

# Evaluation of relaxant effects of the combination of sildenafil and doxazosin in human prostate

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## OBJECTIVES

Lower urinary tract symptoms (LUTS) due to benign prostatic hyperplasia (BPH), are often associated to erectile dysfunction (ED) in aging males. Doxazosin, an  $\alpha$ 1-adrenoceptor antagonist, is indicated in BPH /LUTS patients. The phosphodiesterase 5 inhibitor, sildenafil, is indicated on demand for erectile dysfunction (ED) treatment. Recently sildenafil taken daily has been shown to improve LUTS.

The objective of the study was to assess the relaxant effects of sildenafil, doxazosin and a combination of both on human prostatic tissue

In vitro isometric tension studies using human prostatic tissue were performed to:

- evaluate the effect of sildenafil, doxazosin or a combination of both on phenylephrine (Phe)-induced contractions
- evaluate the effect of sildenafil on doxazosin-induced relaxation on electrical field stimulation (EFS)- induced contractions in prostatic tissue

## MATERIALS & METHODS

### Human prostatic strip preparation

Human prostate samples were obtained from 8 patients undergoing cystoprostatectomy for infiltrating bladder cancer. Prostatic strips were suspended in 5 ml organ chambers filled with Krebs-HEPES buffer containing 118 mM NaCl; 4.7 mM KCl; 1.2 mM MgSO<sub>4</sub>; 1.2 mM KH<sub>2</sub>PO<sub>4</sub>; 2.5 mM CaCl<sub>2</sub>; 4.2 mM NaHCO<sub>3</sub>; 11.1 mM glucose, and 20.8 mM HEPES. Indomethacin (10<sup>-5</sup> M) and dexamethasone (10<sup>-5</sup> M) were also added to the organ bath throughout the experiments to eliminate possible interferences of cyclooxygenase products or induction of NO-synthase. Organ chambers were maintained at 37 °C and continuously bubbled with 95% O<sub>2</sub> and 5% CO<sub>2</sub> to maintain a pH at 7.4.

### In vitro contractile experiments

The tissue preparation were allowed to equilibrate for 60 minutes, while being washed periodically with fresh Krebs-HEPES buffer. Following the equilibration period, the prostatic tissues were primed by the addition to the organ bath of KCl (90 mM, 10 min), washed, and then primed by the addition of Phe at 10<sup>-6</sup> M during 5 min. After the priming period, the strips were washed by fresh Krebs-HEPES solution and allowed to re-equilibrate for 20 minutes.

In a first set of experiments, concentration-response curves (CRC) to phenylephrine (Phe) were performed. Then following a 20-min incubation period with either vehicle, sildenafil (10<sup>-6</sup> or 10<sup>-5</sup> M), doxazosin (10<sup>-6</sup> or 3.10<sup>-6</sup> M) or a combination of sildenafil and doxazosin (10<sup>-6</sup> +10<sup>-6</sup> M or 10<sup>-5</sup>+3.10<sup>-6</sup> M for sildenafil and doxazosin respectively), CRC to Phe was repeated.

In a second set of experiments, prostatic strips were submitted to continuous EFS trains (32 Hz, 5ms, 5s, 300 mA). When a stable response was obtained, strips were incubated with sildenafil 10<sup>-6</sup> M or vehicle and then 2 doses of doxazosin (10<sup>-7</sup> and 10<sup>-6</sup> M) or vehicle were successively added with a 18 min interval between each.

### Data Analysis

For the first set of experiments, results of the 2nd CRC to Phe were expressed in percentage of the maximal value obtained during the first CRC. For each CRC in presence of the tested vehicle or compound(s), a pD<sub>2</sub> value (-log concentration of compound that produces 50 % reduction of the maximal response) and a mean maximal effect (Emax) were evaluated in each experimental condition.

For the second set of experiments, values were expressed in percentage of the maximal contractile response measured during the 5-min period before addition of the first concentration of compound or vehicle. Data were expressed as mean ± SEM for N experiments corresponding to N prostatic samples. Statistical analysis was performed with GraphPad Prism® 4.03 software.

## CONCLUSIONS

Sildenafil and doxazosin have additive relaxant effects on phenylephrine or EFS-induced contractions of isolated human prostatic smooth muscle

These experiments support the need for further clinical research investigation of this combination in BPH patients with ED

## RESULTS

### Effect of sildenafil, doxazosin or a combination of both on phenylephrine-induced contractions on human prostatic strips

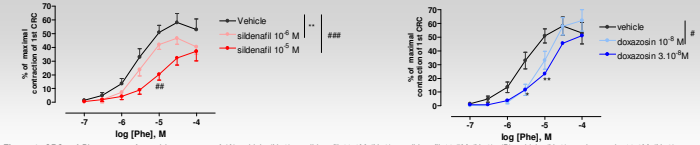
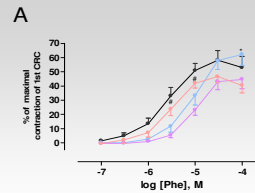


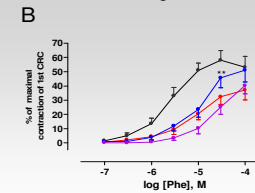
Figure 1: CRCs of Phe were performed in presence of (A) vehicle (N=8) or sildenafil 10<sup>-6</sup> M (N=8) or sildenafil 10<sup>-5</sup> M (N=8); (B) vehicle (N=8) or doxazosin 10<sup>-6</sup> M (N=8) or doxazosin 3.10<sup>-6</sup> M (N=7) on human prostatic strips. \*p<0.01, \*\*p<0.001, two-way ANOVA followed by Bonferroni post-test; #p<0.05, ##p<0.01, ###p<0.001, two-way ANOVA with interaction followed by a modified Student t test with the Bonferroni adjustment for multiple comparisons: ##p<0.01.

Sildenafil dose-dependently inhibited contractions induced by Phe  
As expected, doxazosin dose-dependently inhibited contractions induced by Phe

### Effect of low doses combination



### Effect of high doses combination



### Effect of low and high doses combination

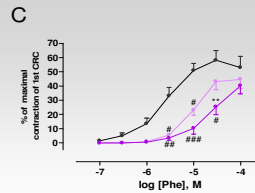


Figure 2: A. CRCs of Phe were performed in presence of vehicle (N=8) or doxazosin 10<sup>-6</sup> M (N=8) or sildenafil 10<sup>-6</sup> M (N=8) or doxazosin 3.10<sup>-6</sup> M (N=7) on human prostatic strips. B. CRCs of Phe were performed in presence of vehicle or doxazosin 3.10<sup>-6</sup> M (N=7) or sildenafil 10<sup>-6</sup> M (N=8) or doxazosin 3.10<sup>-6</sup> M + sildenafil 10<sup>-6</sup> M (N=7) on human prostatic strips. C. Effect of low and high doses combination. \*p<0.01, \*\*p<0.001, two-way ANOVA followed by Bonferroni post-test; #p<0.05, ##p<0.01, ###p<0.001, two-way ANOVA with interaction followed by a modified Student t test with the Bonferroni adjustment for multiple comparisons: ##p<0.01, ###p<0.001.

The combination of sildenafil and doxazosin exerted a dose-dependent greater inhibitory effect on phenylephrine-induced contractions of human prostatic strips than each compound alone

	pD <sub>2</sub>	Emax (%)	N
vehicle	5.64±0.17	58.0±6.7	9
Effect of low doses combination**			
sildenafil 10 <sup>-6</sup> M	5.65±0.16 <sup>††</sup>	46.6±4.3	8
doxazosin 10 <sup>-6</sup> M	4.98±0.11	62.2 ±7.7	8
sildenafil 10 <sup>-6</sup> M + doxazosin 10 <sup>-6</sup> M	4.97±0.09 <sup>††</sup>	44.6±6.6	8
Effect of high doses combination**			
sildenafil 10 <sup>-6</sup> M	5.06±0.18 <sup>††</sup>	37.1±6.9	8
doxazosin 3.10 <sup>-6</sup> M	4.90±0.14 <sup>††</sup>	51.1±8.4	7
sildenafil 10 <sup>-6</sup> M + doxazosin 3.10 <sup>-6</sup> M	4.30±0.12 <sup>†††</sup>	40.3±5.8	7

\*p<0.01, one-way ANOVA followed by Newman-Keuls posttest, †p<0.05, ††p<0.01 versus corresponding combination, §§p<0.01, §§§p<0.001 versus vehicle.

### Effect of sildenafil on doxazosin-induced relaxation on prostatic contractions elicited by EFS

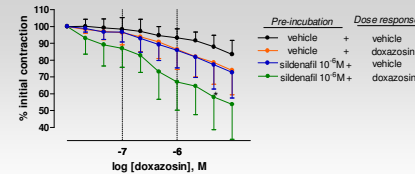


Figure 3: The data are mean ± SEM of N=5 (vehicle-vehicle) or 6 (vehicle+doxazosin; sildenafil 10<sup>-6</sup>M+vehicle; sildenafil 10<sup>-6</sup>M+doxazosin) experiments. \*\*\*p<0.001; \*\*p<0.01, two-way ANOVA followed by Bonferroni post-test; †p<0.05, ##p<0.001, two-way ANOVA with interaction.

Preincubation with sildenafil enhanced the relaxant effect induced by doxazosin on EFS-induced contractions of prostatic strips