Drugs of the Future 2009, 34(8): 635-640

CCC: 0377-8282/2009
DOI: 10.1358/dof.2009.34.8.1401947

REVIEW ARTICLE

β_3-ADRENOCEPTOR AGONISTS FOR THE TREATMENT OF OVERACTIVE BLADDER

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ABSTRACT

Overactive bladder (OAB) is a medical condition that refers to the bothersome symptoms of increased frequency of voiding episodes and involuntary urinary urgency, with or without subsequent urinary incontinence and often associated with nocturia. The disorder afflicts more than 17 million people of both genders in the United States, with a higher prevalence in the elderly population. The etiology of OAB is uncertain, but it is known that voiding in the healthy condition involves bladder contractions triggered by acetylcholine released from parasympathetic nerves, activating postjunctional muscarinic receptors in the detrusor. Muscarinic receptor antagonists are therefore the most common form of therapy prescribed for treating OAB, but their association with mechanistic side effects has prompted the search for new drugs. Functional studies have demonstrated that adrenoceptor agonist-evoked bladder relaxation is mediated primarily by β_3-adrenoceptors (β_3-AR) in humans. These receptors are an attractive target for the treatment of OAB because of their unique ability to produce detrusor relaxation only during the storage phase, which may allow unimpeded bladder voiding without the risk of urinary retention. The recently completed phase II clinical proof-of-concept study BLOSSOM (Beta3-adrenoceptor agonist in Lowering OAB Symptoms Study compared to Oral anti-Muscarinic) revealed that a novel selective β_3-AR agonist (YM-178) was well tolerated and effective in treating OAB symptoms, further supporting the β_3-AR as a valid therapeutic target for OAB treatment.

INTRODUCTION

Overactive bladder (OAB) affects more than 17 million people in the United States (1). OAB is defined by the symptom of urgency (2), which is the complaint of a sudden compelling desire to pass urine. The evaluation of urgency as a sensation is not easy in humans, although several scales are available to measure the intensity of urgency. In animals, "urgency" cannot be used as a measurement, and only a few parameters of physiological endpoints, which are unique to humans as a consequence of urgency, can be measured by urodynamic studies (3). Many neurotransmitters, including acetylcholine, norepinephrine, dopamine, serotonin, excitatory and inhibitory amino acids, adenosine triphosphate, nitric oxide and neuropeptides, are involved in the neural control of the two main phases of micturition, namely storage and the periodic expulsion of urine from bladder, called the voiding phase (4).

Neurons from the brain and spinal cord align together to form a neural control system to coordinate the reciprocal activity of two functional motor units involved in micturition, that is, the urine container (urinary bladder) and the outlet valve formed by the bladder neck, urethra and striated muscles of the urethral sphincter (4). The motor activity of the muscles comprising the anatomy of the bladder and urethral outlet is under the dual control of the twin autonomic branches of parasympathetic and sympathetic nerves, as well as the somatic nerves that contain both afferent and efferent pathways (5). The twin autonomic branches control two separate phases of micturition. Efferent function in the voiding phase is mediated by parasympathetic nerves releasing acetylcholine, which in turn activates postjunctional muscarinic receptors in the detrusor (6). On the other hand, the urine storage phase is governed by sympathetic nerves with inputs from brainstem and cerebral centers. The norepinephrine released by sympathetic nerves innervating the bladder induces bladder relaxation via β-adrenoceptors (β-ARs) (7).

Neuronal reflexes guiding voiding (emptying of the urine container) are mediated by a spinobulbospinal pathway passing through a coordination center (the pontine micturition center) located in the brainstem (4). On the other hand, the reflexes responsible for the storage of urine in the bladder are organized only at the spinal cord level. Furthermore, storage and voiding reflexes in a spinal-intact condition are activated by mechanosensitive A-δ afferents, which convey the afferent information in response to bladder distension (5). Since bladder contractions during the voiding reflex are triggered by an action on muscarinic receptors in the detrusor, muscarinic receptor antagonists are the most common form of pharmacological therapy prescribed for OAB treatment (8). However, these

agents are associated with mechanistic side effects such as drug tolerance and adverse events, including dysuria and dry mouth, which compromise patient adherence (9).

Since the mid-1990s, considerable attention has been focused on the alternative approach of modulating the function of ARs in the efferent limb to prolong the storage phase for providing relief from OAB symptoms. The key requirement for the storage phase is a stable bladder with high compliance (i.e., a relaxed bladder) and closed urethral outlet (10). The β-ARs were identified by genomic cloning of human cells in the late 1980s and have been classified as β_1-AR, β_2-AR and β_3-AR (7) . The β_3-AR, like the other β-ARs, is a seven-transmembrane domain G protein-coupled receptor expressed in several tissues, including adipose tissue, heart, uterus and gut. The β_3-AR modulates different physiological functions and its discovery spawned a search for drugs specifically targeting functional β_3-ARs in human brown and white fat cells for mediating lipolysis and evoking relaxation of the gallbladder, stomach, small intestine, prostate and colon (11, 12). The lipolysis and an increase in energy utilization following activation of β_3-ARs on adipocytes (fat cells) prompted the evaluation of antiobesity effects for several rat β_3-AR-selective agonists, such as N-5984 (Nisshin Pharma) and KRP-204 (Kyorin Pharma), in animal and clinical studies (13, 14). Clinical studies in obese patients revealed the importance of receptor selectivity, as any therapeutic benefits accrued by older-generation β_3-AR agonists were complicated by side effects of tremor and tachycardia, probably mediated by a nonselective action on β_2- and β_1-ARs (15).

EXPRESSION OF β-ARs IN BLADDER

All the subtypes of β-ARs coupled to excitatory G_s and inhibitory G_i proteins have also been identified in the bladder of several species, including humans, namely, β_1-, β_2- and β_3-ARs (7, 16). The predominance of the β_3-AR subtype in human bladder was demonstrated by the finding that 97% of total β-AR mRNA is represented by the β_3-AR subtype (17). Evidence for the predominance of β_3-ARs in the human detrusor revealed by molecular investigations was later supported by functional studies using β_3-AR agonists with higher affinity for human β_3-AR. The β_3-AR agonists developed initially, such as BRL-37344, CL-316243 and CGP-12177A, had higher affinity for rodent β-ARs (18). The lower affinity of these β_3-AR agonists for human relative to rodent receptors seen during in vitro functional studies demonstrated the important species-dependent differences in the pharmacology of β_3-ARs (19). BRL-37344, CL-316243 and CGP-12177A were categorized as partial agonists at human β_3-ARs, and formed the template for the design of potent and highly selective human β_3-AR agonists developed subsequently (20).

It is now well accepted that sympathetic nerves determine the duration of the urine storage phase during the micturition cycle and norepinephrine released from these nerves activates β-ARs in the bladder to relax the detrusor and improve compliance (4). Given the important role of β-ARs in detrusor relaxation and bladder compliance, it has been suggested that the selective activation of bladder β-ARs should provide prophylaxis against involuntary detrusor contractions during the urine storage phase and thereby improve OAB symptoms.

IN VITRO EFFECT OF β_3-AR AGONISTS ON BLADDER

Functional in vitro studies conducted on isolated rat bladder demonstrated that bladder relaxation evoked by β-AR agonists is mediated mainly via β_2- and β_3-ARs in rats (19, 21). In contrast, human bladder relaxation is produced via an action on β_3-ARs and not β_1- or β_2-ARs, demonstrating species-based differences in the expression and function of β-ARs. The functional response of selective agonists acting on β_3-ARs is in agreement with the higher expression levels of β_3-ARs reported from other experiments (17). Functional studies are based on the concentration-dependent relaxation of the resting tension of the human detrusor produced by isoprenaline, which remains unaffected in the presence of selective β_1- (CGP-20712A; 10 μM) and β_2-AR antagonists.

Concentration–response curves for β-AR agonists were obtained by cumulative addition of the appropriate drug to the bathing fluid and β-AR antagonists were added to the bath 30 min before the addition of isoprenaline. A rightward parallel shift of the concentration–relaxation curve for isoprenaline was produced only at the highest concentration of a β_2-AR antagonist (ICI-118551; 10 μM). In contrast, a concentration-dependent rightward shift of the concentration–relaxation curve for isoprenaline was produced by a β_3-AR antagonist (SR-58894A; 0.1-10 μM). The functional experiments on β-AR agonists were conducted in the presence of 1 μM phentolamine, an α-adrenoceptor antagonist, to rule out an action of these agents on α-adrenoceptors.

There has been lot of interest in the application of prodrug technology in designing newer β_3-AR agonists for OAB. One such prodrug developed by MediciNova, a Japanese specialty pharmaceutical company, is MN-246 (formerly TT-138), the active metabolite of which, 138-355, was shown to relax isolated human detrusor muscle removed from cancer patients. Cumulative addition of 138-355 to the bath of stably contracting human detrusor strips induced relaxation, with a pD_2 value of 5.80 ± 0.26. The concentration–relaxation curves of 138-355 were competitively antagonized by SR-59230A, with a pA_2 value of 7.01 ± 0.45 and a Schild slope of 0.72 ± 0.07 (22), which further confirms the specific involvement of the β_3-AR in relaxation of the human bladder. The company is currently testing this compound in a phase I clinical trial for urinary incontinence.

Recently, Astellas published results on the relaxant effects of the company's novel selective β_3-AR agonist YM-178 (mirabegron) in rat and human bladder strips precontracted with carbachol. The efficacy of YM-178 was measured as the EC_{50} in comparison to isoproterenol. In rat bladder strips precontracted with 1 μM carbachol, isoproterenol was more potent, with an EC_{50} of 4 μM compared to 5.1 μM for YM-178. However, in human bladder strips, both YM-178 and isoproterenol concentration-dependently relaxed human bladder smooth muscle strips precontracted with 0.1 μM carbachol with EC_{50} values of 0.78 and 0.28 μM, respectively. Studies with YM-178 further confirmed the subtle species-dependent differences in the pharmacological response to β_3-AR agonists (23).

Muscle relaxation is achieved via stimulation of G_s, which increases cAMP, leading to activation of Ca^{2+}-activated potassium channels (K_{Ca} channels) and hyperpolarization of smooth muscle (Fig. 1) (24). The large-conductance, voltage-dependent K_{Ca} channels are key players in the control of smooth muscle contractility and relaxation

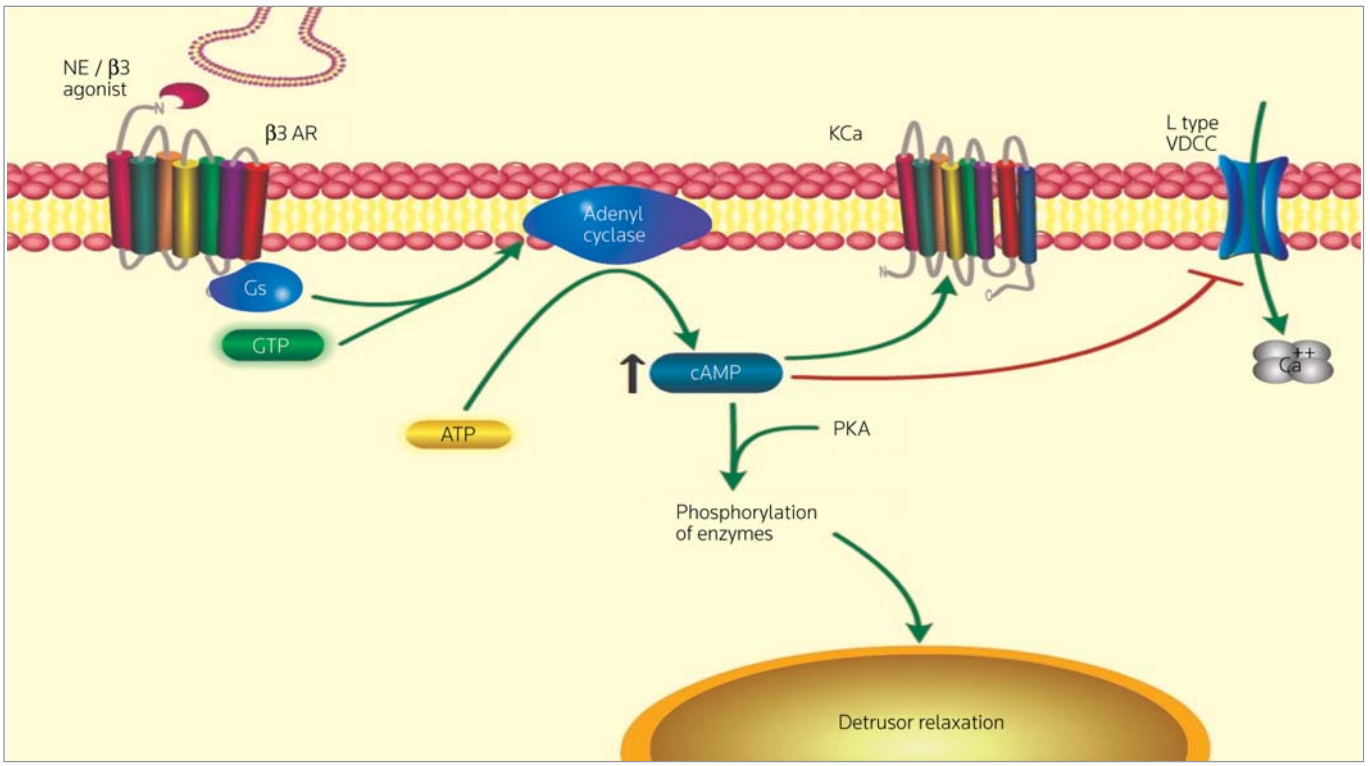

Figure 1. Endogenous norepinephrine (NE) released from sympathetic nerves or exogenous selective β_3-adrenoceptor (β_3-AR) agonist activates β_3-ARs in the bladder to relax detrusor. Relaxation of detrusor muscle is achieved via stimulation of G_s, which increases cAMP, leading to activation of Ca^{2+}-dependent potassium channels (K_{Ca} channels) via PKA and smooth muscle hyperpolarization. The large-conductance, voltage-dependent K_{Ca} channels mediate smooth muscle relaxation by their ability to link local cytosolic free [Ca^{2+}] to hyperpolarizing K^+ outward currents. Detrusor relaxation leads to an increased duration of the urine storage phase and improvement in bladder compliance in overactive bladder.

via their ability to link membrane depolarization and local increases in cytosolic free Ca^{2+} to hyperpolarizing K^+ outward currents (25). As shown in Figure 1, activation of β_3-ARs couples to adenylate cyclase by way of G proteins, leading to an increase in intracellular cAMP levels and subsequent activation of cAMP-dependent protein kinase (PKA). The increase in cAMP production also results in attenuation of cytoplasmic Ca^{2+} concentration by removal of Ca^{2+} from cytoplasm (26). The role of K_{Ca} channels and the requirement of the cAMP/PKA pathway in β_3-AR-mediated detrusor relaxation was demonstrated by studies on isoproterenol-induced relaxation of guinea pig bladder smooth muscles in the presence or absence of a K_{Ca} channel inhibitor (e.g., charybdotoxin and iberiotoxin) and an adenylate cyclase inhibitor (SQ-22536). There was a suggestion of a cAMP-independent pathway that may be involved in β_3-AR-mediated detrusor relaxation (27).

IN VIVO EFFECT OF β_3-AR AGONISTS ON BLADDER

In recent years, compelling in vivo evidence has been reported in the literature to support a role for β_3-ARs in increasing the bladder capacity in conscious and anesthetized rats, as well as in models of pathological bladder instability and hyperreflexia (10, 28). Oral administration of the β_3-AR agonist FK-175 at a dose of 10 mg/kg significantly increased bladder capacity by 158 µL during conscious cystometry in rats with increased urinary frequency induced by ibotenic acid injection. The increase in bladder capacity produced by the β_3-AR agonist was not associated with any change in micturition pressure or threshold pressure during cystometry in the rodent model.

The efficacy of a β_3-AR agonist against bladder instability was evaluated in an obstructed hypertrophied bladder model in rats by cystometry under urethane anesthesia. When compared with other agents (including isoproterenol, verapamil and atropine), oral or i.v. administration of the β_3-AR agonist CL-316243 at a dose of 10 mg/kg significantly increased the voiding interval, bladder compliance and capacity, without any change in the residual bladder volume (28). The amplitude of electrically evoked isovolumetric contractions during cystometry in the presence of acetic acid was significantly smaller after CL-316243 exposure. In comparison with β-AR agonists tested, the dose of CL-316243 that caused positive changes in cystometry did not produce any significant effect on blood pressure and heart rate (29).

Similar results were obtained in our lab with another β_3-AR agonist, CL-314263, on detrusor overactivity caused by partial bladder outlet obstruction (Fig. 2). This disease model was induced by partial obstruction of the urethra using a ligature in female rats 6 weeks prior to treatment. Cystometrograms prior to drug treatment in 6-week-old diseased rats revealed significant nonvoiding contractions, as shown by numerous small-amplitude peaks preceding the big peak of voiding

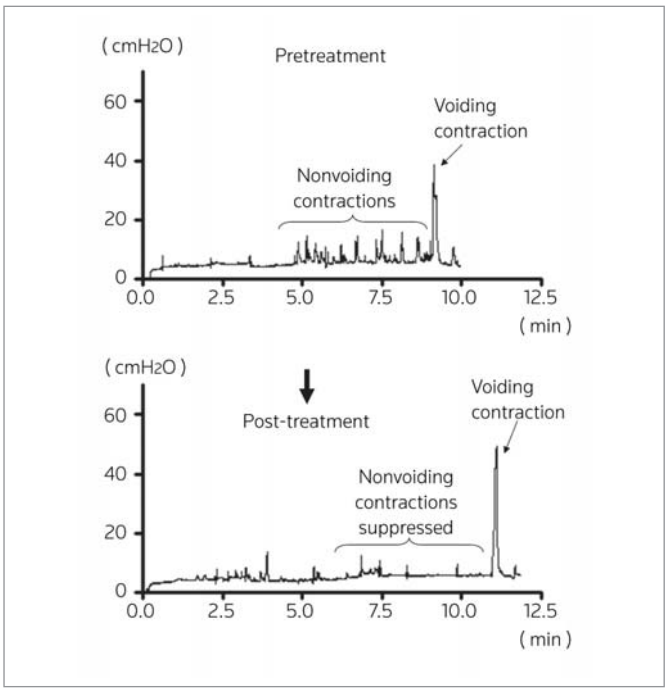

Figure 2. Cystometric effect of acute administration of the β_3-adrenoceptor (β_3-AR) agonist CL-314263 on detrusor overactivity caused by bladder outlet obstruction. Cystometrogram prior to drug treatment in 6-week-old diseased rats revealed significant nonvoiding contractions, as shown by numerous small-amplitude peaks preceding the big peak of voiding contraction (top tracing). Small-amplitude bladder contractions were abolished by the β_3-AR agonist CL-314263 administered at the dose of 1 mg/kg i.v. (bottom tracing). Detrusor relaxation mediated by β_3-ARs suppressed nonvoiding contractions without any effect on the peak micturition pressure of the voiding contraction.

contraction (top tracing). Small-amplitude bladder contractions were reduced by the β_3-AR agonist CL-314263 administered at a dose of 1 mg/kg i.v. Detrusor relaxation mediated by β_3-ARs suppressed the nonvoiding contraction post-treatment without any effect on the peak micturition pressure of the voiding contraction.

Kissei has previously reported the pharmacological effects of its β_3-AR agonist KUC-7483, which is a prodrug, and its active metabolite, KUC-7322. Intravenous administration of KUC-7322 decreased intravesical pressure in anesthetized rats in a dose-dependent manner. In order to test the effect of the prodrug, catheters were implanted in the bladder and stomach of female rats 7 days prior to conscious cystometry. Bladder hyperactivity in conscious rats was induced by continuous bladder infusion of saline containing prostaglandin E_2 (PGE_2; 60 μM), which decreased both the micturition interval and volume. Micturition interval and volume were increased 1 h after intragastric administration of the prodrug KUC-7483 in the dose range of 0.1-10 mg/kg (21). This prodrug was also studied in isolated primate bladder prior to ongoing clinical studies.

The effect of KRP-204, a selective β_3-AR agonist, in suppressing afferents from the lower urinary tract, was evaluated by changes in capsaicin-induced licking behavior of conscious rats. Seven days prior to the experiment, catheters were implanted in the bladder dome for capsaicin instillation, as well as in the stomach or femoral vein for drug administration, in male Wistar rats under halothane anesthesia. All the catheters were tunneled subcutaneously and exteriorized dorsally. Baseline licking behavior was defined as the length of time the rats licked (groomed) their bodies, excluding their paws and tail, during a defined observation time period following saline infusion into the bladder at a rate of 10 mL/h for 30 min. Following saline infusion, 30 μM capsaicin was infused into the bladder via catheter at the same rate, and the duration of licking behavior was measured for 30 min. The duration of licking behavior was significantly reduced by KRP-204 in a dose-dependent manner, with a 75% reduction at the dose of 3 mg/kg. Pretreatment with SR-59230A, a selective β_3-AR antagonist, reduced the effect of KRP-204. The intensity of licking behavior in rats was higher after voiding, suggesting the involvement of urethral afferents rather than bladder afferents in the behavioral response to capsaicin (30).

In a recently completed study, the effects of YM-178 on rhythmic bladder contractions induced by saline bladder filling in anesthetized rats were compared to oxybutynin. Intravenous YM-178 produced a dose-dependent decrease in the frequency of rhythmic bladder contractions without a decrease in the amplitude of rhythmic bladder contractions at doses up to 3 mg/kg. (Transurethral saline infusion during cystometry induces reflex bladder contractions under normal conditions, which follow a rhythm of periodic contractions, where the time interval between two consecutive contractions remains the same, and these are referred to as rhythmic bladder contractions.) In contrast, oxybutynin administered at a 10-fold lower dose of 0.272 mg/kg produced a significant increase in the frequency of rhythmic bladder contractions, with a concomitant decrease in the peak micturition pressure (23).

Several hypotheses may explain the absence of any effect of β_3-AR activation on voiding function. One possible explanation may emerge from the fact that acetylcholine released from parasympathetic nerves during the voiding phase activates both muscarinic acetylcholine M_2 and M_3 receptors located on smooth muscle. The increase in acetylcholine activity in the detrusor mediated by β-ARs can be inhibited by the action of acetylcholine on M_2 receptors during the voiding phase. In addition, stimulation of M_3 receptors activates the phosphatidylinositol/Ca^{2+} recruitment system (31). The hypothesis regarding the inhibition of β-AR-mediated bladder relaxation by M_2 receptors is supported by the increased efficacy of isoproterenol in inducing bladder relaxation in M_2 receptor knockout mice (32). Therefore, the relaxation of bladder smooth muscle in the voiding phase mediated by β_3-AR agonists may be countered by M_2 receptor activation, and such agents may not affect M_3 receptor-mediated bladder contraction.

The next generations of β_3-AR agonists have been designed following the principles of structure–activity relationships for optimizing the potency, selectivity and pharmacokinetic features. One such lipophilic biphenyl benzoic acid derivative with good oral bioavailability and a long plasma half-life was evaluated by Astellas in a dog model of OAB. The new β_3-AR agonist proved effective in reducing the carbachol-induced increase in intravesical pressure in dogs (33).

The results of experiments in commonly used animal models of bladder overactivity suggest that the activation of β_3-ARs increases bladder capacity without influencing bladder contractions or resid-

ual urine volume during the voiding phase. The absence of any effect on bladder contraction is a key characteristic of the β_3-AR agonist class of drugs, which distinguishes them from the antimuscarinic agents. The decrease in the amplitude of rhythmic bladder contractions is a hallmark of antimuscarinic drugs underlying the increase in the interval between voiding episodes for OAB patients (34). It is also important for the successful clinical translation of agonists acting on β_3-ARs expressed in the bladder that they be devoid of any cardiovascular side effects, such as changes in heart rate and blood pressure. Several β_3-AR agonists tested earlier in the clinic were withdrawn owing to adverse changes in heart rate (35).

EFFECT OF β_3-AR AGONISTS ON HUMAN BLADDER

Although the pharmacology of β_3-ARs in the bladder has been known for some time, the clinical testing of drugs targeting these receptors to realize the full therapeutic potential of β_3-AR agonists in humans has only just begun. In recent years, efforts have been made by several pharmaceutical companies to bring this class of drugs to the clinic for the management of OAB. Encouraged by the data on the novel selective β_3-AR agonist YM-178 in preclinical studies (23), its clinical efficacy was tested in comparison to placebo and a tolterodine extended-release (ER) formulation in a randomized, double-blind, parallel-group, proof-of-concept phase II study known as BLOSSOM (Beta3-adrenoceptor agonist in Lowering OAB Symptoms Study compared to Oral anti-Muscarinic), conducted at 31 clinical sites in 6 European countries (36). Patients were enrolled into a single-blind, 2-week placebo run-in period, after which they were randomized to 4 weeks of double-blind treatment with YM-178 100 mg b.i.d. (n = 65 patients) or 150 mg b.i.d. (n = 65), placebo (n = 66) or tolterodine ER 4 mg once daily (n = 64). Efficacy parameters analyzed at the end of the sixth visit included data collected from patient micturition diaries over 3 days preceding each study visit. Efficacy comparison against placebo revealed a statistically significant reduction in mean micturition frequency following twice-daily treatment with 100 and 150 mg YM-178, with a mean difference of 1.0 micturition/24 h on both doses. Treatment with YM-178 at these doses was also significantly better than placebo with respect to the secondary efficacy variables of mean volume voided per micturition, mean number of incontinence episodes, nocturia episodes, urgency incontinence episodes and urgency episodes per 24 h. There were no dose-dependent, clinically relevant adverse events (AEs) and treatment with YM-178 was well tolerated. The most common AEs reported by the study participants were gastrointestinal AEs and headache. The incidence of AEs with YM-178 treatment (39.2%) was less than in the tolterodine active comparator arm (48.4%), but greater than in the placebo group (36.4%). The incidence of gastrointestinal AEs and headache was highest in the tolterodine treatment group (23.4% and 9.4%, respectively), intermediate in the YM-178 treatment group (13.8% and 6.9%, respectively) and lowest in the placebo treatment group (3.0% for both). The results of the first phase II proof-of-concept study showed that a β_3-AR agonist (YM-178) was well tolerated and effective in the treatment of OAB symptoms.

A randomized, double-blind, placebo-controlled phase II study for a β_3-AR agonist (KUC-7483), sponsored by Kissei (ClinicalTrials.gov Identifier: NCT00742833), has been completed in Japan. The 12-week study was conducted in OAB patients who had a disease history of over 6 months. The primary endpoint of the study was the change in daily voiding episodes relative to baseline and the effect on urgency and incontinence episodes are secondary endpoints. Clinical testing of several other β_3-AR agonists, including MN-246, for efficacy in OAB is currently ongoing.

CONCLUSION

Given that β_3-ARs play an important role in relaxation and improvement of compliance of the mammalian bladder, it has been suggested that the selective activation of bladder β-ARs should result in reduction or prevention of involuntary detrusor contractions during the urine storage phase, and thereby improve OAB. In order that new β_3-AR agonists demonstrate efficacy in the clinical situation, several considerations need to be addressed, including bioavailability, lipophilicity to improve β_3-AR activity and selectivity for human versus animal β_3-ARs. The recently completed proof-of-concept study (BLOSSOM) demonstrated the efficacy and safety of a selective β_3-AR agonist (YM-178) in OAB patients. Such agents represent a promising choice for the treatment of OAB with or without lower urinary tract symptoms (LUTS), such as those seen in benign prostatic hyperplasia (BPH). Activation of β_3-ARs prolongs the storage phase, leading to an increase in bladder capacity and a lack of effect on bladder contraction (voiding phase), which leaves emptying of the bladder unimpeded. The discovery of this class of drugs raises hope for OAB patients that drugs with a reduced risk of urinary retention compared to antimuscarinic agents will be available in the near future.

DISCLOSURE

Drs. Yoshimura, Yamaguchi and Chancellor are paid consultants for Astellas Pharma. The other authors declare no conflicts of interest.

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