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SVT-40776, SOLIFENACIN, DARIFENACIN AND TOLTERODINE COMPARATIVE ACTIVITY ON HERG CURRENT (IKR)

Hypothesis / aims of study

Antimuscarinic agents are the first line of treatment for overactive bladder (OAB) It has been suggested that sparing or affecting a specific muscarinic receptor has the potential to be valuable. Higher doses of current antimuscarinic drugs would provide a clinical benefit to patients, providing higher efficacy. Adverse events and heart effects have however limited the use of higher doses. A wider cardiovascular safety ratio, would allow the use of optimal doses in terms of efficacy. Actually the effects of non selective antimuscarinics on heart rate seem to be mediated by blockade of M_2 receptors (1). On the other hand, QT prolongation and concomitant Torsade de Pointes risk is linked to inhibition of the hERG potassium channel in the heart. Thus, it could be stated that the lack of relevant affinity at the M_2 and hERG receptor may be predictive of a safe cardiovascular profile (1).

SVT-40776, a novel selective M_3 receptor antagonist which has recently finished Phase II clinical trials, has shown an in vitro and in vivo M_3:M_2 selectivity of 251-fold and 69-fold respectively (2). The objective of this study was to determine the affinity of SVT-40776 and other antimuscarinic agents for the hERG ion channel transfected in human embryonic kidney (HEK-293) cell line and to establish the expected safety ratio.

Study design, materials and methods

Currents were measured using the whole-cell variant of the patch clamp method. An Axopatch 1-B amplifier (Axon Instruments, Foster City, CA) was used for whole-cell voltage clamping. Creation of voltage clamp pulses and data acquisition were controlled by an IBM PC running pClamp software (Axon Instruments). After rupture of the cell membrane, current kinetics and amplitudes were allowed to stabilize as the cell was dialyzed with internal solution and paced at 0.1Hz (typically 5-7 minutes) and at 3Hz (interpulse interval, pulse duration 400ms). Thus, rate-dependent effects were determined by a train of 20 depolarizing voltage steps at 3Hz from a holding potential of –75mV. Currents were considered stable if currents elicited by a series of voltage pulses given at 0.1Hz were superimposed. Peak hERG current was measured as the maximum outward deflection of the tail current elicited upon return to –40mV. The assay was run at 37ºC.

Experiments were performed in the following order:

Establish whole-cell configuration.
Cell dialyzed and allowed to reach steady-state conditions.
Wash in the first concentration of the drug.
Holding at –75mV pulse at 0.1Hz until steady state block is observed.
Wash in subsequent concentrations of drug.

Affinity for hERG ion channel was compared to affinity for the M3 receptor to determine the safety ratio.

Reagents

SVT-40776, solifenacin, darifenacin and tolterodine were synthesized by SALVAT Medicinal Chemistry Department. Stock solutions were dissolved in DMSO.

Results

Tolterodine, solifenacin, darifenacin and SVT-40776 dose-dependently blocked hERG current. The concentrations tested were in the range of 0.1 to 30 µM, 10µM. Fits of the dose-response data with a Hill equation yielded the IC_{50} values given in Table 1.

Table 1. Effects of tolterodine, solifenacin, darifenacin and SVT-40776 on the HERG channel, M_3 receptor and safety ratio.

Compound	hERG (IC_{50}; nM)	M_3 (Ki; nM) (1)	Safety Ratio
Tolterodine	26	4.1	6
Solifenacin	88.9	7.3	12
Darifenacin	276	3.1	89
SVT-40776	193	0.19	1,072

SVT-40776 did not presented additional rate-dependent blocking of hERG when pacing at 3 Hz when cells were exposed to 1µM.

Interpretation of results

A safety margin of 1072-fold was obtained for SVT-40776 when compared with its therapeutic activity blocking the M_3 receptor. SVT-40776 concentration dependently reduced hERG current amplitude at the concentrations tested with an IC_{50} of 193nM. In contrast, darifenacin, solifenacin, and tolterodine presented an IC_{50} of 276, 88.9 and 26 nM, obtaining a safety margin of 89, 12, and 6-fold vs. the M_3 affinity, respectively. This data referring tolterodine was previously reported (Ki = 17 nM) (3).

Concluding message

SVT-40776 is a selective M_3:M_2 muscarinic receptor antagonist with in vitro and functional selectivity for urinary bladder over cardiac tissues in the order of 200-fold, the highest window compared to the current treatments. Specifically for the hERG channel a safety margin of 1072-fold was obtained. This wide experimental safety window could provide to SVT-40776 a benefit in the

treatment of overactive bladder to avoid any risk of cardiac effect, allowing the use of optimal therapeutic doses to obtain higher efficacy than current antimuscarinic agents.

References
(1) BJU International (2007) 100; 1007-1014
(2) Br J Pharmacol (2002) 136; 45
(3) JPET (2004) 308; 935-940

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Is this a clinical trial?	**No**
What were the subjects in the study?	**HUMAN**
Was this study approved by an ethics committee?	**No**
This study did not require eithics committee approval because	**CELL IN VITRO STUDY**
Was the Declaration of Helsinki followed?	**No**
This study did not follow the Declaration of Helsinki in the sense that	**IS NOT REQUIRED AS HUMANS SUBJECTS WERE NOT USED**
Was informed consent obtained from the patients?	**No**