130

Otsuka A[1], Matsumoto R[1], Suzuki T[1], Yoshikawa S[2], Kobayashi R[2], Kanie S[2], Shinbo H[3], Kurita Y[3], Ozono S[1]
1. *Department of Urology, Hamamatsu University School of Medicine,* ***2.*** *Toray Industries, Inc. Pharmaceutical Research Laboratories,* ***3.*** *Department of Urology, Enshu Hospital*

COMBINATION EFFECT OF B3-ADRENOCEPTOR AGONIST AND MUSCARINIC RECEPTOR ANTAGONIST ON HUMAN DETRUSOR MUSCLE RELAXATION IN VITRO

Hypothesis / aims of study

Many clinical studies have proven that muscarinic receptor antagonists are the most effective pharmacological therapy for overactive bladder (OAB). However, their efficacies have been sometimes limited relieving urgency or urge urinary incontinence in spite of good tolerability and little serious adverse events. Recently, selective β_3-adrenoceptor (AR) agonists have been developed as novel therapeutic agents to treat OAB. As these two agents have different mechanisms of action on relaxing human detrusor muscles, combination therapy of these agents may be considered as an optical pharmacological treatment in case of insufficient efficacy with each monotherapy.

Thus, the aim of this study was to evaluate whether combination of a selective β_3-AR agonist and a muscarinic receptor antagonist was more effective than each agent alone in inhibiting pharmacologically induced contractions of isolated human detrusor strips *in vitro*.

Study design, materials and methods

Human urinary bladder specimens were obtained from patients undergoing total cystectomy for bladder carcinoma. After removal of the urothelium and connecting tissues, the specimens were cut into longitudinal detrusor strips measuring approximately 15×5×5 mm. Detrusor strips were suspended in 10mL organ baths containing pre-gassed Krebs solution. A resting tension of the detrusor strip was 0.5g and isometric tension was recorded.

In the first series of experiments, we examined the additional relaxant effect of one-shot administration of TRK-380 on precontracted detrusor strips with 10^{-6} M carbachol in the presence or absence of propiverine. After precontraction with carbachol had reached a plateau, the detrusor strips were incubated with 3×10^{-6} M propiverine or vehicle for 20 min, following an administration of 10^{-6} M TRK-380 or vehicle for 20 min. At the end of the experiment, 10^{-5} M forskolin was added to obtain the maximal relaxation (100%).

In the second series of experiments, either 40 mM KCl or 10^{-6} M carbachol were used as a contractile agent. After precontraction with KCl or carbachol had reached a plateau, detrusor strips were incubated for 30 min with several different concentrations of propiverine, tolterodine, or vehicle (control). Then, concentration-response curves (CRCs) to TRK-380 were constructed at approximately 10-min intervals in the concentration range of 10^{-9} to 10^{-4} M. After cumulative application of TRK-380, 10^{-5} M forskolin was added to the organ bath.

In the third series of experiments, 10^{-6} M carbachol were used as precontractile agents. After precontraction with carbachol had reached a plateau, detrusor strips were incubated for 30 min with several different concentrations of TRK-380 or vehicle (control). Then, CRCs to either propiverine or tolterodine were constructed at approximately 10-min intervals in the concentration range of 10^{-9} to 10^{-4} M. After cumulative application of either propiverine or tolterodine, 10^{-5} M forskolin was added to the organ bath.

Maximal relaxation response (E_{max}) and pEC_{50} value were statistically compared. A *p* value of less than 0.05 was considered statistically significant.

Results

One-shot administration of either propiverine (3×10^{-6} M), TRK-380 (10^{-6} M), or combination of both exerted the relaxant effects on carbachol-induced contraction. Further, combination had significantly greater relaxant effects in E_{max} than vehicle (control), propiverine, or TRK-380.

Preincubation of propiverine (3×10^{-6} M, 10^{-5} M) or tolterodine (10^{-5} M) decreased the basal tone of KCl-induced contractions before cumulative administration of TRK-380. The presence of either propiverine or tolterodine concentration-dependently caused a parallel leftward shift of the CRC to TRK-380 on KCl-induced contraction. The pEC_{50} value showed significant difference between control and preincubation of either propiverine (10^{-5} M) or tolterodine (10^{-5} M); however, there were no significant differences of the E_{max} between control and preincubation of either propiverine or tolterodine.

Preincubation of propiverine (10^{-6} M, 10^{-5} M) or tolterodine (10^{-8} M, 10^{-7} M) decreased the basal tone of carbachol-induced contractions before cumulative administration of TRK-380. The presence of either propiverine or tolterodine concentration-dependently caused a parallel leftward shift of the CRC to TRK-380 on carbachol-induced contraction. The pEC_{50} value showed significant difference between control and preincubation of either propiverine (10^{-6} M) or tolterodine (10^{-8} M); also, there were significant differences of the E_{max} between control and preincubation of either propiverine (10^{-6} M, 10^{-5} M) or tolterodine (10^{-9} - 10^{-7} M).

Both propiverine and tolterodine produced concentration-dependent relaxation of the human detrusor strips precontracted with carbachol. Preincubation of TRK-380 (10^{-6} M, 10^{-5} M) decreased the basal tone of carbachol-induced contractions before cumulative administration of either propiverine or tolterodine. The presence of TRK-380 concentration-dependently caused a parallel leftward shift of the CRC to either propiverine or tolterodine. The pEC_{50} value of CRCs to either propiverine or tolterodine showed significant difference between control and preincubation of TRK-380 (10^{-6} M in propiverine, or 10^{-6} M, 10^{-5} M in tolterodine); however, there were no significant differences of the E_{max} of either propiverine or tolterodine between control and preincubation of TRK-380.

Interpretation of results

The present study provides the first experimental evidence that the combination of β_3-AR agonist and muscarinic receptor antagonist exerts an additional relaxant effect on human detrusor muscles *in vitro*. These findings indicate that the combination therapy of these agents may have clinical benefit to treat OAB patients with insufficient efficacy by monotherapy alone.

When OAB symptoms persist after initial pharmacological therapy with a muscarinic receptor antagonist, switch to another antimuscarinic agent or flexible dose escalation may be recommended for further treatment of OAB patients [2]. However, the latter therapy may also increase apprehensions in regard to the adverse events such as dry mouth, constipation and blurred vision [3]. Then, combination therapy of β_3-AR agonist and muscarinic receptor antagonist may have more augmented efficacy than monotherapy alone with little apprehensions in regard to the adverse events. Thus, we should purchase the benefit of combination therapy with β_3-AR agonist and muscarinic receptor antagonist in clinical practice.

Concluding message

This experimental finding suggests that the combination therapy of β_3-adrenoceptor agonists and muscarinic receptor antagonists could be more effective than monotherapy alone in relieving OAB symptoms, and provides the experimental support for clinical investigation of the combination therapy to treat patients with OAB. These results strengthen the concept that the combination therapy may become a novel therapeutic strategy for the treatment of OAB.

References

1. Urology (2012) 79;744.e1-7.
2. Expert Opin Pharmacother (2011) 12;1041-55.
3. BMC Urol (2010) 14;10:15.

Disclosures

Funding: This study is partially supported by a grant-in-aid from the Ministry of Education, Culture, Sports, Science and Technology, Japan (21791498, 23592365). **Clinical Trial:** No **Subjects:** HUMAN **Ethics Committee:** the ethics committee of Hamamatsu University School of Medicine **Helsinki:** Yes **Informed Consent:** Yes