

## TRK-380, A NOVEL $\beta_3$ -ADRENOCEPTOR (AR) AGONIST WITH POTENT AGONISTIC ACTIVITY, FUNCTIONALLY RELAXES DETRUSOR STRIPS IN SOME SPECIES INCLUDING HUMANS

### Hypothesis / aims of study

Although adrenergic  $\beta$  receptors ( $\beta$ -ARs) are now subclassified into  $\beta_1$ -,  $\beta_2$ - and  $\beta_3$ -ARs, involvement of  $\beta_3$ -ARs in the relaxation of detrusor has been reported to vary among species (1). In humans as well as monkeys and dogs, it has been demonstrated that the relaxation of detrusor smooth muscle is largely dependent on the activation of  $\beta_3$ -ARs on molecular biological and functional study basis (2, 3). Since  $\beta_1$ - and  $\beta_2$ -ARs have been known to be involved in the cardiovascular events in humans, selective  $\beta_3$ -AR agonists have been highlighted as one of candidate drugs for the treatment of overactive bladder (OAB). The aim of this study is to clarify the agonistic activity of TRK-380 for human  $\beta$ -ARs and the relaxing effect on the detrusor strips from humans, monkeys, dogs and rats.

### Study design, materials and methods

Agonistic activities of human  $\beta$ -ARs were investigated by using SK-N-MC cells ( $\beta_3$ ) and CHO cells expressing human  $\beta$ -ARs ( $\beta_1$  or  $\beta_2$ ). The cells were incubated with various concentrations of compounds and the amount of produced cAMP was measured by cAMP detection kit (PerkinElmer). The maximal response of Isoproterenol was expressed as 100 %, and the negative logarithm of EC<sub>50</sub>, pEC<sub>50</sub>, was calculated from the concentration-response curve of each compound. The detrusor strips of rats (male SD), dogs (male beagle), monkeys (male cynomolgus) and humans (3 patients undergoing total cystectomy due to bladder cancer) were mounted in 10 mL organ bath filled with Krebs-Henseleit solution, which was gassed with 95% O<sub>2</sub> and 5% CO<sub>2</sub>. The preparations were equilibrated for at least 60 min after the establishment of an initial tension of 0.5 g; TRK-380 was then added cumulatively to the organ bath, and concentration-response curves were obtained. The maximal response induced by forskolin (10  $\mu$ M) was expressed as 100 % relaxation. pEC<sub>50</sub> value of each compound was calculated from its concentration-response curve.

### Results

TRK-380 exhibited the agonistic activity for human  $\beta_3$ -ARs, which was almost equal to that of Isoproterenol known to be as a potent and non-selective  $\beta$ -AR agonist. On the other hand, it showed weak or no agonistic activity for human  $\beta_2$ -ARs or  $\beta_1$ -ARs, respectively (Table 1). In isolated detrusor strips, TRK-380 showed a concentration-dependent relaxing effect on the resting tension of human, monkey, dog or rat detrusor with pEC<sub>50</sub> of 7.33 $\pm$ 0.10, 7.34 $\pm$ 0.11, 6.92 $\pm$ 0.11 or 6.27 $\pm$ 0.11, respectively (Fig. 1).

### Interpretation of results

In functional assays with cells expressing each human  $\beta$ -AR and detrusor strips, it was demonstrated that TRK-380 had a potent agonistic activity for human  $\beta_3$ -ARs and that it could functionally relax the human detrusor strips; these activities were almost equal to those of Isoproterenol. In addition, the relaxation response of human detrusor strips by TRK-380 was confirmed to be similar to those of monkeys and dogs rather than that of rats.

### Concluding message

These data suggest that TRK-380 may be a promising compound for the treatment of OAB.

Table 1 Agonistic activity for human  $\beta$ -ARs and the ratio of each compound to Isoproterenol

Compound	$\beta$ 1-ARs		$\beta$ 2-ARs		$\beta$ 3-ARs		Ratio	
	pEC <sub>50</sub> (ratio to Isoproterenol)	Max Res. (%)	pEC <sub>50</sub> (ratio to Isoproterenol)	Max Res. (%)	pEC <sub>50</sub> (ratio to Isoproterenol)	Max Res. (%)	$\beta$ 1/ $\beta$ 3	$\beta$ 2/ $\beta$ 3
Isoproterenol	8.52 ± 0.08 (1.00 ± 0.00)	100.0 ± 0.0	8.52 ± 0.12 (1.00 ± 0.00)	100.1 ± 0.3	6.81 ± 0.08 (1.00 ± 0.00)	100.0 ± 0.0	1.0	1.0
TRK-380	<5 (>2490)	N.D.	5.91 ± 0.08 (419 ± 91)	46.8 ± 3.1	6.83 ± 0.17 (1.00 ± 0.21)	98.3 ± 7.9	>2400	420
Denopamine	6.97 ± 0.10 (36.1 ± 1.8)	84.9 ± 2.9	— (—)	—	— (—)	—	—	—
Procaterol	— (—)	—	8.66 ± 0.06 (0.841 ± 0.281)	82.2 ± 9.7	— (—)	—	—	—
BRL-37344	6.01 ± 0.05 (327 ± 26)	55.7 ± 3.7	6.83 ± 0.06 (57.2 ± 19.2)	50.7 ± 6.5	6.18 ± 0.14 (4.44 ± 0.72)	46.7 ± 6.0	74	13
CL-316,243	<5 (>2490)	N.D.	<5 (>1850)	N.D.	5.00 ± 0.14 (66.1 ± 9.6)	54.9 ± 2.1	>37	>27

—: Not applicable, N.D.: Not determined. Data represents as average ± s.e.m. of three independent experiments.

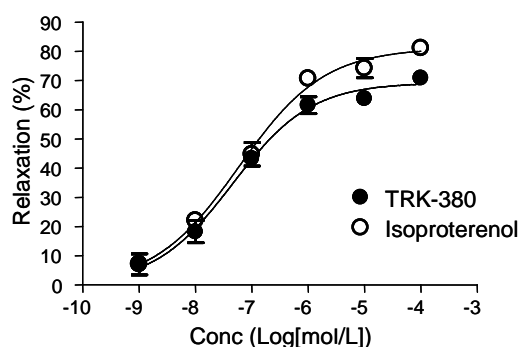


Fig. 1 Effects of TRK-380 and Isoproterenol in resting tension of isolated human detrusor strips (N=3)

#### References

1. Yamazaki Y. et al., Br J Pharmacol (1998) 124: 593-9
2. Yamaguchi O., Urology (2002) 59: 25-9
3. Igawa Y, et al., Br J Pharmacol (1999) 126: 819-25

<b>Specify source of funding or grant</b>	<b>NONE</b>
<b>Is this a clinical trial?</b>	<b>No</b>
<b>What were the subjects in the study?</b>	<b>HUMAN</b>
<b>Was this study approved by an ethics committee?</b>	<b>Yes</b>
<b>Specify Name of Ethics Committee</b>	<b>HUMAN SUBJECTS: The ethical committee of Hamamatsu University School of Medicine; ANIMAL SUBJECTS: The ethical committee of Research &amp; Development Division, Toray Industries, Inc.</b>
<b>Was the Declaration of Helsinki followed?</b>	<b>Yes</b>
<b>Was informed consent obtained from the patients?</b>	<b>Yes</b>