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In vitro formulation optimization of intranasal galantamine leading to enhanced bioavailability and reduced emetic response *in vivo*

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Abstract

The purpose of the current investigation was to optimize an intranasal (IN) galantamine (an acetylcholinesterase inhibitor used for treatment of Alzheimer's disease) formulation using an *in vitro* tissue model, to correlate those results to *in vivo* bioavailability, and to compare emetic response to oral dosing. A design-of-experiments (DOE) based formulation screening employing an *in vitro* tissue model of human nasal epithelium was used to assess drug permeability, tight junction modulation, and cellular toxicity. *In vivo* studies in rats compared pharmacokinetic (PK) profiles of different formulations dosed intranasally.

Finally, studies in ferrets evaluated PK and gastrointestinal (GI) related side effects of oral compared to nasal dosage forms. Galantamine permeation was enhanced without increasing cytotoxicity. Pharmacokinetic testing in rats confirmed the improved drug bioavailability and demonstrated an *in vitro–in vivo* correlation. Compared to oral dosing, IN galantamine resulted in a dramatically lowered incidence of GI-related side effects, e.g., retching and emesis. These findings illustrate that IN delivery represents an attractive alternative to oral dosing for this important Alzheimer's disease therapeutic. To our knowledge, the data herein represent the first direct confirmation of reducing GI-related side effects for IN galantamine compared to oral dosing.

Keywords: *In vitro–in vivo* correlation; Formulation; Galantamine; Nasal drug delivery; Pharmacokinetics

1. Introduction

Intranasal (IN) delivery is suitable for the local and systemic delivery of diverse therapeutic compounds (Wearley, 1991; Sayani and Chien, 1996; Pontiroli, 1998; Romeo et al., 1998; Hussain, 1998; Illum, 2000, 2003, 2004; Song et al., 2004; Turker et al., 2004). Attributes of this approach include a large surface area for delivery and rapid onset of drug levels, potential for direct-to-central nervous system delivery, no first pass metabolism, and non-invasiveness to maximize patient comfort and compliance. Owing to these and other factors, marketed IN formulations exist for a variety of low- and high-molecular-weight drugs (e.g., peptides), and additional products are under development. One of the areas of therapeutic interest in this regard is IN administration of central nervous system (CNS) drugs, for instance opioids (Hussain and Aungst, 1997; Illum et al., 2002; Rudy et al., 2004), benzodiazepines (Olivier et al., 2001), and antimuscarinic agents (Ahmed et al., 2000).

A recent example of a nasally delivered CNS therapeutic is the development of a highly soluble formulation suitable for IN delivery of galantamine (Kays Leonard et al., 2005). This important acetylcholinesterase inhibitor, currently marketed in oral form, is indicated for treatment of Alzheimer's disease (Scott and Goa, 2000; Jann et al., 2002; Evans et al., 2004). Among the advantages for IN delivery is the potential to avoid dose-limiting GI-mediated side effects such as nausea and vomiting, the most common adverse events leading to discontinuation of treatment (Sramek et al., 2000). Additionally, the effect of galantamine on motor and evacuative functions when in contact with intestinal tissue has been documented *in vivo* and *in vitro* (Turiiski et al., 2004). However, currently available literature lacks comparison of GI-related side effects for oral and IN galantamine dosing.

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To pursue such a comparison, it was necessary to provide an IN formulation with sufficiently high drug dose and bioavailability to achieve systemic drug levels similar to administering a therapeutically relevant oral dose. In order to maximize the drug dose, a novel galantamine lactate salt was previously developed with dramatically enhanced (~12-fold) solubility, making IN galantamine administration feasible (Kays Leonard et al., 2005). In order to maximize bioavailability, several model excipients were screened herein to provide an optimized IN formulation with enhanced nasal permeation and low toxicity. Examples of excipients shown to improve nasal permeatrion include bile salts (Aungst and Rogers, 1988; Hosoya et al., 1999; Bagger et al., 2001), alkyl glycosides (Ahsan et al., 2001; Pillion et al., 2002; Nakamura et al., 2002; Mustafa et al., 2004), polymers (e.g., poly-L-arginine (Ohtake et al., 2002), gelatin (Wang et al., 2002), chitosan (Illum et al., 1994; Prego et al., 2005), tight junction modulating peptides (Johnson and Quay, 2005; Chen et al., 2006), lipids and surfactants (Coates et al., 1995; Laursen et al., 1996; Mitra et al., 2000), cyclodextrins (Merkus et al., 1991; Schipper et al., 1993; Matsubara et al., 1995; Marttin et al., 1998), and chelators (Hosoya et al., 1994). Ideally, selected enhancers should exhibit low toxicity for use in chronically administered nasal products.

The current report describes *in vitro* and *in vivo* characterization of various IN galantamine formulations to maximize drug permeation while maintaining low toxicity. The three goals of this work were to: (i) test excipients for improving drug permeation with low toxicity in an *in vitro* tissue model, (ii) extrapolate the *in vitro* results to *in vivo* bioavailability, and (iii) explore the hypothesis of reduced GI-related side effects for IN versus oral dosing in a relevant animal model. To our knowledge, the data herein represent the first direct confirmation of reducing GI-related side effects for IN galantamine compared to oral dosing.

2. Materials and methods

2.1. Materials

Galantamine HBr ($\log P = 1.09$ (Mente and Lombardo, 2005)) was purchased from Tocris Cookson, Inc. (Ellisville, MO, USA) as well as from A.G. Scientific, Inc. (San Diego, CA, USA). Drug was 92–97% pure as determined by HPLC. All other chemicals were purchased from commercial suppliers and were reagent grade or better.

2.2. Production and characterization of galantamine lactate

Galantamine lactate was produced by salt exchange from the hydrobromide salt form on an ion exchange column and confirmed by NMR as described in an earlier report (Kays Leonard et al., 2005). A stability-indicating HPLC assay developed to detect intact galantamine, degradants and impurities was also described previously (Kays Leonard et al., 2005). Unless otherwise noted, galantamine concentrations are reported in mg/ml or μg/ml. Masses are adjusted for purity and reflect only the mass of the galantamine free base (not including mass of counter ion). Osmolality and pH were measured for all formulations.

2.3. Preparation of IN formulations

To prepare formulations, methyl-β-cyclodextrin (Me-β-CD), 0–90 mg/ml, was first added to sterile water for irrigation and mixed with stirring until dissolved, followed by addition of L-α-phosphatidylcholine didecanoyl (DDPC), 0–2 mg/ml, and edetate disodium dihydrate (EDTA), 0–2 mg/ml, in like manner. Galantamine HBr or galantamine lactate was then added to final concentrations of 35, 40, or 80 mg/ml galantamine base. All reported concentrations of galantamine refer to the free base, regardless of the counter ion present. Final galantamine concentration was confirmed by HPLC.

2.4. In vitro permeation studies

Discussion of the utility of human nasal and other epithelial tissue for IN drug discovery and development can be found elsewhere (Dimova et al., 2005). The *in vitro* tissue model employed in the current study was the EpiAirway™ system (MatTek Corp., Ashland, MA, USA). This model consists of primary human upper airway epithelia grown to confluence on a Millicell-CM (Millipore, Bedford, MA, USA) cell membrane (typical surface area was 0.6 cm^2).

The average transepithelial electric resistance (TER) was in the range of 350–650 Ω cm^2. 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 40 μl of test sample, unless otherwise specified, and 1.5 ml of media, respectively. At each time point (15, 60 and 120 min), 150 μl of basolateral media was removed and assayed for drug content and cytotoxicity (via measurement of lactate dehydrogenase (LDH) using a CytoTox 96 Cytotoxicity Assay Kit (Promega, Madison, WI)). The tissue insert was assayed for change in TER, as well as cell viability (via an MTT assay kit from MatTek Corp.). Two controls were applied to the apical side of tissue model: a detergent, Triton X, served as a negative control for cell viability, while media served as a positive control. Further experimental details were previously outlined (Kays Leonard et al., 2005). All experiments and controls were tested in triplicate; data are reported as average ± standard deviation.

Permeation rate was determined by calculating the change in galantamine concentration in the basolateral media over the linear range of the experiment, typically three time points over the period of 15–120 min. Permeation was steady state by the 15 min time point. The apparent permeability constant, P_{app} (cm/s), across the epithelial tissue barrier was calculated according to Eq. (1) as:

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

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) (Youdim et al., 2003).

Design of experiment (DOE) and data analyses were performed using Design Expert 6.0 software (StatEase, Milwaukee, WI). 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.

2.5. In vivo pharmacokinetic studies

Pharmacokinetic (PK) studies in Sprague–Dawley rats adhered to the Principles of Laboratory Animal Care (NIH publication 86-23, revised 1985). Animals were fasted for 12 h prior to dosing. For IN administration, drug was instilled in the right nostril by pipette at a dose volume of 0.05 ml/kg. The animal's head was tilted back slightly at a 15° angle to perpendicular for 3–5 s sufficient to prevent the liquid from leaving the nasal cavity and not to facilitate esophageal administration. The doses given were 1.75 mg/kg. All animals weighed between 0.25 to 0.30 kg. Approximately 1 ml blood was collected per animal per time point (three animals per time point) at pre-dose and 5, 10, 15, and 30 min and 1, 2, 4, 8 and 24 h post-dose. Serial blood draws could not be obtained from one animal because of the volume of blood needed for the assay of galantamine. Therefore, animals bled in each dosing group 1–4 were divided into four sets to collect blood samples. The first set of three animals/sex/group were bled at approximately 5 min and 8 h post-dose; the second set of three animals/sex/group were bled approximately 10 min, 4 and 24 h post-dose; the third set of three animals/sex/group was bled approximately 15 min and 2 h post-dose; the fourth set of three animals/sex/group was bled pre-dose, approximately 30 min and 1 h post-dose.

Ferret studies were conducted following ICH-S7A guidelines (Safety Pharmacology Studies for Human Pharmaceuticals, July 2001). Sixteen ferrets were fasted overnight prior to dosing and fed 30 min pre-dose. All animals weighed between 0.8 and 0.9 kg at start of study. All animals received a single administration of 20 mg/kg galantamine by IN instillation by pipette or by oral gavage at concentrations of 200 and 20 mg/ml, respectively. Each group contained eight animals. For PK analyses, blood was collected (four animals per time point) at pre-dose and at 2.5, 5, 10, 15, 30, min and 1, 2, 4 and 8 h post-dose. Blood was not sampled from each animal due to the time constraints of the earliest time points in the study. Approximately 0.5 ml blood was collected via the jugular vein into vacutaner tubes containing potassium EDTA, placed on ice and centrifuged for 15 min at approximately $1500 \times g$ and 4 °C within 1 h of collection. Plasma was harvested and frozen at −70 °C until bioanalysis.

For emesis studies, the protocol was based on a validated model (Sam et al., 2003; Hasegawa et al., 2002; Rudd et al., 1994; Knox et al., 1993; Yamahita et al., 1997; Mason et al., 2004). Eight naïve ferrets were food deprived overnight for 16 h prior to dosing. The ferrets weighed between 0.75 and 1.00 kg. The ferrets were monitored and observed for episodes of emesis for 4 h after dosing by oral or intranasal galantamine. The time prior to the first episode of retching or vomiting was recorded (latent period), as was the total number of retching and vomiting periods through the observation period. For the purposes of this study, episodes of emesis are defined as rhythmic abdominal contractions associated with oral expulsion of solid or liquid material from the gastrointestinal tract (i.e. vomiting). Episodes of retching were not associated with the passage of material. An episode of retching and/or vomiting was considered separate when an animal changed position in the cage or when the interval between retches and/or vomits exceeded an approximate 5 s period.

Galantamine concentrations in rat and ferret plasma samples were determined following the previously described methodology (Kays Leonard et al., 2005).

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 ± standard error where applicable (note that due to the animal study design discussed above, standard error calculations for certain rat PK parameters, i.e., AUC_{last} and bioavailability, were not applicable).

For the ferret emesis data, the statistical analysis comparison assumed the two sets of counts were from two independent Poisson distributions using a test based on the normal approximation to the exact distribution of the difference in the counts. X and Y represent the counts and the statistical equation used was $Z = (X - Y)/\sqrt{(X + Y)}$ where Z was approximately distributed as a standard normal distribution.

3. Results and discussion

To accomplish the first goal of identifying excipients for improving drug permeation in an *in vitro* tissue model, excipients were explored for their potential to increase drug permeation across the barrier imposed by the nasal mucosal tissue. Among the potential permeation enhancers discussed previously, a combination of representative candidates were chosen from the literature and tested for their ability to enhance galantamine permeation. Specifically, L-α-phosphatidylcholine didecanoyl (DDPC), randomly methylated β-cyclodextrin (Me-β-CD) and disodium edetate (EDTA) were selected as a representative lipid surfactant, cyclodextrin stabilizer, and chelator, respectively (Davis and Illum, 2003). The goal at this probatory stage was not to optimize the formulation, but rather to ascertain if the three excipients affected drug permeation; in a subsequent study the levels of excipients were optimized employing a design-of-experiments (DOE) approach (see below).

For this initial formulation, concentrations of each excipient were selected based on reported values in the literature. DDPC is a representative phospholipid that has been reported to enhance permeation of small molecules and peptides through a reversible opening of tight junctions (Carstens et al., 1993; Röpke et al., 1997). Permeation enhancing effects of DDPC have been reported for concentrations of 1–5 mg/ml (Carstens et al., 1993; Röpke et al., 1997; Vermehren et al., 1997). EDTA, a divalent cation chelator, is thought to affect tight junctions of membrane cells by removing calcium and increasing paracellular transport (Cassidy and Tidball, 1967). Although EDTA has sometimes failed to show permeation enhancement in nasal delivery alone, it has been tested in combination with other enhancers at concentration of 1–5 mg/ml (Natsume et al., 1999;

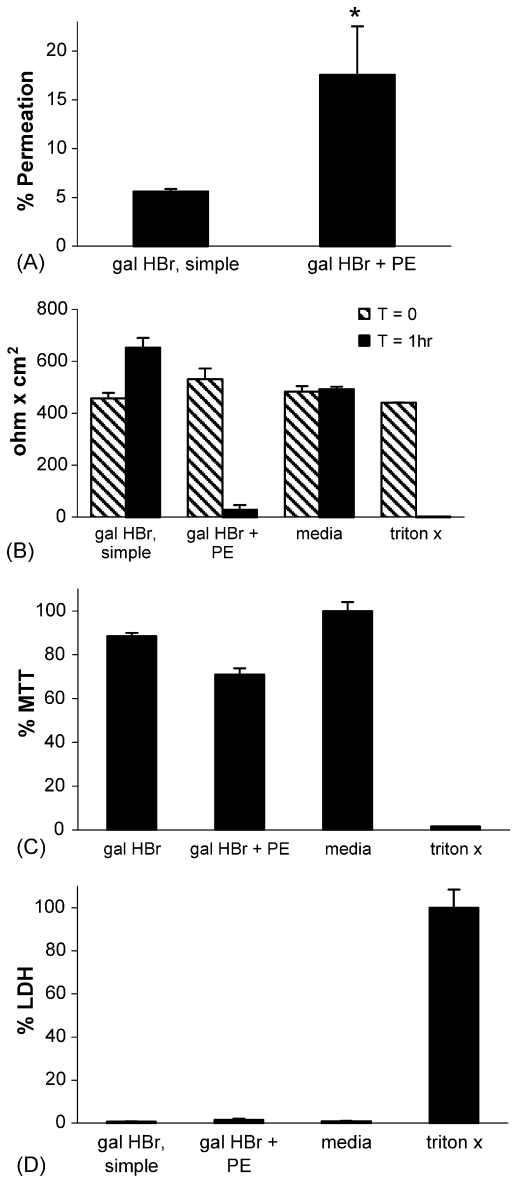

Fig. 1. *In vitro* performance of galantamine HBr (gal HBr) in the absence or presence of permeation enhancers (PE). The gal HBr concentration was 35 mg/ml. (A) Percent of drug transported from apical to basolateral side of tissue barrier after 1 h (asterisk (*) indicates $p < 0.05$). (B) TER before and after apical exposure. (C) Cell viability as assayed by MTT. (D) Cytotoxicity as measured by LDH assay.

Yu et al., 2004). Me-β-CD has been used to stabilize small molecules in nasal solutions and enhance permeation across a nasal mucosa at concentrations of 25–100 mg/ml (Marttin et al., 1997).

In the *in vitro* tissue model, the presence of these three permeation enhancers in combination resulted in substantially increased galantamine HBr permeation (about three-fold) across the epithelial barrier (Fig. 1A). None of the enhancers alone increased drug permeation (data not shown). There was also a dramatic reduction in TER using formulation containing the permeation enhancers (Fig. 1B), consistent with a tight junction opening mechanism. To confirm that the formulation was not toxic to the epithelial membrane, two orthogonal tests were performed. An MTT assay for cell viability (Fig. 1C) and a lactate dehydrogenase (LDH) assay for cytotoxicity (Fig. 1D) were performed and revealed that the formulation was not significantly toxic to the membrane.

Having met the goal of successfully identifying excipients to enhance drug permeation *in vitro*, the next logical step was to confirm the effect *in vivo*. To achieve this, the same formulation containing the permeation enhancers (discussed above) was dosed by the IN route (1.75 mg/kg) in a rat PK model. The relevance of the rat PK model has been established as discussed elsewhere (Monbaliu et al., 2003). Previously, using this same rat model in our earlier studies, PK parameters of a commercially obtained and orally administered galantamine HBr solution were matched by IN delivery of galantamine HBr and exceeded by IN delivery of the novel salt galantamine lactate (Kays Leonard et al., 2005). In the presence of permeation enhancers, the maximal concentration (C_{max}) and area-under-the-curve at the last time point (AUC_{last}) for galantamine HBr dosed intranasally were 410 ± 210 ng/ml and 18,800 ng min/ml, respectively. In contrast, C_{max} and AUC_{last} were 270 ± 250 ng/ml and 11,000 ng min/ml, respectively, in the absence of the permeation enhancers. Therefore, the presence of the permeation enhancers at the levels tested resulted in about a 50–70% increase in the PK parameters C_{max} and AUC_{last}. Furthermore, comparing the absolute bioavailability based upon assay, IN galantamine HBr dosed alone and in the presence of permeation enhancers has an absolute bioavailability of 22% and 41%, respectively, resulting in an 86% increase in bioavailability with the permeation enhancers. Orally delivered galantamine HBr had similar bioavailability to IN galantamine HBr alone at 22% and 23%, respectively. The addition of permeation enhancers to the nasal formulation thus had a 78% increase in bioavailability compared with oral dosing of galantamine HBr alone. This suggests that the addition of permeation enhancers

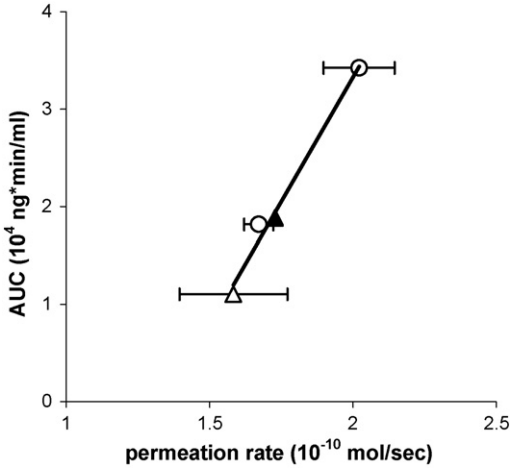

Fig. 2. Correlation between permeation *in vitro* and AUC_{last} *in vivo* for various IN galantamine formulations. The triangles represent data for galantamine HBr (35 mg/ml) in the absence (open triangle) and presence (filled triangle) of permeation enhancers. The circles represent galantamine lactate at 35 and 80 mg/ml in the absence of enhancers (open circles) and were described previously (Kays Leonard et al., 2005) ($n = 12$). The line represents the least squares fit of all data ($R^2 = 0.986$).

can boost the dose delivered *in vivo*, not only matching the therapeutically relevant doses required for treating Alzheimer's disease, but also opening the potential for galantamine as a treatment for other therapeutic areas.

Next, it was of interest to examine the relationship, if any, between *in vitro* permeation and *in vivo* bioavailability in the rat. The data plotted in Fig. 2 result from both the galantamine HBr formulations described above as well as formulations containing the more soluble lactate salt of galantamine alone (without permeation enhancers), described previously (Kays Leonard et al., 2005). The *in vitro* permeation rate of galantamine across the tissue barrier, as determined over the linear range of the experiment, was plotted against the *in vivo* AUC_{last}. As can be seen, the data constitute a reasonable correlation between *in vitro* permeation and *in vivo* bioavailability ($R^2 = 0.986$), suggesting that *in vitro* data are reasonably predictive of *in vivo* behavior.

Having established the utility of Me-β-CD, DDPC and EDTA in the galantamine formulation and the *in vitro–in vivo* correlation, it was next desired to conduct further screening to optimize individual levels of the excipients. Because the lactate salt allows for increased galantamine concentration and shows enhanced *in vivo* and *in vitro* performance relative to galantamine HBr (Fig. 2), further formulation optimization with permeation enhancers was conducted with galantamine lactate. Given the correlation discussed above, it was reasonable to conduct this optimization employing the *in vitro* tissue model.

Towards this goal, three formulation screening rounds were conducted based on a design-of-experiment (DOE) approach. The first round was a three-level full factorial study comprised of 27 formulations; Me-β-CD was varied from 0 to 90 mg/ml and both DDPC and EDTA were both varied from 0 to 2 mg/ml. The concentrations were selected by setting as the mid-point the concentrations of these excipients used in combination with galantamine HBr previously (e.g., formulations tested in Fig. 1). The formulation producing the best permeation was 9 mg/ml Me-β-CD, 2 mg/ml DDPC, and 2 mg/ml EDTA, resulting in a 32.4 ± 1.8% galantamine permeation after 1 h (Table 1). This is a statistically significant improvement over the permeation of galantamine lactate alone ($p = 0.02$). The results from this round also confirm the synergistic effect of the permeation enhancers in combination. When the enhancers were added individually to galantamine, only DDPC showed a slight improvement in permeation enhancement of galantamine alone. Although the concentrations of DDPC used here were well above the critical micelle concentration (CMC) for DDPC (Reynolds et al., 1997), the presence of Me-β-CD solubilizes the phospholipid (Strickley, 2004). Interestingly, increasing the Me-β-CD concentration to 90 mg/ml showed a consistent inhibition of galantamine permeation. Analysis of variance (ANOVA) of this data predicted that the optimal concentration of Me-β-CD with respect to providing permeation enhancement was well below 45 mg/ml, whereas optimal levels of DDPC and EDTA were somewhere between 1 and 2 mg/ml.

Based on the data from the first DOE round, a two-level full factorial DOE with eight formulations was executed in which Me-β-CD was varied between 0 and 9 mg/ml and both DDPC and EDTA were varied between 1 and 2 mg/ml to further investigate the effects of low concentrations of Me-β-CD. Permeation results are summarized in Table 2. Again, the formulation containing 9 mg/ml Me-β-CD, 2 mg/ml DDPC, and 2 mg/ml EDTA ($p = 0.03$ relative to galantamine alone) was among the best performers for enhancing permeation. Based on the second round DOE, the effectiveness of Me-β-CD at lower concentrations became clear. Formulations containing 9 mg/ml Me-β-CD all showed high permeation relative to formulations without the cyclodextrin ($p = 1.0 \times 10^{-6}$).

When ANOVA was performed on the results of these two DOE studies in combination, a modified quadratic model ($p<0.0001$) predicted the optimized formulation would contain 21 mg/ml Me-β-CD, 1.7 mg/ml DDPC, and 2.0 mg/ml EDTA. Because the design space had not yet tested these concentra-

Table 1
Permeation enhancer concentrations tested in the first round of DOE and resulting permeation after 60 min for each formulation

Me-β-CD (mg/ml)	DDPC (mg/ml)	EDTA (mg/ml)	Permeation (%)
0.0	0.0	0.0	14.6 ± 0.4
9.0	0.0	0.0	12.2 ± 0.2
90.0	0.0	0.0	6.5 ± 0.1
0.0	0.0	0.2	13.9 ± 0.3
9.0	0.0	0.2	12.5 ± 0.4
90.0	0.0	0.2	5.6 ± 0.2
0.0	0.0	2.0	12.1 ± 0.6
9.0	0.0	2.0	10.1 ± 0.2
90.0	0.0	2.0	5.5 ± 0.6
0.0	0.2	0.0	14.1 ± 0.5
9.0	0.2	0.0	14.0 ± 0.0
90.0	0.2	0.0	6.1 ± 0.4
0.0	0.2	0.2	15.4 ± 0.2
9.0	0.2	0.2	14.3 ± 1.8
90.0	0.2	0.2	7.2 ± 1.2
0.0	0.2	2.0	12.2 ± 0.3
9.0	0.2	2.0	11.5 ± 0.2
90.0	0.2	2.0	5.6 ± 0.1
0.0	2.0	0.0	15.7 ± 0.1
9.0	2.0	0.0	16.9 ± 0.3
90.0	2.0	0.0	6.6 ± 0.1
0.0	2.0	0.2	16.8 ± 0.5
9.0	2.0	0.2	21.8 ± 0.7
90.0	2.0	0.2	6.0 ± 0.2
0.0	2.0	2.0	21.3 ± 3.6
9.0	2.0	2.0	32.4 ± 1.8
90.0	2.0	2.0	7.1 ± 0.1

Table 2
Permeation enhancer concentrations tested in the second round of DOE and resulting permeation after 60 min for each formulation

Me-β-CD (mg/ml)	DDPC (mg/ml)	EDTA (mg/ml)	Permeation (%)
0.0	0.0	0.0	15.0 ± 0.4
0.0	2.0	2.0	17.8 ± 0.4
9.0	2.0	2.0	31.8 ± 6.6
0.0	2.0	1.0	18.8 ± 0.3
9.0	2.0	1.0	30.6 ± 6.1
0.0	1.0	2.0	17.1 ± 0.4
9.0	1.0	2.0	31.1 ± 1.4
0.0	1.0	1.0	17.0 ± 1.2
9.0	1.0	1.0	28.5 ± 4.9

Table 3
Permeation enhancer concentrations tested in central composite DOE

Formulation			Responses	
Me-β-CD (mg/ml)	DDPC (mg/ml)	EDTA (mg/ml)	Permeation (%)	Clarity[a]
33.7	1.38	2.0	23.6 ± 0.3	+
30.0	1.75	2.0	45.3 ± 2.9	+
30.0	1.00	2.0	17.1 ± 5.3	+
21.0	1.91	2.0	30.0 ± 3.2	−
21.0	1.38	2.0	29.9 ± 1.6	−
21.0	1.38	2.0	24.9 ± 3.0	−
21.0	1.38	2.0	26.1 ± 1.6	−
21.0	1.38	2.0	27.8 ± 2.2	−
21.0	1.38	2.0	23.4 ± 2.0	−
21.0	1.38	2.0	28.6 ± 2.5	−
21.0	0.84	2.0	21.6 ± 1.5	+
12.0	1.75	2.0	27.4 ± 1.2	−
12.0	1.00	2.0	29.1 ± 3.2	−
8.3	1.38	2.0	22.9 ± 5.3	−
0.0	0.00	0.0	13.3 ± 0.9	−

EDTA was held at 2.0 mg/ml in all formulations. Resulting galantamine permeation after 1 h incubation and formulation clarity are shown.
[a] For clarity: (+) visually clear solution and (−) visually turbid solution.

Table 4
ANOVA of permeation enhancers

Source	Sum of squares	d.f.	Mean square	*F* value	*p*-Value
Block	5272	1	5272		
Model	101621	3	33874	10.8	0.002
Me-β-CD	1723	1	1723	0.6	0.477
DDPC	40305	1	40305	12.9	0.006
Me-β-CD × DDPC	59593	1	59593	19.0	0.002
Residual	28159	9	3129		

tions of Me-β-CD or DDPC, the final DOE round was a central composite response surface method (RSM) design in which two factors (in this case, Me-β-CD and DDPC) were varied over five levels. Because EDTA consistently demonstrated enhanced permeation with increasing concentration, its concentration was fixed at 2.0 mg/ml. Table 3 describes the 14 formulations tested in the third and final DOE round. Also included are data for visual clarity of the formulation and the measured amount of galantamine lactate permeation *in vitro* after 1 h incubation. Clarity was a desired characteristic for the galantamine IN solution in order to provide consistent product quality and performance.

The results of the third round were analyzed using a response surface two-factor interaction model, which best fit the data. The permeation values predicted by the model were reasonably predictive of the observed data (adjusted $R^2 = 0.78$), and ANOVA showed that both Me-β-CD and DDPC had a significant impact on permeation (Table 4, p-values <0.05 were considered significant). Although Me-β-CD alone was not predicted to be significant ($p = 0.477$), the interaction of Me-β-CD and DDPC was ($p = 0.002$). The equation which best described the data and accurately predicted optimized permeation is as follows:

$$\text{galantamine permeation } (\mu\text{g}) = 1193.4 - 48.1\ \text{Me-β-CD} - 471.2\ \text{DDPC} + 36.2\ \text{Me-β-CD} \times \text{DDPC} \quad (2)$$

where units of Me-β-CD and DDPC are mg/ml. For galantamine lactate, the model predicted that the following concentrations would maximize permeation enhancement: 30 mg/ml Me-β-CD, 2.0 mg/ml DDPC, and 2.0 mg/ml EDTA. The predicted result closely matches the final optimized formulation, in which DDPC was decreased slightly to ensure clarity of the formulation: 30 mg/ml Me-β-CD, 1.7 mg/ml DDPC, and 2.0 mg/ml EDTA. The results confirmed the important roles of EDTA,

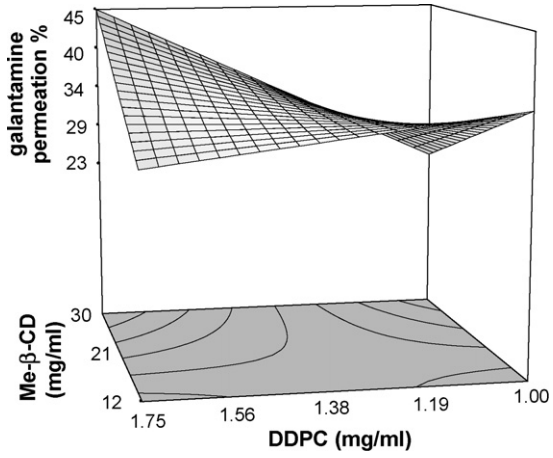

Fig. 3. Third round of DOE screening. Surface response plot for two factor interaction model showing predicted permeation across combinations of Me-β-CD and DDPC concentrations when EDTA is held at 2.0 mg/ml.

DDPC and Me-β-CD in galantamine lactate permeation. Specifically, across the entire concentration ranges tested, increasing EDTA concentrations resulted in increased permeation, while the permeation behavior as a function of DDPC and Me-β-CD concentrations was considerably more complex (Fig. 3).

The optimized formulation for galantamine lactate results in a four-fold increase in galantamine permeation in the *in vitro* model (Fig. 4A). The P_{app} for galantamine lactate alone and in the presence of permeation enhancers is $1.90 \pm 0.78 \times 10^{-6}$ cm/s and $7.58 \pm 2.28 \times 10^{-6}$ cm/s, respectively ($p = 0.04$). As with galantamine HBr, formulations with the lactate salt were tested for their effect on TER (Fig. 4B), cell viability (Fig. 4C), and cytotoxicity (Fig. 4D). As expected, only the formulation containing permeation enhancers reduced TER across the epithelial tissue layer. Cell viability was high in the presence of the optimized formulation as assayed by MTT ($98.4 \pm 8.2\%$) while cytotoxicity was low as assayed by LDH ($12.8 \pm 6.6\%$ relative to the Triton X control). Although the latter data are higher than that for the sample containing galantamine alone, this amount of cytotoxicity in the *in vitro* assay is relatively low, for example ≤20% LDH relative to the Triton X control. While it is anticipated these observations are predictive of low toxicity, *in vivo* toxicology studies are required to confirm the findings.

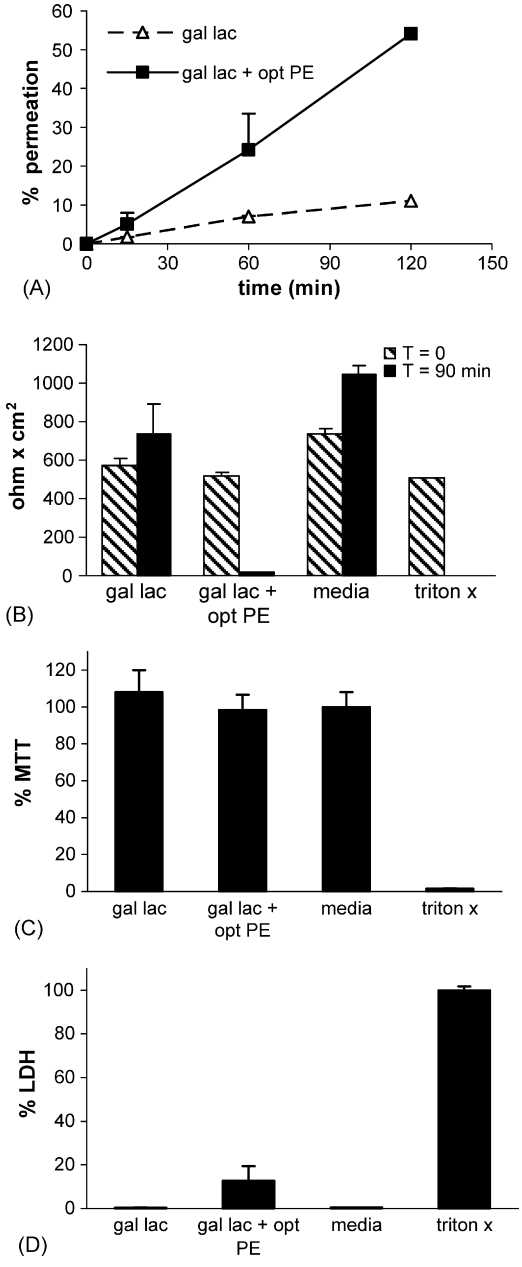

Fig. 4. *In vitro* performance of galantamine lactate (gal lac) with optimized permeation enhancers (+opt PE). (A) Permeation of 80 mg/ml galantamine lactate alone vs. in optimized formulation: 30 mg/ml Me-β-CD, 1.7 mg/ml DDPC, 2 mg/ml EDTA. (B) TER reduction in the presence of galantamine lactate alone vs. in optimized formulation. (C) Cell viability as assayed by MTT. (D) Cytotoxicity as measured by LDH assay.

Having optimized a galantamine lactate formulation with respect to permeation and clarity, the final goal of the current work was to test the hypothesis of reduced GI-related side effects for IN versus oral dosing. To this end, the ferret model was chosen because it is considered a suitable species for predicting emetic response in humans (Yamahita et al., 1997; Hasegawa et al., 2002). To ensure observation of emesis or retching, eight naïve ferrets were dosed at 20 mg/kg either orally or nasally and observed over a 4-h period following dosing. A separate PK study was conducted at the same dose level to compare bioavailability of galantamine in the two dosing routes. Orally, 20 mg/ml galantamine HBr was formulated with the excipients in the commercially available RAZADYNE® oral solution, specifically methyl and propyl parabens, sodium saccharin, sodium hydroxide. For IN administration, the galantamine lactate formulation contained 200 mg/ml galantamine and the optimized levels of permeation enhancers identified following the three DOE *in vitro* screening rounds described above.

The PK profile of the oral versus IN administration is shown in Fig. 5A. The IN galantamine had a shortened T_{max} relative to oral dosing (5 min versus 240 min, respectively) and nearly four-fold increase in C_{max} ($12{,}100 \pm 8000$ ng/ml versus 3200 ± 200 ng/ml). The AUC_{last} and AUC_{inf} was less than the oral route, approximately 70% relative bioavailability. However, the nasal route had a greater $AUC_{0–60}$ and $AUC_{0–120}$ than the oral route, approximately 200% and 128% relative bioavailability, respectively.

In a parallel study, emesis responses were monitored for the oral versus IN galantamine formulations. The emesis study revealed a clearly significant decrease in GI-related side effects when galantamine was administered by the IN route ($p<0.0001$). During the first 4 h after administration, only three emesis and retching events were observed with the IN administration as compared to 34 events with the oral administration (Fig. 5B). This result is interesting considering that within the period where emetic responses were observed, not only was the C_{max} four-fold higher for IN versus oral administration ($12{,}100 \pm 8000$ ng/ml versus 3200 ± 200 ng/ml), but also the drug exposure was higher for IN dosing, e.g., the $AUC_{0–60\ min}$ and $AUC_{0–120\ min}$ were 200% and 128%, respectively, of the value for oral dosing. The absence of nausea in combination with the increased systemic exposure associated with IN administration further confirms that the emetic side effects associated with oral galantamine are

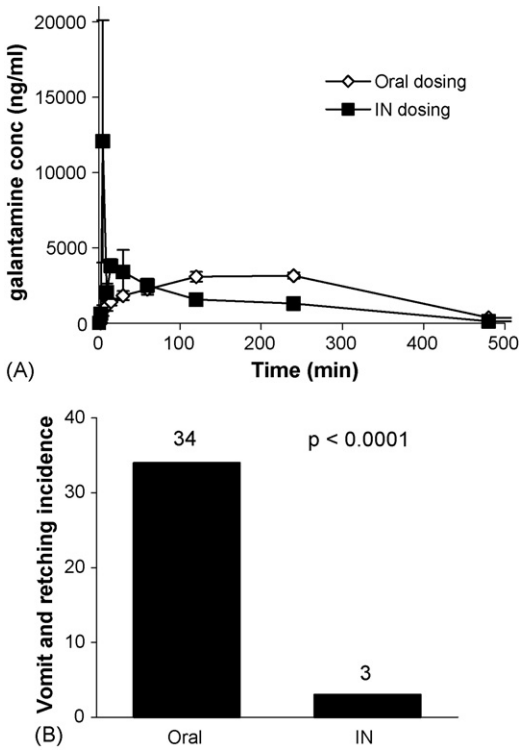

Fig. 5. *In vivo* comparison of the optimized galantamine lactate formulation administered intranasally (200 mg/ml) vs. orally administered RAZADYNE® (20 mg/ml). Ferrets were dosed 20 mg/kg galantamine free base ($n = 8$). (A) Pharmacokinetics. (B) Emetic response.

due to interactions in the GI tract and are not due to systemic exposure of drug.

In summary, this report describes the further development of an IN galantamine formulation. Absorption enhancers were successfully optimized to increase the permeation of galantamine lactate *in vitro*. This optimized formulation was then tested *in vivo* to determine the effects of IN administration of galantamine on GI-related side effects observed with oral delivery. A significant reduction in emesis and retching was observed with the IN administration route. To our knowledge, this study is one of the few that conclusively compares the GI-related side effects of oral versus IN administration for any therapeutic (Cicinelli et al., 1996; Illum et al., 2002), and the first such comparison for the important acetylcholinesterase inhibitor galantamine.

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