Review

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Off-Target Effects of Mirabegron on Muscarinic Receptors

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Abstract

Older adults with multiple diseases are likely to be prescribed multiple medications including anticholinergic agents, which are frequently prescribed to manage conditions such as overactive bladder and chronic obstructive pulmonary disease and Parkinson's disease. Overactive bladder (OAB) has been the subject of increased disease awareness and is a common and significant cause of reduced quality of life, particularly in the elderly. The selective β_3 adrenoceptor agonist, mirabegron was developed for the pharmacological treatment of OAB. Mirabegron has been shown to exert off-target effects on various functional proteins such as muscarinic receptors in rat tissues. This agent may relax the detrusor muscle by activating β_3 adrenoceptors and also antagonizing muscarinic receptors. Mirabegron and antimuscarinics exerted additive effects on muscarinic receptor binding and relaxant responses of cholinergic contractions of the detrusor muscle. Mirabegron excreted in human urine appears to directly attenuate muscarinic receptor-mediated functions in the bladder. Combination therapy of mirabegron and solifenacin in patients with OAB may enhance not only their therapeutic effects on OAB, but also increase the risk of anticholinergic adverse effects. Therefore, the safety of concomitant use of mirabegron and other drugs such as antimuscarinics for elderly patients needs to be carefully considered.

Keywords: mirabegron; off-target effect; anticholinergic effect

1. Anticholinergic Burden

Older adults frequently have many systemic diseases that require treatment with multiple drugs, and, thus, polypharmacy is a significant concern in the management of these individuals. [1–3] More than 600 medicinal products with broad therapeutic ranges have been reported to exhibit anticholinergic activity. [4,5] The age-related alteration in the body's physiology affects significantly pharmacokinetic and pharmacodynamic factors, such as body composition, a change in the volume of distribution for drugs, the reduced clearance of multiple medications and altered sensitivity to neurotrasmitter receptors. In older adults, the renal excretion and hepatic metabolism of drugs are reduced, which may result in their accumulation in tissues, thereby increasing the risk of anticholinergic adverse effects. [6] Since the permeability of the blood-brain barrier increases with age, central adverse effects such as cognitive impairment need to be considered when drugs with anticholinergic effects are prescribed to elderly patients. [7,8] The altered pharmacological sensitivity to the muscarinic receptor blockade may occur by a reduction in the cholinergic reserve and a structural change in muscarinic receptors that may bring a significant impact on the agonist and antagonist receptor binding affinities and on the signal transduction. These alterations increase the

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risk of adverse effects to commonly used medications including anticholinergic drugs in the elderly. The essentiality of a burden scale for anticholinergic accumulation has been emphasized. [9–16]

Yamada det al. [13,14] developed a pharmacological evidence-based anticholinergic burden scale (ABS) for 260 medications used frequently for Japanese elderly patients. In this scale, muscarinic receptor binding activity of each drug was extensively measured by the radioreceptor binding assay using a selective radioligand, [N-methyl-^{3}H]scopolamine chloride (NMS). The anticholinergic burden scale was evaluated by the measurement of muscarinic receptor binding activity of each drug in the consideration of its maximal blood concentrations after the administration at the clinical dose in humans. This scale is the first comprehensive assessment of anticholinergic activities for 260 drugs by pharmacological methods with the consideration of pharmacokinetic properties in humans. According to this scale, 33 drugs were defined as those with strong anticholinergic activity (ABS 3), 37 drugs as those with moderate activity (ABS 2), and 26 drugs as those with weak activity (ABS 1). Other drugs defined as ABS 0 had no muscarinic receptor binding activity even at high concentrations. Kagota et al. [17] investigated functional anticholinergic effects of 60 medications classified as ABS 3 (strong) or 2 (moderate) by the inhibitory effects on the cholinergic (carbachol)-induced contractions in the rat isolated bladder and ileal smooth muscles using the organ bath method. All drugs examined inhibited the muscarinic receptor-mediated smooth muscle contraction in a concentration-dependent manner in the rat isolated bladder and ileum, and their functional activities were positively correlated with muscarinic receptor binding activities. The medication with higher anticholinergic burden score and higher load may cause potentially greater risk of anticholinergic adverse effects in patient with polypharmacy. Therefore, the scoring of anticholinergic burden may predict that adverse effects of different drugs with anticholinergic effects add up in a linear fashion.

Overactive bladder (OAB) has been the subject of increased disease awareness and is a common and significant cause of reduced quality of life, particularly in the elderly. OAB is defined as urinary urgency, usually accompanied by frequency and nocturia, with or without urgency incontinence, in the absence of urinary tract infection or othe obvious pathology. The mainstay of treatment of OAB has been anticholinergic medications. These drugs block muscarinic receptor throughout the body, not only the bladder in the peripheral tissues but in the brain. Antimuscarinic agents such as solifenacin have been utilized as therapeutic agents, but have specific anticholinergic adverse effects, such as dry mouth, constipation, the decline of cognitive function, and an increased residual urine volume. [18] A previous study reported that 70% of patients treated for OAB with antimuscarinics were 61-80 years old. [5] The elderly patients are more susceptible to these anticholinergic adverse effects in the peripheral and central organs, especially as there is increased permeability of the blood brain barrier. The anticholinergic drugs for OAB are able to enter the central nervous system and lead to central side effects. There is increasing evidence that a high anticholinergic load may be linked to the development of cognitive impairment and dementia and increased risk of mortality. Therefore, careful attention should be paid when treating OAB in the elderly.

The selective β_3 adrenoceptor stimulants, mirabegron and vibegron were developed for the pharmacological treatment of OAB. [19–22] Both drugs are considered to stimulate β_3-adrenoceptors in the bladder voiding muscle, resulting in the relaxation of the detrusor smooth muscle. Mirabegron was previously shown to exert off-target effects against various functional proteins such as neurotransmitter receptors, transporters and hepatic enzymes [23–30], as summarized by Dehvari et al. [23] Recently, Yamada et al. [31,32] showed that mirabegron and vibegron exerted antimuscarinic effects in rat tissues by pharmacological procedures. The off-target effects of mirabegron on muscarinic receptors are reviewed herein.

2. Off-Target Effects of Mirabegron on Muscarinic Receptors

Off-target effects are defined as the inhibition or activation of a molecule by a drug that is different from its original target, which generally results in not only undesirable adverse effects, but also unexpected new pharmacological actions or the discovery of new drug targets. [23,33,34]

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Mirabegron has been shown to exert off-target effects on different various functional protein molecules, such as muscarinic M_2 receptors, β_1 adrenoceptors, a_{1A} adrenoceptors, a_{1D} adrenoceptors, drug metabolizing enzymes, cytochrome P450 (CYP2D6, CYP3A4), dopamine transporters, noradrenaline transporters, organic cation transporters, P-glycoprotein, and sodium channel site 2 (Table 1). [23–30] According to the material submitted to the FDA by Astellas Co. Ltd., mirabegron exhibits binding affinity for human M_2 muscarinic receptors (Ki value of 2.1 mM) (U.S. Food and Drug Administration. Pharmacology/Toxicology NDA/BLA Review and Evaluation (NDA 202-611) 2012 [Available from: http://www. accessdata.fda.gov/drugsatfda_docs/nda/2012 /202611Orig1s000PharmR.pdf]). The Australian Department of Health Therapeutic Goods Administration's Australian Public Assessment on Mirabegron (Department of Health Therapeutic Goods Administration: Australian Public Assessment Report for (Mirabegron) (2014) reported that mirabegron bound to muscarinic receptors in muscarinic receptor-expressing cells [29].

Cernecka et al. [35] previously reported that mirabegron inhibited carbachol- or KCl-induced contractions in the rat detrusor muscle, and this relaxant effect was more pronounced for carbachol-induced contractions. Yamada et al. [31,32] demonstrated that both mirabegron and vibegron bound to muscarinic receptors in the bladder and other tissues of rats by the radioligand binding assay using [^{3}H]NMS (Fig. 1 for mirabegron) and that both agents relaxed concentration-dependently the carbachol-induced contractions in the isolated bladder detrusor muscle in the presence of the non-selective β-adrenoceptor antagonist, propranolol. The relaxant activities (EC_{50} values) correlated significantly with their muscarinic receptor binding activities (IC_{50} values) [31,32] in the rat bladder. The relaxation of human and rodent detrusor muscles by mirabegron was characterized by a shallow concentration-effect curve (EC_{50} values in the high nM to low μM range). [18,35,36] A two-site model analysis revealed a high-affinity relaxant response (EC_{50} : 87.3 nM, relative contribution: 44.5%) and low-affinity relaxant response (EC_{50} : 10.7 mM, relative contribution: 55.5%) for mirabegron. [31] The efficacy (EC_{50}) of the low-affinity relaxant response of carbachol-induced contractions by mirabegron was associated closely with its micromolar binding affinity to muscarinic receptors revealed by the competitive inhibition of specific [^{3}H]NMS binding in the rat bladder. These findings suggested the pharmacological antagonism of mirabegron on muscarinic receptors. The mirabegron-induced relaxation of the human detrusor tissue *in vitro* required concentrations that were markedly higher than its affinity to b_3-adrenoceptors and plasma levels during standard dosing. EC_{50} values for the mirabegron-induced relaxation of precontracted human detrusor tissues ranged between 588 nM and 3.9 mM, [19,36] whereas a binding constant of 2.5 nM for b_3-adrenoceptors, and a maximum plasma concentration of 137 nM during standard dosing have been reported. [19,37] The b_3-adrenoceptor specificity of mirabegron has been investigated and different mechanisms have been proposed to account for the attenuation of storage symptoms by mirabegron. [38–40] The mechanisms responsible for the effects of mirabegron on storage symptoms remain unclear. Huang et al. [40] questioned the specificity of mirabegron and proposed other mechanisms to account for improvement of storage symptoms by mirabegron, including muscarinic receptor antagonism, inhibition of cholinergic neurotransmission, or effects on afferent signaling and central nervous system. Therefore, it is possible that the efficacy of mirabegron to relax detrusor muscle results from not only b_3-adrenoceptor activation, but also from antagonism of muscarinic receptors in clinical settings.

M_2 receptor antagonism has been suggested to play a role in the mirabegron-induced relaxation of cholinergic contractions of the detrusor muscle. Yamada et al. [31] demonstrated that the binding affinity of mirabegron was several-fold higher in the bladder and myocardium than in the brain and salivary glands, indicating a higher affinity for the M_2 receptor subtype than for M_3 or M_1 receptors. The higher binding affinity for the M_2 receptor is consistent with findings on muscarinic receptor subtype-expressing cells. [29] M_2 receptor antagonism by mirabegron is considered to amplify the relaxation response by activating β_3 adrenoceptors via increases in cAMP levels. M_2 and M_3 receptor subtypes are expressed in the bladder, and the latter is primarily responsible for contractions of the detrusor muscle. [41,42] The function of the M_2 receptor is considered to indirectly enhance M_3 receptor-mediated contractions by inhibiting relaxation of the detrusor muscle. [43,44] This M_2

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receptor-stimulated inhibition of detrusor muscle relaxation may occur by suppressing adenylate cyclase activation, which may attenuate cAMP production by the adenylate cyclase activation of β_3 adrenoceptor. [43,44] Ehlert et al. [45,46] found that the isoproterenol-induced relaxation of cholinergic contractions by a transmural stimulation of mouse isolated bladder strips was inhibited by the stimulation of M_2 receptors by endogenous acetylcholine. Therefore, the contractile response of the detrusor muscle to muscarinic agonists may be attributed in part to the M_2 receptor-mediated inhibition of the cAMP-increasing b_3 agonist-induced relaxant response. [44] Therefore, M_2 receptor antagonism by mirabegron is considered to amplify the relaxation response by activating β_3 adrenoceptors through increases in cAMP levels. It is assumed that the dual action of β_3 adrenoceptor activation and M_2 muscarinic receptor antagonism by mirabegron occurs in the pharmacotherapy of patients with OAB.

3. Combination Therapy of Mirabegron and Antimuscarinic Agents

Drug combinations are expected generally to exert additive, synergistic or antagonistic pharmacological effects. A systematic review and network meta-analysis by Kelleher et al. [47] showed that the combination of solifenacin (5 mg) and mirabegron (25 or 50 mg) was more effective than mirabegron (50 mg) alone in terms of efficacy in patients with OAB. A detailed examination of the findings obtained showed that anticholinergic adverse events (dry mouth, constipation, visual disturbances) occurred more frequently than with solifenacin alone. A similar finding was reported by Herschorn et al., [48] where combination therapy with both drugs resulted in a higher frequency of dry mouth, constipation, and dyspepsia compared to monotherapy. Therefore, it is considered that the higher incidence of anticholinergic adverse events by combination therapy with mirabegron and solifenacin results partly from the antagonistic effects of mirabegron on muscarinic receptors. Soliman et al. [49] conducted a prospective randomized control study on pediatric patients (190 patients) newly diagnosed with OAB to investigate the efficacy and safety of mirabegron versus solifenacin in the treatment of OAB in children. Dry mouth was reported in 2.8, 10 and 0 % and constipation in 2.8, 11.4 and 1.4% in the mirabegron group (50 mg once daily), solifenacin (5 mg) group, and placebo group, respectively. The incidence of anticholinergic adverse effects was higher with mirabegron than with the placebo. These findings suggest that the combination of mirabegron and an antimuscarinic drug increases the risk of anticholinergic adverse events and also that the muscarinic receptor binding activity of mirabegron is clinically meaningful. A previous study reported that 70% of patients treated with antimuscarinic drugs for OAB were 61-80 years old. [5] They should be paid attention for the central adverse effects such as the decline of cognitive and memory function and depression. Therefore, the anticholinergic effects of mirabegron and antimuscarinics require more detailed investigations for the safety of combination therapy in elderly patients with OAB.

4. Additive Effects of Mirabegron and Antimuscarinic Agents on Muscarinic Receptor Binding and on Relaxation of Cholinergic Detrusor Muscle Contractions

The efficacy and safety of combination therapy with mirabegron and solifenacin compared with monotherapy and placebo in patients with OAB have been reported by Kelleher et al. [47] and Herschorn et al [48]. On the other hand, according to the careful inspection of data concerning anticholinergic side effects, the incidence of anticholinergic adverse events, such as dry mouth and constipation, was found to be higher with combination therapy of solifenacin and mirabegron than with solifenacin alone, suggesting the enhancement of anticholinergic side effects. Based on the clinical observation, very recently, Yamada et al. [50] have investigated whether the combination of mirabegron and antimuscarinics (solifenacin, imidafenacin) exerted additive effects on muscarinic receptor binding and cholinergic contractions of the detrusor muscle in rats. Their data revealed that the muscarinic receptor binding activity of solifenacin in rat tissue was additively enhanced by its

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combination with mirabegron (Fig. 2). Moreover, mirabegron enhanced the relaxant effects of solifenacin on carbachol-induced contractions of rat isolated detrusor muscle strips (Fig. 3). These additive effects on muscarinic receptor binding and functional responses were more pronounced at lower concentrations of solifenacin. These findings may indicate that mirabegron exerted additive effects on antimuscarinic-induced pharmacological actions on muscarinic receptors, which improved therapeutic effects on OAB and also increased the risk of anticholinergic adverse effects. The anticholinergic burden scale of mirabegron was classified as score 2, and that of solifenacin as score 3. [13–15] The additive effects of the anticholinergic burden may contribute to the enhancement by mirabegron of antimuscarinic-induced muscarinic receptor binding and relaxant effects on the cholinergic contractions of rat tissues. Moreover, similar additive effects on muscarinic receptor binding in rat tissues was observed by the combination of mirabegron and imidafenacin, another anticholinergic agent which was frequently used for the therapy of OAB in Japan. [50] The muscarinic receptor binding activities of imidafenacin in rat tissues were additively enhanced by the addition of mirabegron, which was pronounced at lower concentration of imidafenacin. The relaxant effect of cholinergic contraction in the rat smooth muscle by mirabegron and imidafenacin in the presence of propranolol was also additive. Such additive effects of the combination of mirabegron and low concentrations of antimuscarinics in the preclinical study may be associated with the finding by Shin et al. [51] who showed the good efficacy and safety of add-on therapy with low-dose antimuscarinics in patients with suboptimal responses after 4 weeks of mirabegron monotherapy. Collectively, these findings suggest the clinical relevance of the scoring of anticholinergic burden by the combination therapy with mirabegron and other medications with anticholinergic effects in patients with polypharmacy.

In the combination of mirabegron and solifenacin or imidafenacin, Sugaya et al. [52] examined the effect of combining mirabegron and 5-hydroxymethyl tolterodine (an active metabolite of fesoterodine, clinically used anticholinergic agent for the OAB treatment), in a rat model of pelvic congestion. The additive relaxant effects of mirabegron and 5-hydroxymethyl tolterodine were observed in vitro in the electrical field stimulation-induced contractions of bladder strips from pelvic congestion rats. In vivo, bladder capacity was increased significantly by a combination of mirabegron and 5-hydroxymethyl tolterodine, with the combined effect exceeding the sum of the effects of monotherapies. These results indicate that the combination of mirabegron and 5-hydroxymethyl tolterodine causes the potential of synergistic effects in a rat pelvic congestion model.

5. Pharmacokinetics of Mirabegron and Prediction of Human Bladder Muscarinic Receptor Occupancy

Similarities in the muscarinic receptor binding activities of anticholinergic agents used clinically to treat OAB has been reported between rat and human tissues, [14] suggesting negligible differences in the tissue sensitivity of muscarinic receptors between rodents and humans. Using the muscarinic receptor binding activity of mirabegron in the rat bladder and its pharmacokinetic parameters in humans, Yamada et al. [31] estimated human plasma unbound and urinary unbound drug concentrations at clinical doses, from which human bladder muscarinic receptor occupancy was predicted. The absorption of the clinical dose of mirabegron (50 mg/day) after its oral administration to the elderly was rapid, with a maximum plasma concentration of approximately 85 nM and a time to reach T_{max} of 3-4 h. [37] In healthy Japanese male subjects, the mean elimination half-life ($t_{1/2b}$) was 25.1-36.4 h, [53] which was consistent with the range observed in non-Japanese males following single- (27.9-40.6 h) and multiple-dose administration (29.2-36.8 h) in those previous studies. [37,54] Mirabegron accumulated upon once-daily dosing relative to single-dose data. Furthermore, the oral administration of [^{14}C]-labeled mirabegron to rats elevated tissue: plasma radioactivity levels in some organs, with ratios increasing to 20 after its repeated administration, and it was then slowly eliminated from a number of tissues, including the kidney. [23] Pharmacokinetic parameters suggest that urinary mirabegron is significantly concentrated by active tubular secretion and water reabsorption in addition to renal glomerular filtration. The bladder tissue concentration of

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mirabegron was markedly higher than its plasma concentration, while its urinary concentration in the elderly after a single 50 mg dose was predicted to be in the micromolar (mM) range, suggesting that higher concentrations of mirabegron are excreted in the urine after repeated administration. As shown in Figure 4, human urinary unbound concentrations (1.6-8.2 mM) of mirabegron at a clinical dose (50 mg) were significantly (approximately 400-fold) higher than plasma unbound concentrations. [31] As mentioned above, species difference in muscarinic receptor binding affinity of mirabegron in the bladder between rats and humans is small. Based on the assumption of the interstitial concentration of mirabegron in the bladder smooth muscle is close to its urine concentration, the estimation of muscarinic receptor occupancy in the human bladder by mirabegron was performed. According to the predictions by the pharmacokinetics and micromolar receptor binding affinity of mirabegron, muscarinic receptor occupancy was estimated to be 37-76% in the bladders of elderly subjects who received a single 50-mg dose of this drug. [31] Therefore, it is hypothesized that mirabegron excreted in human urine blocks directly muscarinic receptor-mediated functions in the bladder, possibly by simple diffusion across the urothelium during urine storage, contributing to the therapeutic and adverse side effects of this agent.

6. Conclusion

Polypharmacy is a significant concern in the medication and management by anticholinergic medication, particularly in older adults. Moreover, an age-related decline in the renal excretion and hepatic metabolism of drugs results in their accumulation in tissues and increases in the permeability of the blood-brain barrier, indicating a significant increase in the risk of anticholinergic adverse effects in the peripheral and central nervous system, such as cognitive impairment. Mirabegron, β_3 adrenoceptor stimulant clinically utilized to treat patients with OAB, exerts off-target effect on various functional molecules such as neurotransmitter receptors, drug metabolizing enzymes and transports. This drug was shown to antagonize muscarinic receptors that are distinct from its target molecule, the bladder β_3 adrenoceptor, by radioligand receptor binding assay of muscarinic receptors and functional assay in the rat tissues using organ bath method. Therefore, the relaxation of the detrusor muscle by mirabegron may be the result of antagonizing muscarinic receptors as well as activating b_3-adrenoceptors. The prediction by pharmacokinetics and micromolar receptor binding affinity of mirabegron estimated a significant amount of muscarinic receptor occupancy in the bladders of elderly subjects who received clinical dose of mirabegron. Mirabegron and antimuscarinics (solifenacin, imidafenacin) at low concentrations exerted additive effects on muscarinic receptor binding and on the relaxant responses of cholinergic detrusor muscle contractions, and these effects were considered to involve partly in the enhancement of not only their therapeutic effects in patients with OAB, but also anticholinergic adverse effects. The safety of combination therapy for elderly patients with OAB need to be carefully considered. Therefore, concomitant use of mirabegron and drugs having anticholinergic properties such as antimuscarinics may be responsible for the higher incidence of anticholinergic adverse event. Therefore, careful attention should be paid when drugs with anticholinergic effects are prescribed to elderly patients. An evaluation of scoring of the anticholinergic burden in polypharmacy may be clinically significant by using anticholinergic burden scales.

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