Abstract # 1583

Is relaxation of human detrusor by sildenafil relying on PDE 5 inhibition ?

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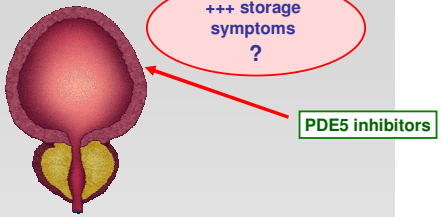
PELVI PHARM

OBJECTIVES

- Clinical studies have reported that phosphodiesterase 5 (PDE5) inhibitors including sildenafil could improve both voiding and storage lower urinary tract symptoms (LUTS) suggestive of benign prostate hyperplasia (BPH) (1-4).
- The mechanism(s) of action responsible for these effects, particularly on storage symptoms, could include a direct relaxing effect of PDE5 inhibitors on the detrusor.

(1) McVary KT, et al J Urol 2007;177:1071-1077; (2) Stief CG, et al Eur Urol 2008;53:1236-1244; (3) McVary KT, et al, J Urol 2007;177:1401-1407; (4) Roehrborn et al., J Urol 2008; Oct;180(4):1228-34.



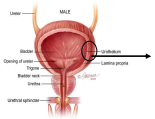
Aim of the study:

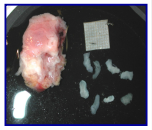
- **Assess the ability of sildenafil to relax human detrusor smooth muscle**
- **Investigate the signaling pathways that could be involved in sildenafil-mediated relaxation**

MATERIALS & METHODS

✓ ***Human detrusor strip preparation***

Human bladder samples were obtained from 20 different patients with no known OAB undergoing cystectomy for bladder cancer. Detrusor strips without urothelium were mounted isometrically at a resting tension of 500 mg in a 5 ml organ bath filled with Krebs-HEPES buffer maintained at 37°C and bubbled with 95%O_2-5%CO_2. The strips were connected to force transducers for isometric tension recordings (Pioden Controls Ltd, UK). Following amplification, the tension changes were computerized via MacLab™/8 using Chart™ 5 software (AD Instruments Ltd).





The experiments, collection and use of any tissue or other samples are carried out in accordance with the Research Plan, all relevant laws, regulations and codes of practice, including having obtaining informed consent of patients in writing.

✓ ***In vitro contractile experiments***

After an equilibration period, concentration-response curves for sildenafil (from 10^{-8}M to 3.10^{-5}M) were generated in the presence or absence of a NO donor, SNAP (S-nitroso-N-acetylpenicillamine 10 µM) and/or selective blockers of the cGMP/ cAMP pathways or K+ channels on carbachol (10^{-6}M)-induced precontration:

ODQ (10 µM) : guanylate cyclase inhibitor
MDL-12,330A (10 µM) : adenylate cyclase inhibitor
Glibenclamide (10 µM) : K_{ATP} channel blocker
Iberiotoxin (30 nM) : BK_{Ca} channel blocker
Apamin (100 nM) : SK_{Ca} channel blocker

In some experiments, strips were pre-contracted KCl (50 mM) and CRCs to sildenafil (10^{-8}M to 3.10^{-5}M) or vehicle were performed.
In another set of experiments, frequency-response curves (FRCs) to electrical field stimulations (EFS) were constructed (2, 4, 8, 16, 32, 48 Hz, 5 ms pulse duration, 10 sec train duration at 300 mA, every 2 minutes). At the completion of the first FRC, bladder strips were washed, and the strips were incubated with sildenafil at 10^{-6} or 10^{-5}M or vehicle. Then, a second FRC was generated with the same EFS parameters than before.

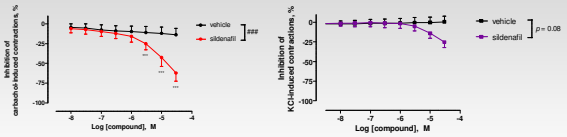
✓ ***Data Analysis***

Results were expressed as a percentage of inhibition of the contractile response to carbachol or KCl. Then, the net relaxing effect of sildenafil was calculated by subtracting the effect of the vehicle. For EFS experiments, results were expressed as a percentage of maximal contraction obtained during the first FRCs. Data were expressed as mean ± SEM for N experiments corresponding to N = bladder samples obtained, from N different patients. Statistical comparisons of the CRC were performed with a two-way ANOVA statistic analysis test and Bonferroni's post-test. In case of interaction between the two factors (concentration and drug) with the two-way ANOVA statistic analysis, a modified Student's t-test with the Bonferroni adjustment for multiple comparisons was performed. P values < 0.05 were considered statistically significant. Statistical analysis was performed with GraphPad Prism® 4.03 software.22

RESULTS

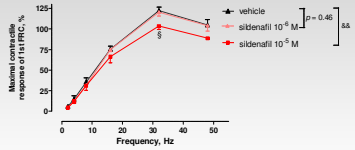
Effect of sildenafil on human detrusor smooth muscle

Effect of sildenafil on carbachol or KCl-induced human detrusor contraction



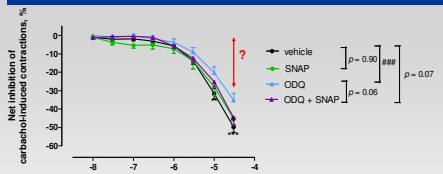
➡ **Sildenafil induced a significant concentration-dependent relaxation of carbachol-induced contraction of human bladder strips when compared to vehicle. In contrast, sildenafil only slightly attenuated KCl-induced detrusor contraction.**

Effect of sildenafil on EFS-induced human detrusor contraction

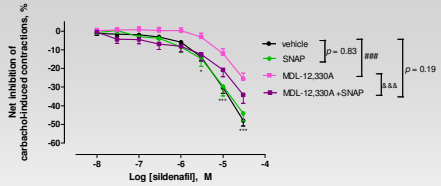


➡ **Sildenafil at 10^{-6}M failed to modify EFS-induced contractions whereas at 10^{-5}M, it significantly inhibited EFS-induced contractions**

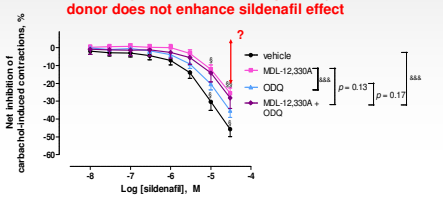
Involvement of the NO/cGMP and the cAMP pathways in sildenafil-induced relaxation



➡ **Relaxation of carbachol-precontracted human bladder by sildenafil involves a cGMP-independent mechanism pathway: Indeed, a NO donor does not enhance sildenafil effect**

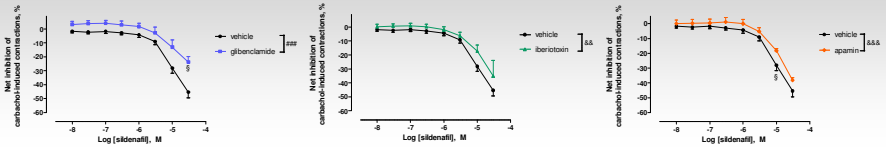


➡ **Sildenafil inhibits carbachol-induced human bladder contractions via a cAMP-dependent mechanism pathway**



➡ **Sildenafil also exerts its inhibitory effect by an other pathway, independent of cGMP or cAMP signaling pathways, since some relaxing activity remains in presence of both MDL 123,330A and ODQ**

Involvement of K+ channels in sildenafil-induced relaxation



➡ **K_{ATP}, BK_{Ca} and SK_{Ca} channels are involved in the relaxation elicited by sildenafil on human detrusor tissue**

CONCLUSIONS

- The present study demonstrates that sildenafil exerts a direct relaxing effect on human detrusor smooth muscle.
- This relaxing effect involves the cAMP-dependent and K+ channels-dependent signaling pathways.
- In contrast, the contribution of the NO-cGMP signaling pathway in sildenafil-induced relaxation appears to be minor.