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Understanding the Role of Adenosine Receptors in the Myofibroblast Transformation in Peyronie's Disease

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ABSTRACT

Background: Peyronie's disease (PD) is a chronic fibrotic disease of the penis affecting a significant number of men worldwide without effective medical treatments. Myofibroblasts are pivotal in the pathogenesis of PD. Adenosine and adenosine receptors have been suggested to be involved in the pathophysiology of fibrosis.

Aim: To understand the role of adenosine receptors in myofibroblast transformation in PD.

Methods: Fibroblasts were isolated from the non-PD tunica albuginea (TA) tissue and PD plaque tissue and were transformed into myofibroblasts using transforming growth factor (TGF)-β1. Quantification of α-smooth muscle actin and adenosine receptors (adenosine receptor A1 [ADORA1], adenosine receptor A2A, adenosine receptor A2B [ADORA2B], and adenosine receptor A3) was performed using immuno-cytochemistry, in-cell enzyme-linked immuno-sorbent assay (ICE), and real-time reverse transcription quantitative polymerase chain reaction. The effect of various adenosine receptor agonists or antagonists on TGF-β1-induced myofibroblast transformation was measured using ICE.

Outcomes: Expression of adenosine receptors in myofibroblasts obtained from human TA and the effect of adenosine receptor ligands on myofibroblast transformation were investigated.

Results: The experiments showed that the protein and messenger RNA levels of α-smooth muscle actin in non-PD TA cells and PD plaque-derived cells were significantly higher in cells exposed to TGF-β1 than those not treated with TGF-β1. 2 of 4 adenosine receptors (ADORA1 and ADORA2B) were found to be expressed in both cell populations. Among various adenosine receptor agonists/antagonist investigated, only ADORA2B agonist, BAY 60-6583, significantly inhibited myofibroblast transformation in a concentration-dependent manner when applied simultaneously with TGF-β1 (IC_{50} = 30 μmol/L).

Clinical Translation: ADORA2B agonists may be clinically efficacious in early-stage PD.

Strengths & Limitations: The strength of this study is the use of primary fibroblasts from human TA. Limitation of the study is the high concentrations of the ligands used.

Conclusion: The effect of an ADORA2B agonist on TGF-β1-induced myofibroblast transformation shows a novel potential therapeutic target for PD if applied during early, non-stable phase of PD. **Mateus M, Ilg MM, Stebbeds WJ, et al. Understanding the Role of Adenosine Receptors in the Myofibroblast Transformation in Peyronie's Disease. J Sex Med 2018;XX:XXX–XXX.**

Key Words: Fibrosis; Transforming Growth Factor; Anti-Fibrotic Therapies; Fibroblast; Cell Culture

INTRODUCTION

Fibrosis can be described as excessive development of fibrous connective tissue, which can occur in various tissue types and organs (eg, kidney, lung, skin, and liver). At a cellular level, tissue resident quiescent fibroblasts and other cells such as endothelial, epithelial cells, and fibrocytes can differentiate into myofibroblasts, which have a crucial role in fibrosis characterized by increased proliferation, increased extra-cellular matrix (ECM) protein production, and contraction.[1,2] Moreover, their persistence (ie, failure to undergo apoptosis) and proliferation

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has been suggested to be one of the hallmarks of chronic fibrosis.[3,4]

Peyronie's disease (PD) is a fibrotic disorder characterized by the formation of plaques within the tunica albuginea (TA) of the penis.[5] Although its etiology is still poorly understood, microvascular trauma has been postulated as the initiating factor.[6] This fibrotic disorder is also characterized by the expression of several cytokines and growth factors, fibrin deposition, and myofibroblast differentiation.[7] In PD, the myofibroblast activity is increased, resulting in increased ECM protein production and eventual plaque formation[7–11] suggesting a pivotal role for myofibroblasts in the pathophysiology of PD.

Inhibition of differentiation of quiescent fibroblasts to profibrotic myofibroblasts has been suggested as a therapeutic approach for fibrosis.[4] Accordingly, we have been investigating potential molecular targets that may be involved in myofibroblast differentiation and small molecule compounds that may inhibit this process.

1 such target that is suggested in pathophysiology of fibrosis is adenosine and its receptors. Adenosine is a ubiquitous purine nucleoside released from cells and tissues under conditions of stress or injury and is generated intra-cellularly and extracellularly from adenine nucleotides, which are then dephosphorylated to adenosine. CD39 and CD73 are 2 cell surface molecules responsible for catalyzing the de-phosphorylation of adenine nucleotides to adenosine in the extra-cellular space.[12,13] Adenosine regulates its effects on tissue re-generation and repair via the interaction with a family of G-protein-coupled receptors: adenosine receptor A1 (ADORA1), adenosine receptor A2A (ADORA2A), adenosine receptor A2B (ADORA2B), and adenosine receptor A3 (ADORA3).[14]

Several studies have shown that adenosine receptors play different roles in acute and chronic injuries. In acute tissue injury, adenosine has been shown to be beneficial, as it is responsible for tissue protection and anti-inflammatory responses[13] (eg, promotion of barrier function and wound healing) in several organs, including kidney,[15] lung,[16] heart,[17] and liver.[18] In contrast to acute states, increased levels of adenosine have been associated with the progression of chronic tissue injuries. In these settings, adenosine has been suggested to promote fibrosis in several organs, such as the heart,[19] skin,[20] liver,[21] lung,[22] penis,[23] and kidney.[24] The adenosine receptors play different roles in the pathogenesis of fibrosis depending on the tissue subtype involved; however, the effects of adenosine are mainly regulated by ADORA2A and ADORA2B.[16] The characterization of these receptors has been investigated in other fibrotic disorders; however, no characterization of these receptors has been carried out in PD. By understanding how adenosine receptors may regulate the response to injury in this specific tissue and by looking at the myofibroblast transformation process, it may provide new insights into the pathophysiology of fibrosis in general and in PD. Furthermore, by targeting the respective pathway and by investigating the effect of selective agonist and antagonist compounds, it may enable avenues to identify potential targets for the treatment of PD and other fibrotic disorders and enhance the resolution of the injury or halt the progression of fibrosis in PD.

The aim of this study was to understand the involvement of adenosine receptors in the myofibroblast differentiation in PD by characterizing the myofibroblast transformation process in TA-derived fibroblasts to identify potential, novel targets for anti-fibrotic therapies.

METHODS

Sample Acquisition

TA tissue samples were acquired from patients undergoing corrective surgery for PD (to be referred to as PD plaque tissue) or invasive penile cancer (to be referred to as non-PD TA tissue) at University College London Hospital (UCLH), United Kingdom. Patients aged between 18–75 years, listed for surgical treatment of PD or penile cancer at UCLH, and able to understand the patient information sheet and to give consent were included in this study. Ethical approval was obtained from local independent research ethics committees (East of England Essex [12-EE-0170] and North of Scotland [15-NS-0051]).

The non-PD TA tissue was obtained from penis of patients with penile cancer (N = 3; average age = 72 ± 10 years). The TA tissue was removed from the proximal side away from the tumor and the tumor had negative margins on histology examination.

The PD TA tissue was obtained from penis of patients with chronic PD (N = 3; average age = 61 ± 6 years). The tissue used in this group was the plaque tissue that was surgically removed and would have otherwise been discarded.

Isolation of Fibroblasts From TA Tissue

Tissue samples were dissected into small pieces, submerged in culture media in 6 well plates and incubated at 37°C, 5% carbon dioxide in a humidified atmosphere for 5–7 days. This method is known as "explant technique" by which pure fibroblast cultures can be obtained.[25] Tissues were removed from the 6 well plates, once cells were observed growing out of the tissue. Cells were then washed 3 times with PBS and fresh, warm medium was added to each well. Cells were incubated at 37°C until they reached 50–70% confluence, after which old medium was removed and cells were washed with PBS. Cells were detached with 0.25% trypsin/EDTA (Thermo Fisher Scientific, Altrincham, United Kingdom) and neutralized with culture media. The cell suspension was transferred to T75 flasks (Thermo Fisher Scientific) and the cells were propagated and maintained up to passage 10.

Real-Time RT-q-Polymerase Chain Reaction

Cells were seeded onto 6 well plates (Thermo Fisher Scientific) at 1.0×10^5 cells/well and incubated in DMEM-F12 medium