BJP BRITISH JOURNAL OF PHARMACOLOGY

BPS BRITISH PHARMACOLOGICAL SOCIETY

British Journal of Pharmacology (2009), 158, 1426–1428

0007-1188/09
www.brjpharmacol.org

COMMENTARY

Vitamin D – a new treatment for airway remodelling in asthma?

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Increased airway smooth muscle (ASM) mass plays a critical role in chronic asthmatic airway remodelling. ASM cell hypertrophy and hyperplasia are likely to contribute to increased ASM mass and a variety of mitogens induce ASM proliferation in cell culture. Recent recognition of widespread vitamin D deficiency and identification of the vitamin D receptor on many cells has implicated vitamin D as a potential therapeutic target for many disorders including cancer, infection and asthma. In this issue of *British Journal of Pharmacology*, Damera *et al.* show that calcitriol, a secosteroidal modulator of vitamin D receptors, inhibited thrombin and platelet-derived growth factor-induced ASM cell proliferation. They also, perhaps surprisingly, show the glucocorticoid dexamethasone to potentiate mitogen-induced ASM proliferation. Their results begin to elucidate the molecular mechanism(s) utilized by calcitriol to inhibit cell proliferation and suggest hyperphosphorylation of retinoblastoma protein and activation of checkpoint kinase 1 (Chk1) as critical to this process. This study identifies inhibition of ASM proliferation as a cellular effect of vitamin D and supports the hypothesis that vitamin D is a potential treatment for airway remodelling in asthma.
British Journal of Pharmacology (2009) **158,** 1426–1428; doi:10.1111/j.1476-5381.2009.00429.x

This is a Commentary on the Research Paper in this issue by Damera *et al.* (pp. 1429–1441). To view this article visit http://www3.interscience.wiley.com/journal/121548564/issueyear?year=2009

Keywords: vitamin D; airway smooth muscle; asthma; airway remodelling; hyperplasia; cell cycle

Abbreviations: ASM, airway smooth muscle; CDK, cyclin-dependent kinase; Chk, checkpoint kinase; EGF, epidermal growth factor; PDGF, platelet-derived growth factor; Rb, retinoblastoma protein; VDR, vitamin D receptor

Airway remodelling plays a critical role in the pathophysiology of chronic asthma, leading to poorly reversible airflow obstruction and thickening of the airway smooth muscle (ASM) plays a key role in this process (Panettieri, 1998). Whether increased thickness of the ASM layer is due to hypertrophy (increased cell size), hyperplasia (increased cell number) or reduced apoptosis of ASM cells is a continuing focus of debate and may be patient-dependent (Bentley and Hershenson, 2008). However, many stimulators of ASM cell growth have been identified using cell culture systems. Many of these are ligands for either receptor serine/threonine kinase signalling, for example platelet-derived growth factor (PDGF), epidermal growth factor (EGF) and basic fibroblast growth factor, or G-protein coupled receptor signalling, for example α-thrombin and endothelin-1 (Hirst, 2000) and utilize extracellular signal-regulated kinase or phosphoinositide-3 (PI3) kinase signalling to increase levels of cyclin D proteins required for cell cycle progression (Bentley and Hershenson, 2008).

β_2 adrenoceptor agonists and glucocorticoids, two well-established asthma therapies, both inhibit mitogen induced proliferation of ASM cells in culture, albeit to varying extents dependent on mitogen and temporal experimental design (Tomlinson *et al.*, 1994; Stewart *et al.*, 1995; Stewart *et al.*, 1997; Fernandes *et al.*, 1999), but there is little evidence these therapies reduce airway remodelling *in vivo*. Furthermore, the current paper by Damera *et al.* (2009) shows the glucocorticoid dexamethasone surprisingly potentiated PDGF and thrombin-induced proliferation of normal ASM cells, while having a small inhibitory effect on asthmatic ASM cells, suggesting the effects may be cell and patient-dependent. Furthermore, Bonacci *et al.* (2003) have shown collagen-enriched cell culture conditions prevent dexamethasone inhibition of ASM cell proliferation. While this cannot explain the overall increased response of ASM cells to dexamethasone in Damera *et al.*, it may be relevant to the difference in response of

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Received 17 June 2009; accepted 22 June 2009

normal and asthmatic cells as the extracellular matrix is altered in asthmatic airways. A detailed study of the different mechanisms of action of dexamethasone on normal and asthmatic ASM cells may identify differences in control of cell proliferation between these cells.

Of particular interest in the paper by Damera *et al.* are their studies showing a potent inhibitory role of vitamin D. Gene polymorphisms in the core nuclear vitamin D receptor (VDR) are associated with childhood and adult asthma (Raby *et al.*, 2004), although the relationship between receptor polymorphisms and the development of asthma remain unclear. The VDR is expressed in ASM cells and upon vitamin D stimulation regulates the expression of many genes. Furthermore, the increased proliferation of ASM cells exposed to serum from asthmatic patients can be inhibited by prior exposure to vitamin D (Song *et al.*, 2007). The current paper by Damera *et al.* strengthens this observation by showing calcitriol, a secosteroidal VDR modulator, to inhibit the proliferation of PDGF and thrombin induced normal and asthmatic ASM cells, and advances it by beginning to elucidate the molecular mechanism responsible for the reduced proliferation. These authors showed calcitriol to inhibit progression of ASM cells to S phase of the cell cycle, without affecting cell apoptosis, and implicated the G0/G1 checkpoint as the point of calcitriol action. They also probed the molecular mechanisms involved, looking for potential candidates in the proliferation pathway. Previous work had implicated p42/44 mitogen-activated protein kinase and the p70 ribosomal protein S6 kinase 1 in EGF and thrombin-induced ASM cell proliferation, but calcitriol had no effect on these pathways. The E2F proteins are a family of transcription factors whose expression regulates the transition into S phase of the cell cycle. Their expression is regulated by growth factors and their target genes include cell cycle regulators including cyclins and cyclin-dependent kinases (CDKs), and therefore seemed a good target for study in the current paper; however, expressions of both 'activator' E2F1-3 and 'inhibitor' E2F4 were not regulated by PDGF, eliminating them from a role in ASM proliferation. Cyclins regulate cell cycle progression via sequential activation of CDKs. The first of these proteins to be upregulated in response to growth factor stimulation is cyclin D1. Despite cyclin D1 upregulation by PDGF in ASM, it was surprisingly unaltered by calcitriol. Upregulation of cyclin D1 results in its association with and activation of CDK4. Active CDK4 phosphorylates the retinoblastoma protein (Rb), causing its dissociation from E2F transcription factors and availability of E2F factor for transcription. Damera *et al.* found Rb hyperphosphorylation in response to PDGF and inhibition of hyperphosphorylation by calcitriol suggesting that this was involved in the mechanism of its effect. In an attempt to further understand how calcitriol modulates Rb phosphorylation, the authors investigated expression levels of various CDK inhibitors and CDK activators (cell division cycles (Cdc) phosphatases remove inhibitory phosphate groups from CDKs) but were unable to show PDGF or calcitriol modulation of these proteins. It is not entirely clear why the authors did not look into the activity of CDK enzymes themselves but instead looked at the activity of checkpoint kinase 1 (Chk1), a kinase that causes G2 cell cycle arrest. Nevertheless, their results vindicated this approach as Chk1 inhibition mirrored the inhibitory effect of calcitriol on ASM cell proliferation and calcitriol inhibited Chk1 activity as indicated by Chk1 phosphorylation. Chk1/2 have recently been shown to phosphorylate Rb, albeit to create a cell cycle inhibitory complex with E2F (Inoue *et al.*, 2007) and it would be interesting to establish whether Chk1 can regulate Rb phosphorylation and whether inhibition of Chk1 mimics the inhibitory effects of calcitriol on Rb phosphorylation.

The studies in the paper by Damera *et al.* are important as they begin to uncover a novel mechanism of cell cycle regulation in ASM cells. They confirmed the finding of others that ASM cells from asthmatic patients proliferate more readily in response to mitogens and in light of the increased smooth muscle cell mass in asthmatic airways, altered ASM cell proliferation may be an important target for drug development. While Damera *et al.* showed no difference in the extent of growth inhibition by calcitriol between normal and asthmatic ASM cells, it would be of interest to determine whether the same cell cycle control mechanisms exist in the two cell types. The differing effects of dexamethsone on normal and asthmatic ASM cell growth suggest differences may be present.

It remains debatable whether vitamin D represents a viable treatment option for asthma. Vitamin D deficiency and asthma prevalence coexist in certain populations; in cross-sectional studies, vitamin D insufficiency has correlated with low pulmonary function; and maternal vitamin D intake during pregnancy inversely associates with asthma (at age 5 years) (Erkkola *et al.*, 2009). However, a study in northern Finland reported vitamin D supplementation in the first year of life increased the risk of asthma by age 31 years (Ginde *et al.*, 2009) and high maternal vitamin D levels during pregnancy were associated with increased likelihood of childhood eczema and asthma (at age 9 years) (Devereux *et al.*, 2009). Interventional trials of vitamin D supplementation are required before firm conclusions can be drawn and this may be delayed by differences in opinion on what is an adequate vitamin D intake. What is clear is that future studies into the effects of vitamin D on cellular functions are essential to clarify any potential role for vitamin D as a therapeutic option for asthma.

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