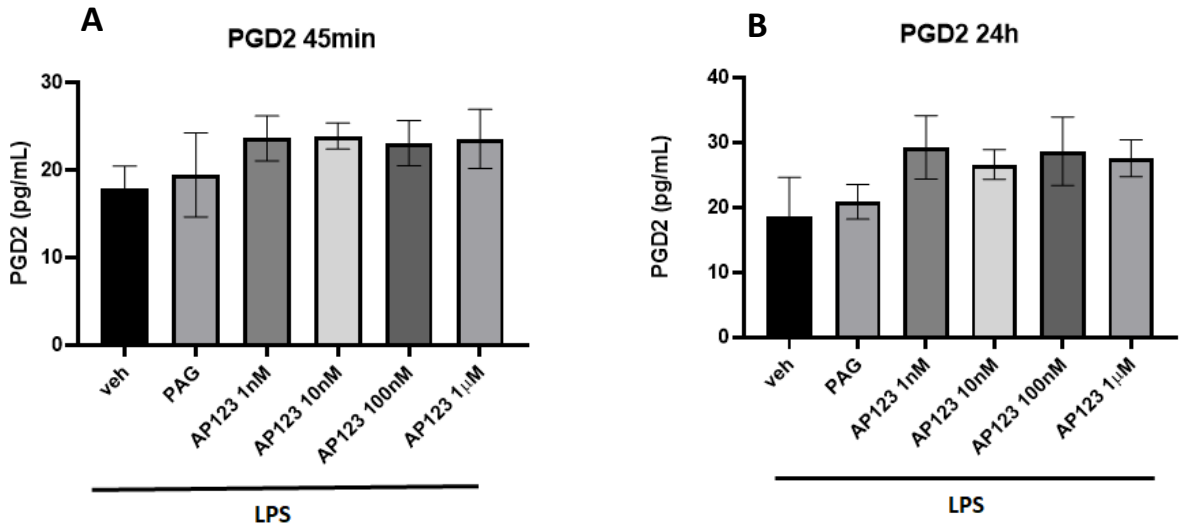

Fig.3. Levels of PGD_2 following treatment with PAG or AP123 at 45 min (A) and 24h (B)

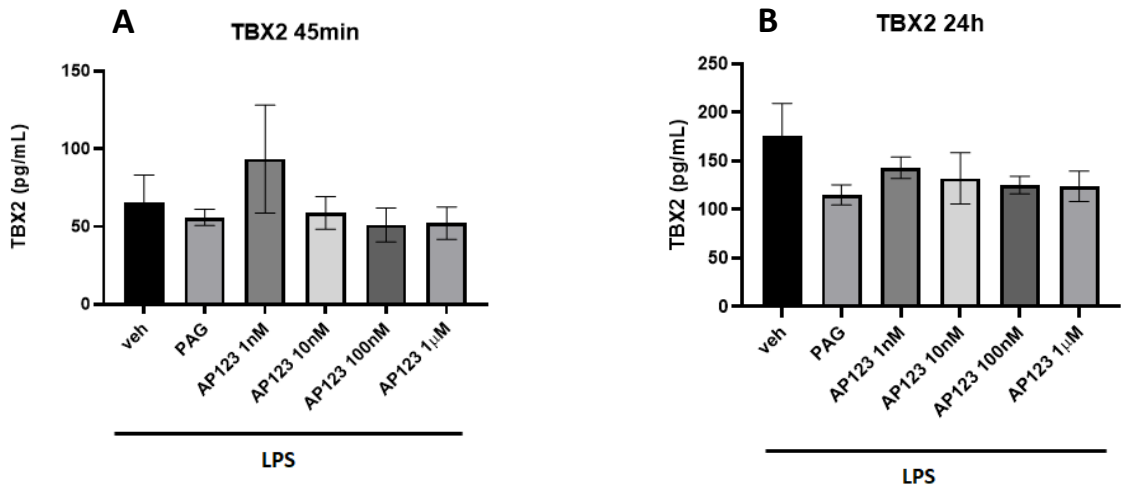

Fig.4 Levels of TBX_2 following treatment with PAG or AP123 at 45 min (A) and 24h (B)

Overall, our preliminary results highlight that H_2S seems to be somehow involved in the generation of PGs and that AP123 can modulate the levels of some prostaglandins in a different manner. In particular, the reducing effect observed on the levels of the pro-inflammatory mediators at high concentrations and dependently from administration time led us to hypothesize a possible antinflammatory/proresolutive effects of AP123. However, this idea still need further studies in order to be confirmed, including the analysis of specific SPMs.

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