135

Kumano S[1], Aizawa N[1], Ogawa S[1], Homma Y[2], Watanabe S[3], Takahashi N[3], Tajimi M[3], Igawa Y[1]
1. *Department of Continence Medicine, The University of Tokyo graduate School of Medicine, Tokyo, Japan,* ***2.*** *Department of Urology, The University of Tokyo graduate School of Medicine, Tokyo, Japan,* ***3.*** *RaQualia Pharma Inc., Aichi, Japan*

EFFECTS OF RQ-00311610, A NOVEL T-TYPE CALCIUM CHANNEL INHIBITOR, ON BLADDER FUNCTION AND BLOOD PRESSURE IN RATS WITH BLADDER OUTLET OBSTRUCTION

Hypothesis / aims of study

Both L-Type Ca^{2+} channels (L-Ca) and T-type of Ca^{2+} channels (T-Ca) have been found in human detrusor smooth muscle cells (1). The former are high voltage-activated and involve active contractions, whereas the latter are low voltage-activated and initiate spontaneous Ca^{2+} oscillations and spontaneous action potentials (1). In a previous report, overexpression of T-Ca on bladder tissue was shown at the mRNA level in rats with bladder outlet obstruction (BOO), and whole-cell patch-clump recordings showed that $NiCl_2$, a T-Ca inhibitor, blocked action potentials evoked by depolarization in bladder tissue isolated from BOO rats (2). T-Ca also distribute in dorsal root ganglionic (DRG) cells and contribute to neuronal excitability by initiating burst firing of action potentials of the DRG cells in rats (3). We hypothesized that T-Ca may have an important role in generating detrusor overactivity (DO) in BOO rats. We developed a novel T-Ca inhibitor, RQ-00311610 (RQ), and investigated the effects of RQ on bladder function and blood pressure in the BOO rats.

Study design, materials and methods

For the affinity investigation of ion channels, measurement of Ca^{2+} influx/ functional assay and binding assay with human or rat cultured cell and rat tissues were performed. For the *in vivo* study, female Sprague-Dawley rats were used. Under anesthesia with pentobarbital sodium (30 mg/kg intraperitoneally), a 3-0 nylon ligature was placed around the proximal urethra and an adjacent 1.1-mm diameter steel rod tied with urethra, and then steel rod was removed. Sham-operated (SHAM) rats underwent similar procedures without urethral ligation. Six weeks after surgery, the nylon suture around urethra was removed and a polyethylene catheter (PE-50) was implanted into the bladder. Two days after the implantation, under isoflurane anesthesia, intravenous catheter (PE-50) for drug-administration and intra-arterial catheter (PE-50) for blood pressure measurement were placed into left jugular vein and left carotid artery, respectively. Saline at room temperature was instilled into the bladder at a rate of 6 ml/hr in SHAM rats and at 18 ml/hr in BOO rats because of larger bladder in BOO rats. After waiting for stabilization of blood pressure and bladder pressure, RQ was administrated intravenously at 10 mg/kg. Mean blood pressure and each cystometric parameters were measured and analyzed before and after drug-administration for 30 minutes, and non-voiding contractions (NVCs) were defined as bladder contractions, the amplitudes of which were more than 3 cmH_2O, observed for 4 minutes before micturition.

Results

RQ has great selectivity for T-Ca compared with mibefradil and NNC 55-0396 that are conventionally used (Table 1). Five SHAM rats and 6 BOO rats were used. The bladder weight in the BOO rats was significantly greater than that in the SHAM rats (BOO: 879.8 ± 36.2 mg vs. SHAM: 240.5 ± 4.5 mg). After the administration of RQ, both voided volume and bladder capacity were significantly increased in the BOO rats but not in the SHAM rats. Neither number nor amplitudes of NVCs changed after the RQ-administration (Figure 1B and 2 A-D). None of the other cystometric parameters, such as basal pressure, threshold pressure and micturition pressure, changed significantly in either the SHAM or BOO rats after the RQ-administration (data not shown). After the RQ-administration, mean blood pressure was decreased in the both groups, and this response was more remarkable and significant in the BOO rats (Figure 2 E).

Interpretation of results

The results of the present study showed that the T-Ca inhibitor, RQ increased voided volume and bladder capacity without significant effects on NVCs in the BOO rats, but not in the SHAM rats. These results suggest that T-Ca may not have a major role in physiological control of bladder function in normal rats, but in rats with BOO and that T-Ca may contribute to modulation of the bladder mechanosensation as reflected in the increases in voided volume and bladder capacity. Further studies are needed to clarify whether upregulation of T-Ca in the afferent nerves contributes to these findings. The negligible effects of RQ on NVCs in BOO rats suggest that T-Ca on detrusor smooth muscle cells may not be directly involved in generating NVCs in BOO rats as the NVCs has been reported to be myogenic.

Concluding message

The present results demonstrate that a novel T-type calcium channel inhibitor, RQ-00311610, can increase voided volume and bladder capacity in BOO rats. These findings may give us a new insight of treatment of OAB associated with bladder outlet obstruction.

Table 1. Selectivity and affinity of each drug on ion channels

	RQ-00311610	Mibefradil	NNC55-0396
human T-Ca Ca^{2+} influx: IC_{50}	110 nM	330 nM	130 nM
rat T-Ca Ca^{2+} influx: IC_{50}	170 nM	210 nM	130 nM
rat brain L-Ca binding: IC_{50}	>10,000 nM	-	-
human N-Ca Ca^{2+} influx: IC_{50}	9,800 nM	580 nM	680 nM
rat N-Ca Ca^{2+} influx: IC_{50}	28,000 nM	790 nM	880 nM

Rat plasma protein binding	6.0%	0.8%	0.17%

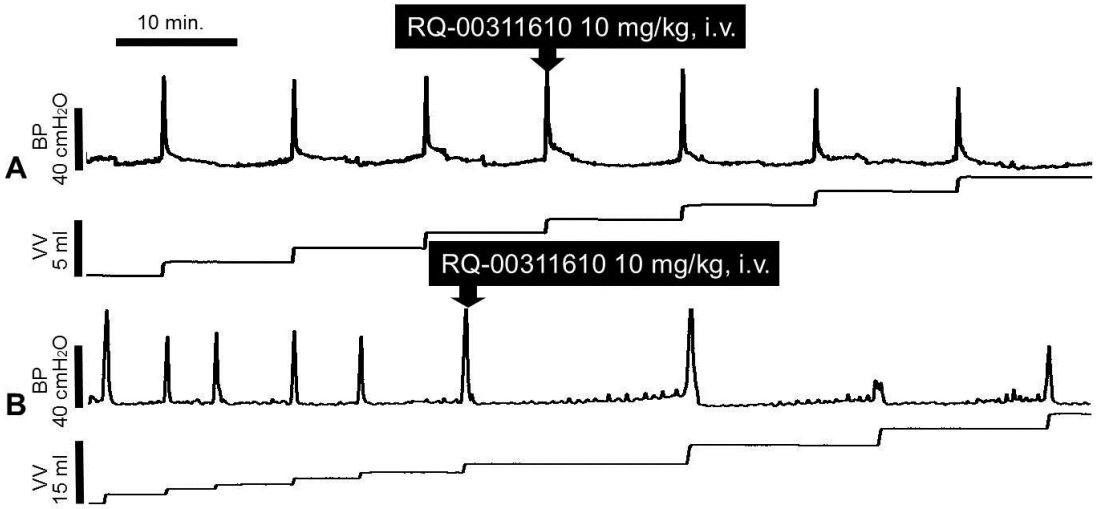

Figure 1. Bladder pressure (BP) and voided volume (VV) of a SHAM rat (A) and a BOO rat (B) during bladder filling with saline before (Base) and after RQ-administration.

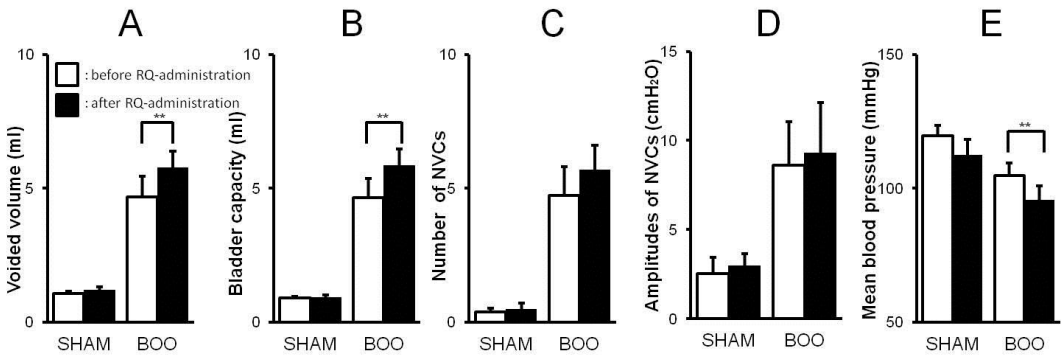

Figure 2. Responses to intravenous administration of RQ-00311610 on SHAM rats (left) and BOO rats (right). The values are expressed as mean ± S.E.M. (SHAM n=5; BOO n=6). ***p*<0.01: significant difference between before and after RQ-administration (paired Student's *t*- test).

References

1. Cell Calcium. 2009 May;45(5):456-64.
2. Neurourol Urodyn. 2007;26(6):870-8.
3. Proc Natl Acad Sci U S A. 1989 Sep;86(17):6802-6.

Disclosures

Funding: Research Grant from RaQualia Pharma Inc. **Clinical Trial:** No **Subjects:** ANIMAL **Species:** Rat **Ethics Committee:** Animal Ethics Committee, The University of Tokyo Graduate School of Medicine