

PRE-CLINICAL PHARMACOLOGY OF RO1151240, A SELECTIVE ALPHA1A/L-ADRENOCEPTOR PARTIAL AGONIST BEING DEVELOPED FOR THE TREATMENT OF STRESS URINARY INCONTINENCE

Aims of Study

RO1151240 (N-[6-chloro-3-(4,5-dehydro-1-H-imidazole-2-yl-methoxy)-2-methylphenyl]-methanesulfon-amide HCl) is a selective $\alpha_{1A/1L}$ -adrenoceptor (AR) *partial* agonist being developed for the treatment of stress urinary incontinence. RO1151240 was specifically designed as a partial agonist to minimise effects on the vasculature while retaining effects on urethral and bladder neck smooth muscle.

In order to demonstrate the partial agonism of RO1151240, the $\alpha_{1A/1L}$ -AR full agonist amidephrine was used as a reference standard in pre-clinical studies.

Methods

In Vitro:

Radioligand Binding: The affinity (pK_i) of RO1151240 was determined using [3 H]-prazosin in membranes prepared from CHO-K1 cells expressing human recombinant α_{1A} -, α_{1B} - and α_{1D} -ARs. Assays were conducted in physiological buffer at 37° C.

Functional Assays: Inositol-phosphates accumulation and calcium-imaging (FLIPR) assays were used to determine the potency (pEC_{50}) of amidephrine and RO1151240 and the intrinsic activity of these agonists relative to noradrenaline at human recombinant α_{1A} -, α_{1B} - and α_{1D} -ARs expressed in CHO-K1 cells. Assays were conducted in physiological buffer using established protocols (*Br. J. Pharmacol.*, 1997, 121: 1127; Current Protocols in Pharmacology Online, 2002, John Wiley & Sons, Inc., Unit 9.2).

In Vivo: The effects of amidephrine and RO1151240 on urethral smooth muscle tone and blood pressure were evaluated in anaesthetised and conscious micropigs.

Anaesthetised Micropigs: Female Yucatan micropigs (18-36 kg) were anaesthetised with pentobarbital (12 mg/kg/hr) and instrumented with vascular cannulae for blood pressure (BP) measurements and compound administration. Following an abdominal incision, the ureters were externalised and the bladder was cannulated via the dome with a water-filled balloon cannula positioned in the proximal urethra. Non-cumulative, intravenous dose-response curves were constructed after administration of RS 42206 (300 μ g/kg, iv; non-selective α_2 -AR antagonist), propranolol (100 μ g/kg, iv) and chlorisondamine (200 μ g/kg, iv) and were primed with phenylephrine (10 μ g/kg, iv) to insure proper cannulae placement. Changes in intra-urethral pressure (IUP; cmH_2O) and BP (mmHg) were measured.

Conscious Micropigs: Female Yucatan micropigs (20-26 kg) were chronically instrumented under aseptic conditions. A strain gauge transducer was sutured onto the proximal urethra. The wire leads were tunnelled subcutaneously and secured on the pig's flank. Blood pressure was measured via a telemetry device whose cannula was inserted in the femoral artery. Non-cumulative, intravenous dose-response curves were constructed by administering compounds via a jugular vein vascular access port. Changes in urethral tension were expressed as a percentage of a phenylephrine prime (10 μ g/kg, iv) whereas absolute changes in diastolic BP (mmHg) were measured.

Results

In Vitro:

Radioligand Binding: The pK_i values (mean \pm SEM) of RO1151240 for human α_{1A} -, α_{1B} - and α_{1D} -ARs are 7.39 ± 0.07 , 5.80 ± 0.10 and 5.19 ± 0.07 , respectively (12 determinations from 2 separate studies). Thus, based on affinity, RO1151240 is approximately 30-fold selective for the α_{1A} -AR subtype.

Functional Studies: RO1151240 and amidephrine acted as selective α_{1A} -AR agonists in both inositol phosphates accumulation and FLIPR assays. The potency (pEC_{50}) and intrinsic activity relative to

noradrenaline were as follows (mean \pm SEM):

Amidephrine

- Inositol phosphates accumulation (n=4): $pEC_{50} = 6.27 \pm 0.12$, intrinsic activity = 1.01 ± 0.03
- FLIPR (n=19): $pEC_{50} = 7.48 \pm 0.02$, intrinsic activity = 0.98 ± 0.01

RO1151240

- Inositol phosphates accumulation (n=4): $pEC_{50} = 6.79 \pm 0.04$, intrinsic activity = 0.31 ± 0.02
- FLIPR (n=5): $pEC_{50} = 7.61 \pm 0.04$, intrinsic activity = 0.54 ± 0.01

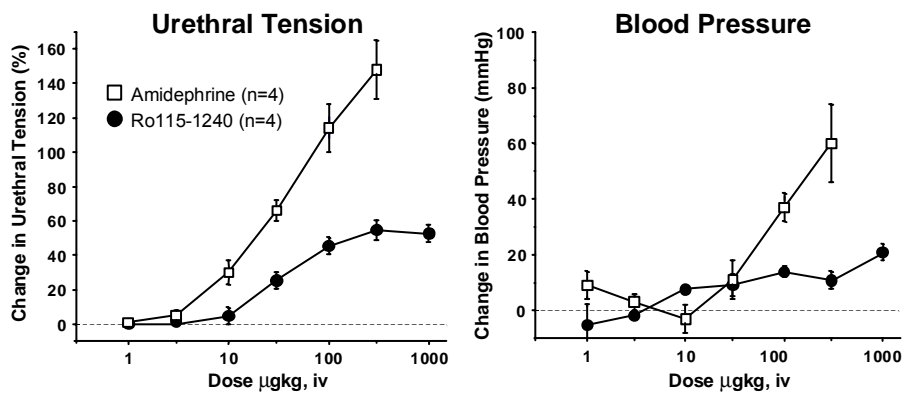
In Vivo:

Anaesthetised Micropigs: RO1151240 and amidephrine (n=4) produced dose-dependent increases in IUP and BP. The potency (ED_{50}) and maximal responses (MAX) were as follows (mean \pm SEM):

Amidephrine (n=4): IUP: $ED_{50} = 39.5 \pm 8 \mu\text{g/kg}$, MAX = $51.0 \pm 3 \text{ cmH}_2\text{O}$; BP: $ED_{50} = 39.1 \pm 5 \mu\text{g/kg}$, MAX = $95.3 \pm 8 \text{ mmHg}$

RO1151240 (n=4): IUP: $ED_{50} = 41.3 \pm 5 \mu\text{g/kg}$, MAX = $21.3 \pm 3 \text{ cmH}_2\text{O}$; BP: $ED_{50} = 35.6 \pm 6 \mu\text{g/kg}$, MAX = $33.0 \pm 5 \text{ mmHg}$.

Conscious Micropigs: The effects of amidephrine and RO1151240 on urethral tension and blood pressure are shown below. RO1151240 produced minimal effect on blood pressure at a doses (30-300 $\mu\text{g/kg}$) producing significant, dose-dependent elevations in urethral tension.



Conclusions

The present study details *in vitro* and *in vivo* studies for the selective α_{1A} -AR agonists RO1151240 and amidephrine. These data suggest the following:

- 1) α_{1A} -AR Agonists are able to increase both urethral and vascular smooth muscle tone in anaesthetised micropigs.
- 2) RO1151240 is a partial agonist relative to amidephrine and the endogenous neurotransmitter noradrenaline.
- 3) As a partial agonist, RO1151240 is capable of increasing urethral smooth muscle tone with minimal effects on blood pressure in conscious micropigs.

A compound with this profile may have therapeutic utility in patients with stress urinary incontinence.