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1. SALVAT

BLADDER SELECTIVITY OF SVT-40776: A PHARMACOKINETIC / PHARMACODYNAMIC (PK/PD) ANALYSIS BASED ON PRECLINICAL AND CLINICAL STUDIES.

Hypothesis / aims of study

SVT-40776 is a new muscarinic antagonist exhibiting high selectivity for human M3 over M2 receptors [1, 2, 3]. The compound is currently on Phase II clinical testing for overactive bladder treatment. It is well known that the same muscarinic receptor subtype (M3) regulates both bladder contractibility and salivary flow production. This fact has hampered to obtain a tissue selective agent with marked bladder preferential activity. This also explains why one of the most common adverse reactions associated with muscarinic blockade in humans is dry mouth. SVT-40776 has shown a very high selectivity ratio (23-fold) between bladder and salivary gland in functional studies.

The aim of this study was to determine the effective plasma concentrations of SVT inhibiting detrusor contractibility in animals and to investigate if these plasma levels were able to induce dry mouth in human volunteers.

Study design, materials and methods

Effective dose in mice: Groups of animals received a single oral dose (0.3 to 50 mg/kg) of SVT-40776 or vehicle. Mice were sacrificed 3h later, detrusor strips were dissected and placed in 25 mL organ baths containing Krebs solution maintained at 37ºC and aerated with 95%O2/5%CO2. The Krebs solution routinely contained indomethacin (30 μM). Pure isometric transducers were used for all experiments. A pA2-equivalent dose (pA2-ED) was calculated using the oral doses instead of bath concentrations for each agent. Briefly, curves obtained from vehicle-treated animals were assigned as 'control curves' and the EC50 of carbachol (CCh) determined. Shifts on the EC50 of CCh in tissues coming from treated animals respect to control curves were calculated. Maximal tissue responses were determined using KCl (90 mM).

Effective plasma concentration in mice: SVT-40776 was administered orally at 0.5, 1, 5 and 10 mg/kg. Blood was collected at 3h post administration. Blood samples were centrifuged (1000 g; 4ºC; 10 min) and plasma analysed for SVT-40776 by LC-ESI/MS/MS.

Plasma protein binding: The binding of SVT-40776 to plasma proteins was evaluated *in vitro.* Blood was obtained from male CD-1 mice and healthy human subjects. SVT-40776 was prepared in ethanol and dried under N2. Plasma from the two species was added to yield final concentrations of 0.1 μg/mL. The protein binding of SVT-40776 was evaluated using an ultra-filtration method. The total and unbound fractions were determined by LC-ESI/MS/MS

PK and tolerability data in human volunteers: Total maximum plasma concentrations (Css,max,total) were determined in the steady state after multiple oral once daily doses of SVT-40776 (0.125, 0.25 and 0.5 mg) in 24 postmenopausal healthy volunteer females aged 50-70 years (6 active : 2 placebo per dose level). Css,max values were correlated with the occurrence of dry mouth.

Results

The obtained results are summarized in Tables 1 and 2. The effective oral dose of SVT-40776 (defined as pA2-ED) was 0.6 (0.4-1.1) mg/kg. The calculated effective total plasma levels at the same period of time (3h after administration) in mice were 1.3 ng/mL nM. SVT-40776 showed an extensive binding to plasma proteins (98.6 ± 0.1%) in mice. Thus, the calculated effective free plasma levels of SVT-40776 were 18 pg/mL (Table 1).

Two groups of volunteers were obtained according with their Css,max,total values: group 1: < 1.5 ng/mL; group 2: > 1.5 ng/mL. Css,max,free values were calculated considering that SVT-40776 was bound to human plasma proteins in a 97.6 ± 0.5 % (Table 2). Interestingly, no volunteer with Css,max,free lower than 36 pg/mL (range 8-36 pg/mL) reported dry mouth. Dry mouth only appeared at higher plasma concentrations (Table 2).

Table 1. Effective total and free plasma levels of SVT-40776 in mice (see text for details).

pA2-ED (mg/kg p.o.)	Total Plasma levels at pA2-ED (ng/mL)	Protein bound (%)	**Free Plasma levels at pA2-ED (pg/mL)**
0.6	1.3	98.6	**18**

Table 2. Relationship between plasma levels (Css,max) of SVT-40776 and reported dry mouth in human volunteers (see text for details).

	Group	Css,max,total (ng/mL)	Protein bound (%)	**Css,max,free (pg/mL)**	**# Total volunteers**	**# Volunteers with Dry Mouth**
PLACEBO	0	--	--	**--**	**6**	**0**
SVT-40776	1	< 1.5	97.6	**< 36**	**8**	**0**
	2	> 1.5	97.6	**> 36**	**10**	**6**

Interpretation of results

- Since M3 receptors are located in the cell membrane, free plasma levels are an excellent estimation of the amount of drug interacting with the therapeutic target.
- Efficacy data obtained in mice strongly suggest that the Effective Therapeutic Plasma Concentration Free ($ETPC_{FREE}$) in humans must be approximately 18 pg/mL.
- No cases of dry mouth were reported in groups 0 (placebo) and 1 (Css,max,free below 36 pg/mL). All volunteers belonging to group 1 were treated with daily doses of 0.125 or 0.250 mg of SVT-40776.
- Dry mouth episodes were only reported in volunteers with supratherapeutic plasma levels much higher than the expected ETPC.
- In addition to SVT-40776 selectivity, its reduced metabolism rate, its low intradaily fluctuation and its small inter-individual variations are probably contributing to its safety profile.

Concluding message

Predicted therapeutic doses of SVT-40776, obtained after PK/PD analysis, administered to human volunteers exhibit a dry mouth incidence similar to placebo.

This finding strongly indicates a promising clinical profile of SVT-40776 in terms of functional selectivity for bladder smooth muscle over salivary gland that must be confirmed in patients.

References

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