Trospium chloride has no effect on memory testing and is assay undetectable in the central nervous system of older patients with overactive bladder

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SUMMARY

Background: Muscarinic receptors in the brain play an important role in cognitive function, especially memory, and there is growing awareness that specific antimuscarinic drugs for overactive bladder (OAB) may have adverse central nervous system (CNS) effects. Selection of an antimuscarinic OAB drug with reduced potential for CNS effects could be especially beneficial in the elderly people, in whom even the modest cognitive impairment may negatively affect independence. Purpose: The purpose of the study is to determine if trospium chloride is assay detectable in the CNS of older adults with OAB and to assess whether deterioration of memory occurs in these individuals. Methods: Twelve cognitively intact older adults (≥65–75 years old) with OAB were given extended-release trospium chloride 60 mg once daily over a 10-day period to achieve plasma steady-state levels. Standardised memory testing (Hopkins Verbal Learning Test-Revised and Brief Visuospatial Memory Test-Revised) was performed predose and postdose. Cerebrospinal fluid (CSF) and plasma samples were drawn on day 10 and assayed for trospium chloride. Results: Trospium chloride levels in all the CSF samples (n = 72) of all participants were assay undetectable (<40 pg/ml) on day 10 at steady-state peak plasma concentration, and was not assay detectable in human CSF concurrent with simultaneous clinical cognitive safety measures. Repeat memory testing revealed no significant net drug effect on learning or recall. Conclusions: This is the first study to investigate for the presence of an orally dosed OAB antimuscarinic in the human brain, performed by assaying for concentrations of trospium chloride and correlating with simultaneous clinical cognitive safety measures in older adults. Specifically, ER trospium chloride was not assay detectable in human CSF concurrent with peak steady-state plasma concentration, and concomitantly showed no decline in performance on tests of learning and memory over a 10-day period.

What’s known
Evidence suggests that the treatment for OAB with muscarinic receptor antagonists may result in clinically important impairment of cognition. On the basis of the quaternary amine structure and the corresponding cationic charge, it has been predicted that trospium chloride would not be able to cross the human blood–brain barrier (BBB).

What’s new
This is the first study to investigate for the presence of an orally dosed OAB antimuscarinic in the human brain, performed by assaying for concentrations of trospium chloride and correlating with simultaneous clinical cognitive safety measures in older adults. Specifically, ER trospium chloride was not assay detectable in human CSF concurrent with peak steady-state plasma concentration, and concomitantly showed no decline in performance on tests of learning and memory over a 10-day period.

Introduction

The mainstay of pharmacological treatment of the overactive bladder syndrome (OAB) is the anticholinergic therapy using muscarinic receptor antagonists (tertiary or quaternary amines) (1). Muscarinic receptors in the brain play an important role in cognitive function, especially memory, and there is growing awareness that specific antimuscarinic drugs for OAB may have adverse central nervous system (CNS) effects (2,3). Therefore, selection of an antimuscarinic OAB drug with reduced potential for CNS effects could be especially beneficial in the elderly people, in whom even the modest cognitive impairment may negatively affect independence (2–4).

The current state of the art for assessing cognitive changes is neuropsychological testing. In response to multiple case reports concerning the negative CNS effects of specific antimuscarinic agents for OAB (5–7), validated neuropsychological tests have been used to study cognition with this class of agents (8,9). However, given the lack of sensitivity of neuropsychological testing, the issue of drug penetration into the CNS has still not been fully addressed.

The primary blood–brain barrier (BBB) defends integrate both physiochemical barriers and molecular
Subjects and Methods

Overall research design
The trial was a single-centre, non-comparative, prepost dose intervention phase IV study (NCT00863551), and it was approved by an institutional review board (Aspire IRB, La Mesa, CA, USA). All subjects gave written informed consent to participate in the study.

Diagnosis and subject selection criteria
Fourteen subjects were recruited via advertising, screened and 12 met all of the following inclusion criteria: men or women 65–75 years of age, within ±20% of ideal body weight for height and frame size and weighing <90 kg; non-smoker (no tobacco products for 6 months before the screening visit); stable health, based on history, physical examination, vital signs, stable 12-lead electrocardiogram and clinical laboratory tests; serum creatinine ≤2.0 mg/dl; OAB syndrome (minimum of 8 or more toilet voids and 1 urinary urgency event per day); Folstein Mini-Mental State Examination score ≥25; 20 minimum HVLT-R score 1 standard deviation below the mean for subject’s age (15). One subject failed screening – subject 13 (abnormal ECG), and one (subject 4) withdrew consent before initiation of study (withdrew consent before initiation because of the concern that study was too invasive). No study subject withdrew from the study once dosed with Sanctura XR 60 mg (Allergan, Irvine, CA, USA) daily.

Participants received extended release (ER) trospium chloride 60 mg (Allergan, Lot No. 0800807) once daily with 240 ml of water fasted, 1 h before a meal, at home, for 9 days (to achieve steady-state) and were then admitted to the test facility. On day 10, following an overnight fast, the final dose of trospium chloride was administered. Pill count was used to measure medication compliance. Cerebrospinal fluid (CSF) samples were obtained by means of a spinal catheter, predose (0 h) on day 10 and 2, 5, 7, 12, and 24 h after the dose. Two millilitres of deadspace tubing volume was removed followed by a 2 ml volume of CSF for trospium chloride assay. Plasma samples were obtained predose on day 10 (0 h) and 5 and 24 h after the dose.

Cerebrospinal fluid and plasma samples were frozen and kept at approximately −20°C pending shipment on dry ice to Prevalere Life Sciences Inc. (Whitesboro, NY, USA) for assay. The samples were analysed using a validated turbo ion spray high performance liquid chromatography tandem mass spectrometric (LC/MS/MS) method with a lower limit of detection of 40 pg/ml. Trospium and the internal standard, clidinium, were extracted from human sample (0.2 ml) using solid-phase extraction, which employed a carboxypropyl bonded phase (CBA) cartridge. The adsorbed trospium and clidinium were eluted from the CBA cartridge using 1 ml of 0.1% HCL in methanol. After evaporation, the analytes were reconstituted in 0.2 ml of 50 : 50 methanol : water. Ten to 20 μl of the reconstituted sample was injected onto the LC-MS/MS system (Sciex [Sciex LLC, Foster City, CA, USA] API 3000 LC-MS with turbo ionspray ionisation). Trospium and clidinium were resolved on Zorbax Eclipse (Quantum Analytics, Foster City, CA, USA) XDB-C8 2.1 × 50 mm, 3.5 μm column using a mobile phase consisting of mixture of methanol : 5 mmol/l ammonium acetate (70 : 30 v/v) flowing at the rate of 0.075–0.4 ml/min. Trospium and clidinium were monitored in the positive ion MRM mode at transitions of 392.3/163.9 and 352.0/142.0 (m/z), respectively.

Memory testing was performed using both the Hopkins Verbal Learning Test-Revised (HVLT-R) and the Brief Visuospatial Memory Test-Revised (BVMT-R) (15,16). These instruments have been validated to measure verbal and visual memory in clinical research trials. The literature supports both the reliability, and construct validity of these tests as measures of learning and recall (7,17–19). The testing was performed at screening (to familiarise subjects with the test), day 0 (baseline measurement), day 9 (to control for potential effects of anxiety related to the spinal catheter and to minimise practice effects of repeated measurements) and day 10 (to correlate with CSF measurement). Training in memory test administration procedures and quality
assurance of memory test scoring were performed by the second author.

**Criteria for evaluation**

The assay detectable CNS penetration of trospium chloride and the associated memory effect were the criteria evaluated. Specifically, the concentration of trospium chloride in CSF day 10, 5 h postdose, (peak plasma steady-state) was the primary end-point measured to identify the quaternary amine penetration of the BBB. Plasma trospium chloride levels (0, 5 and 24 h postdose) served to confirm achievement of steady-state and also provided evidence of systemic absorption.

The change from baseline (day 0, predose) to steady-state trospium chloride drug levels (day 10, 5 h postdose) and the memory test finding were secondary study end-points. Memory testing was performed using the HVLT-R and the BVMT-R. For both of these measures, test administration involved three free recall learning trials (the sum of which constituted the total recall score) followed by a single delayed recall trial. The total recall score reflects the subject’s ability to learn, whereas, the delayed recall score provides a measure of recent memory.

**Statistical methods**

The primary and secondary clinical study results were analysed in a descriptive manner. For the primary end-point, pre and postdose trospium chloride CSF concentration, the numbers of patients involved was determined to achieve 80% power to detect a twofold difference. Memory testing was a secondary end-point, in which the trial was designed to detect clinically meaningful changes via the reliable change index.

For the memory testing, a reliable change index was utilised to assess the significance of changes in memory scores on repeat testing (19). For the HVLT-R total recall score, the change in score would need to exceed 6.43 to surpass the reliable change index (note: a change in score exceeding 6.43 would reach significance at a p < 0.05 considering the effects of practice and test-retest reliability) (18,19). The reliable change score for HVLT-R delayed recall is 3.23 (18,19). The absolute difference in raw score required to achieve the reliable change index for the BVMT-R at 0.05 significance is as follows:

- Total Recall: ≥ 6, Delayed Recall: ≥ 2 (16).

**Ethics**

The studies were performed according to the principles of the Declaration of Helsinki and its amendments and the principles of Good Clinical Practice. All subjects provided written informed consent prior to study participation. The clinical study protocols, protocol amendments, informed consent documents and other appropriate study-related documents were reviewed and approved by an Institutional Review Boards (IRB).

**Results**

**Participant characteristics**

The study subjects were primarily Caucasian (9 of 12, 75%). The gender distribution of the study population was five men and seven women. Subjects’ mean age was 69 years (median 68 years, range 65–74 years). The study subjects’ co-morbidities included: OAB (12), arthritis (3), myopia (3), insomnia (2), gastroesophageal reflux (2), chronic headache (2), hypertension (2), diabetes mellitus (1) memory loss (1), urinary tract infection (1), dyslipidaemia (1), constipation (1), depression (1), hearing loss (1), cataracts (1) and incontinence (1).

**Pharmacokinetic results**

The pharmacokinetic results are summarised in Table 1, which displays the summary statistics derived from the bio-analytical data for trospium in CSF and plasma. No measurable trospium (<40 pg/ml) was found in CSF at any sampling time (0, 2, 5, 7, 12, and 24 h postdose) in any subject (n = 12), including the primary end-point, day 10, 5 h postdose. The observed plasma levels document systemic absorption of ER trospium chloride and, based on two successive trough concentrations before and after the day 10 dose, that steady state had been achieved (Table 1). If steady-state trospium chloride had crossed the BBB, it would be expected that it would be assay detectable in the CSF.

**Cognitive test results**

Individual HVLT-R total and delayed recall scores at baseline and day 10 are displayed in Figures 1 and 2, respectively. By the reliable change score criteria described in the statistical methods above, none of the observed predose (day 0) to postdose (day 10) total or delayed recall individual HTLV-R scores indicates a significant decline in learning or memory on trospium chloride. One subject, no. 2 Figure 2, unexpectedly showed a significant improvement in delayed recall. In no subject was the numerical change from baseline in total recall score as large as 6.43, defined as the reliable change score for this measurement.

As a result of a procedural error in administration of the BVMT-R, the postdose test scores were considered invalid after undergoing quality assurance.
The BVMT-R results indicate marked improvement in BVMT-R total recall (learning) and delayed recall (memory), which is likely a result of exposure to the same designs on all three occasions; therefore, invalidating the results.

Safety results
Adverse events (AEs) were reported in 10 of the 12 subjects. In total, 27 AEs were reported. The maximum severity grade of these events was 'mild' in 19 of the events and 'moderate' in the remaining 8. No AEs were classified as severe or serious, and none required discontinuation from study drug. Five of the 27 reported AEs are considered to be possibly or probably related to study drug.

The most frequently reported AE was headache (seven subjects). The headaches were mild to moderate in all instances and likely unrelated to study drug. Headache occurred 1–3 days before \((n = 2)\), on day of \((n = 2)\) and 2–10 days after the lumbar puncture \((n = 3)\). Nausea, accompanied by emesis, was reported by three subjects. Other events occurred less frequently: constipation, dry mouth, flatulence and low back pain, each in two subjects; folliculitis, change in taste, increased appetite and anxiety, each in one subject. In every case, subjects were noted to have recovered from the events with little to no intervention.

Discussion
This is the first study to investigate for the presence of an orally dosed OAB antimuscarinic in the human brain, performed by assaying for concentrations of trospium chloride and correlating with simultaneous clinical cognitive safety measures in older adults. Specifically, ER trospium chloride was not assay detectable in human CSF concurrent with peak steady-state plasma concentration, and concomitantly showed no decline in performance on tests of learn-

| Cerebrospinal fluid | Mean ± SD (pg/ml) | Minimum (pg/ml) | Maximum (pg/ml) | Median (pg/ml) |
|---------------------|-------------------|----------------|----------------|---------------|
| Trough concentration, before day 10 dose | 522.2 ± 328.9 | 158 | 1205 | 496 |
| Concentration at presumed \(T_{\text{max}}\) (5 h postdose day 10) | 925.4 ± 477.7 | 312 | 1817 | 765 |
| Trough concentration, 24 h after day 10 dose | 648.8 ± 353.8 | 249 | 1488 | 537 |

CV = Coefficient of variation.
Tropism is undetectable in the older human CNS

The safety profile of tropism chloride recorded in this study population was satisfactory, and it was consistent with class effects and previously reported findings (21). The results of both pharmacological and memory tests support the hypothesis of a lack of significant CNS penetration for the quaternary amine tropism chloride.

Evidence suggests that the treatment for OAB with muscarinic receptor antagonists may result in clinically important impairment of cognition (2–4). Memory deficits with oxybutynin and tolterodine have been reported (5–7). Clinical studies have also compared the effects of antimuscarinic OAB agents on sleep physiology and quantitative electroencephalogram (qEEG) (22). Todorova et al. (22) compared tolterodine (2 mg twice daily), tropism (15 mg three times daily), oxybutynin (5 mg three times daily) and placebo on daytime EEG. Of the three agents, oxybutynin was found to significantly alter qEEG. Diefenbach and co-workers reported oxybutynin altered sleep architecture, and polysomnography specifically, oxybutynin was found to significantly increase the latency of rapid eye movement sleep latency. Tropism and tolterodine did not (23,24). Additional physiological findings of sedation, neuropsychiatric events (hallucinations, psychosis, concentration and orientation problems) have been associated with oxybutynin (25). As a result, the risks of drowsiness, hallucinations and dizziness have been incorporated into the prescribing information for oxybutynin (dittaran/oxbytyn XL on line) (26).

Central nervous system penetration by antimuscarinic agents is an important prerequisite for the assumed mechanism of cognitive disruption. The BBB, formed by the endothelial cells that line cerebral capillaries, plays an important role in maintaining the microenvironment for reliable neuronal signalling. In contrast to peripheral capillary beds, the brain endothelial cells have contiguous tight junctions between cells, lack fenestrations and exhibit very low levels of endocytosis/transcytosis (10). Consequently, molecular transport across the BBB is forced to take a transcellular route. Small gaseous molecules such as O₂ and CO₂ can diffuse freely across the lipid membranes of the endothelium, as can small, lipophilic agents such as ethanol. Selective transport of required nutrients is mediated by specific transport systems on the luminal and abluminal endothelial membranes, and numerous efflux transporters (e.g. P-gp) have been identified within the BBB that serve to extrude back into the circulation any unwanted substrates that have managed to enter into the epithelial cell (27).

The concentration of drug in the CNS is a function of the equilibrium between drug penetration of the BBB, efflux from the CNS and the pharmacokinetic parameters of drug distribution and metabolism. A drug’s ability to penetrate the CNS is based on molecular size, polarity and lipid solubility (11,12). The quaternary ammonium compounds are hydrophilic and polar and have been predicted to have a reduced ability to diffuse across the BBB. The permeability of the BBB can be increased by a number of conditions – including, increasing age (>45) (28), use of particular medications (29), comorbid diseases, stress (30) and P-gp transport dysfunction; P-gp drug–drug interaction and P-gp genetic polymorphism (31). Evidence now exists that tropism chloride is a P-gp ligand, and in addition to its hydrophilic qualities, the MDR1 efflux pump is involved in restricting the CNS access of tropism chloride but not oxybutynin (12,27). These CNS equilibrium determining variables, in aggregate, are unique, complex and difficult to predict on a per patient basis.

The advanced age and co-morbidities of the human subjects in this study provides evidentiary support that the quaternary amine tropism chloride is both assay undetectable in the CNS of human subjects at risk for increased BBB permeability and without detectable memory impairment. However, there are limitations to this study. The tropism chloride assay had a limit of detection of <40 pg/ml. To place this level into context, oxybutynin levels in animals have been reported 30- to 40-fold (300–400 pg/g) above the lower limit of detection for the tropism assay (27). Tropism levels below the limits of detection may exist. As the study was designed as a ‘proof of concept’, the sample size is small (n = 12), and the conclusion is a ‘negative’ finding. The conclusion that tropism did not significantly penetrate the CNS in the absence of a positive result for either detection of tropism in the CNS, or for detection of a significant cognitive change, would be strengthened by the inclusion of a positive control, which would be detectable in the CNS, and in addition have measurable cognitive effects. In addition, consistent with clinical practice, more research is required in patients with varying degrees of altered baseline cognition or dementia and more severe co-morbidities including neurological conditions. A patient population with cognitive impairment with and without dementia or other specific neurological conditions may perform differently in the presence of tropism chloride. The reliable change index was used to screen for pre and postdose changes in cognition, but not to necessarily compare groups with and without drug, and the analysis may have
benefited from an additional measure of visual memory function (BVMT-R results were invalidated by quality assurance because of re-administration of the same test version).

Conclusions

On the basis of the quaternary amine structure and the corresponding cationic charge, it has been predicted that trospium chloride would not be able to cross the human BBB. As a result of the complexities of the ageing human BBB, this concept required human clinical trial validation. The current trial, although small, is the first to support the lack of trospium chloride penetration in older adults, with OAB and stable comorbid disease, who are at increased risk for drug penetration across the BBB. The correlation of this pharmacokinetic finding with the memory test findings supports the pharmacodynamic antimuscarinic hypothesis – if an antimuscarinic does not cross the blood brain barrier, it should not cause cognitive impairment. Carefully designed, comparator trials are a logical next step in the evaluation and interpretation of these preliminary findings.

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Author contributions

David R. Staskin is a consultant and speaker for Allergan, Astellas, Pfizer and Watson. Gary Kay is a consultant for Allergan, Astellas, Pfizer, Novartis and Watson. Cara Tannenbaum is a consultant for Allergan, Pfizer, Astellas, Watson, Johnson and Johnson. Kavitha Bhavi is an employee of Allergan. John Lin is an employee of Allergan. Michael G. Oefelein is an employee of Allergan.

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