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Palea S[1], Guilloteau V[1], Brandolini L[2], Guardia M[1], Rollet K[1], Guerard M[1], Bianchini G[2], Lluel P[1], Aramini A[2]
***1.** URObsphere, **2.** Dompé S.p.A.*

DFL 14817, A NEW POTENT AND SELECTIVE TRPM8 ANTAGONIST FOR THE TREATMENT OF URINARY BLADDER DISORDERS

Hypothesis / aims of study

Transient receptor potential ion channel melastatin subtype 8 (TRPM8) is activated by cold and cooling agents such as menthol and icilin. The discovery of TRMP8 in urinary bladder led to the hypothesis that this receptor could be linked to the bladder cooling reflex, a clinical test for inducing the micturition reflex by instillation of cold saline into the bladder of patients. This reflex is negative in normal adults but may be unmasked in pathological situations. More recently, it was found that intravesical instillation of menthol facilitated the micturition reflex in conscious rats. Taken together, these findings strongly support the hypothesis that TRPM8 antagonists could be useful to block exaggerated afferent stimulation in pathological situations, therefore suppressing bladder overactivity.

Our effort to find small molecule TRPM8 antagonists started with a high-throughput screen of our in-house library that led to a number of chemical series, the most promising of these was the naphthalene series. Optimization of this chemical scaffold led to the discovery of some novel selective TRPM8 antagonists. The aim of this study was to compare a new selective TRPM8 antagonist (DFL14817) with compounds known from the literature, namely compound 5 from J&J (Cpd 5) as well as BCTC, previously described as a mixed TRMP8-TRPV1 antagonist. These molecules were tested intravenously and intravesically in the isovolumetric model in anesthetized rats, a simple model to evaluate the efficacy of drug candidates to decrease bladder overactivity.

Study design, materials and methods

TRPM8 channel inhibition was quantified with the Ascent Fluoroskan assay by measuring Ca^{2+} influx induced by TRPM8 agonists like icilin (30 □M) or capsazepine (50 □M) on recombinant receptors expressed in HEK293 cells. Then, ionomycin 1 □M (to induce the maximum intracellular signal), EGTA 6 mM (to induce the minimum intracellular signal) and $CaCl_2$ 8mM (for final induction of intracellular signal) were added to cells, respectively, and Ca^{2+} mobilization was measured.

For *in vivo* studies, adult female Wistar rats were anesthetized with urethane. Ureters were ligated and sectioned. A catheter was inserted through the urinary meatus into the bladder before urethral ligature. Compounds were administered by intravenous (iv) or intravesical (ives) routes. For i.v. administration, the bladder was filled in steps of 0.1 ml of saline every 5 min until the occurrence of rhythmic bladder contractions (RBC). After a 30 min control period, DFL14817 (10 mg/kg), BCTC (0.1, 0.3 and 1 mg/kg) or their vehicles were administered, then the effect of drugs was followed for 60 min. For each group, Micturition frequency (MF), Amplitude of Micturition (AM) and time of RBC inhibition were measured. For i.ves. administration, first the bladder was filled, 3 times every 5 min, with 100 µL of DFL14817 (0.3mg and 2.268 mg), or Cpd 5 (0.3 and 1.5 mg) or vehicle, then with 100 µL of saline every 5 min until the occurrence of RBC. A maximal volume of 3 mL was infused. For each group, Threshold Volume (mL) was measured. Statistical analysis was performed by Student *t*-test and one-way ANOVA.

Results

DFL14817 was identified as the most potent and selective TRPM8 antagonist in the naphthalene series with an IC_{50} = 4 nM. For comparison, IC_{50} values for Cpd 5 and BCTC were 10 nM and 540 nM, respectively.

DFL14817, Cpd 5 and BCTC, each at 10 µM, were inactive versus M2, M3 muscarinic, TRPA1 and TRPV4. However, as previously reported, BCTC was quite potent at TRPV1 (IC_{50} = 19 nM) whereas DFL 14817 and Cpd 5 were inactive.

Intravenous administration: After DFL14817 (10 mg/kg, i.v.) administration, the inhibition time of RBC was significantly higher than inhibition time observed after vehicle administration (526±106 *versus* 204±63 sec) ($p<0.05$ unpaired Student *t*-test, Table 1). DFL14817 significantly decreased MF 0-30 min post-administration and was without effect on AM (data not shown). Intravenous administration of vehicle did not modify MF and AM (data not shown). BCTC significantly increased the inhibition time only at the higher dose compared to vehicle ($p<0.01$ one way ANOVA, Table 1). BCTC significantly reduced MF from a dose of 0.3 mg/kg and did not modify AM (data not shown).

Table 1: Effects of intravenous administration of BCTC, DFL14817 and their vehicles on time of inhibition in anesthetized female rats.

Exp. group (i.v. route)	BCTC Vehicle N=6	BCTC 0.1 mg/kg N=6	BCTC 0.3 mg/kg N=6	BCTC 1 mg/kg N=6	DFL14817 Vehicle N=8	DFL14817 10 mg/kg N=8
Time of inhibition (sec)	198±83	560±50	730±162	1762±463^{++}	204.4±63	526±105 $^{\$}$

++ $p<0.01$ *versus* vehicle group; one way ANOVA followed by Newman-Keuls test
$ $p<0.05$ *versus* vehicle group; unpaired Student *t*-test

Intravesical administration:

When DFL14817 was administered at 0.3 mg/rat i.ves., RBC occurred in 4 out of 5 rats. DFL14817 at 2.268 mg/rat abolished the occurrence of RBC. Indeed, the maximal volume of filling (3 mL) was reached without the occurrence of RBC. Compared to

vehicle, the threshold volume after DFL14817 was significantly higher at the two doses tested ($p<0.01$ one way ANOVA, Table 2). Cpd 5 also significantly increased ThV compared to vehicle but only at a dose of 1.5 mg/rat ($p<0.05$ one way ANOVA, Table 2).

Table 2: Effects of intravesical administrations of DFL14817, Cpd 5 and the common vehicle on ThV in anesthetized female rats.

Exp.Group (i.ves. route)	Vehicle N=8	DFL14817 0.3 mg/rat N=5	DFL14817 2.298 mg/rat N=7	Cpd 5 0.3 mg/rat N=4	Cpd 5 1.5 mg/rat N=8
ThV (mL)	0.70±0.09	1.94±0.4**	3.0 ***	0.8±0.07	1.56±0.34*

*p<0.05 ** p<0.01 *versus* vehicle group; one way ANOVA followed by Newman-Keuls test

Interpretation of results

Our results show that BCTC and DFL14817 reduced RBC frequency when administered intravenously, whereas both Cpd 5 and DFL14817 inhibited the occurrence of RBC in a dose-dependent manner when administered intravesically. Interestingly, DFL14817 seems more potent than Cpd 5 after i.ves administration. These results suggest that DFL14817 inhibited the bladder afferent pathway. The fact that DFL14817 i.v. had no effect on AM suggests that it selectively affects the afferent arm of micturition reflex with no action on the efferent pathway. This finding is important since it suggests that DFL14817 would not induce urinary retention in the clinic, a dangerous side-effect for a medical treatment of a chronic condition, like urinary incontinence.

Concluding-message

Selective and potent TRPM8 antagonists are promising as a new pharmacological treatment for overactive bladder disorders.

Disclosures

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