European Journal of Pharmacology ∎ (∎∎∎∎) ∎∎∎–∎∎∎

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Pulmonary, gastrointestinal and urogenital pharmacology

Silodosin and tadalafil have synergistic inhibitory effects on nerve-mediated contractions of human and rat isolated prostates

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ARTICLE INFO

Article history:
Received 12 May 2014
Received in revised form
9 September 2014
Accepted 14 September 2014

Keywords:
Alfa-1A-adrenoceptor
Phosphodiesterase 5
Inhibition
Contraction
Prostate
Smooth muscle

ABSTRACT

Lower urinary tract symptoms (LUTS) in men with benign prostatic hyperplasia (BPH) are associated with erectile dysfunction. Alpha-1-adrenoceptor antagonists are effective drugs for treating symptomatic BPH. Clinical data show improvements in LUTS by phosphodiesterase 5 inhibitors. This study aimed to evaluate effects of silodosin, a highly selective α_{1A}-adrenoceptor antagonist, alone or in combination with the phosphodiesterase 5 inhibitor tadalafil on contractions of isolated human and rat prostates. In organbath studies, effects of increasing concentrations of silodosin (1 nM–1 μM) and tadalafil (100 nM–100 μM) on contractions by electrical field stimulation or phenylephrine of human and rat prostate strip preparations were investigated. The combination silodosin and tadalafil reduced electrically-induced contractions of human prostate preparations better than single drugs alone. At any frequencies (1–32 Hz), inhibitory effects of combined therapy (*P*-values vs single drug) in human tissue were 26–42% (1 nM silodosin + 100 nM tadalafil; $P<0.05$), 40–58% (10 nM silodosin + 1 μM tadalafil; $P<0.001$–0.05), 56–67% (100 nM silodosin + 10 μM tadalafil; $P<0.01$–0.05), and 33–55% (1 μM silodosin + 100 μM tadalafil $P<0.01$–0.05). Similar findings were obtained in rat prostate preparations. In human and rat prostate tissue, the drug combination exerted similar inhibitory effect on phenylephrine contractions as silodosin alone. Silodosin plus tadalafil had greater potency than each drug alone to inhibit prostate contractions to electrical field stimulation but not to phenylephrine. This study supports the clinical application of a combination of an α_{1A}-adrenoceptor antagonist and a phosphodiesterase 5 inhibitor for symptomatic BPH and suggests that the drug combination requires endogenous nerve-activity for optimal effect.

1. Introduction

The importance of the α_1-adrenoceptor for regulation of the tonus of the prostate is utilized in α_1-adrenoceptor antagonists that have a central position in the pharmacological management of benign prostatic hyperplasia (BPH)-related lower urinary tract symptoms (LUTS) (Andersson and Gratzke, 2007; McVary et al., 2011). Several studies have demonstrated that the α_{1A}-adrenoceptor subtype is predominantly expressed in human normal and hyperplastic prostate tissue (Andersson and Gratzke, 2007). Silodosin is the only clinically available α_1-adrenoceptor antagonist that exhibits relevant selectivity vs the α_{1A}-adrenoceptor over α_{1B}- (593-fold) and α_{1D}-adrenoceptors (57-fold) (Russo et al., 2011). Compared to placebo, silodosin improved voiding and storage symptoms and maximum urinary flow rate, and exhibited greater improvement in voiding symptoms than tamsulosin, that exhibits some selectivity for the α_{1A}- and α_{1D}-adrenoceptors vs α_{1B}-adrenoceptors (Andersson and Gratzke, 2007; Wu et al., 2013). Other α_1-adrenoceptor antagonists that are used in male LUTS, e.g. terazosin, doxazosin, and alfuzosin, are non-subtype α_1-adrenoceptor-selective (Andersson and Gratzke, 2007).

Altered activity of the nitric oxide (NO)/cyclic guanosine monophosphate (cGMP) pathway of the prostate and outflow region of the bladder has been suggested related to LUTS with or without BPH (Andersson et al., 2011; Hedlund, 2005). Prostatic

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http://dx.doi.org/10.1016/j.ejphar.2014.09.030
0014-2999/

tissue from men with BPH or from spontaneously hypertensive rats that develop BPH-like changes express less neuronal NO-synthase that may be linked to increased prostate tonus (Andersson et al., 2011; Bloch et al., 1997; Yono et al., 2007). The human or rat prostate stroma also expresses the cGMP-dependent protein kinase and the cGMP-degrading phosphodiesterase 5 that are downstream regulators for NO/cGMP activities (Uckert et al., 2001; Waldkirch et al., 2007; Morelli et al., 2011). Suggesting tonus-regulatory functions for NO/cGMP-mediated signals of the prostate, NO-donors and phosphodiesterase 5 inhibitors relax isolated human prostate with concomitant increase in cGMP tissue levels (Uckert et al., 2001; Kedia et al., 2006). Several clinical trials have studied effects of phosphodiesterase 5 inhibitors on LUTS in BPH (Gacci et al., 2012). Recently, the phosphodiesterase 5 inhibitor tadalafil was approved for treatment of LUTS in men with BPH and erectile dysfunction, and has been shown to have significant effect on symptoms vs placebo, and similar efficacy on symptoms and urinary flow rate as tamsulosin (Broderick et al., 2010; Porst et al., 2011; Oelke et al., 2012). Even so, the effect by phosphodiesterase 5 inhibitors on flow rate, as a surrogate parameter for pharmacological targeting of motor functions of the outflow region, has been debated (Gacci et al., 2012; Andersson et al., 2011). It has been reported that a combination of a phosphodiesterase 5 inhibitor and a non-selective α_1-adrenoceptor antagonist can have additive effects on prostate contractions and perhaps on flow rate (Oger et al., 2009, 2010; Angulo et al., 2012; Gacci et al., 2012). No information is available on effects by combining the α_{1A}-adrenoceptor selective antagonists silodosin and a phosphodiesterase 5 inhibitor. The aim of the current study was to assess if silodosin and tadalafil had additional effects on contractile functions of isolated rat and human prostate compared to either compound alone.

2. Material and methods

2.1. Tissues and ethical approval

In accordance with the regulations of the local Institutional Ethical Committee and after patient-informed consent, prostatic tissue was obtained from 40 male patients (age: 67 ± 2 years) who underwent radical prostatectomy or radical cystectomy due to prostate or bladder cancer. A dedicated uro-pathologist examined all specimens and excised a cone-shaped piece of normal non-malignant tissue from the peripheral region to the transitional and periurethral region. Tissue specimens were immediately placed in an ice-cold organ protective Krebs solution (for composition see below) and transported to the laboratory for additional processing. Only tissues free from malignant changes were included. All specimens were functionally evaluated within 1 h.

Prostate tissues were also obtained from 30 male Sprague-Dawley rats (225–250 g) (Charles River). Animals were housed in a pathogen-free facility (12:12 h light:darkness cycle with free access to food and water) at our Institution and in accordance with the European Community guidelines, and with the approval of the Institutional Ethical Committee.

2.2. Functional studies

Preparations ($3 \times 3 \times 6$ mm^3) were dissected from the prostate. Silk ligatures were applied at both ends of the preparations that were mounted in 5 ml aerated (95% O_2 and 5% CO_2) tissue baths (37 °C, pH 7.4), containing Krebs solution that was routinely replaced with fresh Krebs solutions every 30 min. Isometric tension was registered with a Grass Polygraph model 7E (Grass Technologies, West Warwick, RI, USA). Human prostate preparations were stretched to a tension of approximately 5 mN and left to equilibrate for 60 min to attain a mean stable tension of 2.6 ± 0.1 mN ($n=40$). Rat prostate preparations were stretched to a tension approximately 2 mN and left to equilibrate for 45 min to obtain a mean stable tensions of 1.3 ± 0.1 mN ($n=30$).

The viability of the preparations was verified by addition of a 60 mM K^+ solution to the organ baths that resulted in contractile responses amounting to 0.65 ± 0.08 mN ($n=40$) for human preparations, and 0.43 ± 0.03 mN ($n=30$) for rat preparations. No differences were obtained for different treatment groups (not shown).

Transmural activation of nerves was performed by electrical field stimulation with two platinum electrodes, placed in parallel to the strips in the organ baths as previously described (Hedlund et al., 1997). After voltage-titration at 20 Hz, single square-wave pulses at supramaximum voltage (40 V) and with a duration of 0.5 ms were delivered by a Grass S48 stimulator. The train duration was 5 s and the train interval 120 s. Contractile responses were determined at various frequencies (1–32 Hz) and effects by silodosin (1 nM–1 μM), tadalafil (100 nM–100 μM), or silodosin (1 nM–1 μM)+tadalafil (100 nM–100 μM) were studied.

Concentration–response curves to phenylephrine (10 nM–100 μM) at baseline were also constructed before and after addition of silodosin (1 nM–1 μM), tadalafil (100 nM–100 μM), or silodosin+tadalafil.

Drugs were added in separate concentrations and allowed to equilibrate for 30 min before the next activation by electrical field stimulation or by the next concentration–response curve to phenylephrine was performed. Hereafter, the preparations were washed with fresh Krebs solution and the next higher concentrations of the investigated drugs were added. Separate control experiments with the different vehicles were performed.

2.3. Drugs and solutions

The composition of Krebs solution was the following (mM): NaCl 119, KCl 4.6, $CaCl_2$ 1.5, $MgCl_2$ 1.2, $NaHCO_3$ 15, NaH_2PO_4 1.2, glucose 5.5. A K^+ solution (60 mM) was used, in which the NaCl in the normal Krebs solution was replaces by KCl.

The following drugs were used: phenylephrine (Sigma Aldrich, Milan, Italy), silodosin and tadalafil (Recordati, Milan, Italy). Phenylephrine was dissolved in water, silodosin in ethanol (Merck Chemicals, Darmstadt, Germany), and tadalafil in dimetylsulfoxid (Sigma Aldrich).

2.4. Calculations

Values are given as mean plus standard error of the mean. The $-\log IC_{30}$ values denote the concentration of drugs that produced 30% inhibition of electrically-induced contractions. Synergy analysis of the drug combination concentration–response effect was evaluated at 4 and 16 Hz for the two drugs as single treatments and in combination. The data were used to calculate the IC_{30} values as well as the combination index (CI) according the Chou–Talalay equation (Chou and Talalay, 1984). CI values = 1, > 1, < 1 indicate additive, antagonistic, and synergistic interactions, respectively. The $-\log EC_{30}$ values denote the concentration of phenylephrine that caused 30% of maximum contraction. The $-\log IC_{30}$ and the $-\log EC_{30}$ were determined by linear interpolation. A one-way ANOVA (followed by Newman–Keuls posthoc test) was used for statistical comparison of efficacy between different drugs. A one-way ANOVA (followed by Newman–Keuls posthoc test) was used for comparison of $-\log EC_{30}$ before and after drug exposure. An unpaired *t*-test was used for comparisons of $-\log IC_{30}$ or $-\log EC_{30}$ values for single drug exposure to the corresponding values for the same compound in combination with the other test compounds. A

Please cite this article as: Buono, R., et al., Silodosin and tadalafil have synergistic inhibitory effects on nerve-mediated contractions of human and rat isolated prostates. Eur J Pharmacol (2014), http://dx.doi.org/10.1016/j.ejphar.2014.09.030