

Development of a Novel High-Concentration Galantamine Formulation Suitable for Intranasal Delivery

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ABSTRACT: The goal of the current study was to develop an intranasal (IN) formulation of the acetylcholinesterase inhibitor galantamine, an important therapeutic for treating Alzheimer's disease. To allow for delivering a therapeutically relevant dose, it was necessary to greatly enhance drug solubility. Various approaches were examined to this end, including adding co-solvents, cyclodextrins, and counterion exchange. Of these, the latter, for example, replacement of bromide ion with lactate or gluconate, resulted in a dramatic drug solubility increase, more than 12-fold. NMR confirmed the molecular structure of new drug salt forms. An *in vitro* epithelial tissue model was used to assess drug permeability and cellular toxicity. *In vitro*, galantamine lactate formulations performed as well as or better than their hydrobromide (HBr) counterparts with respect to drug permeation across the epithelial membrane with minimal toxicity. *In vivo* studies in rats compared pharmacokinetic (PK) profiles of different formulations. The *in vivo* studies confirmed that IN galantamine achieves systemic blood levels comparable to those of conventional oral administration. Both the *in vitro* and *in vivo* data support the feasibility of IN administration of this important drug. © 2005 Wiley-Liss, Inc. and the American Pharmacists Association *J Pharm Sci* 94:1736–1746, 2005

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INTRODUCTION

Dementia, the acquired global impairment of cognitive function, affects approximately 5% of people over 65 years of age; the majority of these cases are due to Alzheimer's disease.¹ Currently, the first-line treatments for Alzheimer's symptoms are acetylcholinesterase inhibitors such as rivastigmine, donepezil, and galantamine.^{1–3} Among these, galantamine (Figure 1) has been shown to exhibit the dual mechanism of acetylcholinesterase inhibition and allosteric modulation of nicotinic acetylcholine receptor.^{4–6}

Similar to other orally delivered acetylcholinesterase inhibitors, galantamine has a clinically significant level of mechanism-based gastrointestinal (GI) side effects including nausea and vomiting.⁷ The influence of galantamine on motor and evacuative functions when in contact with intestinal tissue has been documented *in vivo* and *in vitro*.⁸

An alternative approach would be to deliver galantamine intranasally. Intranasal (IN) delivery has the potential to reduce GI side effects by administering a drug systemically while avoiding contact with the GI tract.^{9,10} Furthermore, the potential for IN administration to directly deliver a drug to the central nervous system has been widely investigated.^{11–23} For example, improved delivery to the brain via the IN route has been reported for some small molecules^{12,14,15,17} as well as peptides

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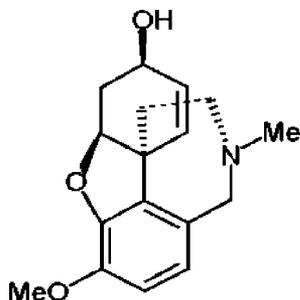


Figure 1. Molecular structure of galantamine.

and proteins.^{13,18–20} IN delivery has utility for administration of central nervous system drugs such as opioids,^{10,24,25} benzodiazepines,²⁶ and antimuscarinic agents.²⁷

To this end, the current report focuses on developing formulations of galantamine suitable for IN delivery. To achieve this goal in a solution formulation, it was necessary to increase drug solubility, accomplished via salt exchange, in order to intranasally deliver relevant doses. *In vitro* studies were conducted to examine stability, and an epithelial tissue model was employed to investigate the novel IN galantamine formulation's cellular toxicity and delivery of the drug across a mucosal tissue barrier. Finally, *in vivo* studies compared the pharmacokinetics of the IN *versus* oral galantamine formulations.

MATERIALS AND METHODS

Materials

Galantamine hydrobromide (HBr) was purchased from Tocris Cookson, Inc. (Ellisville, MO) as well as from A. G. Scientific, Inc. (San Diego, CA). Drug was 92–97% pure as determined by HPLC. Q Sepharose FF was obtained from Amersham Biosciences (Piscataway, NJ). Sulfolobus cyclodextrin was obtained from CyDex, Inc. (Lenexa, KS). Random methyl- β -cyclodextrin was purchased from Sigma (St. Louis, MO). Galantamine HBr internal standard (¹³C,²H₃-galantamine) was generously provided by Janssen Pharmaceutica N. V. (Beerse, Belgium). All other chemicals were purchased from commercial suppliers and were reagent grade or better.

Characterization of Galantamine Formulations

A stability-indicating HPLC assay was developed to detect intact galantamine, degradants, and

impurities. A volume of 25 μ L, diluted in mobile phase (1.5% acetonitrile in 50 mM ammonium formate, pH 3.0) was loaded on a Waters Symmetry Shield, C18, 5 μ m (25 \times 0.46 cm) column using a Waters Alliance 2695 LC system (Waters Corp., Milford, MA) and a gradient LC method with detection at a wavelength of 285 nm. The column temperature was 30°C. Flow rate was 1.3 mL/min. Mobile phase A was 50 mM ammonium formate, pH 3.0. Mobile phase B was acetonitrile. For purity determination, a gradient was run with 1.5% mobile phase B for the first 17 min, followed by a linear increase to 35:65 A:B (v:v) at 32 min. Data for galantamine are presented as concentration and % purity (peak area of compound divided by area of all galantamine-associated peaks). For quantitation only (no purity assessment), the same HPLC system was used, except the method was isocratic (1.5% mobile phase B for 20 min). Unless otherwise noted, galantamine concentrations are reported in mg/mL or μ g/mL and masses are adjusted for purity and reflect only the mass of the galantamine free base (not including mass of counter ion). Osmolarity and pH were measured for all formulations.

Accelerated stability was assessed by storage of 1 mL samples at 25, 40, or 80°C with a pH of 2.5, 5.2, or 10.0, respectively. Formulations were stored in silanized 1-cc amber glass bottles (SGD Pharma, Inc., New York, NY) covered with trifol lined caps (O'Burke Company, Union, NJ).

Galantamine Solubility Assays

Solubility of galantamine was determined at equilibrium in various formulations described in the results section. Briefly, galantamine was slowly added to 1 mL of a solution in mg quantities and mixed at ambient conditions, unless otherwise specified, until particles remained visible, signifying that the solution was saturated. Upon reaching equilibrium, the soluble portion was assayed by HPLC as described above to determine the concentration of galantamine in solution. The same approach was used for all subsequent dissolution experiments.

Galantamine Salt Exchange

An anion exchange column was packed in an XK50/60 column body (Amersham Biosciences, Piscataway, NJ) with 1 L Q Sepharose Fast Flow (FF) resin. Between uses, the column was stored in 20% ethanol. A peristaltic pump controlled flow

rate. The column was run at ambient temperature. All buffers were degassed before use. Prior to use, six column volumes of purified water were run through the column.

The column was primed at a flow rate of 9–12 mL/min with four column volumes of 1 M sodium lactate or sodium gluconate or until the conductivity of the eluted material matched that of the buffer being loaded on the column. To remove excess lactate or gluconate ions and residual sodium ions, the column was washed with six column volumes of purified water (the running buffer) until the conductivity dropped below 30 $\mu\text{S}/\text{cm}$. A galantamine HBr solution of ~ 30 mg/mL (including the mass of galantamine and HBr) was loaded on the column at a flow rate of 9–12 mL/min. The molar ratio of Q Sepharose resin to galantamine HBr was 20:1. Bromide ion bound to Q Sepharose and the released lactate or gluconate eluted with galantamine in 0.5–0.9 column volumes typically, with the peak elution around 0.7 column volumes. Eluant was collected as 8 mL fractions and monitored for conductivity, absorption at 285 nm, pH, and bromide ion concentration. The pooled fractions containing galantamine were freeze dried in a lyophilizer and then dissolved at high concentration (250–400 mg/mL) in water. The concentrated stocks were stored at refrigerated or frozen conditions until use.

NMR

Samples were freeze dried and then reconstituted in D_2O . ^1H and ^{13}C NMR were conducted on a 400 MHz NMR. Data collection was performed at room temperature with 16 scans for ^1H and 1062 scans for ^{13}C spectra. Data were analyzed using a commercial software package (WinNUTS, Acorn NMR, Inc., Livermore, CA).

In Vitro Permeation Studies

The EpiAirwayTM system (MatTek Corp., Ashland, MA) was the *in vitro* tissue model used to mimic nasal epithelia. This model consists of primary human upper airway epithelia grown to confluence on a Millicell-CM (Millipore, Bedford, MA) cell membrane (typical surface area was 0.6 cm^2).

The average transepithelial electric resistance (TER) was in the range of $350\text{--}650\ \Omega\cdot\text{cm}^2$. All experiments and controls were tested in triplicate; data are reported as average \pm standard deviation.

Upon receipt, tissues were incubated in serum-free media at 37°C for 24 h. For *in vitro* studies, samples were incubated at 37°C with gentle shaking (100 rpm) for 2 h. The typical apical and basolateral milieu were 10 μL of test sample, unless otherwise specified, and 1.0–1.5 mL of media, respectively. At each timepoint, 150 μL of basolateral media was removed and assayed for drug content by HPLC (as described above), cytotoxicity via measurement of lactate dehydrogenase (LDH) using a CytoTox 96 Cytotoxicity Assay Kit (Promega, Madison, WI), and cell viability with an MTT assay kit.

Permeation rate was determined by calculating the change in galantamine concentration in the basolateral media over the linear range of the experiment, typically 0–60 min of the incubation time. Equation 1 was used to determine the permeability constant, P_{app} (cm/s), across the epithelial membrane,

$$P_{\text{app}} = V_r / (A \times C_d) \times (\Delta C_r / \Delta t) \quad (1)$$

where V_r = volume of basolateral media (cm^3), A = membrane surface area (cm^2), C_d = apical concentration at start of experiment, and $\Delta C_r / \Delta t$ = change in concentration of drug in basolateral media over time (s).²⁸

Data from all experiments were compared by analysis of variance and the Student's *t*-test. A *p*-value of ≤ 0.05 was considered significant.

In Vivo Studies

Pharmacokinetic (PK) studies were performed on Sprague–Dawley rats and adhered to the Principles of Laboratory Animal Care (NIH publication 86–23, revised 1985). All animals were fasted for 12 h prior to dosing. For oral dosing, the animals were dosed by gavage at 5 mL/kg. For the animals dosed intranasally, the test products were instilled in the right nostril at a dose volume of 0.05 mL/kg. Following nasal dosing, the animal's head was tilted upward for a period of time sufficient to prevent the liquid from leaving the nasal cavity. The doses given were 1.75 mg/kg and 4.0 mg/kg for both oral and nasal galantamine.

Blood samples were collected on day 1 at approximately $t = 0$ (predose), 5, 10, 15, and 30 min and 1, 2, 4, 8 and 24 h after dosing. The twelve animals in each dosing group 1–6 were divided into four sets to collect blood samples: The first set of three animals/group at 5 min and 8 h post dose; the second set at 10 min, 4 hr and 24 hr post dose; the third set at 15 min and 2 h post-dose; the fourth

set at pre-dose, 30 min and 1 h post-dose. The last time point sampled for each rat was terminal. Blood samples were chilled until centrifugation at 4°C. Following centrifugation, the plasma was separated and frozen at -70°C until bioanalytical analysis. Galantamine concentration in plasma samples was determined following the method reported by Verhaeghe et al. employing a galantamine HBr internal standard (^{13}C , $^2\text{H}_3$ -galantamine).²⁹

PK calculations were performed using WinNonlin software (Pharsight Corporation, Version 4.0, Mountain View, CA) employing a non-compartmental model approach. Data are presented as mean \pm standard error.

RESULTS AND DISCUSSION

Due to solubility and dose volume limitations, the commercially available form of galantamine, that is, the HBr salt, is not suitable for delivery as an IN solution. The typical oral dose for galantamine is 8 mg, which would require at least an 80 mg/mL solution for a single IN dose (at the typical IN spray volume of 100 μL). However, the solubility of galantamine HBr in water is only about 35 mg/mL.³⁰ Therefore, increasing galantamine solubility is critical to establishing the feasibility of IN administration. To this end, the initial variables tested for their potential to increase drug solubility included pH, solvents, solubility enhancers, and process conditions.

The pH of the currently marketed liquid formulation of galantamine HBr was measured

to be about 5.2. To determine if pH and/or buffer strength would affect solubility, galantamine HBr was saturated in citrate buffer with strengths ranging from 10 to 100 mM and pH spanning 5.0 to 7.5. The data indicate that the various pH buffering conditions tested did not significantly enhance galantamine HBr solubility. This is not altogether surprising since the pK_a of galantamine HBr is 8.3.^{30,31} There was a very slight trend towards increased solubility at pH 7.5 with increasing buffer strength (Figure 2); however, the galantamine concentration did not achieve the minimum target of 80 mg/mL.

Next, some common, pharmaceutically acceptable co-solvents were tested. For example, galantamine HBr was added to solutions containing up to 10% glycerin or 1% poly(ethylene glycol) (MW \sim 300 Da). For the co-solvents and conditions tested, no improvements in galantamine solubility were observed (Figure 2).

Yet another approach examined was manipulation of process conditions, specifically, increasing temperature. After dissolution at 37°C, galantamine HBr solubility increased slightly from 33 to 40 mg/mL (Figure 2). Although this method resulted in a modestly increased galantamine concentration, such solutions were prone to drug precipitation over time at lower temperature (4–25°C), suggesting that the concentrations achieved were super saturated for the lower temperatures.

Another common approach used in the pharmaceutical industry to improve solubilization is the addition of cyclodextrins in the formulation.

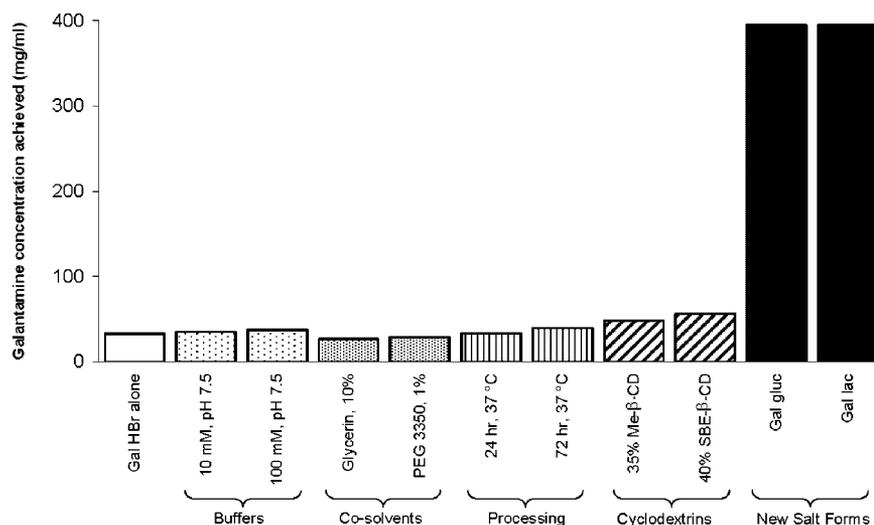


Figure 2. Solubility of galantamine under various conditions, including after exchange to galantamine gluconate (Gal gluc) and galantamine lactate (Gal lac) salt forms.

Acting as a chaperone, cyclodextrins can interact with and shield hydrophobic portions of drugs, thus improving their apparent solubility.^{32,33} Cyclodextrins have been reported to enhance the IN delivery of a variety of therapeutic compounds from small molecules to macromolecules.^{34–36} Because a 1:1 molar ratio is generally assumed for the association of cyclodextrin to a small molecule, the cyclodextrin must be present in excess of the saturating concentration of the small molecule in order to significantly enhance solubility.

Two cyclodextrins were tested in the current study: randomly methylated- β -cyclodextrin (Me- β -CD) and sulfobutylether- β -cyclodextrin (SBE- β -CD). Both of these cyclodextrins can be found in marketed products: Me- β -CD in a European IN product (i.e., AERODIOL[®]) and SBE- β -CD in multiple injectable drug formulations in the United States (e.g., VFEND[®] IV). In this study, the cyclodextrins were first dissolved in solution before addition of galantamine HBr. Dissolution of galantamine at 37°C resulted in increased drug solubility in the presence of the various cyclodextrin formulations tested. For instance, at the maximum levels of cyclodextrin tested, 40% (w/v) SBE- β -CD and 35% Me- β -CD, galantamine solubility was increased to 57 and 49 mg/mL, respectively (data depicted in Figure 2).

In order to further understand the relationship between galantamine and the cyclodextrins, phase-solubility diagrams were constructed (Figure 3). Phase-solubility diagrams are frequently used to calculate stoichiometry of drug/cyclodextrin complexes.^{32,37} Linear diagrams indicate that the complexes are first order with respect to cyclodextrin and first or higher order with respect to the drug. The strength of the complex,

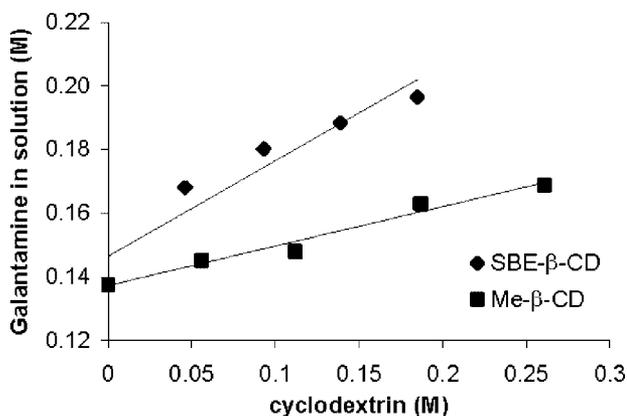


Figure 3. Phase solubility of galantamine relative to cyclodextrin concentration.

quantitated as an apparent complexation constant ($K_{1:1}$) can be calculated from the slope and intercept (S_0 or intrinsic solubility) of a line drawn through the points on the graph,⁽³⁷⁾ following equation (2):

$$K_{1:1} = \text{Slope}/(S_0(1 - \text{Slope})) \quad (2)$$

In the case of galantamine, the strength of the complex, expressed as the complexation constant, $K_{1:1}$, was found to be 1.0/M for Me- β -CD and 2.9/M for SBE- β -CD. Although some improvement in apparent solubility was observed, these relatively weak complexation constants necessitated exploration of other strategies for formulation of IN galantamine.

Because of the limited success achieved with the aforementioned traditional routes to enhancing galantamine solubility, yet another approach was explored, namely salt exchange. It was hypothesized that the counter ion plays an important role in influencing galantamine solubility. The traditional counter ion, bromide, is a chaotropic species that interacts weakly with water; in this case, ionic interactions are preferred over hydration, which results in limited drug solubility.^{38–40} It was further hypothesized that a less electronegative anion (e.g., lactate) would interact more strongly with water, and result in preferential hydration and increased galantamine solubility. Lactate not only contains multiple hydroxyl groups for hydrogen bonding with water, but also has carboxyl groups, which can spread the negative charge across π orbitals. Thus, it was envisioned that employing such hydroxycarboxylic anions would be a promising approach for improving galantamine solubility. One exemplary case is that of the increased solubility observed for lactate over halide salt forms of chlorhexidine.⁴¹

To test the concept, an anion exchange column comprised of Q Sepharose was utilized. The quaternary amine group of Q Sepharose readily binds both weak anions, such as lactate, and strong anions, such as bromide. Briefly, the column was initially loaded until saturation with a sodium salt of the new counterion (e.g, lactate). Galantamine HBr was then applied to the column at an excess (20:1) molar ratio of resin-to-galantamine, allowing for binding of bromide to the resin with concomitant elution of the new galantamine salt from the column.

Representative elution data are depicted in Figure 4 (conductivity and absorbance at 285 nm shown for elution of galantamine lactate). The pooled fraction (spanning the range of approximately

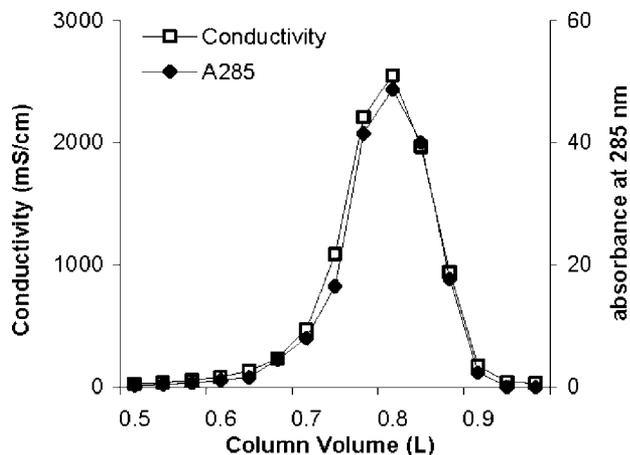


Figure 4. Representative graph of conductivity and absorbance at 285 nm for galantamine elution from salt exchange column. Eluant from a Q Sepharose FF column was monitored by both conductivity (to measure salt) and absorbance at 285 nm (to measure galantamine).

0.5–0.9 column volumes) was typically pH 6.2. The salt exchange process was consistently high-yield (>90% drug recovered with no impact on drug purity) and efficient (bromide ion reduction was >500 fold, or $99.97\% \pm 0.05\%$ complete). The pool of eluted material was dried on a lyophilizer and then reconstituted in varying amounts of water in order to determine the upper solubility limit for the new salt.

The data (Figure 2) indicate a remarkable increase in galantamine solubility when exchanged to the lactate salt, up to about ~400 mg/mL, representing more than an order of magnitude (>12-fold) increase compared to the starting HBr salt. In a further confirmation, the maximum solubility of galantamine after exchange of bromide to gluconate (another hydroxycarboxylic anion) was also found to be ~400 mg/mL (Figure 2). In both cases, the solubility achieved was fivefold higher than the aforementioned goal for making an IN galantamine formulation practical. The studies described in the remainder of this report focus of further development of the lactate salt form of galantamine for IN administration.

To confirm its molecular structure, dried galantamine lactate was analyzed by ^1H and ^{13}C NMR. Figure 5 shows the ^1H NMR spectra of galantamine lactate, galantamine HBr, and sodium lactate. Comparing the galantamine HBr and sodium lactate ^1H NMR spectral data, it is apparent that each molecule provides a distinct

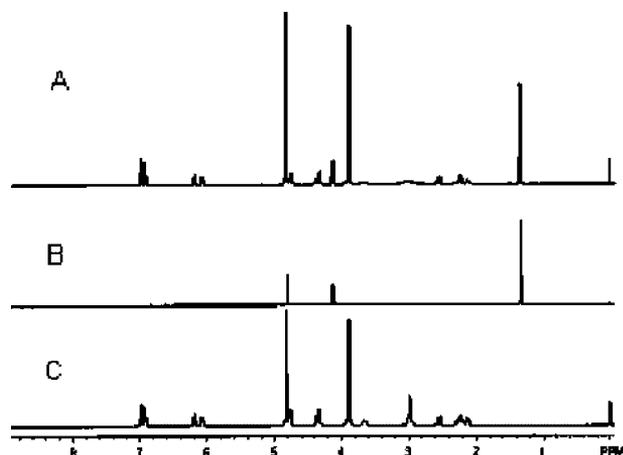


Figure 5. ^1H NMR of (A) galantamine lactate, (B) sodium lactate, and (C) galantamine HBr.

signature of their proton peaks. The data for galantamine lactate clearly show the presence of both sets of proton peaks, thus confirming that galantamine and lactate were present. Integration of the ^1H NMR and ^{13}C NMR trace of galantamine lactate confirmed a 1:1 molar ratio of galantamine-to-lactate.

Another important question was whether drug stability was affected by change in salt form. To address this, an accelerated stability study was conducted for both salt forms. Galantamine lactate and galantamine HBr were dissolved in water to 15 mg/mL and the pH was adjusted to 2.5, 5.2 and 10.0. Samples at each pH were placed at 25°C, 40°C, and 80°C. HPLC analysis for content and purity at days 1, 7, 28 and at 2 months demonstrated that galantamine lactate exhibited comparable or superior stability when compared to galantamine HBr under the conditions tested. For instance, after 2 months storage at temperatures of 25–40°C there was no detected drop in purity for either the lactate or HBr salt (data not shown). Similar to galantamine HBr, the lactate salt experienced significant degradation at time points earlier than 2 months only under the combination of extreme pH (pH 2.5 or 10) and temperature (80°C) conditions. For instance at pH 10 and 80°C, galantamine HBr and galantamine lactate both showed a decrease in purity to 31.6% and 29.5%, respectively, in 2 months.

In addition to testing stability, it was also important to compare the two galantamine salts in an *in vitro* tissue model, the next step towards IN formulation development. *In vitro* testing allows for toxicity assessment of the drug formulations via cell viability and cytotoxicity assays. Cell

viability, as measured by the MTT assay, for both galantamine lactate ($94.7\% \pm 6.7\%$) and galantamine HBr ($100.4\% \pm 3.1\%$) at the formulation concentrations of 35 mg/mL was the same as the media control ($100.0\% \pm 4.5\%$). More than doubling the galantamine lactate concentration (80 mg/mL) applied to the cells did not significantly reduce cell viability ($91.9\% \pm 6.1\%$). Cytotoxicity, as measured by activity of LDH in the basolateral media, was correspondingly low. The LDH assay results for galantamine HBr at 35 mg/mL and galantamine lactate at 35 or 80 mg/mL demonstrated low cytotoxicity ($2.5\% \pm 0.5\%$ of the Triton X control). These data suggest that galantamine lactate exhibits low cellular toxicity comparable to its marketed HBr counterpart.

The *in vitro* tissue model was also used to optimize drug permeation conditions across the epithelial barrier. For instance, a range of pH conditions were tested between pH 4.0 and pH 7.0 (spanning that of the marketed oral galantamine HBr solution, pH ~ 5.2). For both HBr and lactate salts, drug permeation of galantamine increased with increasing pH (Figure 6; $p < 0.0001$). Therefore, galantamine formulations for further *in vitro* and *in vivo* studies were adjusted to pH 6.7 ± 0.3 .

Finally, the permeation rates for galantamine HBr and lactate were determined. The following formulations were applied to the apical side of the epithelial tissue: 35 mg/mL galantamine HBr, 35 mg/mL galantamine lactate, and 80 mg/mL galantamine lactate. Tissues were incubated with gentle shaking at 37°C for 120 min. At 15, 30, 60, and 120 min after applying the formulations, the

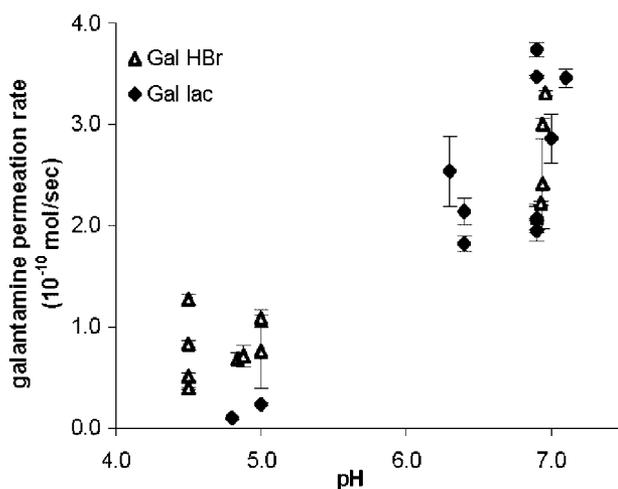


Figure 6. Effect of formulation pH on galantamine HBr (Gal HBr) and galantamine lactate (Gal lac) permeation rate ($p < 0.0001$).

basolateral media was sampled to determine the amount of galantamine permeation.

Analysis of galantamine in the basolateral media revealed that the lactate salt permeates epithelial tissue at a slightly higher rate than the HBr salt at 35 mg/mL concentration (Figure 7A). Interestingly, increasing the concentration of galantamine lactate more than twofold (to 80 mg/mL) resulted in a lower than expected increase in permeation. These data illustrate that galantamine permeation is non-linear with respect to the amount of drug applied to the tissue, and thus permeation may be approaching a saturation limit.

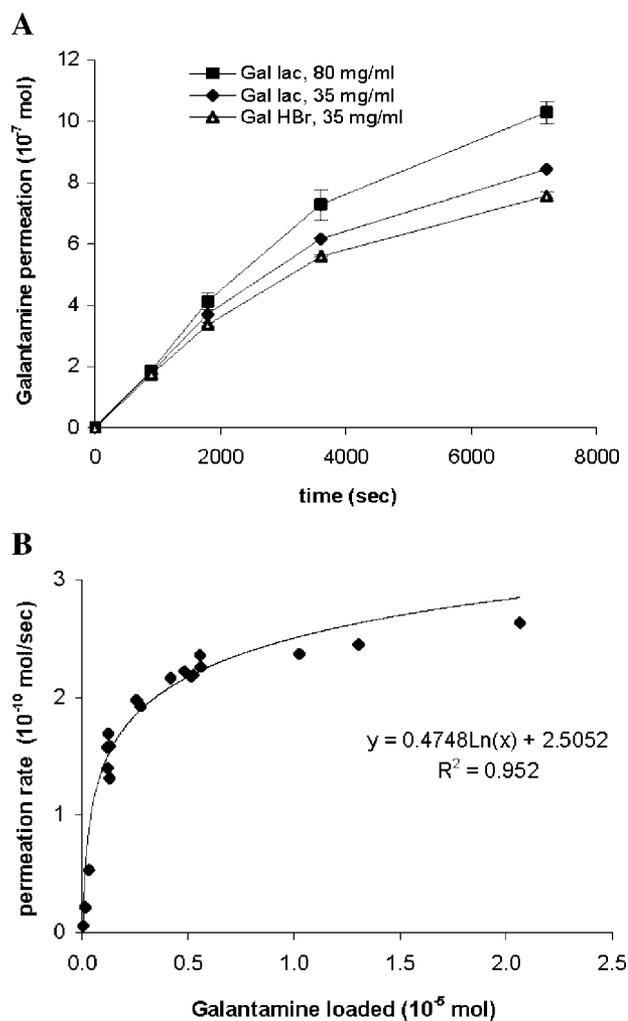


Figure 7. (A) Time course of permeation for galantamine HBr (Gal HBr) and galantamine lactate (Gal lac) across epithelial tissue *in vitro*. (B) *In vitro* permeation rate versus amount galantamine lactate loaded on the apical side of the tissue.

Such saturation would be consistent with permeation across the epithelial layer via facilitated diffusion or by active transport.⁴² To further explore the mechanism of drug transport, a wider range (0.1 to 6.4 mg or 0.3 mM to 22.3 mM) of galantamine lactate was applied to the apical side of the epithelial tissue, and drug concentrations in the basolateral media were measured at multiple time points. To load more than 1.6 mg (5.6 mM) galantamine on the tissue, the loading volume was increased to 40 μ L. This did not affect cell viability adversely (data not shown). Permeation rates were calculated for each of the loading amounts (Figure 7B). The P_{app} for galantamine lactate was found to be $1.83 \pm 0.86 \times 10^{-6}$ s/cm. The data suggest a permeation mechanism which was limiting at high amounts of galantamine loaded. These findings support the observations about drug permeation noted in preceding experiment (data shown in Figure 7A).

Having established feasibility of IN galantamine *in vitro*, the next logical step was to conduct *in vivo* PK studies. The rat is commonly employed as a PK model for IN studies. In addition, it has been reported that PK in this species resembles delivery in humans (for repeated oral administration).⁴³ The objective was to compare oral *versus* IN administration, and also to compare PK behavior for the two different drug salt forms (bromide *vs.* lactate). Six groups were dosed as follows: IN 35 mg/mL galantamine HBr (1.75 mg/kg), oral 0.35 mg/mL galantamine HBr (1.75 mg/kg), IN 35 mg/mL and 80 mg/mL galantamine lactate (1.75 mg/kg and 4.0 mg/kg, respectively), and oral 0.35 mg/mL and 0.8 mg/mL galantamine lactate (1.75 mg/kg and 4.0 mg/kg, respectively).

The data reveal that, for the same dose, the overall PK performance for oral and IN administration were similar. Generally, the T_{max} for IN delivery was between 5 and 15 min, while the T_{max} for oral administration was about 15 min. For a given dose, IN delivery was able to achieve a maximum serum level (C_{max}) and an overall area-under-the-curve (AUC_{last}) comparable to oral administration. For instance, Figure 8A depicts PK data for oral *versus* IN administration of galantamine HBr at the same dose of 1.75 mg/kg. A similar PK behavior was observed following either delivery route: the AUC_{last} and C_{max} after oral dosing was 13288 min \cdot ng/mL and 190 ng/mL, respectively, while these PK parameters were 11031 min \cdot ng/mL and 268 ng/mL, respectively, for IN administration. A similar observation was

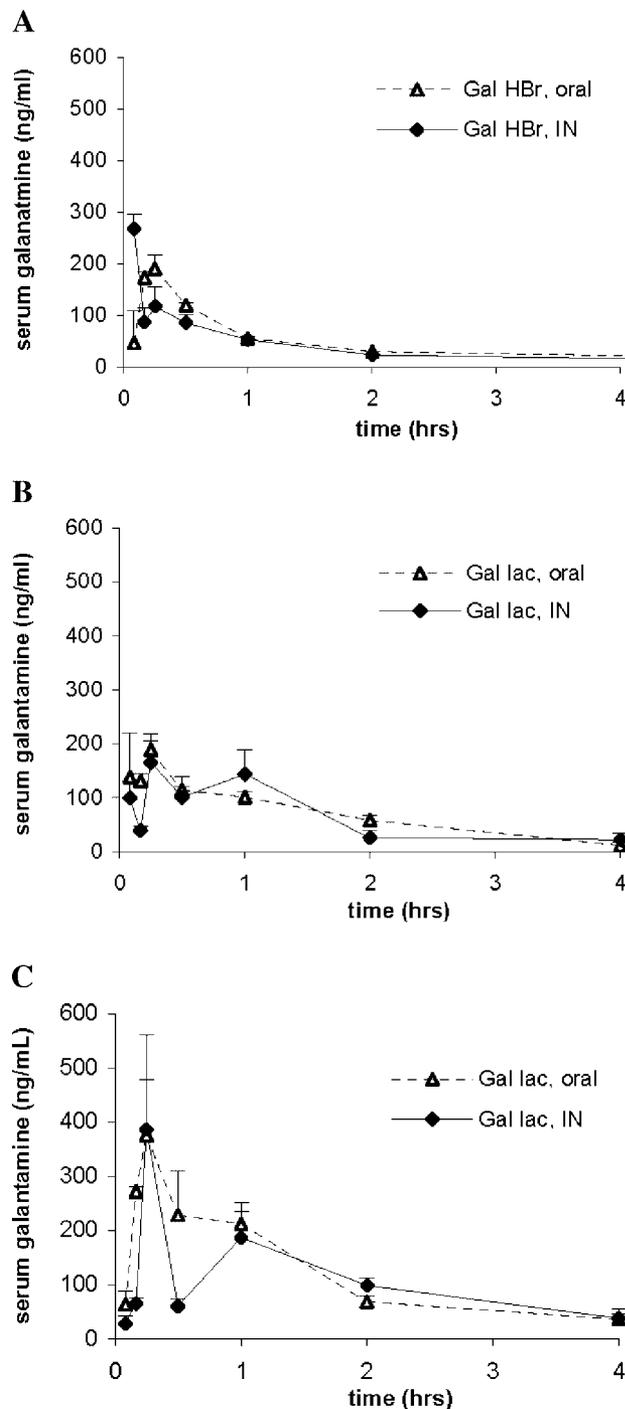


Figure 8. Serum levels of galantamine following administration of various formulations in rats. (A) Galantamine HBr (Gal HBr) dosed at 1.75 mg/kg, (B) Galantamine lactate (Gal lac) dosed at 1.75 mg/kg. (C) Gal lac dosed at 4.0 mg/kg. Open triangles and closed diamonds represent data for oral and IN administration, respectively.

made for dosing the alternative salt, galantamine lactate, at this same dose (Figure 8B: C_{max} for oral and IN dosing were 189 and 165 ng/mL, respectively) and at a higher dose of 4.0 mg/kg (Figure 8C: C_{max} for oral and IN dosing were 376 and 386 ng/mL, respectively).

Similar to the *in vitro* findings discussed above, the *in vivo* performance of galantamine lactate was comparable or superior to that of the conventional HBr salt. For example, when given by IN route in rats at 1.75 mg/kg, galantamine lactate exhibits an AUC_{last} of 18170 min · ng/mL compared to 11031 min · ng/mL for the same IN dose of the HBr salt (compared Figures 8A and B). At an even higher dose of 4.0 mg/kg of the galantamine lactate formulation, there was a concomitantly higher AUC_{last} of 34227 min · ng/mL; due to solubility limitations it was not possible to deliver a galantamine HBr solution via IN route as a comparator at this dose.

CONCLUSION

The primary hurdle to demonstrating the feasibility of IN galantamine, namely, achieving a therapeutically relevant dose in a single administration, has been successfully met by exchange of the conventional HBr salt to a novel lactate salt. Specifically, this approach allowed for *in vivo* dosing of an 80 mg/mL drug solution, or 4 mg/kg in the rat, which resulted in a concomitant increase in C_{max} and AUC_{last} compared to dosing a solution of 35 mg/mL, representing the approximate solubility limit of the conventional salt form (compare PK data shown in Figure 8C and B). As delineated at the outset of this report, a concentration of 80 mg/mL is the minimum required to achieve an 8 mg dose in a single IN spray in humans. Formulation of galantamine as a hydroxycarboxylic salt allows for concentrations up to 400 mg/mL drug.

Finally, it was important to understand the relationship between *in vitro* and *in vivo* performance. The same three galantamine formulations dosed in rats were also examined using the cell tissue model. These data are presented in Figure 9 as the correlation between permeation rate across the model epithelia tissue and the AUC_{last} upon IN administration. The data, although limited, suggest that the *in vitro* model is a reasonable predictor of *in vivo* behavior. To our knowledge, this report represents the first description of a feasible IN formulation of the important acetyl-

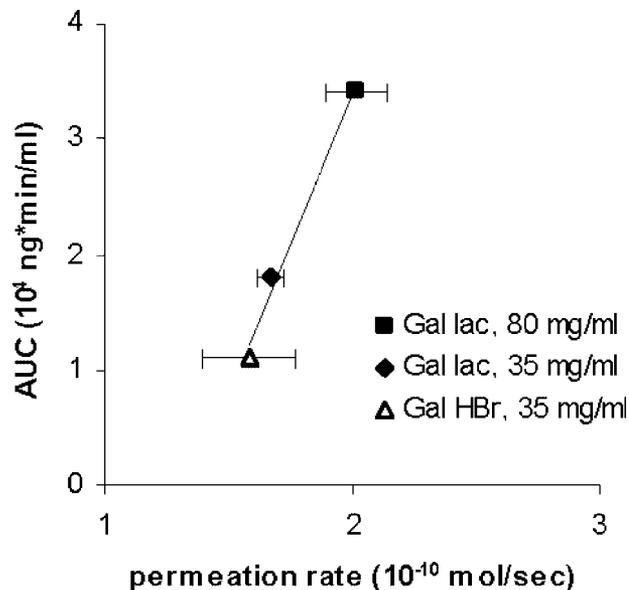


Figure 9. Correlation between permeation *in vitro* and AUC_{last} *in vivo* for IN galantamine formulations. The line represents the least squares fit ($R^2 = 0.987$), suggesting that *in vitro* data are reasonably predictive of *in vivo* behavior.

cholinesterase inhibitor galantamine and such a corresponding *in vitro*–*in vivo* correlation.

ABBREVIATIONS

HBr	hydrobromide
GI	gastrointestinal
IN	intranasal
PK	pharmacokinetic
Me- β -CD	random methyl-beta-cyclodextrin
SBE- β -CD	sulfobutylether-beta-cyclodextrin
TER	transepithelial electrical resistance
LDH	lactate dehydrogenase
MTT	a tetrazolium salt (2, 3-bis-(2-methoxy-4-nitro-5-sulphophenyl)-2H-tetrazolium-5-carboxanilide, disodium salt)

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