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Salcedo C[1], Balsa D[1], Enrich A [1], Davalillo S [1], Pellicer T[1], Lagunas C [1], Catena J[1], Fernández-Serrat A[1], Farrerons C [1], Fernández A G[1]
1. Laboratorios SALVAT

SVT-40776, A NEW SELECTIVE M3 MUSCARINIC ANTAGONIST: HUMAN RECEPTOR BINDING PROFILE AND BLADDER EFFECTS IN THE GUINEA PIG

Aims of Study

Detrusor overactivity incontinence (OAB) is a highly prevalent condition characterised by an increased urinary frequency, urgency and urge incontinence. Muscarinic receptor antagonists are the most widely used therapeutic agents for OAB, but cardiovascular effects related with M_2 blocking can limiti their clinical use. The aims of the study was to determine the effect of SVT-40776, a novel substituted quinuclidine derivative with high M_3 receptor affinity, on the different human muscarinic receptors through radioligand binding assays and to evaluate its activity on the intravesical and arterial pressure in anaesthetised animals.

Methods

Binding Study: Membranes containing human muscarinic receptors (M_1-M_5) were obtained from Receptor Biology Inc. Competition studies: [^{3}H]-NMS binding to the membranes was carried out in 96-well filter plates, containing glass fiber type B at room temperature for 1 h. Radioligand binding to muscarinic receptors was determined in the absence and presence of increasing concentrations of antagonists. Non-specific binding was determined in the presence of 10 µM of atropine. At the end of the incubation time, the binding reaction was terminated by vacuum filtration using the Millipore vacuum manifold; filters were washed three times and dried. Scintillation liquid was added to the filters, and the retained radioactivity was quantitated in a scintillation counter (Microbeta, Wallac). The data points derived from the specific binding of [^{3}H]-NMS were analysed using a non-linear curve-fitting programme (GraphPad Prism®, GraphPad Software Inc.). Binding parameters were obtained as the best-fit values for the data using the least-squares method. Reagents: SVT-40776, oxybutynin, darifenacin, solifenacin and tolterodine were dissolved in DMSO to prepare a concentrated stock solution of 10 mM.

In vivo Study: Animals were anaesthetised by urethane 1.5 g/kg i.p.. A polyethylene catheter (PE-50) was implanted in the bladder via urethra and the bladder empted of urine. Carotid artery and jugular vein were cannulated to register arterial pressure (AP) and as administration route respectively. Bladder and carotid catheters were connected to pressure transducers (Transpac IV) and analysed using PowerLab® Software (ADI System). Through carotid, a baseline AP of 59.8 ± 1.7 mmHg was registered. Bladder was filled with 2.8 ml of saline to obtain a mean pressure of 58.5 (± 2.8) mmHg, which induced regular spontaneous contractions. After obtaining a stable response, the compound was administered by intravenous bolus followed by a cumulative consecutive dose-response protocol (15 min between doses or when stable contractions were obtained). AP was measured on the first 5 min post-dose periods. Amplitude from all bladder contractions (intravesical pressure) was measured during the 15 min period between doses and an amplitude mean calculated for baseline and for each dose. Percentage of variation was calculated vs. baseline effect. Reagents: Concentrated stock solutions of 10 mM were prepared for all the agents. SVT-40776 and solifenacin were diluted in saline. Oxybutynin and tolterodine were diluted in distilled water. Darifenacin was diluted in 10% DMSO in distilled water. Successive dissolutions were prepared with saline.

Results

Binding Study:

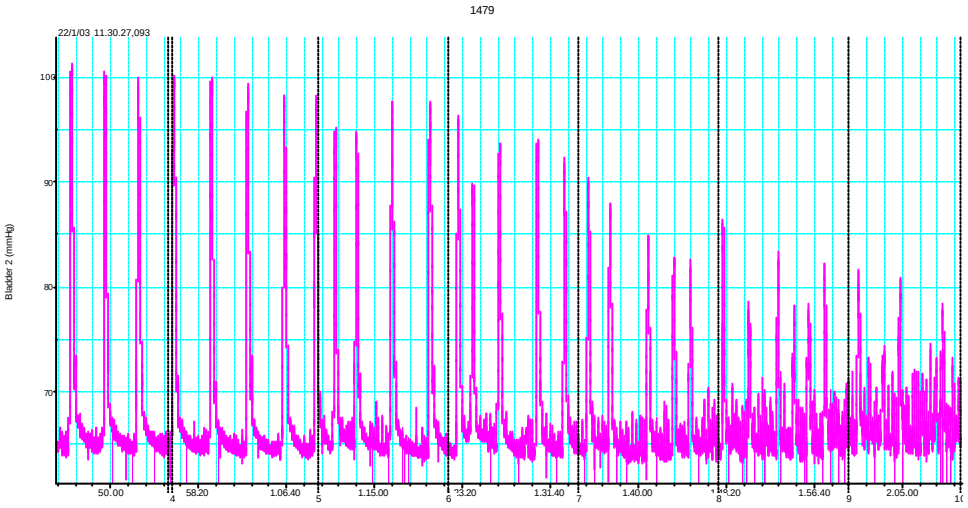
Table 1. Binding results at M_1-M_5 recombinat human muscarinic receptors

Compound	Affinity (Ki, nM)	Ratio

	hM_3	M_1/M_3	M_2/M_3	M_4/M_3	M_5/M_3
Oxybutynin	1.6 ± 0.3	2.4	5.7	1.2	3.3
Tolterodine	4.13 ± 1.7	0.6	0.5	0.4	0.6
Darifenacin	3.05 ± 0.2	16.6	29.1	6.9	2.4
Solifenacin	7.3 ± 1.4	0.3	5.9	0.3	0.7
SVT-40776	0.18 ± 0.09	2.0	213.7	1.5	2.1

In vivo Study:

Graph 1: Representative trace of dose-response inhibition of spontaneous bladder contractions by SVT-40776



Graph events: **4** = 1 nmols/kg; **5** = 3 nmols/kg; **6** = 10 nmols/kg; **7** = 30 nmols/kg; **8** = 100 nmols/kg; **9** = 300 nmols/kg.

Table 2. Comparative results in the guinea pig anaesthetised model.

Compound	**Intravesical Pressure Inhibition ED25** (nmol/kg i.v.)	**Arterial Pressure Increase ED25** (nmol/kg i.v.)
Oxybutynin	181.1	>3000
Tolterodine	299.2	820.4
Darifenacin	53.2	>1000
Solifenacin	200.9	>3000
SVT-40776	17.1	>3000

Conclusions

SVT-40776 exhibits high affinity, in the sub-nanomolar range, for the human M_3 muscarinic receptor, being, indeed, the most potent ligand among all the reference compounds assayed.

SVT-40776 shows the highest selectivity of human M_3 *versus* the M_2 subtype, among all the reference antagonists tested.

All these agents are able to inhibit spontaneous bladder contractions. SVT-40766 is the most potent compound inhibiting these contractions, at the very low doses of 17.1 nmols/kg i.v..

All this agents, apart of tolterodine, do not affect arterial pressure in this model even at the very high dose of 1000-3000 nmols/kg i.v..