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LOSS OF PROSTAGLANDIN E₂ RELEASE FROM IMMORTALIZED UROTHELIAL CELLS OBTAINED FROM INTERSTITIAL CYSTITIS PATIENT BLADDERS

Prerna Rastogi¹, Alice Rickard¹, Nikolay Dorokhov¹, David J. Klumpp², and Jane McHowat¹

¹Department of Pathology, Saint Louis University School of Medicine

²Department of Urology, Northwestern University Feinberg School of Medicine

Abstract

Interstitial cystitis (IC) is associated with increased activated mast cell numbers in the bladder and impairment of the barrier function of the urothelium. We stimulated immortalized urothelial cells derived from the inflamed region of IC bladders (SR22A or SM28 abn) or from healthy bladders (PD07i or PD08i) with tryptase and measured phospholipase A₂ (PLA₂) activity and the resultant release of arachidonic acid and prostaglandin E₂ (PGE₂). Tryptase stimulation of either PD07i or SR22A resulted in similar increases in PLA₂ activity and arachidonic acid release. However, tryptase stimulation of SR22A and SM28 abn did not result in a significant increase in PGE₂ release when compared to the increase in PGE₂ release from tryptase-stimulated PD07i and PD08i cells. Expression of mRNA for cyclooxygenase-2 (COX-2) and prostaglandin E synthase was lower and mRNA for 15-hydroxyprostaglandin dehydrogenase was higher in SR22A compared to PD07i, suggesting that both decreased synthesis and increased metabolism is responsible for the lack of a PGE₂ response in tryptase stimulated SR22A cells. Since PGE₂ is a cytoprotective eicosanoid, the failure to produce this metabolite in cells isolated from the IC bladder may represent an increased susceptibility to damage by pro-inflammatory stimuli.

Introduction

Interstitial cystitis (IC) is a chronic painful bladder condition of unknown etiology that is characterized by an increase in the number of activated mast cells, urothelial permeability changes and neurogenic inflammation (25). Mast cells are the primary effectors of type 1 IgE mediated immune reaction and release a battery of stored and newly formed mediators upon activation including biogenic amines such as histamine, neutral proteases such as tryptase, chymase, cathepsin and carboxypeptidase, arachidonic acid metabolites, chemokines and cytokines (25). Bladder biopsies from patients diagnosed with IC have demonstrated an increase in the number of IL-6 positive mast cells (14,21) and mast cell mediators such as tryptase (1) and histamine (39) are detected in the urine of IC patients.

In a previous study, we have demonstrated that activation of urothelial cell calcium-independent phospholipase A₂ (iPLA₂) by tryptase leads to membrane phospholipid hydrolysis and rapid release of arachidonic acid (22). Bisoxygenation of free arachidonic acid catalyzed by either cyclooxygenase (COX)-1 or COX-2 results in formation of

Corresponding author: Jane McHowat, Department of Pathology, Saint Louis University School of Medicine, Doisy Research Building, 3rd Floor, 1110 S. Grand Blvd., St. Louis, MO 63104, Phone: 314-977-9295, Fax: 314-977-8499, jane.mchowat@tenethealth.com.

prostaglandin H₂ (PGH₂), which is the precursor of all prostanoid products including prostaglandins, prostacyclin and thromboxanes (30). Further metabolism of PGH₂ by prostaglandin E synthase (PTGES) converts PGH₂ to PGE₂, which exerts its effects via a family of heterotrimeric G-protein coupled receptors. The actions of PGE₂ can be pro- or anti-inflammatory depending on the expression pattern of the PGE receptors in each tissue. In the bladder, PGE₂ has been shown to be increased in carcinogenesis, overactive bladder and urinary tract infections (11,12,27,35,36). However PGE₂ is widely accepted to be cytoprotective in the epithelium and is involved in wound repair and cell motility. Since IC is associated with impairment of urothelial integrity, it is compelling to suggest that this prostaglandin may be beneficial in this disease.

In this study, we stimulated urothelial cells isolated from a normal bladder or an IC bladder with trypase and examined activation of iPLA₂ and the resultant arachidonic and PGE₂ release. We now report that PGE₂ release is impaired in urothelial cells isolated from an IC bladder and discuss the results in the context of a possible link between decreased PGE₂ release and impaired cytoprotection and wound healing properties of the urothelium in IC.

Materials and Methods

Materials

Recombinant human skin β tryptase was obtained from Promega Corp., Madison, WI. Antibody for PAR-2 was obtained from Santa Cruz Biotech, Santa Cruz, CA. PGE₂ assay kit was obtained from R&D systems (Minneapolis, MN). For RT-PCR the Versagene RNA purification kit was obtained from Gentra Systems, Inc., Superscript was from Invitrogen. Taqman gene expression arrays were from Applied Biosystems. We have carried out sequence analysis and confirmed that the information given to us by Applied Biosystems will recognize our gene of interest.

Culture of the urothelial cell lines

Normal pediatric bladder epithelial (PD07i and PD08i) and inflamed region of bladder epithelium from a patient with interstitial cystitis (SR22A and SM28 abn) cell lines were generated by immortalization with a retrovirus encoding the oncoproteins E6 and E7 of human papillomavirus type 16 and selected for stable integration of the retroviral pro virus with G418. Expanded cultures were grown in EpiLife Media (Cascade Biologics, Inc. Portland, OR) with calcium (0.06mM), growth factor supplements provided by the manufacturer and penicillin (20U/ml)/streptomycin (100mg/ml) (Sigma Chemical Company, St.Louis, MO).

Characterization of epithelial cells by cytokeratins, ZO-1 and E-cadherin

PD07i and SR22 cell cultures were fixed and examined for an anti-epithelial keratin AE1/AE3 mixture and E-cadherin as previously described (22). To detect ZO-1 protein, cultured cells were washed in phosphate buffered saline, 1 mM calcium chloride, 37° C, fixed in cold methanol, washed, and refrigerated until the time of staining. Cells were treated on ice with 0.5% Triton X-100, 10 mM piperazine ethane sulfonic acid, 50 mM NaCl, 300 mM sucrose and 3 mM MgCl₂, pH 6.8 for 2 minutes, washed, blocked, and incubated with primary antibodies to ZO-1 (Invitrogen Corporation, Frederick, MD) or non-immune serum. Following treatment with Alexa Fluor secondary antibody (Molecular Probes, Eugene, OR), samples were viewed by confocal microscopy [MRC 1024: Bio-Rad, Hercules, CA]. All confocal images were analyzed using ImageJ software and Image Pro Plus (MediaCybernetics, Silver Spring, MD).

Measurement of phospholipase A₂ activity

SR22A and PD07i were grown to confluence in 100 mm tissue culture dishes. The surrounding medium was removed and replaced with ice-cold buffer containing (in mmol/l) 250 sucrose, 10 KCl, 10 imidazole, 5 EDTA, and 2 DTT with 10% glycerol, pH=7.8 (PLA₂ assay buffer). The cell suspension was sonicated and PLA₂ activity was assessed by incubating enzyme (200 µg of cellular protein) with 100µM (16:0; [³H] 18:1) plasmenylcholine substrate (specific activity~150 dpm/pmol) in assay buffer containing 100 mM Tris, pH=7.0, and 10% glycerol with 4 mM EGTA at 37°C for 5 min in a total volume of 200 µl. Reactions were terminated by the addition of 100 µl butanol and released radiolabeled fatty acid was isolated by TLC and quantified by liquid scintillation spectrometry.

Measurement of arachidonic acid release

SR22A and PD07i were grown to confluence in 35 mm tissue culture dishes. Arachidonic acid release was determined by measuring [³H] arachidonic acid released into the surrounding medium from cells prelabeled with 3 µCi of [³H] arachidonic acid (specific activity 100 Ci/mmol; Perkin-Elmer Life Sciences, Boston, MA) per culture dish for 18 h. Cells were washed three times with HEPES buffer containing (in mmol/l) 133.5 NaCl, 4.8 KCl, 1.2 CaCl₂, 1.2 MgCl₂, 1.2 KH₂PO₄, 10 HEPES (pH 7.4), 10 glucose, and 0.36% bovine serum albumin and incubated at 37°C for 15 min before addition of inhibitors and/or tryptase. At the end of stimulation, radioactivity in the medium and cells was quantified by liquid scintillation spectrometry.

Measurement of PGE₂ release

Cells were grown to confluence in 16 mm tissue culture dishes. Cells were washed twice with Hanks balanced salt solution (HBSS) containing (in mmol/l) 135 NaCl, 0.8 MgSO₄, 10 HEPES (pH=7.6), 1.2 CaCl₂, 5.4 KCl, 0.4 KH₂PO₄, 0.3 Na₂HPO₄, and 6.6 glucose. After washing, 0.5 ml of HBSS with 0.36% bovine serum albumin was added to each culture well. Cells were then stimulated with the appropriate tryptase concentrations and PGE₂ release was measured using an immunoassay kit (R&D Systems, Minneapolis, MN).

PAR-2 surface expression assay

SR22A and PD07i were cultured to confluence in 16mm culture dishes. Cultures were fixed in a solution containing 127mM NaCl, 5mM KCl, 1.1mM NaH₂PO₄, 2mM MgCl₂, 5.5mM Glucose, 20mM PIPES, and 1 % paraformaldehyde at 4°C overnight. Cells were washed with phosphate buffered saline, blocked in Tris- buffered saline containing 0.8% bovine serum albumin, 0.1% Tween, and 0.5% gelatin and incubated with primary antibody to PAR-2 (1:50, Santa-Cruz Biotechnology, Inc.) followed by incubation with HRP conjugated secondary antibody (1:5000). At the end of incubation secondary antibody was removed and 3,3',5,5', tetramethyl benzidine was added. Cells were incubated for 30 min. in the dark, the reaction stopped by 8N sulfuric acid and color development measured at 450nm.

Real Time PCR

Total RNA from both PD07i and SR22A cells was extracted using the Versagene RNA purification kit (Gentra Systems, Inc), and first strand cDNA synthesized using Superscript II (Invitrogen). All PCR were performed using TaqMan® Gene Expression Assays (Applied Biosystems) on a 7300 Real-Time PCR System (Applied Biosystems). The cycling conditions were as follows: 50°C for 2 min, 95°C for 10 min, followed by 40 cycles at 95°C for 15 s, 60°C for 1 min. Experiments were performed in triplicate for each data point. For all experiments, controls without templates were included. Reactions were then amplified with IQ SYBR Green supermix (Bio-Rad). The fold change in mRNA level for each protein

of interest was calculated using the $\Delta\Delta\text{CT}$ method against either 18s RNA or beta glucuronidase.

Results

To verify the epithelial origin of PD07i and SR22 cells used in this study, we examined the cell morphology and the presence of cytokeratins, ZO-1 tight junction associated protein, and E-cadherin (Figure 1). Taken together, the results confirmed the presence of these proteins in both cell lines and determine that the cell lines originated from epithelial cells of the bladder (Figure 1).

Confluent monolayers of PD07i and SR22A cells were stimulated with trypsin for up to 30 minutes and PLA₂ activity was measured in the absence of calcium using (16:0, [³H]18:1) plasmenylcholine as substrate. Trypsin stimulation resulted in an increase in PLA₂ activity in both PD07i and SR22A cells that returned to unstimulated values by 15 minutes (Figure 2). Significant increases in PLA₂ activity occurred after 1 minute of trypsin stimulation in PD07i, whereas the increase in activity was delayed and smaller in SR22A (Figure 2). Since PLA₂ activity was measured in the absence of calcium, this activity represents calcium-independent PLA₂ (iPLA₂). We pretreated PD07i and SR22A with bromoenol lactone (BEL, a selective iPLA₂ inhibitor, 2 μM , 10 mins) prior to trypsin stimulation and determined that the increase in PLA₂ activity was inhibited (data not shown), further supporting our hypothesis that iPLA₂ is involved.

To investigate whether the delay in iPLA₂ increased activity in SR22A cells was related to a difference in protease activated receptor-2 (PAR-2) expression on the cell surface, the receptor density and cell number was determined in confluent monolayers of PD07i and SR22A. A measure of the cell surface expression of PAR-2 was achieved by developing an immunoassay that used an antibody to the extracellular amino terminus of the PAR-2 in non-permeabilized monolayers. This allows selective assessment of the cell surface receptor expression independent of intracellular PAR-2 stores. Cell surface expression of PAR-2, as measured by absorbance @ 450 nm, was found to be 40% less in SR22A when compared to PD07i (Figure 3). We fixed representative monolayers of SR22A and PD07i cells with methanol, stained them with hematoxylin and eosin and counted the number of SR22A and PD07i in a defined area in order to estimate the number of cells per unit area in each culture. We found that there were $46 \pm 4\%$ ($n=3$) more SR22A cells per unit area compared to PD07i. Taken together, these data suggest that the cell surface expression of PAR-2 per cell is lower in SR22A when compared to PD07i and may explain the delay and attenuation of the trypsin-induced PLA₂ response. However, although sequence analysis of the epitope of the PAR2 antibody has been compared to the human protein database and shown to have no significant sequence similarity with any other protein (personal communication with Santa Cruz Biotechnology, Santa Cruz, CA), we do not have experimental evidence that the antibody doesn't cross-react with other receptors. Therefore, our results should be taken in light that this antibody may have identified other PAR on the urothelial cell surface as well.

The delay in iPLA₂ activity in SR22A compared to PD07i was reflected in the delay in [³H] arachidonic acid release from trypsin stimulated SR22A and PD07i (Figure 4). However, after 10 mins of trypsin stimulation, there was no significant difference in the amount of arachidonic acid released from each cell line. The amount of [³H] arachidonic acid release from unstimulated PD07i or SR22A was not significantly different between time 0 and 30 minutes (data not shown).

Stimulation of confluent monolayers of PD07i with 20 ng/ml trypsin resulted in a 4-fold increase in PGE₂ release into the surrounding medium whereas PGE₂ release from SR22A

was approximately 1.5-fold greater than unstimulated values (Figure 5). This difference in the magnitude of PGE₂ release was also observed at both 2 ng/ml and 200 ng/ml tryptase (Figure 5). To determine whether the increase in PGE₂ release in immortalized urothelial cells from normal bladder was not affected by the immortalization process, we stimulated PD07i, PD08i and primary human urothelial cells (HUR) with 20 ng/ml tryptase for up to 30 minutes. As shown in Figure 6, increases in PGE₂ release were similar between the three samples. Tryptase stimulation of an additional cell line obtained from IC bladder (SM28 abn) failed to show any increase in PGE₂ release over 30 minutes (Figure 7). Taken together, these data indicate that PGE₂ release from immortalized cells isolated from IC bladders is significantly decreased when compared to the responses from normal bladder urothelial cells and that the responses in normal immortalized cells are comparable to that observed in primary urothelial cell cultures.

The low levels of PGE₂ release seen with SR22A could be ascribed either to a decrease in synthesis of PGE₂ by prostaglandin E synthase or an increase in degradation by 5-hydroxy prostaglandin dehydrogenase (15-HPGD) to 13,14 dihydro-15-keto PGE₂. Using real time PCR, we observed a decrease in mRNA for cyclooxygenase-2 (COX-2) and PGE synthase (PGES) and an increase in mRNA for 15-HPGD in SR22A when compared to PD07i (Table 1). These data suggest that both decreased PGE₂ synthesis by COX-2 and PGES and increased metabolism by 15-HPGD may contribute to the decreased PGE₂ accumulation in tryptase-stimulated SR22A cells.

Discussion

Interstitial cystitis (IC) is primarily a disease of the urothelium in which impaired urothelial cell barrier function can lead to penetration of urinary solutes into the bladder wall, leading to pain and inflammation (6,20). Alterations in paracellular permeability and expression of specific tight junction proteins have been demonstrated in cultured IC bladder epithelial cells (40). Further, bladder epithelial cells in IC patients produce anti-proliferative factor (APF) (11). APF treatment causes significant increases in paracellular permeability of normal bladder cells in culture and decreased expression of zonula occludens-1 and occludin (40). Thus, APF may contribute to the impaired urothelial cell barrier, leading to pain and inflammation. In addition, mast cells play a central role in the pathogenesis of IC. Several mediators that are indicative of mast cell activation, including IL-6 (13,16), histamine (1) and tryptase (34) are elevated in the urine of IC patients and can come into contact with urothelial cells. Bladder mast cells can be activated as a result of the impaired barrier function and resultant leakage of urinary contents into the bladder wall, thus setting up a cycle of inflammation.

In these studies, we used urothelial cells from normal and IC patients that were immortalized using HPV E6E7, which endows epithelial cells with extended lifespan in vitro. While E6 and E7 are known to interact with cell cycle regulators, the resulting perturbations are nonetheless permissive for epithelial differentiation. Indeed, the HPV life cycle requires epithelial differentiation for amplification of the viral genome and induction of late gene expression. Furthermore, the immortalization induced by E6E7 is distinct from the transformed phenotype associated with carcinoma cell lines that are capable of substrate-independent growth in soft agar and tumor formation in nude mice. Immortalization of urogenital epithelial cultures results in cell lines that retain the capacity to express tissue-specific differentiation markers in multiple culture models of epithelial differentiation, including 3D organotypic raft cultures (15,18). Immortalized urothelial cell lines also undergo responses to pathogenic insult that mimics the in vivo kinetics of the murine urothelium (16,17). Most importantly, immortalized urothelial cells retained the same pattern of differentiation-induced expression of the cyclin-CDK inhibitors p21 and p27 as

the parental, primary urothelial cultures (18). In this study, we have demonstrated that immortalized cells derived from normal bladder biopsies demonstrate a similar PGE₂ release response to tryptase stimulation when compared to primary human urothelial cells, suggesting that the biochemical responses to tryptase stimulation are not changed by the immortalization process. Zhang et al (2005) have shown cultured IC cells have significantly less ZO-1 expression compared to cultured normal cells. In contrast our results showed similar staining in the normal and IC derived cell lines (figure 1). This difference in observations between the two laboratories could possibly be due to sample variability. IC patient sample variability may be due to several factors, including severity of the disease, duration of the disease, initiating etiology, and hormonal status of the patient at the time the original sample was taken.

In a previously published study, we demonstrated iPLA₂ activation and increased release of arachidonic acid and PGE₂ from human urothelial cells stimulated with thrombin or tryptase (22). In the present study, activation of iPLA₂ and the subsequent release of arachidonic acid from tryptase stimulated PD07i and SR22A was similar to the results we obtained previously with primary cells from human adult urothelium (22). However, the time course of iPLA₂ activation and arachidonic acid release was slightly delayed in SR22A when compared to that of PD07i. Further studies suggested that there is a smaller number of PAR-2 expressed on the cell surface of SR22A when compared to PD07i. In our previously study (22), immunoblot analysis demonstrated the presence of both PAR-1 and PAR-2 in isolated human urothelial cells and all four PAR are present in the J82 human urothelial cell line (3). Saban et al have demonstrated the presence of all four PAR in the mouse bladder (23) and observed that urothelial PAR-2 are downregulated in inflammation (3). The authors suggested that the downregulation of PAR-2 may be due to continued PAR activation and that the downregulation may contribute to inflammation since PAR-2 ligands have been proposed to be anti-inflammatory in inflammatory bowel disease (4,5). We now report that PAR-2 cell surface expression is significantly lower in urothelial cells isolated from an IC bladder when compared to those from a normal bladder in the absence of receptor activation. Real time PCR data indicated that mRNA expression for both PAR-1 and PAR-2 was significantly decreased in SR22A when compared to PD07i (Table 1), indicating that there is not increased production and turnover of these receptors in SR22A cells. Thus, our data indicate that in urothelial cells isolated from an IC bladder, there may be a permanent change in the downregulation and cell surface expression of PAR-2 that is independent of receptor activation. Whether the initiating event is long term cleavage of PAR-2 by increased mast cell tryptase in the early stages of this disease remains to be determined.

Despite the downregulation of cell surface expression of PAR-2, tryptase stimulated increases in iPLA₂ activity and arachidonic acid release remained similar between the two cell types. Resting PLA₂ activity in SR22A was slightly higher than that in PD07i, but did not reach significance. Bladder instillation of PAR activating peptides have been shown to result in upregulation of iPLA₂ in cytoplasmic extracts from the mouse bladder mucosa (24). Resting iPLA₂ activity and arachidonic acid release were higher in SR22A and activation of iPLA₂ and arachidonic acid were delayed in SR22A when compared to PD07i, suggesting that there may be some long term alteration in PLA₂ activation or expression in SR22A.

The most dramatic difference between the two cell types is clearly the lack of PGE₂ accumulation in SR22A and SM28 abn cells. Despite increased arachidonic acid production in response to tryptase, there was little subsequent metabolism to PGE₂. Real time PCR data demonstrate that this may a result of a combination of downregulation of COX-2 and PGES and upregulation of HPGD, resulting in both decreased synthesis and increased metabolism of PGE₂ in SR22A cells. Among prostaglandins, PGE₂ is the most widely produced in the body and exhibits the most versatile actions. It can have both pro- and anti-inflammatory

actions that are regulated by the expression of PGE receptors in the relevant tissues (for review, see 32). Prostaglandin E₂ is involved in a wide spectrum of biological activities, including pain, pyrexia, platelet aggregation, airway contraction and bronchodilation, bone resorption, ovulation and fertilization, vasodilation, modulation of cytokine and chemokine production and gastric and duodenal cytoprotection. Increased PGE₂ release in the bladder has been associated with several diseases, including bladder carcinogenesis (27,29), overactive bladder (11,12), cyclophosphamide-induced cystitis (19) and urinary tract infections (35). This is the first study to describe a decrease in PGE₂ release in bladder disease.

Prostaglandin E₂ is widely considered to have a cytoprotective role in epithelium (2,26,31,37,38). Bladder biopsies from patients with IC can demonstrate epithelial denudation. Following injury, the process of regeneration involves spreading and migration of cells at the wound edge into the denuded area to re-epithelialize the wound site. Extracellular matrix components, growth factors and eicosanoids all play a role in the process of wound healing and repair. Prostaglandins are known to favor cell migration and wound healing in a variety of tissues including corneal endothelium (9), umbilical cord endothelium (8), keratinocytes (10), skin (33), intestinal epithelium (41) and airway epithelium (26). Our finding that SR22A and SM28 abn cells fail to release PGE₂ in response to tryptase may represent a mechanism whereby the urothelium in the IC bladder is not protected in settings where there is mast cell activation and suggest that the failure to produce PGE₂ may contribute to delayed wound healing in the IC bladder.

The decrease in PGE₂ release from SR22A appears to be a result of both decreased synthesis by COX-2 and/or PGES and increased metabolism by PGDH. Increased PGE₂ production associated with increased COX-2 expression has been observed in bladder cancer and in rat bladder inflammation (27,35,36). Membrane-bound PGE₂ synthase-1 mRNA is increased in bladder carcinogenesis (27). However, we observed a significance decreases in both COX-2 and PGES mRNA levels in SR22A when compared to PD07i. Prostaglandin dehydrogenase has been identified previously in the urothelium and shown to be decreased in the ureter during obstruction, contributing to the accumulation of prostaglandins (7). More recently, PGDH has been implicated in maintaining urothelial differentiation (34). Urothelial cells treated with siRNA for PGDH showed lower expression of E-cadherin (34), although whether this was a direct result of increased PGE₂ accumulation remains to be evaluated. It is becoming increasingly apparent that IC may be associated with distinctive changes in the urothelium at both the structural and molecular level. A recent study suggested that urothelial cells in the IC bladder may follow an aberrant differentiation program leading to altered synthesis of several proteins involved in barrier function (28). Our study suggests that in addition to these proteins, alterations in COX-2, PGES or PGDH and the resultant loss of PGE₂ production in IC could further contribute to loss of protection and wound repair of the urothelium.

In conclusion, this study demonstrates that urothelial cells derived from the inflamed area of the bladder of IC patients fail to release PGE₂ in response to tryptase stimulation. This may represent a key impairment in the normal protection and repair of the urothelium during inflammatory events in the progression of this disease.

Acknowledgments

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REFERENCES

1. Boucher W, el-Mansoury M, Pang X, Sant GR, Theoharides TC. Elevated mast cell tryptase in the urine of patients with interstitial cystitis. *Br. J. Urol.* 1995; 76:94–100. [PubMed: 7648070]
2. Chapman JT, Otterbein LE, Elias JA, Choi AM. Carbon monoxide attenuates aeroallergen-induced inflammation in mice. *Am. J. Physiol.* 2001; 281:L209–L216.
3. D'Andrea MR, Saban MR, Nguyen NB, Andrade-Gordon P, Saban R. Expression of protease-activated receptor-1, -2, -3, and -4 in control and experimentally inflamed mouse bladder. *Am. J. Pathol.* 2003; 162:907–923. [PubMed: 12598324]
4. Fiorucci S, Distrutti E. Role of PAR-2 in pain and inflammation. *Trends Pharmacol Sci.* 2002; 23:153–155. [PubMed: 11931979]
5. Fiorucci S, Mencarelli A, Palazzetti B, Distrutti E, Vergnolle N, Hollenberg MD, Wallace JL, Morelli A, Cirino G. Proteinase-activated receptor 2 is an anti-inflammatory signal for colonic lamina propria lymphocytes in a mouse model of colitis. *Proc. Natl. Acad. Sci.* 2001; 98:13936–13941. [PubMed: 11717450]
6. Graham E, Chai TC. Dysfunction of bladder urothelium and bladder urothelial cells in interstitial cystitis. *Curr. Urol. Reports.* 2006; 7:440–446.
7. Jerde TJ, Mellon WS, Fischer SM, Liebert M, Bjorling DE, Nakada SY. Suppression of 15-hydroxyprostaglandin dehydrogenase messenger RNA concentration, protein expression, and enzymatic activity during human ureteral obstruction. *J. Pharmacol. Exp. Ther.* 2004; 309:398–403. [PubMed: 14718596]
8. Jiang H, Weyrich AS, Zimmerman GA, McIntyre TM. Endothelial cell confluence regulates cyclooxygenase-2 and prostaglandin E₂ production that modulate motility. *J. Biol. Chem.* 2004; 279:55905–55913. [PubMed: 15485847]
9. Joyce NC, Joyce SJ, Powell SM, Mekli B. EGF and PGE₂: effects on corneal endothelial cell migration and monolayer spreading during wound repair in vitro. *Curr. Eye Res.* 1995; 14:601–609. [PubMed: 7587307]
10. Kaneko F, Zhang JZ, Maruyama K, Nihei Y, Ono I, Iwatsuki K, Yamamoto T. Prostaglandin I₁ analogs, SM-10902 and SM-10906, affect human keratinocytes and fibroblasts in vitro in a manner similar to PGE₁: therapeutic potential for wound healing. *Arch. Dermatol. Res.* 1995; 287:539–545. [PubMed: 7487139]
11. Keay S, Zhang CO, Trifillis AL, Hise MK, Hegel JR, Jacobs SC, Warren JW. Decreased ³H-thymidine incorporation by human bladder epithelial cells following exposure to urine from interstitial cystitis patients. *J. Urol.* 1996; 156:2073–2078. [PubMed: 8911393]
12. Kim JC, Park EY, Hong SH, Seo SI, Park YH, Hwang T-K. Changes of urinary nerve growth factor and prostaglandins in male patients with overactive bladder symptom. *Int. J. Urol.* 2005; 12:875–880. [PubMed: 16323980]
13. Kim JC, Park EY, Seo SI, Park YH, Hwang T-K. Nerve growth factor and prostaglandins in the urine of female patients with overactive bladder. *J. Urol.* 2006; 175:1773–1776. [PubMed: 16600756]
14. Lotz M, Villiger P, Hugli T, Koziol J, Zuraw BL. Interleukin-6 and interstitial cystitis. *J. Urol.* 1994; 152:869–873. [PubMed: 8051739]
15. Klumpp DJ, Forrestal SG, Karr JE, Mudge CS, Anderson BE, Schaeffer AJ. Epithelial differentiation promotes the adherence of type 1-piliated *Escherichia coli* to human vaginal cells. *J. Infect. Dis.* 2002; 186:1631–1638. [PubMed: 12447740]
16. Klumpp DJ, Rycyk MT, Chen MC, Thumbikat P, Sengupta S, Schaeffer AJ. Uropathogenic *Escherichia coli* induces extrinsic and intrinsic cascades to initiate urothelial apoptosis. *Infect. Immun.* 2006; 74:5106–5113. [PubMed: 16926402]
17. Klumpp DJ, Weiser AC, Sengupta S, Forrestal SG, Batler RA, Schaeffer AJ. Uropathogenic *Escherichia coli* potentiates type 1 pilus-induced apoptosis by suppressing NF-kappaB. *Infect. Immun.* 2001; 69:6689–6695. [PubMed: 11598039]
18. Mudge CS, Klumpp DJ. Induction of the urothelial differentiation program in the absence of stromal cues. *J. Urol.* 2005; 174:380–385. [PubMed: 15947694]

19. Nakahara T, Kubota Y, Saito M, Sakamoto K, Ishii K. Protease-activated receptor-2-mediated contraction of urinary bladder is enhanced in cyclophosphamide-treated rats. *N-S Arch. Pharmacol.* 2004; 369:212–219.
20. Parsons CL. The role of the urinary epithelium in the pathogenesis of interstitial cystitis/prostatitis/urethritis. *Urology.* 2007; 69:9–16. [PubMed: 17462486]
21. Peecker R, Enerback L, Fall M, Aldenborg F. Recruitment, distribution and phenotypes of mast cells in interstitial cystitis. *J. Urol.* 2000; 163:1009–1015. [PubMed: 10688040]
22. Rickard A, McHowat J. Production of phospholipid metabolites following cleavage of protease-activated receptors on human urothelial cells. *Am. J. Physiol.* 2002; 283:F944–F951.
23. Saban R, D'Andrea MR, Andrade-Gordon P, Derian CK, Dozmorov I, Ihnat MA, Hurst RE, Davis CA, Simpson C, Saban MR. Mandatory role of proteinase-activated receptor 1 in experimental bladder inflammation. *BMC Physiol.* 2007; 7:4. [PubMed: 17397548]
24. Saban R, D'Andrea MR, Andrade-Gordon P, Derian CK, Dozmorov I, Ihnat MA, Hurst RE, Simpson C, Saban MR. Regulatory network of inflammation downstream of proteinase-activated receptors. *BMC Physiol.* 2007; 7:3. [PubMed: 17397547]
25. Sant GR, Kempuraj D, Marchand JE, Theoharides TC. The mast cell in interstitial cystitis: Role in pathophysiology and pathogenesis. *Urology.* 2007; 69:34–40. [PubMed: 17462477]
26. Savla U, Appel HJ, Sporn PHS, Waters CM. Prostaglandin E₂ regulates wound closure in airway epithelium. *Am. J. Physiol.* 2001; 280:L421–L431.
27. Shi Y, Cui L, Chen J, Pan H, Song L, Cheng S, Wang X. Elevated prostaglandin E₂ level via cPLA₂--COX-2--mPGES-1 pathway involved in bladder carcinogenesis induced by terephthalic acid-calculi in Wistar rats. *Prost. Leukotr. Ess. Fatty Acids.* 2006; 74:309–315.
28. Slobodov G, Feloney M, Gran C, Kyker KD, Hurst RE, Culkin DJ. Abnormal expression of molecular markers for bladder impermeability and differentiation in the urothelium of patients with interstitial cystitis. *J. Urol.* 2004; 171:1554–1558. [PubMed: 15017219]
29. Smakman N, Schaap N, Snijckers CM, Borel Rinkes IH, Kranenburg O. NS-398, a selective cyclooxygenase-2 inhibitor, reduces experimental bladder carcinoma outgrowth by inhibiting tumor cell proliferation. *Urology.* 2005; 66:434–440. [PubMed: 16098373]
30. Smith WL, De Witt DL, Garavito RM. Cyclooxygenases: structural, cellular, and molecular biology. *Annu. Rev. Biochem.* 2000; 69:145–82. [PubMed: 10966456]
31. Stenson WF. Prostaglandins and epithelial response to injury. *Curr. Opin. Gastroenterol.* 2007; 23:107–110. [PubMed: 17268236]
32. Sugimoto Y, Narumiya S. Prostaglandin E receptors. *J. Biol. Chem.* 2007; 282:11613–11617. [PubMed: 17329241]
33. Talwar M, Moyana TN, Bharadwaj B, Tan LK. The effect of a synthetic analogue of prostaglandin E₂ on wound healing in rats. *Ann. Clin. Lab. Sci.* 1996; 26:451–457. [PubMed: 8879363]
34. Tseng-Rogenski S, Lee I-L, Gebhardt D, Fischer SM, Wood C, Park JM, Liebert M. Loss of 15-hydroxyprostaglandin dehydrogenase expression disrupts urothelial differentiation. *Urology.* in press.
35. Wheeler MA, Hausladen DA, Yoon JH, Weiss RM. Prostaglandin E₂ production and cyclooxygenase-2 induction in human urinary tract infections and bladder cancer. *J. Urol.* 2002; 168:1568–1573. [PubMed: 12352459]
36. Wheeler MA, Yoon JH, Olsson LE, Weiss RM. Cyclooxygenase-2 protein and prostaglandin E₂ production are upregulated in a rat bladder inflammation model. *Eur. J. Pharmacol.* 2001; 417:239–248. [PubMed: 11334856]
37. Xiao Z-L, Biancani P, Behar J. Role of PGE₂ on gallbladder muscle cytoprotection of guinea pigs. *Am. J. Physiol.* 2004; 286:G82–G88.
38. Yamato M, Nahahama K, Kotani T, Kato S, Takeuchi K. Biphasic effect of prostaglandin E₂ in a rat model of esophagitis mediated by EP1 receptors: relation to pepsin secretion. *Digestion.* 2005; 72:109–118. [PubMed: 16172547]
39. Yun SK, Laub DJ, Weese DL, Lad PM, Leach GE, Zimmern PE. Stimulated release of urine histamine in interstitial cystitis. *J. Urol.* 1992; 148:1145–1148. [PubMed: 1404625]

40. Zhang CO, Wang JY, Koch KR, Keay S. Regulation of tight junction proteins and bladder epithelial paracellular permeability by an antiproliferative factor from patients with interstitial cystitis. *J. Urol.* 2005; 174:2382–2387. [PubMed: 16280852]
41. Zushi S, Shinomura Y, Kiyohara T, Minami T, Sugimachi M, Higashimoto Y, Kanayama S, Matsuzawa Y. Role of prostaglandins in intestinal epithelial restitution stimulated by growth factors. *Am. J. Physiol.* 1996; 270:G757–G762. [PubMed: 8967486]

FIGURE 1.

Characterization of epithelial cells in PDO7i (Panel A) and SR22 (Panel B) cell cultures. **1)** confluent monolayer of cells exhibiting epithelial cobblestone appearance. **2)** subconfluent, single cells stained for AE1, AE3 epithelial specific cytokeratins. **3-4)** stratified cell cultures stained for ZO-1 and E-cadherin, respectively.

FIGURE 2.

Activation of phospholipase A₂ in PD07i (filled squares) and SR22A (open circles) following tryptase stimulation (20 ng/ml). Phospholipase A₂ activity was determined by measurement of the release of radiolabeled fatty acid following incubation of enzyme (200 µg of cellular protein) with 100µM (16:0; [³H] 18:1) plasmenylcholine substrate in assay buffer containing 4 mM EGTA at 37°C for 5 min. Data are shown as mean ± SEM for 3 separate cell cultures. * p<0.05, ** p<0.01 when compared to unstimulated values.

FIGURE 3.

Expression of PAR-2 on the cell surface of confluent monolayers of PD07i & SR22A. Cell surface expression was determined on non-permeabilized confluent monolayers using an antibody to the extracellular amino terminus of PAR-2 and an immunoassay as described in Methods. Data are shown as means + SEM for 12 samples from 3 separate cell cultures. ** $p < 0.01$ when comparing the two cell lines

FIGURE 4.

[³H] arachidonic acid release from PD07i (filled squares) and SR22A (open circles) following tryptase stimulation (20 ng/ml). [³H] arachidonic acid released into the surrounding medium was measured after cells were prelabeled with 3 μ Ci of [³H] arachidonic acid per culture dish for 18 h. Radioactivity in the medium and cells was quantified by liquid scintillation spectrometry. Data are shown as mean $\pm$ SEM for 3 separate cell cultures. * $p < 0.05$, ** $p < 0.01$ when compared to unstimulated values.

FIGURE 5.

Prostaglandin E₂ release from PD07i (upper panel) and SR22A (lower panel) following tryptase stimulation at 2 ng/ml (●), 20ng/ml (□) or 200 ng/ml(Δ). PGE₂ release was measured using an immunoassay kit (R&D Systems, Minneapolis, MN). Data are shown as mean ± SEM of 6 separate cell cultures. * p <0.05; ** p <0.01 when compared to unstimulated values.

FIGURE 6.

Prostaglandin E₂ release from PD07i, PD08i and primary human urothelial cells (HUR) following 20 mg/ml tryptase stimulation. PGE₂ release was measured using an immunoassay kit (R&D Systems, Minneapolis, MN). Corresponding PGE₂ release in cell cultures incubated with the tryptase vehicle diluted to the same concentration as in the tryptase-stimulated cell cultures (Promega Corp., Madison, WI) are indicated by the dotted lines. Data are shown as mean $\pm$ SEM of 3 separate cell cultures. ** $p < 0.01$ when compared to unstimulated values.

FIGURE 7.

Prostaglandin E₂ release from PD07i, PD08i, SR22A and SM28abn following tryptase stimulation (20 ng/ml). Release from each unstimulated cell line is represented by the corresponding symbols and dotted lines. Data for stimulated samples are shown as mean $\pm$ SEM of 3 separate cell cultures. $P < 0.05$, ** $p < 0.01$ when compared to unstimulated values. Data for unstimulated cell cultures are means from 3 separated cell cultures. For graphic clarity, SEM for unstimulated cultures is not noted.

Table 1

Fold change in gene expression in SR22A when compared to PD07i. Experiments were performed in triplicate from 3 separate cell cultures. Data are for the fold change in mRNA level between each cell type in 3 separate cell cultures, calculated using the $\Delta\Delta\text{CT}$ method. Relative mRNA expression for each gene was compared to that of beta glucuronidase

Gene Name	Fold Change compared to PD07i
Protease-activated receptor-1	– 3.3 ± 1.3
Protease-activated receptor-2	– 3.2 ± 1.0
Cyclooxygenase-2	– 4.0 ± 1.0
Prostaglandin E synthase	– 6.7 ± 0.1
15-hydroxyprostaglandin dehydrogenase	11.3 ± 3.0