Sildenafil

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Oxidative stress linked to vascular damage plays an important role in the pathogenesis of systemic sclerosis (SSc). Indeed, vascular damage at nailfold capillaroscopy in patients with Raynaud's Phenomenon (RP) is a major risk factor for the development of SSc together with presence of specific autoantiobodies. Here we investigated the effects of the phosphodiesterase type 5 inhibitor (PDE5i) sildenafil, currently used in the management of RP, in modulating the proinflammatory response of dermal fibroblasts to oxidative stress in vitro. Human fibroblasts isolated by SSc patients and healthy controls were exposed to exogenous reactive oxygen species (ROS) (100μM H_2O_2), in the presence or not of sildenafil (1μM). Treatment with sildenafil significantly reduced dermal fibroblasts gene expression and cellular release of IL-6, known to play a central role in the pathogenesis of tissue damage in SSc and IL-8, directly induced by ROS. This reduction was associated with suppression of STAT3, ERK, NF-κB and PKB/AKT dependent pathways. Our findings support the notion that the employment of PDE5i in the management of RP may be explored for its efficacy in modulating the oxidative stress induced proinflammatory activation of dermal fibroblasts in vivo and ultimately aid in the prevention of tissue damage in SSc.

Keywords: Systemic sclerosis ; Oxidative stress ; Inflammation ; PDE5 inhibitors

1. Introduction

Sildenafil belongs to the class of drugs inhibiting phosphodiesterase type 5 (PDE5i) commonly used to treat erectile dysfunction, Raynaud's phenomenon, and pulmonary arterial hypertension [1]. PDE5 is a group of ubiquitously present enzymes that hydrolyze cyclic guanosine monophosphate (cGMP) to its inactive form GMP. This cyclic nucleotide plays a prominent role in the regulation of important cellular functions, and PDE5i can therefore elicit a variety of effects [2][3]. The capacity of PDE5i to inhibit cytokine release has been already observed [4][5]. In particular, sildenafil has been shown to have an immunomodulating ability in human immune cells and cardiomyocytes subjected to inflammatory stimuli [4][5]. However, to date, this potential mechanism of action has never been explored in SSc. In this study, we showed for the first time that the PDE5i sildenafil exerts an inhibitory effect on IL-6 and IL-8 gene expression and is released into the culture medium of SSc fibroblasts exposed to ROS. Numerous reports have shown that both IL-6 and IL-8 levels are elevated in culture supernatants of dermal fibroblasts and serum from patients with SSc [6][7]. Consistent with these findings, we observed that SSc fibroblasts cultured in a pro-oxidant environment showed a significant increase not only in IL-6 and IL-8 gene expression, but also their secretion in the medium. It remains to be investigated whether this could be the result of persistent exposure to pro-oxidants and/or of the reduced antioxidant capacity of these cells [8]. Interestingly, sildenafil did not show effects on IL-8 secretion in healthy fibroblasts. In a previous study, performed in patients affected by diabetic cardiomyopathy, we showed that sildenafil could counteract IL-8 release in consequence of a "cut-off" value [5]. Particularly, only patients with a circulating cytokine level above this "cut-off" were responsive to sildenafil treatment with a significant decrease of the chemokine. By contrast, patients with IL-8 below the "cut-off" value were not sensitive to this PDE5i. It is likely that the IL-8 level in healthy fibroblasts was not sufficient to reach the cut-off value, determining a different sensitivity to sildenafil.

2. History and Development

As suggested by numerous authors, IL-6 and IL-8 may have a direct effect on regulating tissue fibrosis and endothelial damage [7]. In particular, IL-6 is a pleiotropic pro-inflammatory cytokine capable of stimulating SSc fibroblasts to differentiate and proliferate, causing collagen overproduction and fibrosis [9]. IL-8 is a chemoattractant cytokine responsive to oxidative stress that unlike others has distinct target specificity for neutrophils [10]. The persistent neutrophil activation determines neutrophils accumulation in different body districts (e.g., lung), promoting the genesis of interstitial fibrosis, which is one of the most dreaded clinical manifestations of SSc [11][12]. Indeed, a neutrophil-derived gene signature has been shown to be one of the top discriminants in SSc vs. healthy control blood and a major biological marker of clinical

improvement [13]. To begin to dissect the potential mechanism by which sildenafil can modulate IL-8 and IL-6 expression, we analyzed the modulation of proteins such as STAT3, ERK, NF-κB, and PKB/AKT, known to be involved in ROS-mediated signaling. Firstly, we observed a greater modulation of these molecules in SSc compared with healthy fibroblasts, supporting the already proposed notion that SSc fibroblasts may have a reduced ability to counteract the redox-balance [14][15]. Importantly, the presence of sildenafil significantly reduced the phosphorylation levels of these proteins. We believe that, despite not offering a complete explanation, these initial observations do inform and warrant future studies aimed to define the molecular mechanisms underlying this novel biological effect of sildenafil. In this sense, it would be worth exploring the extent to which this effect is directly mediated by cyclic nucleotide hydrolysis inhibition or by independently elevating levels of cAMP and cGMP or modulating ion channels in tissue fibroblasts [16]. In conclusion, we believe that our study, although in vitro and on a limited set of samples, has a strong potential impact. Sildenafil is one of the commonly used drugs in the management of Raynaud's phenomenon, and given the epidemiological observations strongly indicating that patients with Raynaud's phenomenon and ANA are at high risk of developing SSc, the dissection of the mechanisms underlying the PDE5i-induced modulation of proinflammatory and profibrotic cytokines following ROS may pave the way to extending the scope of treatment with sildenafil in patients at risk of developing SSc from simple management of Raynaud's phenomenon to a pre-disease-modifying agent.

References

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