Abstract: The present invention relates to combinations of phosphodiesterases (PDEs) and muscarinic receptors or beta adrenergic receptors and the pharmacology of PDE inhibitors and muscarinic receptor antagonists or beta adrenergic receptors.

Title: COMBINATION OF PDE5 INHIBITORS WITH MUSCARINIC RECEPTOR ANTAGONISTS
**Combination of PDE5 inhibitors with Muscarinic Receptor antagonists**

**Technical field of the invention**

The present invention relates to combinations of phosphodiesterases (PDEs) and muscarinic receptors and the pharmacology of PDE inhibitors and muscarinic receptor antagonists. More particularly, the invention is related to combinations of PDE5 inhibitors and muscarinic receptor antagonists.

The present invention relates further to combinations of phosphodiesterases (PDEs) and beta adrenergic receptors and the pharmacology of the combinations of PDE inhibitors and beta adrenergic receptor agonists. More particularly, the invention is related to combinations of PDE5 inhibitors and beta-3 adrenergic receptor agonists.

**Background of the invention**

Ovaractive Bladder (OAB) is a term, defined by the International Continence Society (Abrams 2002), and is a symptomatic diagnosis. OAB is usually defined as urgency with or without incontinence, frequency and nocturia. Urgency is often described as sudden and compelling desire to pass urine which could be accompanied by the involuntary leakage of urine (UUI). In addition patients display higher frequency with more then 8 voidings during day-time and nocturia, with more then 1 voiding during sleeping-time. Urge Urinary Incontinence (UUI) is the most bothersome symptom of OAB and is associated with a significantly reduced quality of life of the patients (Jackson 1997).

The overall prevalence in OAB in Western Europe is 16 and 17% in men and women, respectively, with similar finding in the US and an increase of the incidence with age (Milsom 2001, Stewart 2003). The pathophysiology of this disease is not fully understood and seemed to be multi-factorial. The voiding process is tightly regulated by neural circuits in the brain and the spinal cord, coordinating the activity of the smooth muscle in bladder and urethra. Thus, various neurological disorders and aging unbalance this well regulated interplay resulting in detrusor overactivity (DO) (Andersson 2004). Moreover urge incontinence may be due to intrinsic detrusor myogenic abnormalities like spontaneous phasic activity and detrusor instability (Kumar 2005).

Besides local application of Botulinustoxin (BTX) or Resiniferatoxin (RTX) by direct injection in the bladder or instillation (Sahai 2006; Patterson 2006), the main treatment option is based on muscarinergic-receptor antagonists (antimuscarinics). Antimuscarinics prevent acetylcholine-induced contraction of the detrusor muscle during filling phase mainly by blocking postsynaptic M3 receptors on the bladder smooth muscle (Wang 1995, Andersson 2004). However efficacy of
these drugs is limited, they show a slow onset of action and cause adverse effects such as dry mouth or constipation (Herbison 2003, Chappie 2005). Due to this reasons the compliance of these drugs is low and there are intense efforts to discover and develop new treatment options of OAB and UUI. A broad variety of drugs acting on different targets, i.e. Purinergic Receptors, NK-3 receptors, beta-3 receptors, TRP-channels, K+ channels, GABA-b receptors, Serotonin receptors, were tested which are currently under preclinical and early clinical evaluation within this indication (Yoshimura 2002). The hints that Phosphodiesterase (PDEs), especially PDE-5, could be used for the treatment of OAB and UUI are rare and only indirect. It has been shown that PDE-5 inhibitors reduced the tonus of the bladder muscle (Ueckert 2001, Tinel 2006) and reduced the Lower Urinary Tract Symptoms (LUTS) in rats in vivo (Tinel 2006). This was paralleled by findings in BPH patients which reported significant reduction of LUTS after treatment with Sildenafil (McVary 2006; Kaplan 2006; Mulhall 2006) and Tadalafil (Roehrborn 2006) and Vardenafil (Neuser, personal communication). Since LUTS comprise urgency, frequency and nocturia LUTS in BPH patients is similar to UUI in OAB patients (Chappie 2006). Both, LUTS and UUI symptoms are originated within the bladder and characterized by Detrusor Overactivity (Chappie 2006). This could explain why the M-3 antagonist tolterodine which is normally used in OAB patients, if used in BPH patients with LUTS, significantly improved the symptoms (Lee 2004, Kaplan 2006). Thus, one might assume if PDE-5 inhibition which reduced LUTS in BPH patients might be able to reduce UUI symptoms in OAB patients. This is further supported since both, PDE-5 inhibitors and antimuscarinics target the bladder muscle, reducing the bladder tone (Ueckert 2001, Tinel 2006) and therefore - at least in part - could decrease detrusor overactivity. In addition, dilatation of the bladder by cGMP) allows prolonged and smoother filling of the bladder, which might increase bladder capacity and result in reduction of the micturition frequency after PDE-5 inhibition. Our experiments now showed that combination of the cGMP elevation with PDE5 in combination with the well established standard therapy with Antimuscarinics resulted in unexpected and significant higher efficacy of the combination of PDE5 inhibitors and Antimuscarinics, when compared to each single treatment.

Our experiments using distinct preclinical in vitro and in vivo model now showed that PDE5 inhibition in combination with Muscarinic Receptor antagonists is a very effective treatment for detrusor overactivity, overactive bladder (OAB), different forms of incontinence i.e. urge urinary incontinence (UUI); and also of Lower Urinary Tract Symptoms (LUTS) and symptoms associated with Benign Prostate Hyperplasia (BPH). Combinations of PDE5 inhibition with beta3 activation resulted in increased efficacy in vitro and in vivo. In addition cyclic AMP (cAMP) plays an important role in regulating smooth muscle tone. cAMP production in the bladder can be stimulated by agonists of the GS-coupled beta3 adrenergic receptor, which is the predominant beta adrenergic receptor on the bladder smooth muscle cells.
It has now been found, that the elevation of the cGMP level via PDE5 inhibitor-treatment and the blocking of the Muscarinic Receptor results in unexpected and significant higher efficacy, compared to each single treatment, if combinations of PDE5 inhibitors and beta3 agonists are used in the treatment and that the elevation of the cGMP level and cAMP level results in unexpected and significant higher efficacy, compared to each single treatment, if combinations of PDE5 inhibitors and beta3 agonists are used in the treatment.

It has further been found that combinations of PDE5 inhibitors with beta3 agonists are very effective for the treatment of detrusor overactivity, overactive bladder (OAB), different forms of incontinence i.e. urge urinary incontinence (UUI); and also of Lower Urinary Tract Symptoms (LUTS) and symptoms associated with Benign Prostate Hyperplasia (BPH). Those combinations of PDE5 inhibitors with beta3 agonists resulted in over-additively increased efficacy in vitro and in vivo tests. Therefore this combination will extend the treatment options of the aforementioned diseases significantly. Thus the mRNA expression of PDE-5 and beta-3 in the bladder and in the lower urinary tract is nearly semi-quantitated using the combinations according to the invention.

PDE5 according to the invention are for example inhibitors selected from the group of PDE5 inhibitors consisting of, Sildenafil, Tadalafil, Udenafil, Dasantafil, Avanafil, SLx2101 and LAS34179 whereas Vardenafil is the preferred PDE5 inhibitor.

Muscarinic Receptor antagonists according to the invention are for example inhibitors selected from the group of Muscarinic Receptor Antagonists consisting of, Oxybutinine, Trospium, Tolerodin, Solifenacin, Fesoterodin and Darifenacin.

Beta 3 receptor agonists according to the invention are for example agonists selected from the group consisting of BRL-37344, CI-316234, YM-178, Solabegron, and Ritobegron whereas 2-((2,3-Dihydroxypropyl)Aminomethyl)chroman derivates are preferred beta 3 receptor agonists.

These PDE5 inhibitors, muscarinic receptor antagonists and beta 3 receptor agonists are known in the art.

The PDE5 inhibitors and muscarinic receptor antagonists can be combined in the dose range of 1 to 20mg once daily of the PDE5 inhibitor and 1 to 15mg once daily of the muscarinic receptor antagonist.

The PDE5 inhibitors and beta 3 receptor agonists can be combined in the dose range of 1 to 20mg once daily of the PDE5 inhibitor and 0.1 to 10mg once daily of the beta3 agonist.
The combinations according to the invention can be used for the preparation of medicaments for the treatment of a disease comprised in a group of diseases consisting of Benign Prostate Hyperplasia (BPH), Bladder Outlet Obstruction (BOO), Lower Urinary Tract Symptoms (LUTS), genitourinary disorders comprising neurogenic bladder syndrome (overactive bladder - OAB, Interstitial Cystitis - IC), urinary incontinence (UI) like mixed-, urge, stress- or overflow incontinence (MUI; UUI, SUI, OUI), pelvic pain.

The combinations according to the invention can further be used for the preparation of medicaments for the treatment of other urological disorders and in particular and with substantial advantage in the treatment of benign and malign disorders of the organs consisting the genitourinary system of female and male, renal diseases like acute or chronic renal failure, immunologically mediated renal disease like renal transplant rejection, lupus nephritis, immune complex renal disease, glomerulaopathies, nephritis, toxic nephropathy, obstructive uropathies and erectile dysfunction.
Examples

Example 1

Tissue preparation: Wistar Rats (200-250g) are euthanized due to the German Law for the protection of laboratory animals. Bladder tissue is removed and placed in ice-cold Krebs-Henseleit buffer of following composition (in mmol/l): NaCl 112, KCl 5.9, CaCl₂ 2.0 MgCl₂ 1.2, NaH₂PO₄ 1.2, NaHCO₃ 25, glucose 11.5. Four equally sized longitudinal strips of approximately 2 mm x 10 mm are cut from the bladder body.

Recording of mechanical activity in the bladder strip assay: The preparations are transferred to 20 ml organ baths containing Krebs-Henseleit solution equilibrated with 95% O₂, 5% CO₂ at 37°C. The strips are mounted between two hooks by means of two clips. For recording of isometric tension one of the hooks is connected to a force transducer which is in turn linked to an amplifier and chart recorder. The other hook is attached to a movable unit, permitting precise adjustment of preload tension. All tissues are then given a 60 min equilibration period during which they are washed and the resting tension is adjusted to 1 g every 20 min. After the equilibration period, each experiment is started by exposing the preparation to K⁺ (50 mmol/l) Krebs-Henseleit solution. The procedure is repeated 3 times and the tissues are washed at least three times between each contraction. The bladder strips are then pre-contracted using K⁺ (50 mmol/l) Krebs-Henseleit solution. When the contraction is stabilized, an accumulative dose response curve of the compound tested is constructed. The stabilized contraction induced by K⁺ (50 mmol/l) Krebs-Henseleit solution is defined as 100% tension. The relaxation is expressed as percentage tension.

Results: The effects of a PDE5 inhibitor, i.e. Vardenafil, a muscarinic receptor antagonist, i.e. Tolterodine are tested on isolated and pre-contracted rat bladder strips as described above. Vardenafil at 1µM and the muscarinic receptor antagonist at 1µM relaxed the rat bladder by 82±5% and by 61±3 % respectively. In contrast the combination of Vardenafil at 1µM and the muscarinic receptor antagonist at 1µM led to a significant and enhanced relaxation by 98±3% of the rat bladder strip (Figure 1) completely reversing the pre-contraction of the bladder strip. These results show that combinations of PDE5 inhibitors, i.e. Vardenafil and muscarinic receptor antagonists i.e. Tolterodine acted on the bladder and are superior to mono-therapy.
Example 2

**Tissue sampling and RNA preparation:** Male (m) and Female (fin) Sprague Dawley rats with a body weight between 200-250 g are used for tissue collecting. The rats are briefly anaesthetized with a mixture of 5% Isofluurane (Baxter S.A.) in a carrier with 70% N₂O and 30% O₂, and than euthanized by decapitation. The abdomen is opened by a midline incision and the bladder is quickly removed. The bladder is dissected in the bladder neck, referred as bladder Trigone (T) and bladder detrusor muscle (DM). The tissues are frozen in liquid N₂ and stored until RNA preparation. Total RNA is isolated using RNeasy mini columns (QIagen Inc.) and further purified by DNase digestion.

PDE5 and beta3 adrenergic Receptor (beta3 AR) mRNA quantification: The mRNA expression of the PDE5 and beta3 AR in the male and female rat bladder trigone (termed m-T and fm-T) and rat bladder detrusor muscle (termed m-DM and fin-DM) is measured by real time quantitative PCR (TaqMan-PCR, Heid 1996). Therefore 1 μg of total RNA are transcribed into cDNA with Superscript II RT cDNA synthesis kit (Gibco, Inc) according to the manual of the supplier. The mRNA for the PDEs is measured by real-time quantitative RT-PCR on an ABI Prism 7700 sequence detection instrument (Applied Biosystems, Inc.). Specific Sequences for forward and reverse primers as for the fluorogenic probe of PDE5 and beta3 AR mRNA are designed by Primer Express 1.5 Software (Applied Biosystems, Inc.). During PCR amplification, 5’ nucleolytic activity of Taq polymerase cleaves the probe separating the 5’ reporter fluorescent dye from the 3’ quencher dye. The threshold cycle, Ct, which correlates inversely with the target mRNA level, is measured as the cycle number at which the reporter fluorescent emission increases 10 standard deviations above background level. As housekeeping gene, beta-action is quantified as described above, using as forward primer 5’-accttcaacacccagcca-3’, reverse primer 5’-cagtggtacgaccagggca-3’ and fluorescent probe 5’-6AFM-acgtgtagccaggtcgttggcc-TAMARA-3’. The mRNA levels are corrected for beta-actin mRNA levels and calculated as relative expression using comparative Ct-method.

**Results:** The results showed that there is substantial PDE-5 mRNA (Figure 2) and beta3 AR mRNA (Figure 3) expression in the bladder, hi addition no gender differences between male and female in either PDE5 or beta 3AR mRNA can be detected. Finally the detrusor muscle and the trigone mRNA expression of PDE5 and beta3 AR is not significant different in this two regions of the bladder. (Figure 2, Figure 3). The expression profile demonstrates that PDE5 and beta3 AR mRNAs are abundant in the bladder, therefore inhibitors of PDE5 and beta3 agonists could act on the bladder tone. Moreover treatment with PDE5 inhibitors and beta3 agonists are efficacious in both genders to a similar extent since no differences of PDE5 or beta3 mRNA in males and females can be detected. Since the mRNA expression of beta3 mRNA and PDE5 is not significant different
in trigone and detrusor muscle a pronounced efficacy of both, PDE5 inhibitors and beta3 agonists can be expected. Thus, inhibitors of PDE-5 or agonists of beta3 AR, but in particular combinations of both, PDE5 inhibitors and beta3 AR agonists, such as Vardenafil in combination with beta3 AR agonists, such as 2-((2,3-Dihydroxypropyl)Aminomethyl)chroman derivates reduce bladder tone synergistically.

Example 3

**Tissue preparation:** Male Beagle dogs (15-16kg) are euthanized due to the German Law for the protection of laboratory animals. Bladder tissue is removed and placed in ice-cold Krebs-Henseleit buffer of following composition (in mmol/l): NaCl 112, KCl 5.9, CaCl2 2.0 MgCl2 1.2, NaH2PO4 1.2, NaHCO3 25, glucose 11.5. Four equally sized longitudinal strips of approximately 2 mm x 10 mm are cut from the bladder body.

**Recordine of mechanical activity in the bladder strip assay:** The preparations are transferred to 20 ml organ baths containing Krebs-Henseleit solution equilibrated with 95% O2, 5% CO2 at 37°C. The strips are mounted between two hooks by means of two clips. For recording of isometric tension one of the hooks is connected to a force transducer which is in turn linked to an amplifier and chart recorder. The other hook is attached to a movable unit, permitting precise adjustment of preload tension. All tissues are then given a 60 min equilibration period during which they are washed and the resting tension is adjusted to 1 g every 20 min. After the equilibration period, each experiment is started by exposing the preparation to K+ (50 mmol/l) Krebs-Henseleit solution. The procedure is repeated 3 times and the tissues are washed at least tree times between each contraction. The bladder strips are then pre-contracted using K+ (50 mmol/l) Krebs-Henseleit solution. When the contraction is stabilized, an accumulative dose response curve of the compound tested is constructed. The stabilized contraction induced by K+ (50 mmol/l) Krebs-Henseleit solution is defined as 100% tension. The relaxation is expressed as percentage tension.

**Results:** The effects of a PDE5 inhibitor, i.e. Vardenafil, a beta3 AR agonist, i.e. a 2-((2,3-Dihydroxypropyl)Aminomethyl)chroman derivate and combinations thereof (PDE5 inhibitor, i.e. Vardenafil + beta3 agonist i.e. a derivate of 2-((2,3-Dihydroxypropyl)Aminomethyl)chroman) are tested on isolated and pre-contracted dog bladder strips as described above. Vardenafil at 1µM and the beta 3 agonist at 1µM relaxed the dog bladder by 29±1% and by 34±2% respectively. In contrast the combination of Vardenafil at 1µM and the beta3 AR agonist at 1µM led to a significant and enhanced relaxation by 46±3% of the dog bladder strip (Table 1). These results show that combinations of PDE5 inhibitors, i.e. Vardenafil and beta3 AR agonists i.e. 2-((2,3-Dihydroxypropyl)Aminomethyl)chroman derivates acted on the bladder and are superior to mono-therapy.
Table 1:

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Relaxation [in % ± SEM]</th>
</tr>
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<tbody>
<tr>
<td>Vardenafil</td>
<td>29 ± 1</td>
</tr>
<tr>
<td>beta3 AR agonist</td>
<td>34 ± 2</td>
</tr>
<tr>
<td>Vardenafil + beta3 AR agonist</td>
<td>46 ± 3</td>
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</tbody>
</table>
What is claimed is:

1. A pharmaceutical composition containing at least one PDE5 inhibitor and at least one muscarinic receptor antagonist or at least one beta 3 receptor agonists.

2. A pharmaceutical composition according to claim 1 containing a PDE5 inhibitor selected from the group consisting of Vardenafil, Sildenafil, Tadalafil, Udenafil, Dasantafil, Avanafil, SLx2101 and LAS34179.

3. A pharmaceutical composition according to claim 1 containing a muscarinic receptor antagonist selected from the group consisting of Oxybutinin, Trospium, Tolterodin, Solifenacin, Fesoterodin and Darifenacin.

4. A pharmaceutical composition according to claim 1 containing a beta 3 receptor agonists selected from the group consisting of BRL-37344, Cl-316234, YM-178, 2-((2,3-Dihydroxypropyl)Aminomethyl)chroman derivates, Solabegron and Ritobegron.

5. Use of a combination of at least one PDE5 inhibitor and at least one muscarinic receptor antagonist or at least one beta 3 receptor agonists for the treatment of a disease comprised in a group of diseases consisting of Benign Prostate Hyperplasia (BPH), Bladder Outlet Obstruction (BOO), Lower Urinary Tract Symptoms (LUTS), genitourinary disorders comprising neurogenic bladder syndrome (overactive bladder - OAB, Interstitial Cystitis - IC), urinary incontinence (UI) like mixed-, urge, stress- or overflow incontinence (MUI; UUI, SUI, OUI), pelvic pain and other urological disorders.

6. Use of a combination of at least one PDE5 inhibitor and at least one muscarinic receptor antagonist or at least one beta 3 receptor agonists for the treatment of benign and malign disorders of the organs consisting the genitourinary system of female and male, renal diseases like acute or chronic renal failure, immunologically mediated renal disease like renal transplant rejection, lupus nephritis, immune complex renal disease, glomerulaopathies, nephritis, toxic nephropathy, obstructive uropathies and erectile dysfunction.

7. Use of a combination of at least one PDE5 inhibitor and at least one muscarinic receptor antagonist or at least one beta 3 receptor agonists for the preparation of medicaments for the treatment of a disease comprised in a group of diseases consisting of Benign Prostate
Hyperplasia (BPH), Bladder Outlet Obstruction (BOO), Lower Urinary Tract Symptoms (LUTS), genitourinary disorders comprising neurogenic bladder syndrome (overactive bladder - OAB, Interstitial Cystitis - IC), urinary incontinence (UI) like mixed-, urge, stress- or overflow incontinence (MUI; UUI, SUI, OUI), pelvic pain and other urological disorders.

8. Use of a combination of at least one PDE5 inhibitor and at least one muscarinic receptor antagonist or at least one beta 3 receptor agonists for the preparation of medicaments for the treatment of benign and malign disorders of the organs consisting the genitourinary system of female and male, renal diseases like acute or chronic renal failure, immunologically mediated renal disease like renal transplant rejection, lupus nephritis, immune complex renal disease, glomerulaopathies, nephritis, toxic nephropathy, obstructive uropathies and erectile dysfunction.
The effects of PDE-5 inhibitors and Antimuscarinics on bladder contractility were tested \textit{in vitro} in an organ bath assay using rat bladder tissue.
Figure 2:

Relative PDE5a mRNA Expression [rE] in bladder of male and female Sprague dawley rats, data are mean + SEM, n=10

Figure 3:

Relative beta3 AR mRNA Expression [rE] in bladder of male and female Sprague dawley rats, data are mean + SEM, n=10