Soluble Guanylate Cyclase Activators to Treat Benign Prostatic Hyperplasia and associated LUTS

A.J. Kanai[a,*], **K.-E. Andersson**[b], **L.A. Birder**[a], **C.H. Fry**[c]

[a]University of Pittsburgh, Department of Medicine, Renal-Electrolyte Division, USA

[b]Lund University, Division of Clinical Chemistry and Pharmacology, Lund, Sweden

[c]University of Bristol, School of Physiology, Pharmacology, and Neuroscience, UK

Abstract

This review summarises the presentations during a workshop session entitled "The Use of Soluble Guanylate Cyclase Activators to Treat Benign Prostatic Hyperplasia, Obstruction and Fibrosis – Mechanistic Concepts and Clinical Implications" at the International Continence Society (ICS) 2021 Melbourne Virtual meeting. Benign prostatic hyperplasia (BPH) is a highly prevalent condition that can result in bladder outflow obstruction (BOO) and development of lower urinary tract symptoms (LUTS), and by 80 years of age is present in about 75% of men. Current pharmacological therapies include a-adrenoceptor antagonists, $5a$-reductase inhibitors, and the phosphodiesterase type 5 (PDE5) inhibitor, tadalafil. The efficacy of tadalafil suggests a role for nitric oxide (NO•) through activation of soluble guanylate cyclase (sGC) and production of cyclic guanosine 3'5'-monophosphate (cGMP), a cyclic nucleotide that relaxes smooth muscle, reduces neurotransmitter release and also acts as an antifibrotic agent. Patient refractoriness to tadalafil may be, for example, due to sGC inactivation due to oxidative stress. The workshop discussed the superiority of cinaciguat, an sGC activator that functions even when the enzyme is oxidised, over PDE5 inhibitors, and potentially its use in combination with agents that reduce formation of reactive oxygen species.

Keywords

*Corresponding author. ajk5@pitt.edu (A.J. Kanai).

1. Introduction

This review is a summary of the workshop cited above. There were four separate presentations covering current and emerging pharmacotherapies for BPH/LUTS and the mechanistic aspects of BPH pathophysiology with a focus on NO -cGMP signalling and fibrosis.

2. Pharmacological options to treat BOO and LUTS associated with BPH

BPH is one of the most common clinical conditions in ageing men. In the fourth decade of life, it is demonstrable in 30%–40% with a steady increase of prevalence to 70%–80% in those older than 80 years [1]. Benign prostatic syndrome (BPS), comprising LUTS alone or secondary to BPH obstructing the urethra (BOO), is equally prevalent in the aging male and represents a major health care concern in most western countries. The causative agents and factors of BPH/LUTS are incompletely understood and probably multifactorial. However, there is evidence for a three-factor pathology, namely: hyperplastic and hypertrophic bulk prostate growth of the gland; an adrenergically-driven increase of prostate stromal muscle tone; and fibrosis that decreases compliance of both prostate and bladder. Several pathways have been implicated in the pathogenesis of BPH, involving androgens, oestrogen and insulin, as well as inflammation and proliferative reawakening of stem cells [2]. *In vitro* studies show that an increase of intraurethral pressure with BPH is related to contraction of smooth muscle in the prostate, prostatic capsule and prostatic urethra. Thus, about 40% of total urethral pressure in patients with benign prostatic hypertrophy has been attributed to a-adrenoceptor mediated tone, with most of the remainder due to static pressure resulting from the greater prostatic bulk [3]. A consequence of greater BOO due to BPH is remodelling of the bladder itself. Available, but limited, evidence from human studies shows a structured time-course of changes: initial bladder hypertrophy; a compensatory increase of detrusor contractile function in the voiding phase (often in combination with filling phase detrusor overactivity); followed by a functional decompensation phase (detrusor underactivity) [4]. Current therapeutic treatments [5,6] to minimise BPH progression and associated LUTS include: a-adrenoceptor antagonists, $5a$-reductase inhibitors, muscarinic receptor antagonists, β3-adrenoceptor agonists, PDE5 inhibitors and their combinations. A recent systematic review and meta-analysis evaluated if newer a1-adrenoceptor antagonists introduced to treat BPH-associated LUTS offered advantages over established agents (*i.e.*, tamsulosin, alfuzosin, doxazosin). None of the newer drugs, or their combinations, was more effective than current a1-adrenoceptor antagonist monotherapy, when evidence was sufficient to compare [7].

However, conventional drugs are not effective in all patients and thus, several potential new and alternative drug treatments have been developed and are under evaluation, which include: selective cannabinoid receptor agonists; a luteinising hormone-releasing hormone antagonist, cetrorelix; a pro-apoptotic protein injectable, fexapotide trifutate; a hexokinase inhibitor, ionidamine; vasopressin and tachykinin receptor modulators; and a·vitamin D3 receptor modulator, elocalcitol [6].

Moreover, the NO·/cGMP signalling pathway is a target for drug development in this context, either through agents that donate NO·; upregulate the target enzyme, sGC, to enhance cGMP generation; or reduce cGMP removal with phosphodiesterases (specifically PDE5) inhibitors such as sildenafil and tadalafil. The role of cGMP-driven and associated pathways to reduce pathological structural and functional changes to prostate and bladder was a central theme of the workshop.

3. sGC activators and the treatment of BPH progression and BOO/LUTS

The PDE5 inhibitor, tadalafil, is approved to treat LUTS with BPH [9] suggesting a key role in BPH pathogenesis for the NO•/sGC/cGMP signalling pathway. However, PDE5 inhibitor refractoriness can develop for several reasons including NO•-producing (nitrergic) nerve damage and decreased NO• production, and inflammation-related oxidation of the heme group on sCG, normally maintained in a reduced state by the cofactor, cytochrome-b5-reductase 3 (CYB5R3, Fig. 1A). Several sGC activators, such as cinaciguat (BAY 58–2667), have been developed to enhance sGC activity even in an oxidised state or in the absence of NO [10] and their effect on LUT function of aged mice was evaluated [8]. sGC was expressed more in the outflow tract than in detrusor suggesting its importance as a site of action in NO•/sGC/cGMP signalling. Moreover, cinaciguat had only small and transient (1-h) cardiovascular effects with oral gavage suggesting a positive safety profile. Aged mice (24 months) demonstrated a functional BPH/BOO phenotype, compared to adult animals (9 months), with low, delayed voiding responses and elevated intravesical pressures as measured by telemetric cystometry (Fig. 2). This was consistent with histological and molecular data from the outflow tract that showed urethral constriction (Fig. 3), increased prostate weight, greater collagen deposition and cellular hyperplasia. All changes in aged animals were attenuated by daily oral treatment with cinaciguat for two weeks, without effect on serum testosterone levels. The superiority of cinaciguat over PDE5 inhibitors was confirmed by it reversing an overactive cystometric profile in CYB5R3 smooth muscle knock-out mice, where the PDE5 inhibitor, sildenafil, was ineffective. The proposed mechanism for the development of BPH/LUTS and sites of action for sGC are shown in Fig. 1B. In particular, enhanced cGMP production attenuates the TGF-β dependent transition of fibroblasts to a more collagen-producing myofibroblast phenotype. Thus, the aged male mouse is a suitable model for BPH-induced BOO/LUTS and cinaciguat is superior to a PDE5 inhibitor to reduce obstruction and its consequent effects on bladder function.

The importance of fibrosis as a pathological end-point in BPH-induced BOO/LUTS is shown in Fig. 4. Age is associated with increased fibrosis in the rat bladder wall (Fig. 4A, B), as well as greater stiffness (reduced compliance) of uncontracted tissue, as seen by a steeper stress–strain function (Fig. 4D). Treatment with cinaciguat, with the same régime that reversed cystometric dysfunction, reduced the fibrosis content of the aged bladder wall and normalised the stiffness to that measured in the control adult (Fig. 4C, D).

4. PNPase inhibition, oxidative damage, fibrosis and age-related lower urinary tract dysfunction

Despite having different etiologies, most chronic fibrotic disorders are associated with persistent production of a number of factors, including reactive oxygen species that stimulate extracellular matrix (ECM) production. In part this may be due to sGC inactivation as discussed above and so strategies that reduce the extent of oxidation should also be useful to maintain adequate cGMP activity.

There is emerging evidence that changes to the activity of purine nucleosidase phosphorylase (PNPase) may lead to an abnormal urinary bladder purine metabolome, which in turn causes bladder and urethral inflammation, oxidative injury and cellular damage. This phenomenon has been demonstrated in aged rats which exhibit prolonged voiding intervals and decreased contractile indices compared to younger rats. These changes could be normalised by treatment with a PNPase inhibitor, 8-aminoguanine (8-AG) [11] (Fig. 5). Furthermore, aged rat bladders exhibited altered collagen alignment and biomechanical responses to distention *versus* younger animals. 8-AG treatment in aged rats returned collagen structure to that resembling young rat bladders (Fig. 6). Therefore, inhibition of PNPase could be used to manipulate the urinary bladder purine metabolome that may prevent age-associated deterioration of the LUT and decrease the burden of LUT diseases. Whether combination therapy of sGC activators and 8-AG represents an added advantage to the use of either alone remains to be evaluated.

5. cGMP-dependent pathways to regulate smooth muscle function and neurotransmission

Cyclic nucleotides, such as cGMP, are essential signalling molecules that mediate a plethora of functions in different cell types generally by activation of protein kinase G, including regulation of smooth muscle contraction and control of transmitter release from autonomic nerves. The contractile state of smooth muscles is a myosin-regulated system and is determined by the degree of myosin light chain (MLC) phosphorylation, in turn dependent on the activity of MLC kinases or phosphorylases. cGMP importantly relaxes smooth muscle, including that of the prostate [12], by decreasing the MLC phosphatase and Ca^{2+} sensitivity of the contractile machinery and is well-summarised in several texts [13]. A less well documented action of cGMP is its ability to regulate differentially transmitter release from post-ganglionic fibres supplying lower urinary tract tissues. In the context of BPH-induced LUTS this has been investigated in detrusor muscle, but equivalent experiments with prostate stromal tissue are awaited. A feature of post-ganglionic neurotransmission is dual release of ATP along with either acetylcholine (ACh) or noradrenaline for respective parasympathetic and sympathetic fibres. In the context of human overactive bladder, supplied by parasympathetic fibres, only ACh is functionally active in the normal bladder, but with overactive bladder ATP has an additional excitatory role, rendering its release as a useful potential drug target [14]. The two transmitters have a differential frequency range for release; ATP at lower stimulation rates. Agents that raise cGMP levels, such as cinaciguat or sildenafil, reduce low frequency contractions but leave those elicited by

high frequencies unaffected consistent with blockade of ATP, but not ACh release. This may be directly corroborated by measurement of nerve-mediated ATP or ACh release when cGMP is raised: ATP release is greatly attenuated, but ACh is unaffected (Fig. 7). The differential regulation of purinergic and cholinergic co-transmission offers a potential route to selectively manipulate transmitter release in the human bladder that is associated with overactive pathologies, whilst leaving physiological transmitter release more intact [15,16].

6. Summary and conclusion

This workshop considered the potential role of NO•-sGC-cGMP signalling in the prostate, outflow tract and bladder as a potential drug target to prevent progression of BPH and subsequent pathologies associated with outflow tract obstruction and bladder overactivity. Raised cGMP levels induced by activation of sGC activity, with activators such as cinaciguat, or reduction of its breakdown by PDE5 inhibitors, can influence not just smooth muscle contractility in these regions, but differentially reduce release of neurotransmitters associated with the overactive bladder. Moreover, the antifibrotic activity of raised cGMP levels will normalise tissue compliance, to also reduce BOO by an action on the prostate and urethra and facilitate bladder filling. A potential weak point in this system is decreased sGC activity due to oxidative stress, but this can be minimised by using cinaciguat over sildenafil, potentially in combination with PNPase inhibitors that reduce the generation of oxidative stress.

Acknowledgements

References

[1]. Egan KB, The epidemiology of benign prostatic hyperplasia associated with lower urinary tract symptoms: Prevalence and incident rates, Urol. Clin. North. Am 43 (2016) 289–297, 10.1016/j.ucl.2016.04.001. [PubMed: 27476122]

[2]. Devlin CM, Simms MS, Maitland NJ, Benign prostatic hyperplasia - what do we know? BJU Int. 127 (2021) 389–399, 10.1111/bju.15229. [PubMed: 32893964]

[3]. Lloyd GL, Marks JM, Ricke WA, Benign prostatic hyperplasia and lower urinary tract symptoms: What is the role and significance of inflammation? Curr. Urol. Rep 20 (2019) 54, 10.1007/s11934-019-0917-1. [PubMed: 31377881]

[4]. Bosch R, Abrams P, Averbeck MA, Finazzi Agro E, Gammie A, Marcelissen T, Solomon E, Do functional changes occur in the bladder due to bladder outlet obstruction? ICI-RS 2018 Neurourol. Urodyn 38 (Suppl 5) (2019) S56–S65, 10.1002/nau.24076. [PubMed: 31278801]

[5]. Uckert S, Kedia GT, Tsikas D, Simon A, Bannowsky A, Kuczyk MA, Emerging drugs to target lower urinary tract symptomatology (LUTS)/benign prostatic hyperplasia (BPH): focus on the prostate, World J. Urol 38 (2020) 1423–1435, 10.1007/s00345-019-02933-1. [PubMed: 31506747]

[6]. Yu ZJ, Yan HL, Xu FH, Chao HC, Deng LH, Xu XD, Huang JB, Zeng T, Efficacy and side effects of drugs commonly used for the treatment of lower urinary tract symptoms associated with benign prostatic hyperplasia, Front. Pharmacol 11 (658) (2020) 10.3389/fphar.2020.00658.

[7]. Dahm P, Brasure M, MacDonald R, Olson CM, Nelson VA, Fink HA, Rwabasonga B, Risk MC, Wilt TJ, Comparative effectiveness of newer medications for lower urinary tract symptoms

attributed to benign prostatic hyperplasia: A systematic review and meta-analysis, Eur. Urol 71 (2017) 570–581, 10.1016/j.eururo.2016.09.032. [PubMed: 27717522]

[8]. Zabbarova IV, Ikeda Y, Kozlowski MG, Tyagi P, Birder LA, Chakrabarty B, Perera SK, Dhir R, Straub AC, Sandner P, Andersson KE, Drake MJ, Fry CH, Kanai AJ, Benign prostatic hyperplasia/obstruction ameliorated using a soluble guanylate cyclase activator, J. Pathol 256 (2022) 442–454, 10.1002/path.5859. [PubMed: 34936088]

[9]. Gacci M, Andersson KE, Chapple C, Maggi M, Mirone V, Oelke M, Porst H, Roehrborn C, Stief C, Giuliano F, Latest evidence on the use of phosphodiesterase type 5 inhibitors for the treatment of lower urinary tract symptoms secondary to benign prostatic hyperplasia, Eur. Urol 70 (2016) 124–133, 10.1016/j.eururo.2015.12.048. [PubMed: 26806655]

[10]. Sandner P, Stasch JP, Anti-fibrotic effects of soluble guanylate cyclase stimulators and activators: A review of the preclinical evidence, Respir. Med 122 (Suppl 1) (2017) S1–S9, 10.1016/j.rmed.2016.08.022. [PubMed: 28341058]

[11]. Birder LA, Wolf-Johnston A, Wein AJ, Cheng F, Grove-Sullivan M, Kanai AJ, Watson AM, Stoltz D, Watkins SC, Robertson AM, Newman D, Dmochowski RR, Jackson EK, Purine nucleoside phosphorylase inhibition ameliorates age-associated lower urinary tract dysfunctions, JCI Insight 5 (2020) 10.1172/jci.insight.140109.

[12]. Kedia G, Uckert S, Scheller F, Chigogidze T, Managadze L, Jonas U, Truss MC, In vitro functional responses of isolated normal human prostatic tissue to compounds interacting with the cyclic guanosine monophosphate pathway, Urology 67 (2006) 1292–1297, 10.1016/j.urology.2005.12.002. [PubMed: 16678889]

[13]. Puetz S, Lubomirov LT, Pfitzer G, Regulation of smooth muscle contraction by small GTPases, Physiol. (Bethesda) 24 (2009) 342–356, 10.1152/physiol.00023.2009.

[14]. McCarthy CJ, Ikeda Y, Skennerton D, Chakrabarty B, Kanai AJ, Jabr RI, Fry CH, Characterisation of nerve-mediated ATP release from bladder detrusor muscle and its pathological implications, Br. J. Pharmacol 176 (2019) 4720–4730, 10.1111/bph.14840. [PubMed: 31430833]

[15]. Bayliss M, Wu C, Newgreen D, Mundy AR, Fry CH, A quantitative study of atropine-resistant contractile responses in human detrusor smooth muscle, from stable, unstable and obstructed bladders, J. Urol 162 (1999) 1833–1839, https://www.ncbi.nlm.nih.gov/pubmed/10524944. [PubMed: 10524944]

[16]. Chakrabarty B, Ito H, Ximenes M, Nishikawa N, Vahabi B, Kanai AJ, Pickering AE, Drake MJ, Fry CH, Influence of sildenafil on the purinergic components of nerve-mediated and urothelial ATP release from the bladder of normal and spinal cord injured mice, Br. J. Pharmacol 176 (2019) 2227–2237, 10.1111/bph.14669. [PubMed: 30924527]
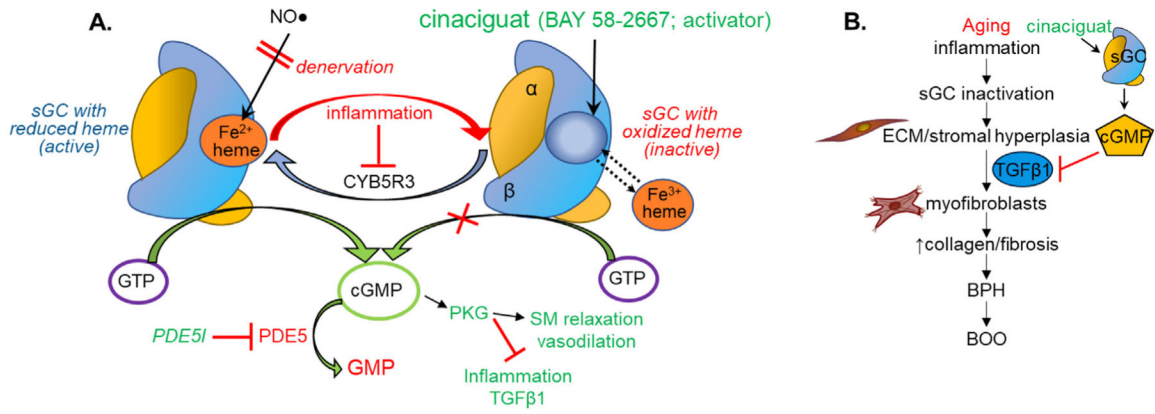
Fig. 1. Regulation of sGC and the Proposed Action of Cinaciguat in Treating Prostatic Hyperplasia and Fibrosis.

(**A**) The reductase CYB5R3 plays a critical role in maintaining the active state of sGC with reduced heme (Fe^{2+}) for NO•-driven cGMP production. However, oxidative stress can downregulate CYB5R3 activity, which results in heme oxidation and insensitivity of sGC to NO•. This can be corrected by sGC activators (*e.g.*, cinaciguat) that do not require NO• or heme for cGMP production as they are heme analogues. PDE5 inhibitors are structural analogs of cGMP and competitive antagonists of PDE5 while cinaciguat is an allosteric activator of sGC. (**B**) Putative mechanism for the development of BPH/BPO through inactivation of sGC (with reduced cGMP formation) and the consequent TGFα1-mediated, myofibroblast-dependent fibrosis and hyperplasia of prostate tissue. Activation of sGC with cinaciguat will restore cGMP levels and attenuate the fibrosis pathway. This figure is published data from [8].
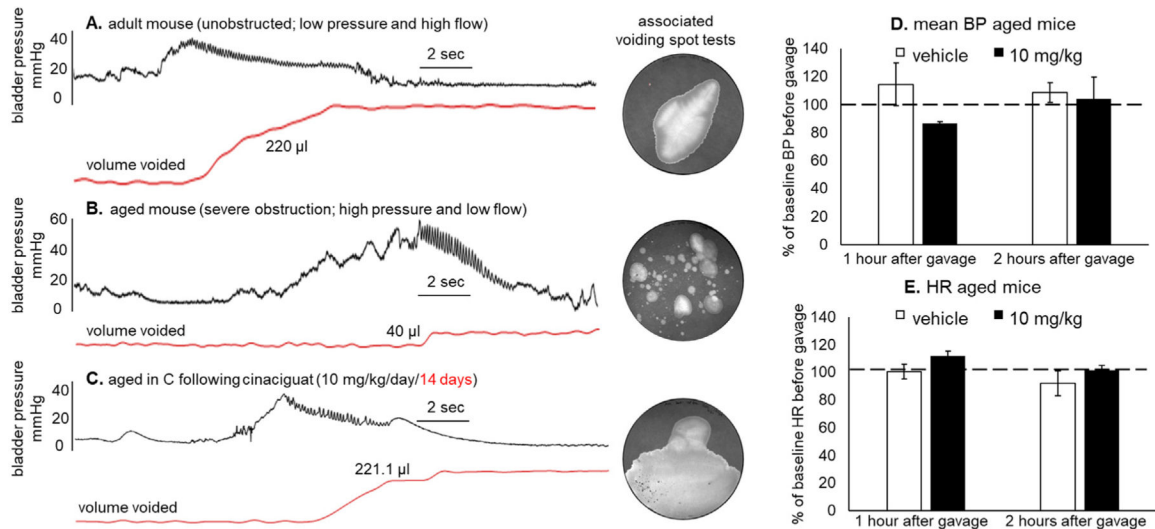
Fig. 2. Cinaciguat Reversed BOO in Aged Mice with only Nominal Effects on Blood Pressure and Heart Rate.

(**A–C**) Telemetric bladder pressure, void (red trace) recordings with associated maximum flow rate at peak pressure, and respective voiding spot tests, from (**A**) unobstructed adult, (**B**) severely obstructed aged animal, (**C**) aged animal in B after 2 weeks of cinaciguat treatment (10 mg/kg/day). Values of voided volume and peak flow rate are shown by each void tracing. (**D**) Mean arterial BP (percentage of baseline) from aged animals gavaged with vehicle or 10 mg/kg cinaciguat, and (**E**) HR measurements from the same animals shown in (D) (Mean ±SD). Data were averaged over at least three separate recordings performed every other day. Measurements in D–E were taken before drug administration, and 1 and 2 h after gavage (aged n=3, adult n=6). This figure is published data from [8].
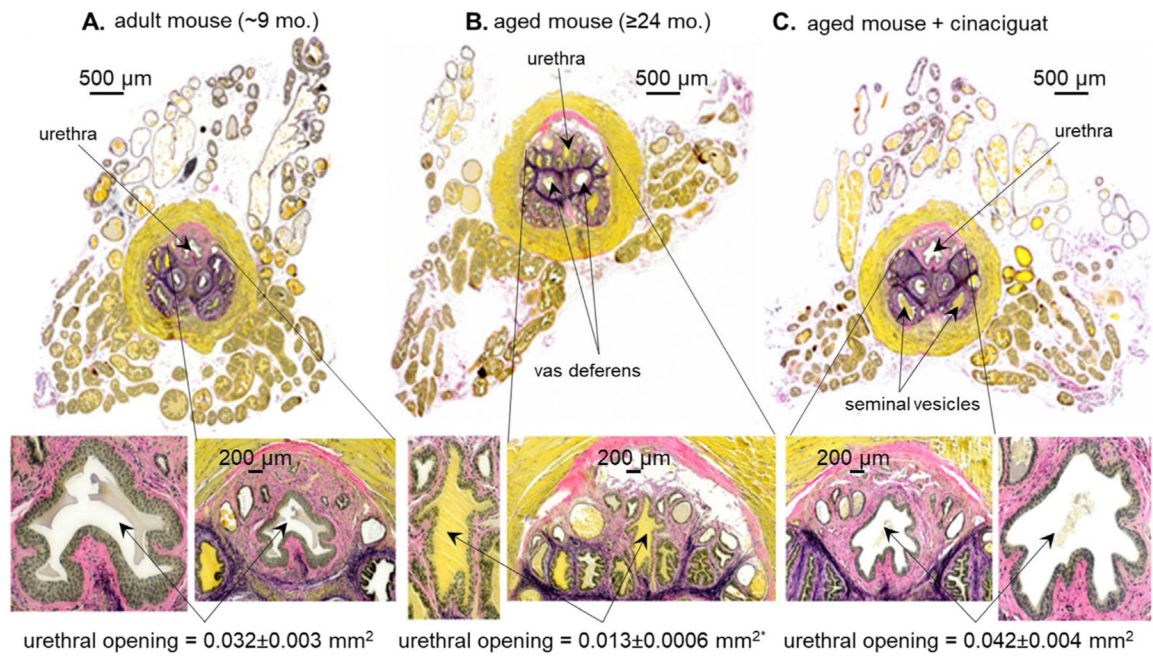
Fig. 3. Bladder Outlet Obstruction in the Aged Mouse and its Reversal Using Cinaciguat.
Verhoeff van Gieson stain to highlight the differences in urethral opening size in: (**A**) Adult (yellow inclusions in the lumen is trapped seminal fluid) versus (**B**) Aged with hyperplasia and (**C**) Aged mouse treated with cinaciguat (10 mg/kg/day/14 days). Bottom panels are expanded views of prostatic urethras. Pink is collagen, yellow is muscle and black is elastin. This figure is published data from [8].
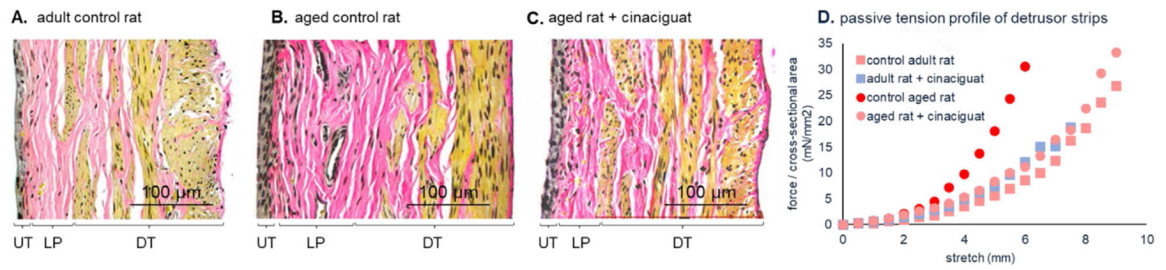
Fig. 4. Cinaciguat Reverses Bladder Fibrosis and Increases Compliance.
Verhoeff van Gieson stain for collagen in bladder wall sections from (**A**) adult, (**B**) aged
and (**C**) aged animals + cinaciguat (10 mg/kg/day/2 wks). (**D**) Passive tension profiles from
adult/aged animals gavaged with cinaciguat or vehicle. This figure is unpublished data.
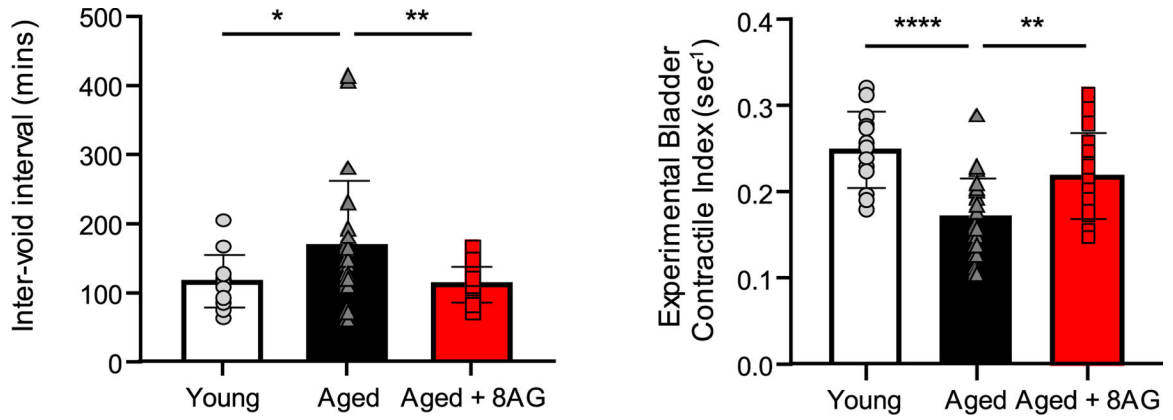
Fig. 5. PNPase Inhibition Restores Bladder Function to a Younger State.
Aging alters bladder storage and voiding function in aged Fischer 344 rats showing increases in the inter-void interval and decreases in the contractile index (*i.e.*, voiding efficiency). 8-aminoguanine (8-AG, 5 mg/kg/day) was administered for 6 weeks in the drinking water resulting in restored bladder function similar to younger animals. This figure is published data from [11].
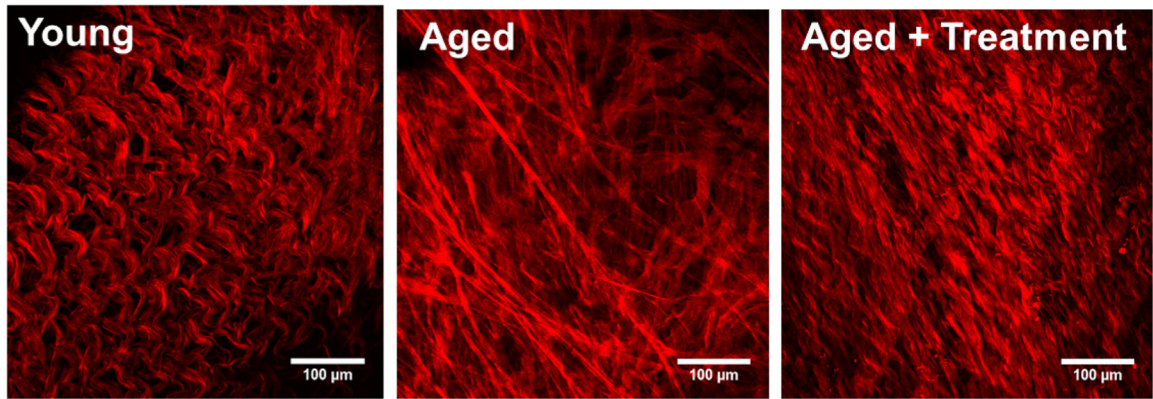
Fig. 6. Aging Alters Bladder Collagen Biomechanics.

Age-related LUTS are prevalent and increased oxidative stress and declining mitochondrial activity are hallmarks of the aging process. These changes can upregulate inflammatory factors that affect bladder structure and function. Increasing uro-protective while simultaneously decreasing uro-damaging metabolites (by inhibiting PNPase) may correct and even reverse age-associated changes in bladder dysfunction. Multiphoton images from young, aged and aged rodent treated with PNPase inhibitor. 8-AG (5 mg/kg/day) was administered for 6 weeks in the drinking water. Treatment of aged rats with PNPase inhibitor reduces 'uro-damaging' levels of hypoxanthine/xanthine. In turn, this improves bladder compliance by increasing collagen fibre tortuosity or 'waviness'. This figure is published data from [11].
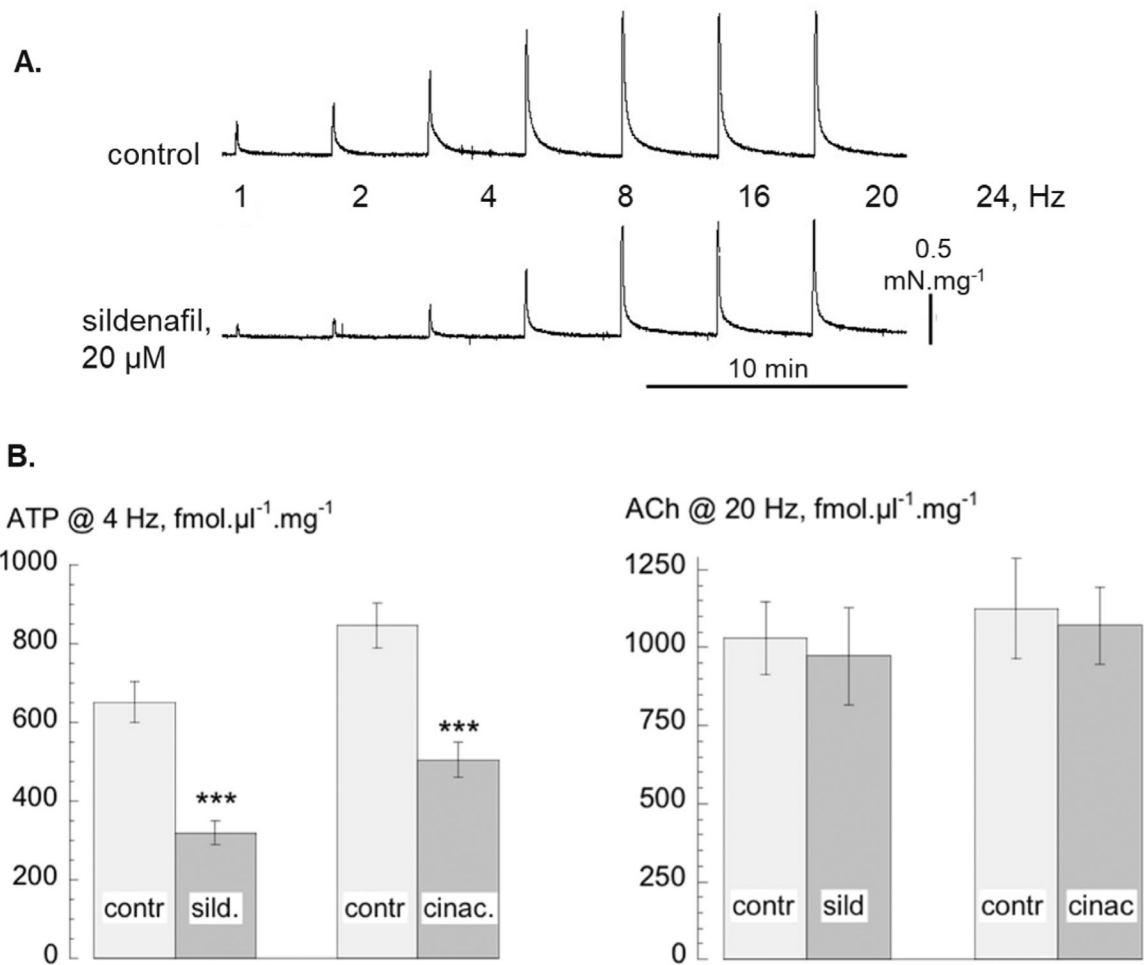
A.

B.

Fig. 7. PDE5 Inhibitor and sGC Activator Decrease Presynaptic Transmitter Release in Isolated Mouse Bladder Strips.
(**A**) tension trace from increasing frequencies of electrical field stimulation of mouse bladder strip under control conditions and in the presence of 20 μM sildenafil in the superfusate.
(**B**) Electrochemical recordings of ATP and acetylcholine (ACh) release from electrically stimulated mouse bladder strips under control conditions and in the presence of sildenafil (20 μM) or cinaciguat (10 μM). This figure is published data from [16].