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Upregulation of both PDGF-BB and PDGF-BB receptor in human bladder fibroblasts in response to physiologic hydrostatic pressure☆

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Abstract *Objective:* Bladder outlet obstruction can lead to the deposition of extracellular matrix and a resultant decrease in bladder wall compliance. Platelet-derived growth factor (PDGF) is a potent mitogen for fibroblasts and can increase the deposition of extracellular matrix. We attempt to determine if the expression of PDGF-BB and its receptor are altered in human bladder fibroblasts and bladder smooth muscle cells when exposed to hydrostatic pressures in the physiologic range.
Materials and methods: Cultured human bladder fibroblasts and smooth muscle cells were evaluated in vitro by using a novel device that controls for hydrostatic pressure. Cells were exposed to pressures of 20 and 40 cmH_2O for up to 72 h. Western blot analyses and RT-PCR were performed to evaluate expression of both PDGF-BB and PDGF-BB receptor.
Results: PDGF-BB and its receptor increased up to 22-fold and 8-fold, respectively, when human bladder fibroblasts were exposed to 40 cmH_2O sustained hydrostatic pressure, while at 20 cmH_2O the effect was minimal until 72 h. mRNA for the PDGF-BB receptor in human bladder fibroblasts increased in comparison to control. Western blot analyses demonstrated that exposure of human bladder smooth muscle cells to a sustained hydrostatic pressure of 20 and 40 cmH_2O for up to 72 h did not alter expression of either PDGF-BB or its receptor.

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Conclusions: Both PDGF-BB and its receptor in human bladder fibroblasts were upregulated in a time- and pressure-dependent manner after as little as 24 h exposure to pressures of $\leq$40 cmH_2O. Our results provide support for a potential role of both PDGF-BB and its receptor in bladder fibrosis secondary to increased intravesical pressure. Newer selective PDGF receptor antagonists may prove beneficial in preventing bladder wall fibrosis in patients with either anatomic or functional bladder outlet obstruction.

Introduction

Fibrosis can occur in nearly every organ system including lung, liver, kidney, intestine, heart, skin, bone marrow and bladder due to a variety of pathologic insults. This insult can be in the form of mechanical injury as seen in vascular hypertension. Chronic exposure of vessels to high blood pressure is characterized by cellular hypertrophy and deposition of extracellular matrix. In all of these fibrotic responses, the underlying mechanism involves the proliferation of mesenchymal cells and the subsequent deposition of collagen and other extracellular matrix proteins by these cells leading to progressive scarring and loss of organ function [1].

Several growth factors such as epidermal growth factor and insulin-like growth factor are physiologically important in bladder fibrosis secondary to bladder outlet obstruction. An additional growth factor, platelet-derived growth factor (PDGF), is a major mitogen for fibroblasts, smooth muscle cells and several other cell types, mainly of mesenchymal origin. PDGF exerts its effects on target cells by activating two structurally related protein tyrosine kinase receptors (PDGF-α and PDGF-β). PDGF-activated PDGF-β receptors mediate cell growth, actin reorganization, chemotaxis, Ca^{2+} mobilization and inhibition of apoptosis [2]. PDGF-BB is also a potent mitogen for rat ureteral and human bladder smooth muscle cells [3].

Elevated hydrostatic pressure secondary to anatomic or functional bladder outlet obstruction occurs in many pediatric urologic disorders including posterior urethral valves, neurogenic bladder and voiding dysfunction. The bladder's responses to obstruction are numerous. There is profound smooth muscle cell hypertrophy, fibroblast and urethelial cell hyperplasia, and extracellular matrix deposition. The clinical consequences of the histological changes and altered compliance of the bladder include a small functional capacity with elevated urine storage pressures [4,5].

We hypothesized that bladder cells may increase PDGF-BB secretion and over-express the PDGF-BB receptor (PDGF-BB R) secondary to elevated hydrostatic pressure, and thereby play an important role in the histological changes experienced with bladder outlet obstruction. This led us to investigate whether exposure of bladder fibroblasts and smooth muscle cells to elevated hydrostatic pressure results in altered expression of PDGF-BB and/or PDGF-BB R.

Materials and methods

Cell isolation

Bladder fibroblast cells were obtained from full-thickness bladder biopsies taken at the time of ureteroneocystostomy in a 9-year-old girl with negative urine cultures and a normal urodynamic evaluation. The urothelium was separated from the underlying detrusor muscle by using sharp dissection. The muscle was minced into fine pieces and placed on the dry surface of a 10-cm round culture dish. The tissue pieces were air dried for 5 min, before gentle addition of 10 ml smooth muscle cell basal media (Clonetics), supplemented with fetal bovine serum (5%), insulin (5 μg/ml), human recombinant fibroblast growth factor β (2 ng/ml), human recombinant epidermal growth factor (0.5 ng/ml), gentamicin (15 μg/ml) and amphoteracin (7.5 ng/ml). These explants were left undisturbed for 4 days before adding fresh media and were passaged at 90% confluence. Cells at passage 1 were characterized by immunohistochemistry, staining positive for vimentin, but negative for alpha smooth muscle actin, cytokeratin-7 and von Willebrand Factor.

Bladder smooth muscle cells were obtained from Clonetics (Cambrex Bio Science Walkersville, Inc.). These cells test positive for alpha smooth muscle actin and negative for von Willebrand Factor VIII.

Application of elevated hydrostatic pressure to the cell culture

Bladder fibroblasts and bladder smooth muscle cells were plated at a density of 7.5×10^3 per cm^2