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Inhibition of HIF Reduces Bladder Hypertrophy and Improves Bladder Function in Murine Model of Partial Bladder Outlet Obstruction

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Abstract

Purpose—Posterior urethral valves are the most common cause of partial bladder outlet obstruction in the pediatric population. However, to our knowledge the etiology and the detailed mechanisms underlying pathological changes in the bladder following partial bladder outlet obstruction remain to be elucidated. Recent findings suggest that hypoxia and associated up-regulation of HIFs (hypoxia-inducible factors) have a key role in partial bladder outlet obstruction induced pathology in the bladder. We examined the effects of pharmacological inhibition of HIF pathways by 17-DMAG (17-(dimethylaminoethylamino)-17-demethoxygeldanamycin) in pathophysiological phenotypes after partial bladder outlet obstruction.

Materials and Methods—Partial bladder outlet obstruction was surgically created in male C57BL/6J mice. The animals received oral administration of 17-DMAG or vehicle daily starting from the initiation of obstruction up to 5 days. Sham operated mice served as controls. Bladders were harvested from each group 2, 4 and 7 days postoperatively, and analyzed for histological and biochemical changes. Bladder function was assessed by in vitro muscle contractility recordings.

Results—Partial bladder outlet obstruction caused a significant increase in the bladder mass accompanying enhanced collagen deposition in the bladder wall while 17-DMAG treatment suppressed those increases. Treatment with 17-DMAG attenuated the degree of up-regulation of HIFs and their target genes involving the development of tissue fibrosis in obstructed bladders. Treatment with 17-DMAG improved the decreased responses of obstructed bladder strips to electrical field stimulation and KCl.

Conclusions—In vivo 17-DMAG treatment decreased partial bladder outlet obstruction induced pathophysiological changes in the bladder. HIF pathway inhibition has a potential clinical implication for the development of novel pharmacological therapies to treat bladder pathology associated with partial bladder outlet obstruction.

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Keywords

urinary bladder neck obstruction; anoxia; 17-(dimethylaminoethylamino)-17-demethoxygeldanamycin; hypoxia-inducible factor 1; muscle; smooth

Posterior urethral valves are the most common cause of congenital lower urinary tract obstruction in the pediatric population with an estimated incidence of 1/5,000 to 1/25,000 live births.^{1,2} Despite advanced medical and surgical interventions posterior urethral valves remain a clinical challenge due to progression to end stage renal disease in at least a third of the patients.² Surgical treatments relieve the anatomical obstruction. However, pathological changes in the bladder generated during the period of PBOO, such as DSM hypertrophy, denervation, remodeling of ECM components and alterations to urothelium, are responsible for long-lasting voiding dysfunction after initial treatment. Experimental animal models of PBOO mimic the clinical phenotypes, demonstrating similar pathophysiological alterations in obstructed bladders.^{3,4} While the underlying mechanisms of prolonged voiding dysfunction after surgical treatment are not fully understood, increasing evidence suggests that ischemic hypoxia has an important role in the progression of bladder dysfunction following PBOO.⁵⁻⁸ In agreement with these findings previous studies demonstrated that PBOO triggers up-regulation of HIFs, which are major mediators of the hypoxic response, in patients and in animal models.^{9,10}

HIF-1 and HIF-2 are closely related protein complexes composed of HIF-1 α and HIF-2 α , respectively, which form heterodimers with a constitutively expressed HIF-1 β subunit.¹¹ Under normoxic conditions the HIF- α subunit is rapidly degraded whereas under hypoxic conditions it is stabilized and up-regulated.¹² HIFs activate the transcription of a variety of genes that encode proteins participating in a broad range of physiological processes to adapt to hypoxic conditions, including angiogenesis, cell proliferation, cell cycle control, cell death and energy metabolism.¹³

HIF pathways have been shown to contribute to pathological changes in bladders with PBOO, although the detailed underlying molecular mechanisms are still under debate.^{7,10,14} This diversity of suggestions for HIF mediated mechanisms in PBOO induced bladder pathology might have been caused by the fact that HIFs involve various cell physiological responses as well as by differences among studies, including PBOO duration and severity, and species, age and gender of the animals used.^{5,7,8,10,14,15} To date it is still not clear whether and how inhibition of the HIF pathways may affect pathophysiological changes in bladders that develop due to PBOO.

An increasing number of novel therapeutic agents have been designed to inhibit HIF pathways, mainly to modulate tumor pathogenesis.¹⁶ One of these drugs is 17-DMAG. It has been reported to inhibit the activity of HIFs through its ability to block the molecular chaperone HSP90, which interacts with HIF- α subunits and is needed for HIF activation in hypoxia.^{17,18} In this study we investigated potential therapeutic effects of 17-DMAG on pathophysiological changes in the bladder wall after an acute period of PBOO.

METHODS

Animals and Experimental Groups

Six-week-old male C57BL/6J mice underwent surgical ligation of the proximal urethra to induce PBOO as previously described.⁸ Sham operated animals without urethral ligation served as controls. All animals received a single dose of carprofen (5 mg/kg) intraperitoneally for analgesia daily 0 to 3 days after surgery. The mice had free access to food and water postoperatively. Sham operated and PBOO mice were divided into 3 subgroups, which received 100 μ l water (placebo/vehicle) or 17-DMAG (3 or 15 mg/kg body weight, LC Laboratories®) via oral gavage daily from days 0 to 5 postoperatively. Thus, the experimental groups of 8 mice each were Sham plus P (placebo), Sham plus T (3 mg/kg 17-DMAG), Sham plus 15 mg/kg 17-DMAG, PBOO plus P, PBOO plus T and PBOO plus 15 mg/kg 17-DMAG.

The mice were sacrificed 2, 4 and 7 days postoperatively, respectively. Body, kidney and bladder weight were recorded. Bladders from animals sacrificed 4 days after surgery were subjected to histological, molecular and physiological evaluations. The University of Colorado Anschutz Medical Campus institutional animal care and use committee approved all animal experiments.

Analyses

Histological—Paraformaldehyde fixed paraffin sections (5 μ m) of the bladders from each group were used. Masson trichrome staining and immunostaining were performed as previously described.⁸ Areas of collagen in the DSM layer and the DSM in trichrome stained sections were measured and are expressed as the collagen-to-DSM ratio. The supplementary table (<http://jurology.com/>) lists specific antibody information.

Gene Expression—Total RNA was isolated from the bladders and used for cDNA synthesis. We performed quantitative real-time polymerase chain reaction as previously described.⁸ Expression levels of each gene were calculated as fold changes based on Ct values. Data were normalized to the housekeeping gene *18s rRNA*.

In Vitro Detrusor Contractility Measurements

Freshly isolated bladders from 3 mice per experimental group were cut into 2 halves longitudinally. Each strip (about 3 $\times$ 6 mm) was placed in an organ bath (Radnoti, Monrovia, California) filled with oxygenated Tyrode's buffer at 37°C. Setup and pretest conditioning were performed as previously described.¹⁹ Tissues were subjected to several tests, including the contractile response to EFS (70 V and 32 Hz), high KCl solution (125 mM, which was substituted for NaCl in Tyrode's buffer) and the muscarinic receptor agonist CCh (0.1 to 10 μ M). Contractile parameters were measured with LabChart®, version 7.1.2. Force measurements were performed and analyzed as previously described.¹⁹

Statistical Analysis

Differences significant on ANOVA were analyzed by the 2-tailed Student t-test with $p < 0.05$ considered statistically significant. ANOVA and the t-test were performed with VassarStats (<http://vassarstats.net/>) and Excel® 2007, respectively.

RESULTS

Increased Bladder and Kidney Weight Induced by PBOO

PBOO caused a significant increase in bladder and kidney weight compared to that in the Sham plus P group at days 2, 4 and 7, and 2 and 4 postoperatively, respectively. Daily treatment with 17-DMAG prevented the weight increase in both tissues (see table). No difference in body weight was detected among the groups. Mean $\pm$ SEM bladder weight progressively increased by $36\% \pm 7\%$, $49\% \pm 9\%$ and $54\% \pm 22\%$ at days 2, 4 and 7 postoperatively, respectively, in the PBOO plus P group. Relative kidney weight increased by a mean of $31\% \pm 4\%$ at day 2 postoperatively and gradually returned to normal in the PBOO plus P group. Bladders from mice that received sham operation plus 15 mg/kg 17-DMAG became significantly smaller compared to bladders from the Sham plus P group at days 4 and 7 postoperatively while bladder histology revealed a trend toward decreased DSM layer thickness (data not shown). We speculated that this compound caused undesired side effects in the bladders and also possibly in the kidneys when used under nonpathological conditions. Therefore, we discontinued the high dose and subsequently focused on low dose treatment (3 mg/kg).

PBOO Induced Bladder Morphological Changes

Masson trichrome staining demonstrated that PBOO caused significantly enhanced collagen deposition between and within DSM fascicles in the bladders as previously reported in animal and human studies (fig. 1, A). The collagen-to-DSM ratio was significantly increased in obstructed bladders compared to bladders in the Sham plus P group (mean 0.07 ± 0.01) while 17-DMAG significantly alleviated the increase in collagen deposition (mean $\pm$ SEM 0.22 ± 0.03 and 0.11 ± 0.02 in the PBOO plus P and PBOO plus T groups, respectively) (fig. 1, B). This suggests that the compound prevented fibrosis in obstructed bladders.

Hif-1 α and Hif-2 α Protein Expression in Obstructed Bladders

Immunohistochemical analyses revealed the induction of Hif-1 α and Hif-2 α proteins following PBOO as previously reported (fig. 2).^{5,8,10} Decreased intensity of immunostaining signals for Hif-1 α and Hif-2 α were observed in bladders from the PBOO plus T group. This indicated that 17-DMAG at least partly blocks HIF pathways by preventing the accumulation of Hif proteins in obstructed bladders.

PBOO Induced Increase in HIF Target Gene mRNA Expression

Quantitative real-time polymerase chain reaction revealed significant 2.1-fold to 7.7-fold up-regulation of the HIF transcription target genes *Tgfb*, *Glut1*, *Vegf*, *Flt-1* and *Timp-1* in the bladders 4 days after PBOO induction compared to bladders in the sham operated group (fig. 3). All of these genes have been reported to have key roles in PBOO induced bladder

pathogenesis.^{5,7,8,10,20} Treatment with 17-DMAG decreased PBOO induced up-regulation of the *Tgfb*, *Glut1*, *Flt-1* and *Timp-1* genes whereas the expression level of *Vegf* was similar to that in the placebo group (2.8-fold vs 2.2-fold increase vs the Sham plus P group). Many stimuli, including growth factors, hormones and cytokines, regulate *Vegf* expression at the transcriptional level.²¹ Thus, we speculate that other transcription factors contributed to up-regulation of this gene, partly compensating for the inhibition of HIF pathways. These results confirmed that 17-DMAG blocked HIF transcriptional activity in the bladders following PBOO.

Detrusor Contractility in Obstructed Bladders

Bladder strips from PBOO mice showed a significant decrease in detrusor contractility to EFS compared to bladder strips from the Sham plus P group upon normalization of the response to strip weight. Maximal contractile force in response to high KCl was also significantly lower in the PBOO plus P group compared to other groups (fig. 4). This observation suggests that PBOO induced alterations in DSM contractility as well as in nerve dependent responses upon EFS. Treatment with 17-DMAG partially restored muscle contractility in obstructed bladders in response to EFS (mean $\pm$ SEM 58% $\pm$ 17% vs 25% $\pm$ 5% in the PBOO plus P group) and KCl (87% $\pm$ 5% vs 51% $\pm$ 7% in the PBOO plus P group with the Sham plus P group considered 100%). These results suggest that 17-DMAG is efficient in preserving contractile function of the DSM during PBOO development. We confirmed that 17-DMAG did not affect DSM contractility by testing bladder strips from the Sham plus P group in Tyrode's buffer containing 17-DMAG (3 mg/ml).

To investigate the effects of the treatment on muscarinic receptor dependent contractility we examined the concentration-response relationship of bladder strips upon application of CCh. Cumulative addition of CCh to the bath solution triggered concentration-dependent contractions. Contraction amplitude reached levels similar to those observed for KCl at the maximal tested concentration of CCh in all groups, confirming the presence of functional muscarinic receptors in the obstructed bladders.

DISCUSSION

In this study we sought to clarify the role of HIFs in obstructed bladder pathology and investigate potential mechanisms of hypoxia mediated voiding dysfunction. We did this by treating mice with PBOO using the HIF inhibitor 17-DMAG, a water soluble analogue of geldanamycin, and its derivative 17-AAG. Geldanamycin and 17-AAG inhibit HSP90, which promotes productive folding and stabilization of many proteins, including HIF- α subunits.²² These compounds block the adenosine triphosphate binding site of HSP90, interfering with its interaction with client proteins and rendering the client protein susceptible to aggregation, misfolding and subsequent degradation.²³

Phase I and II trials proved that these drugs have potent antitumor activity but those studies also revealed unfavorable properties such as tissue toxicity and water insolubility.²⁴ While 17-DMAG retains the ability to block HSP90 function in the same manner as geldanamycin and 17-AAG, it has more desired pharmaceutical properties such as water solubility, good

oral bioavailability and lower toxicity.¹⁷ In patients with several types of cancer 17-DMAG was clinically evaluated using various schedules, doses and routes of administration.²⁴

Based on previous studies in animal models and phase I trials^{24,25} we tested oral doses of 17-DMAG at 3 and 15 mg/kg daily, resulting in total doses of up to 18 and 90 mg/kg, respectively, in our experiments. We observed no change in physical appearance or behavior of the treated animals. Consistent with previous reports^{3,8,26,27} enlarged bladders and enhanced collagen deposition in the bladder wall were observed in the PBOO plus P group (see table and fig. 1). This suggests the presence of DSM hypertrophy/hyperplasia and ECM remodeling in the bladders.

There was no visible change in the physical appearance or behavior of mice in the 17-DMAG treated groups, although a daily dose of 15 mg/kg caused a significant decrease in bladder weight in sham operated mice 4 and 7 days postoperatively. We speculate that when applied at high dose, 17-DMAG may affect some basal cellular processes regulated by HSP90 target proteins in nonpathological bladders in which HIF pathways are not activated. We cannot exclude the possibility of some side effects that were unobservable from the appearance and behavior of the animals and the gross morphology of the major organs. However, no adverse effects were detected in sham operated or PBOO mice upon treatment with the lower dose of 17-DMAG while the agent improved pathological outcomes in bladders with PBOO. From these results we consider the recommended dose to be 3 mg/kg daily, resulting in a weekly dose of 18 mg/kg, equivalent to approximately 1.46 mg/kg in humans. This result is in agreement with the recommended dose established in the phase I study of 1.14 to 2.16 mg/kg per week.²⁴

Analysis of gene expression demonstrated that PBOO caused up-regulation of several HIF target genes, including *Vegf*, *Flt-1*, *Glut-1*, *Tgfb* and *Timp-1*, suggesting activation of HIF pathways (fig 3). These genes encode the proteins involved in the regulation of angiogenesis, energy metabolism and fibrosis, which are all closely associated with pathological changes induced by PBOO.^{5,8,10} Treatment with 17-DMAG improved PBOO induced morphological changes and suppressed up-regulation of the HIF target genes, confirming the inhibitory effect of this drug on HIF signaling in obstructed bladders.

PBOO impairs the ability of the bladder to store and empty urine.^{3,27,28} Initially the DSM undergoes transient decompensation in response to PBOO, which triggers subsequent molecular alterations associated with compensatory hypertrophy/hyperplasia to produce and maintain the contractile force of the DSM needed to overcome increased urethral resistance during micturition.²⁸ Prolonged PBOO ultimately changes the DSM into a decompensated state characterized by an increased amount of ECM with an altered composition, leading to a dysfunctional bladder with low compliance and compromised voiding ability.

In this study bladder strips from PBOO mice responded to exogenously applied CCh to a level similar to that of the contractile amplitude evoked by high KCl, in contrast to a profound decrease in response to EFS. These results suggest that PBOO causes partial denervation of the DSM, which in turn leads to a compensatory increase in sensitivity to muscarinic receptor agonist in the damaged DSM. This is demonstrated by the reduced

response to EFS, likely through up-regulation of muscarinic receptors as previously reported.²⁹ Daily treatment with 17-DMAG improved DSM contractility in response to EFS and KCl in obstructed bladders, providing evidence that HIF pathways have a functional role in bladder innervation and in the DSM. The fact that 17-DMAG preserved the nerve mediated DSM contraction triggered by EFS after PBOO is in line with a previous study describing hypoxia mediated axonal damage and the active role of HIF in neuronal viability.³⁰

CONCLUSIONS

The current study provides direct evidence of the involvement of HIF pathways in the progression of PBOO induced bladder dysfunction as confirmed by improved DSM contractility of obstructed bladders treated with 17-DMAG. We evaluated the effects of 17-DMAG during the acute phase of PBOO development. Therefore, further investigations are required to evaluate outcomes of long-term treatment with 17-DMAG for PBOO induced voiding dysfunction. Our data suggest that HIF pathway inhibition may serve as a potential target toward the development of novel pharmacological approaches to improve PBOO associated pathophysiological changes in the affected urinary tract.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations and Acronyms

17-AAG	17-allylamino-17-demethoxygeldanamycin
CCh	carbachol
DSM	detrusor smooth muscle
ECM	extracellular matrix
EFS	electrical field stimulation
Flt-1	Flt-1/VEGF receptor 1
Glut1	glucose transporter 1
HSP90	heat shock protein 90
PBOO	partial bladder outlet obstruction
Tgf-β	transforming growth factor-β

Timp-1	tissue inhibitor of metalloproteinase-1
Vegf	vascular endothelial growth factor

References

1. Casella DP, Tomaszewski JJ, Ost MC. Posterior urethral valves: renal failure and prenatal treatment. *Int J Nephrol*. 2012; 2012:351067. [PubMed: 21860792]
2. Yiee J, Wilcox D. Abnormalities of the fetal bladder. *Semin Fetal Neonatal Med*. 2008; 13:164. [PubMed: 18053783]
3. Levin RM, High J, Wein AJ. The effect of short-term obstruction on urinary bladder function in the rabbit. *J Urol*. 1984; 132:789. [PubMed: 6147424]
4. Sibley GN. The physiological response of the detrusor muscle to experimental bladder outflow obstruction in the pig. *Br J Urol*. 1987; 60:332. [PubMed: 3690205]
5. Ghafar MA, Anastasiadis AG, Olsson LE, et al. Hypoxia and an angiogenic response in the partially obstructed rat bladder. *Lab Invest*. 2002; 82:903. [PubMed: 12118092]
6. Buttyan R, Chichester P, Stisser B, et al. Acute intravesical infusion of a cobalt solution stimulates a hypoxia response, growth and angiogenesis in the rat bladder. *J Urol*. 2003; 169:2402. [PubMed: 12771806]
7. Galvin DJ, Watson RW, O'Neill A, et al. Hypoxia inhibits human bladder smooth muscle cell proliferation: a potential mechanism of bladder dysfunction. *Neurourol Urodyn*. 2004; 23:342. [PubMed: 15227652]
8. Iguchi N, Hou A, Koul HK, et al. Partial bladder outlet obstruction in mice may cause E-cadherin repression through hypoxia induced pathway. *J Urol*. 2014; 192:964. [PubMed: 24647082]
9. Koritsiadis G, Stravodimos K, Koutalellis G, et al. Immunohistochemical estimation of hypoxia in human obstructed bladder and correlation with clinical variables. *BJU Int*. 2008; 102:328. [PubMed: 18384635]
10. Ekman M, Uvelius B, Albinsson S, et al. HIF-mediated metabolic switching in bladder outlet obstruction mitigates the relaxing effect of mitochondrial inhibition. *Lab Invest*. 2014; 94:557. [PubMed: 24589856]
11. Wang GL, Jiang BH, Rue EA, et al. Hypoxia-inducible factor 1 is a basic-helix-loop-helix-PAS heterodimer regulated by cellular O₂ tension. *Proc Natl Acad Sci U S A*. 1995; 92:5510. [PubMed: 7539918]
12. Jaakkola P, Mole DR, Tian YM, et al. Targeting of HIF- α to the von Hippel-Lindau ubiquitylation complex by O₂-regulated prolyl hydroxylation. *Science*. 2001; 292:468. [PubMed: 11292861]
13. Ke Q, Costa M. Hypoxia-inducible factor-1 (HIF-1). *Mol Pharmacol*. 2006; 70:1469. [PubMed: 16887934]
14. Sawada N, Yao J, Hiramatsu N, et al. Involvement of hypoxia-triggered endoplasmic reticulum stress in outlet obstruction-induced apoptosis in the urinary bladder. *Lab Invest*. 2008; 88:553. [PubMed: 18347581]
15. Drzewiecki BA, Anumanthan G, Penn HA, et al. Modulation of the hypoxic response following partial bladder outlet obstruction. *J Urol*. 2012; 188:1549. [PubMed: 22910264]
16. Semenza GL. Defining the role of hypoxia-inducible factor 1 in cancer biology and therapeutics. *Oncogene*. 2010; 29:625. [PubMed: 19946328]
17. Egorin MJ, Lagattuta TF, Hamburger DR, et al. Pharmacokinetics, tissue distribution, and metabolism of 17-(dimethylaminoethylamino)-17-demethoxygeldanamycin (NSC 707545) in CD2F1 mice and Fischer 344 rats. *Cancer Chemother Pharmacol*. 2002; 49:7. [PubMed: 11855755]
18. Minet E, Mottet D, Michel G, et al. Hypoxia-induced activation of HIF-1: role of HIF-1 α -Hsp90 interaction. *FEBS Lett*. 1999; 460:251. [PubMed: 10544245]

19. Hypolite JA, Chang S, Wein AJ, et al. Protein kinase C modulates frequency of micturition and non-voiding contractions in the urinary bladder via neuronal and myogenic mechanisms. *BMC Urol.* 2015; 15:34. [PubMed: 25896919]
20. Yang L, Liu R, Wang X, et al. Imbalance between matrix metalloproteinase-1 (MMP-1) and tissue inhibitor of metalloproteinase-1 (TIMP-1) contributes to bladder compliance changes in rabbits with partial bladder outlet obstruction (PBOO). *BJU Int.* 2013; 112:E391. [PubMed: 23305285]
21. Pages G, Pouyssegur J. Transcriptional regulation of the Vascular Endothelial Growth Factor gene—a concert of activating factors. *Cardiovasc Res.* 2005; 65:564. [PubMed: 15664382]
22. Katschinski DM, Le L, Schindler SG, et al. Interaction of the PAS B domain with HSP90 accelerates hypoxia-inducible factor-1 alpha stabilization. *Cell Physiol Biochem.* 2004; 14:351. [PubMed: 15319539]
23. Jez JM, Chen JC, Rastelli G, et al. Crystal structure and molecular modeling of 17-DMAG in complex with human Hsp90. *Chem Biol.* 2003; 10:361. [PubMed: 12725864]
24. Jhaveri K, Taldone T, Modi S, et al. Advances in the clinical development of heat shock protein 90 (Hsp90) inhibitors in cancers. *Biochim Biophys Acta.* 2012; 1823:742. [PubMed: 22062686]
25. Hollingshead M, Alley M, Burger AM, et al. In vivo antitumor efficacy of 17-DMAG (17-dimethylaminoethylamino-17-demethoxygeldanamycin hydrochloride), a water-soluble geldanamycin derivative. *Cancer Chemother Pharmacol.* 2005; 56:115. [PubMed: 15791458]
26. Kim JC, Yoon JY, Seo SI, et al. Effects of partial bladder outlet obstruction and its relief on types I and III collagen and detrusor contractility in the rat. *Neurourol Urodyn.* 2000; 19:29. [PubMed: 10602246]
27. Kato K, Monson FC, Longhurst PA, et al. The functional effects of long-term outlet obstruction on the rabbit urinary bladder. *J Urol.* 1990; 143:600. [PubMed: 1968106]
28. Juan YS, Cuang SM, Jand MY, et al. Basic research in bladder outlet obstruction. *Incont Pelvic Floor Dysfunct.* 2011; 5:1.
29. Kato K, Wein AJ, Kitada S, et al. The functional effect of mild outlet obstruction on the rabbit urinary bladder. *J Urol.* 1988; 140:880. [PubMed: 3418826]
30. Vangeison G, Carr D, Federoff HJ, et al. The good, the bad, the cell type-specific roles of hypoxia inducible factor-1 alpha in neurons and astrocytes. *J Neurosci.* 2008; 28:1988. [PubMed: 18287515]

Figure 1.

Histological analysis of collagen deposition in bladders of experimental mice. *A*, bladders from sham operated controls and mice with PBOO that received placebo or 3 mg/kg 17-DMAG at day 4 postoperatively. Enhanced collagen deposition (blue areas) was observed in between and within detrusor muscles (red areas) in bladders from PBOO mice treated with placebo. Bladders from PBOO mice treated with 17-DMAG looked comparable to those from sham operated controls. Masson trichrome stain, scale bars indicate 100 μ m. *B*, collagen-to-DSM ratio showed significant increase in collagen in PBOO plus P mice while 17-DMAG significantly decreased collagen deposition in muscle layer of obstructed bladders ($p = 0.011$). Values represent mean $\pm$ SEM of 7 sections per group with DSM area per section considered 1.0. Asterisks indicate $p < 0.01$ vs Sham plus P group.

Figure 2.

Immunohistochemical analysis of Hif-1 α (A) and Hif-2 α (B) in bladders from sham operated and PBOO groups that received placebo or 3 mg/kg 17-DMAG at day 4 postoperatively. Decreased Hif-1 α and Hif-2 α immunoreactive signal intensity (brown areas) were observed in bladders from PBOO plus T group compared to strong staining in PBOO plus P group. Faint immunostaining was observed for both proteins in bladders from sham operated groups. Stained sections were analyzed in 3 mice per experimental group for reproducibility. Scale bars indicate 50 μ m.

Figure 3.

Expression analysis of HIF target genes in bladders after PBOO. Significant increases in mRNA expression levels normalized to *18s rRNA* gene were noted for TGF- β (*Tgfb*), *Glut1*, *Vegf*, *Flt-1* and *Timp-1* genes in bladders from PBOO plus P mice. Values represent mean $\pm$ SEM fold difference in expression level of each gene in bladders from PBOO mice vs bladders from sham operated groups 4 days postoperatively. Single asterisk indicates $p < 0.05$ vs Sham plus P group. Double asterisks indicate $p < 0.01$ vs Sham plus P group.

Figure 4. Peak amplitude of bladder smooth muscle contractions in response to EFS, high KCl and CCh. *A*, representative raw traces of DSM contractions in response to EFS, KCl and CCh in Sham plus P, PBOO plus P and PBOO plus T groups. *B*, bladder muscle contraction peak amplitude in response to EFS and KCl normalized to that in Sham plus P group. Values represent mean $\pm$ SEM of 3 mice per group. Asterisk indicates $p < 0.05$.

Body, kidney and bladder weight in 8 mice each in sham operated and experimental groups

17-DMAG (mg/kg)	Mean $\pm$ SEM Wt		
	Body (gm)	Bladder (mg)	Kidney (mg)
<i>Postop day 2</i>			
Sham operation:			
0	19.4 $\pm$ 0.7	20.4 $\pm$ 1.0	242 $\pm$ 13
3	18.5 $\pm$ 0.7	18.9 $\pm$ 0.8	237 $\pm$ 7
15	19.2 $\pm$ 0.5	18.2 $\pm$ 1.0	242 $\pm$ 6
PBOO:			
0	18.7 $\pm$ 0.4	26.7 $\pm$ 1.5 *	303 $\pm$ 9 *
3	19.2 $\pm$ 0.6	20.4 $\pm$ 1.6 †	269 $\pm$ 14 †
15	19.1 $\pm$ 0.6	18.8 $\pm$ 1.1 †	254 $\pm$ 9 †
<i>Postop day 4</i>			
Sham operation:			
0	20.1 $\pm$ 0.7	20.6 $\pm$ 1.1	257 $\pm$ 8
3	19.2 $\pm$ 0.6	19.3 $\pm$ 1.0	250 $\pm$ 6
15	19.6 $\pm$ 0.4	17.0 $\pm$ 1.0 *	237 $\pm$ 6
PBOO:			
0	20.4 $\pm$ 0.5	31.3 $\pm$ 2.0 *	303 $\pm$ 15 *
3	19.8 $\pm$ 0.8	20.0 $\pm$ 1.1 †	264 $\pm$ 5 †
15	19.3 $\pm$ 0.4	17.8 $\pm$ 0.8 †	250 $\pm$ 8 †
<i>Postop day 7</i>			
Sham operation:			
0	20.3 $\pm$ 0.3	19.2 $\pm$ 0.9	257 $\pm$ 8
3	20.0 $\pm$ 0.4	18.8 $\pm$ 0.7	240 $\pm$ 4
15	20.0 $\pm$ 0.4	15.2 $\pm$ 0.3 *	234 $\pm$ 5
PBOO:			
0	20.8 $\pm$ 0.7	28.6 $\pm$ 3.0 *	271 $\pm$ 12
3	19.5 $\pm$ 0.8	20.7 $\pm$ 0.8 †	247 $\pm$ 3 †
15	19.9 $\pm$ 0.4	18.0 $\pm$ 1.3 †	244 $\pm$ 7

* Significantly different vs Sham plus P (0 mg/kg) group (p <0.05).

† Significantly different vs PBOO plus P and PBOO plus T (3 mg/kg) groups, and Sham plus 15 mg/kg 17-DMAG (p <0.05).