Classical Activation of Macrophages and Vardenafil

Vardenafil (Levitra; GlaxoSmithKline, UK) is a member of a class of highly selective inhibitors of cGMP-dependant phosphodiesterase (PDE) type 5, a class of drugs which includes sildenafil, tadalafil and udenafil, which is approved for the treatment of pulmonary arterial hypertension and erectile dysfunction.[1] Sildenafil (Viagra; Pfizer Inc, USA), vardenafil, and udenafil, but not tadalafil (Cialis; Eli Lilly, USA), inhibit PDE6 with substantially lower affinities than those for PDE5A. Tadalafil has been shown to potently inhibit PDE11A activity still much less potently than it does PDE5A.[2] The selectivity and successful application of these PDE inhibitors as treatment of erectile dysfunction and more recently for pulmonary hypertension has validated the emergence of PDEs as drug targets.[1, 3] It is also likely that potential new applications for these drugs will emerge as PDE involvement in new diseases, signalling pathways and cellular processes is identified.

Cystic Fibrosis (CF) is a monogenic disorder resulting from mutations in the CF transmembrane conductance regulator (CFTR, ABCC7) gene which encodes the main chloride channel expressed in epithelia of respiratory, digestive and reproductive systems.[4] Majority CF patients (70%) carry at least one allele of F508del-CFTR and manifest defective airway mucociliary clearance, persistent infection and inflammation leading to end-stage lung damage. Recent reports have provided evidence that phosphodiesterase type 5 (PDE5) inhibitors, including vardenafil, rescued F508del-CFTR trafficking and corrected deficient chloride transport activity and are potential agents to attenuate exaggerated lung inflammatory responses in CF.[5-9]

PDE are expressed ubiquitously in inflammatory cells and regulate intracellular cyclic nucleotide (cAMP/CGMP) levels and as such, selective PDE inhibitors offer novel therapeutic strategies for treatment of various inflammatory diseases including asthma, inflammatory bowel disease, chronic obstructive pulmonary disease, psoriasis and CF. For example, PDE4 is a major cyclic AMP metabolizing enzyme in inflammatory cells, and PDE4 inhibitors produce a wide range of anti-inflammatory and immunomodulatory effects; they reduce neutrophil trafficking to sites of inflammation, release of neutrophil products, leukotriene B_4 (LTB_4), IL-8, reactive oxygen species and promote neutrophil apoptosis. [10-13] Poschet et al demonstrated that pharmacological inhibition of PDE5 attenuated CF pro-inflammatory responses; they showed that in cell models and airway of mice infected with Pseudomonas aeruginosa, sildenafil reduces bacterial adherence and neutrophil lung inflammation, respectively.[8] Lubamba et al found that vardenafil attenuates the expression of pro-inflammatory cytokines (e.g. IL-1B) and chemokines (e.g. monocyte/macrophage chemo-

attractant CCL-2) and cell infiltrates in the bronchoalveolar lavage of both wild type and F508del-CF mice [9]

Macrophages are mononuclear immune cells derived from monocytes. Different subspecialties of macrophages exist in various organs or locations throughout the body.[14] They play a key role in both innate and adaptive immunity and phagocytise invading microorganisms as well as apoptotic and necrotic cells of the host, secrete mediators of inflammation, and present antigens to T cells.[15] In response to various signals of exogenous and endogenous origin, macrophages polarize or undergo activation, and acquire different functional properties - leading to pro-inflammatory (M1) or anti-inflammatory (M2) phenotypes.[16, 17] The polarized macrophages may differ in terms of receptor expression, cytokine production, effector function and chemokine repertoires and show considerable plasticity between the two extreme phenotypes. M1 macrophages are characterized by high capacity to present antigen; high interleukin-12 (IL-12), IL-23, IL-1 and IL-6 production and consequent activation of a polarized type I response; and high production of toxic intermediates [nitric oxide (NO), reactive oxygen intermediates (ROI)]. [18, 19] Potent key activators of the macrophage response such as interferon-γ (IFN-γ), alone or together with microbial endotoxins (e.g. lipopolysaccharide (LPS)) or cytokines (e.g. tumour necrosis factor (TNF)), bind to macrophage receptors (e.g. CD14, TLR) and drive classical activation or M1 polarization of macrophages with potentiated cytotoxic and anti-tumoural properties. Consequently, classically activated M1 macrophages are potent effector cells which kill microorganisms and tumour cells and produce copious amounts of proinflammatory cytokines. However, uncontrolled release of these mediators can be dangerous for the host tissue – a typical example being the airway of CF patients.

In this issue of the Journal (pp----), Noel et al [20] provide evidence that macrophages are the target effector cells through which vardenafil modulates CF airway inflammation. Previous studies had shown that macrophages contribute significantly to the exaggerated inflammation in CF lung disease; macrophages from CF mice display exaggerated pro-inflammatory responses [21], and the differentiation pattern of cultured alveolar and peritoneal macrophages in F508del-CFTR^{EUR} mice is altered towards a pro-inflammatory status (M1) [22]. Noel et al [20] evaluated pro-inflammatory responses driven by M1 polarization in lung macrophages from F508del-CFTR and wild-type mice and showed exaggerated pro-inflammatory responses in CF macrophages. Treatment with vardenafil downregulated the overresponses of M1 markers (NOS-2 and TNF-α) in CF cells. In this regard, it is interesting that increased cGMP was previously shown to inhibit release of TNFα and nitric oxide in cells of myeloid lineage activated with LPS. [23, 24] Intracellular accumulation of cGMP can be achieved by stimulating its formation (i.e. by means of guanylyl cyclase agonists) or by

inhibiting its degradation (i.e. by means of phosphodiesterase (PDE) inhibitors). By catalyzing the hydrolysis of guanosine 3' 5' cyclic monophosphate (cGMP), the enzyme regulates the intracellular levels of the cGMP second messenger (Figure 1). Using siRNA experiments, Noel et al [20] confirmed a role for PDE5 in modulating inflammatory responses of CF M1 activated macrophages. Interestingly, they also observed that the effect of vardenafil to downregulate M1 polarization requires CFTR expression and was not observed in $cftr^{-/-}$ null mice indicating that M1 overresponses in CF are related to the loss-of-function of CFTR. The precise nature of interaction between PDE5 and CFTR or CFTR mutation and macrophage polarisation is as yet not understood. Interestingly, although the mechanism by which CFTR regulates the major innate immune response pathways is still unclear, expression of CFTR on the membrane inhibits the pro-inflammatory signaling via inflammatory (TNFα, TLR or IL-1β) pathways, which regulate NF-κB activation and IL-8 secretion. [25]

The activation of intracellular signaling pathways in activated macrophages leads not only to synthesis of nitric oxide (NO) and the proinflammatory cytokines, TNFα, IL-6, Il-1β but to elevated expression of other proteins to facilitate fast development of an inflammatory response. For example, PDE4B is up-regulated by LPS and is responsible not only for cAMP hydrolysis and thereby removing the anti-inflammatory effects of cAMP but for synthesis of TNFα induced by LPS [26, 27]. On the other hand, cyclic GMP (cGMP) has been shown to inhibit hydrolysis of cAMP by PDE3 and is involved in the regulation of expression of inflammatory cytokines in macrophages. [28] See Figure 1.

Human macrophages have been shown to contain PDE1, PDE3, PDE4, and PDE5.[28, 29] Until now, partly due to the absence of specific tools or inhibitors, the identity and significance of cGMP-regulated PDEs that are actively expressed or functional in activated macrophages was unknown. The findings by Noel et al have highlighted a cGMP-PDE5 lesion or dysregulation in CF and should lead to full characterisation of the proteins involved in cGMP signaling and M1 macrophage polarisation or activation. Increasing evidence indicates that cGMP plays an important role in regulating essential functions of macrophages such as chemotaxis, phagocytosis, cytotoxicity, and synthesis of inflammatory mediators [23, 24, 27, 30-32], and thus, controls the macrophage inflammatory response. Eleven families of phosphodiesterases have been identified in mammalian tissues and classified according to amino acid sequence, substrate specificity, pharmacological properties and tissue distribution; among them - five (PDE1, PDE2, PDE3, PDE10, and PDE11) hydrolyze both cAMP and cGMP, three (PDE4, PDE7, and PDE8) are cAMP-specific, and only three (PDE5, PDE6, and PDE9) are exclusively cGMP-specific. [33] The activity profile of PDE's which hydrolyze cGMP and are regulated by cGMP will also need to be determined in polarised macrophages. Interestingly, analysis of peritoneal macrophage PDE activity profile shows soluble PDE5

activity is low and undetectable in the particulate fraction of the macrophage.[34, 35] It has been suggested PDEs whose total activity level is low could still contribute significantly to cellular function by controlling an important microdomain within the cell. [36-38] Thus involvement of PDE5 with CFTR, a transmembrane protein resident in the phagosome and plasma membrane in macrophages [39, 40], suggests PDE5 might exist in a multi-protein complex with CFTR in a discrete compartment within the cell to limit the spread of the second messenger cGMP. Interestingly, in rat jejenum, cGMP effect on CFTR-dependent fluid secretion was inhibited by PKG inhibitors. [41]

Finally, the finding by Noel et al that regulation of PDE5 by vardenafil modulates macrophage function and exaggerated inflammatory response in CF will stimulate further research in this area. For example, the precise cGMP targets and signalling pathway(s) that lead to downregulation of M1 macrophage pro-inflammatory markers (NOS-2 and TNF-α) in CF, following vardenafil treatment, remain to be identified. Identification and characterization of these signaling pathways may elucidate additional potential therapeutic targets.

Figure Legends

Figure 1. Potential down-stream effectors of PDE5 inhibition by vardenafil in macrophage. NO – Nitric Oxide, sGC – soluble guanyl cyclase, AC – adenylyl cyclase. GAFs - cGMP binding domains. PKA/PKG can be cross activated by cAMP and cGMP. Compounds such as vardenafil that rescue CFTR to the cell surface or lipid-rafts can block NF-κB mediated chronic inflammation.

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