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Brain Targeting of a Water Insoluble Antipsychotic Drug Haloperidol via the Intranasal Route Using PAMAM Dendrimer

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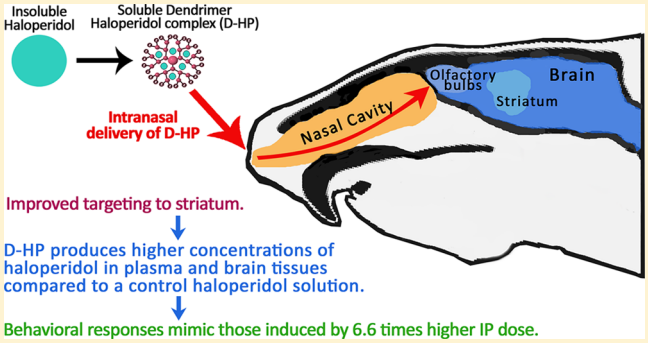
ABSTRACT: Delivery of therapeutics to the brain is challenging because many organic molecules have inadequate aqueous solubility and limited bioavailability. We investigated the efficiency of a dendrimer-based formulation of a poorly aqueous soluble drug, haloperidol, in targeting the brain via intranasal and intraperitoneal administration. Aqueous solubility of haloperidol was increased by more than 100-fold in the developed formulation. Formulation was assessed via different routes of administration for behavioral (cataleptic and locomotor) responses, and for haloperidol distribution in plasma and brain tissues. Dendrimer-based formulation showed significantly higher distribution of haloperidol in the brain and plasma compared to a control formulation of haloperidol administered via intraperitoneal injection. Additionally, 6.7 times lower doses of the dendrimer−haloperidol formulation administered via the intranasal route produced behavioral responses that were comparable to those induced by haloperidol formulations administered via intraperitoneal injection. This study demonstrates the potential of dendrimer in improving the delivery of water insoluble drugs to brain.



KEYWORDS: *brain targeting, intranasal administration, dendrimers, nanotechnology, haloperidol, drug delivery*

1. INTRODUCTION

The development of new neurotherapeutics is limited because of the challenges associated with transport of molecules across the blood brain barrier (BBB) and due to poor aqueous solubility of most of the new drug candidates. It has been reported that 98% of all small molecules and almost all large proteins and genes are unable to permeate across the BBB due to its unique barrier properties.[1] An important determining factor for efficient transport across the BBB is the phenomenon of lipophilicity, which is ability of a molecule to dissolve in lipids. As lipophilicity increases, however, aqueous solubility is reduced. This limits dissolution of the drug and its capacity for transport in systemic circulation. Poor transport of therapeutics across the BBB necessitates the systemic administration of very large doses to achieve therapeutic concentration in the brain. This exposes nontarget sites outside the central nervous system (CNS) to unnecessarily large concentrations of a drug, which can increase the risk of adverse effects. Inadequate aqueous solubility of most of the organic molecules also presents a bottleneck for development of novel therapeutics. Approximately 40% of drug candidates are rejected by the pharmaceutical industry due to poor aqueous solubility and consequently poor systemic bioavailability.

Intranasal (IN) delivery is distinguished among the various strategies currently available for drug targeting. It is noninvasive and reduces the exposure of nontarget sites to therapeutic substances, thus increasing efficiency and safety of drug delivery. Drug molecules can be targeted to the brain from the nasal cavity through the olfactory nerve pathway and trigeminal nerve pathway, therein bypassing the BBB. In addition to this, drug molecules can be systemically absorbed from the nasal cavity and can subsequently permeate the BBB if the drug molecules have sufficient lipophilicity.[2]

IN drug delivery is advantageous for water-soluble molecules, particularly biomolecules like proteins, which have poor bioavailability in brain tissues through oral or parenteral routes.[3,4] However, most small molecules that are used for therapy of CNS disorders have limited aqueous solubility. As such, they need to be formulated in nanoparticles or emulsions, solubilized using micellar solubilization or complexation with dextrans in order to be delivered to the brain via the IN route.[2,5] Although use of nanoparticles has been reported to

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improve the delivery of drugs to the brain via the IN route in several studies,[6−9] transport of large sized particles, especially larger than 200 nm, is not efficient due to poor paracellular and intracellular transport.[7]

Among various drug delivery systems, dendrimers due to their small size (less than 10 nm) are likely to have more efficient paracellular and transcellular transport across the BBB,[7,10] which makes them ideal carriers for targeting water insoluble drugs to the brain via IN administration. Dendrimers are discrete nanostructures/nanoparticles which are synthesized beginning with a core, and grown in concentric layers to produce stepwise increases in size. The unique extent of control of size and surface properties have led to the emergence of a "nanoperiodic" concept which proposes nanoparticle structure control and the engineering of "critical nanoscale design parameters" (CNDPs) as a strategy for optimizing pharmacokinetics, pharmacodynamics, and site-specific targeting of disease.[11]

Dendrimers have been reported to enhance the aqueous solubility of drugs by forming a complex with them,[12,13] which would provide a high concentration of diffusible drug at the nasal area. The dendrimers can be selected so as to a have positive charge on the surface that would also lead to increased transport of drugs to the brain as positively charged nanoparticles have been reported to have increased association with mucus as well as greater cell uptake.[7,10]

Intranasal delivery of the water-soluble radiolabeled siRNA−dendrimer complexes (dendriplexes) incorporated into *in situ* forming mucoadhesive gels shows increased radioactivity in the brain as compared to that obtained following IN delivery of si RNA incorporated in the similar mucoadhesive gels.[14] However, to date there is no report on using dendrimers for targeting water-insoluble drugs to the brain via intranasal administration and how this route of administration compares with intraperitoneal administration of the same formulations.

In the present study, we explored the potential of polyamidoamine dendrimers (PAMAM) with amine surface groups for targeting haloperidol to the brain following IN and IP (intraperitoneal) administration. Haloperidol (Figure 1), a

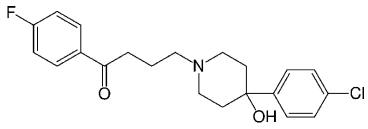

Figure 1. Chemical structure of haloperidol (IUPAC name: 4-[4-(4-chlorphenyl)-4-hydroxypiperidin-1-yl]-1-(4-fluorophenyl)butan-1-one).

classic antipsychotic drug, has limited solubility in water and therefore cannot be administered intranasally or intraperitoneally without solubility enhancement or formulation into some nanoscopic carrier systems. Haloperidol is also well documented to cause catalepsy and motor suppression in adult rats on acute administration,[15] which can be used as behavioral confirmation of successful delivery to the brain. Dendrimer entrapment was used to enhance the solubility of haloperidol by more than 100-fold, and the resulting formulation was assessed to determine whether such a modality can be used for targeting inherently water-insoluble drugs to the brain via IN administration.

2. MATERIALS AND METHODS

2.1. Materials. PAMAM dendrimer 1,4-diaminobutane core, amine terminated, Gen 5.0 was purchased from Nanosynthons, Mt. Pleasant, MI (USA). Haloperidol, Tween-20, ethanol, and ammonium acetate were purchased from Sigma (USA). High performance liquid chromatography (HPLC) mobile phase and supplies were purchased from Caleden Laboratories and Waters. [^{3}H]NPA with a specific activity of 60 Ci/mmol was obtained from Vitrax Radiochemicals (Placentia, CA).

2.2. Methods. *2.2.1. Preparation of the Dendrimer−Haloperidol Formulation.* 20 mg (equivalent to 0.694 μM) of PAMAM dendrimer (1,4-diaminobutane core, polyamidoamine, amine terminated, Gen 5.0) was dissolved in 1.6 mL of deionized water, and the pH was adjusted to 7.4 using 0.1 N HCl. To the dendrimer solution were added 0.4 mL of ethanol, 40 μL of Tween 20, and 5 mg of haloperidol. The resulting suspension was sonicated for 30 s and incubated overnight (16 h) at 37 °C in a shaker at 250 rpm Following incubation, the suspension was filtered through 0.2 μm syringe filters to separate insoluble haloperidol from the solubilized drug solution to obtain final dendrimer−haloperidol formulation (D-HP) and stored at 4 °C.

A blank formulation containing dendrimers was prepared similarly without the addition of haloperidol (dendrimer−vehicle, D-Veh). Control haloperidol formulation (HP-A) for IP administration was adopted from Igarashi et al.[16] and was prepared by dissolving 1 mg/mL haloperidol in water containing 0.2% acetic acid.

2.2.2. Estimation of Haloperidol Content in the Dendrimer−Haloperidol Formulation. Dendrimer formulations were diluted with 2% acetic acid in methanol, and haloperidol content was estimated according to the HPLC method reported by Igarashi et al.[16] using Waters 2695 separation module coupled to Waters 2489 UV detector and Novapak C18 column (4 μm particle size, internal diameter 3.9 mm, length 150 mm). Mobile phase consisted of 10 mM ammonium acetate in water, acetonitrile, methanol, and glacial acetic acid in the ratio 57.2:22:20:0.8 respectively. Injection volume was 20 μL, and flow rate of the mobile phase was 0.5 mL per minute; estimation of haloperidol was carried out at 254 nm. A standard curve was prepared in methanol containing 2% acetic acid with haloperidol concentrations 10, 50, 100, 500, and 1000 μg/mL.

2.2.3. Effect of Haloperidol Entrapment on Size and Zeta Potential of Dendrimers. To assess the effect of haloperidol complexation on size distribution and zeta potential of dendrimers, blank dendrimer formulation (D-Veh) and dendrimer−haloperidol formulation (D-HP) were analyzed for particle size distribution and zeta potential using Zetasizer Nano ZS (Malvern).

2.2.4. In Vitro Release of Haloperidol from the Dendrimer−Haloperidol Formulation. In vitro release of haloperidol from dendrimer formulation was determined in release medium consisting 0.1 N HCl (pH 2.0) and 20 mM PBS (pH7.4). 0.5 mL formulations were transferred to a dialysis membrane with a 10 kDa cutoff, and the tube was immersed in 40 mL of release medium. The volume of release medium was sufficient to completely dissolve haloperidol present in the sample. 0.1 N HCl was used as one of the release media to determine the drug release in a simulated gastric fluid, as one group of animals received formulation orally. Release medium was stirred at 250 rpm at room temperature. At different time

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intervals, 0.5 mL samples were withdrawn from the release medium for analysis of drug released and replenished with fresh medium.

2.2.5. Receptor Binding Evaluation of the Dendrimer−Haloperidol Formulation. Competitive receptor binding experiments were performed in triplicate to investigate the ability of haloperidol, D-HP, and D-Veh to displace dopamine D2 receptor agonist, [^{3}H]-*N*-propylnorapomorphine ([^{3}H]NPA) binding from dopamine D_2 receptors in bovine striatal membranes. Bovine striatal membranes were prepared as previously described by Kazmi et al.[17] where freshly dissected calf bovine striatum was homogenized and centrifuged in buffer to isolate cell membranes. 100 μg of striatal membrane was incubated in 500 μL of assay buffer (50 mM Tris-HCl, 1 mM EDTA, 0.1 mM PMSF, 5 mM $MgCl_2$, 0.1 mM DTT, 100 μg/mL bacitracin, 5 μg/mL soybean trypsin) for 3 h at 25 °C in the presence of [^{3}H]NPA and concentrations ranging from $10^{-14.5}$ M to 10^{-4} M of either haloperidol, D-HP, or D-Veh. Specific binding was defined as the difference between the radioactivity bound in the absence and presence of 10 μM sulpiride (Sigma). Following the 3 h incubation, reaction mixtures were subjected to rapid filtration onto filter disks. Filter disks were placed in plastic scintillation vials with 4 mL of scintillation fluid and allowed to equilibrate for at least 12 h. Finally, scintillation vials were counted in a Beckman LS5000 liquid scintillation counter (model LS5 KTA). The [^{3}H]NPA binding was compared between assays using the IC_{50}.

2.2.6. In Vivo Evaluation Assessed through Behavioral Response of the Dendrimer−Haloperidol Formulation. Developed D-HP formulation was evaluated for in vivo behavioral response in Sprague−Dawley rats (Charles River, St. Constant, Quebec) following IN, IP, and oral administration. Rats weighing 250−350 g were procured and housed at the McMaster University Central Animal Facility. All protocols were approved by the Animal Research Ethics Board of McMaster University and were in compliance with the Canadian Council on Animal Care.

Animals were divided into five groups with a total of six animals per group. Group 1 was administered HP-A IP at a dose equivalent to 2 mg/kg body weight.[9] Group 2 was administered D-HP IP at 2 mg/kg. Equivalent volumes of D-Veh were administered IP to the third group as a negative control. Group 4 received the D-HP formulation via IN administration. However, since the total volume that could be administered via the IN route did not exceed 120 μL (30 μL in each nostril administered twice with 4−5 min interval between each administration), lower doses (0.3 mg/kg) were administered via the IN route. Animals in group 5 were administered D-HP formulation containing haloperidol equivalent to that administered IN (0.3 mg/kg) via oral administration, to test the possibility that IN administered dose was being swallowed by the rats. Catalepsy and motor suppression were evaluated on different days, with at least a three-day interval between the evaluations. Dendrimer concentrations in the formulation were 10 mg/mL. Maximum dose of dendrimers administered per rat through the IP route was less than 6 mg, and through the IN route, maximum dose was less than 1 mg.

All the groups were lightly anesthetized to the same degree (when the animals were unresponsive to physical manipulation) using isofluorane and to the same duration (8−10 min) during the administration of the different formulations. For IN administration, the formulation was administered by pipetting aliquots of 30 μL in each nostril, with 3−4 min intervals between each administration. For oral administration, formulation was delivered directly into the gastrointestinal tract by gavage through a cannula. IP administration was performed with a single injection into the intraperitoneal space.

Immediately after formulation administration, animals were transferred to AccuScan computerized cages (AccuScan Instruments, Columbus, OH) and multidirectional movements were recorded by the computerized system locomotion chambers and locomotion was recorded with a laser-assisted apparatus for 2 h. For the assessment of catalepsy, 30 and 60 min following the administration, the front paws of the rat were gently placed in an extended position on a 10 cm high horizontally mounted metal bar, and the amount of time spent maintaining this abnormal position was measured.[18] The animals were tested in triplicate with 30-s intervals, and the maximum intensity of the cataleptic response was recorded in seconds.[19] Rats were given a rated score (1−3) based upon time spent on the bar (0 = less than 20 s, 1 = 20−60 s, 2 = 60−120 s, 3 = 120−180 s).

2.2.7. Estimation of Haloperidol Distribution in Brain Tissues and Plasma. After the behavioral tests, animals were rested for 1 week to allow for complete excretion of haloperidol. They were administered formulations, keeping the routes and doses similar to that used for behavioral testing. After 1 h of administration of formulations, rats were anesthetized using isoflurane and decapitated. Tissue samples from the olfactory bulb, striatum and cerebellum were collected and stored in dry ice and subsequently at −70 °C before the actual estimation. Blood was collected using the cardiac puncture method in heparinized tubes and centrifuged at 5000 rpm for 10 min at 4 °C, and plasma was separated. Separated plasma was stored at −70 °C until analysis.

Haloperidol was extracted from the tissues according to the procedure described by Igarashi et al.[16] 0.2 mL of ice cold 1.15% (w/v) KCl solution and 0.3 mL of 2% (v/v) acetic acid in methanol were added to the tissue samples, and the tissues were homogenized first using a hand homogenizer and then by ultrasonic homogenization for 30 s thrice with 30-s intervals. Samples were centrifuged at 13000 rpm for 10 min at 4 °C, and the supernatants were used for the estimation of haloperidol.

For the estimation of haloperidol in plasma, 0.8 mL of 2% (v/v) acetic acid in methanol was added to 0.2 mL of plasma and the solution was added and mixed by vortexing for 1 min. The samples were then centrifuged at 13000 rpm for 10 min at 4 °C and the supernatants were separated for analysis.

Standard curves for the estimation of haloperidol in brain tissues or plasma were prepared by adding a haloperidol solution in 2% (v/v) acetic acid in methanol containing 1.6−400 ng of haloperidol to the homogenate obtained from 0.2 g of brain tissues or 0.2 mL of plasma, and extraction was carried out as described above.

The plasma and brain tissue extracts were analyzed by liquid chromatography electrospray time-of-flight mass spectrometry (LC−ESI-TOF-MS). Haloperidol was separated using an Agilent 1200 C HPLC system (Agilent, Canada) attached to a Microtof II (Bruker) TOF MS. 2 μL was injected into the Eclipse Plus C18 RRHD 1.8 μm column, 2.1 × 100 mm (Agilent). Separation was carried out at 25 °C. The mobile phase consisted of 10 mM ammonium acetate in water, acetonitrile, methanol, and glacial acetic acid in the ratio 57.2:22:20:0.8 respectively, and the flow rate was 0.2 mL/min. For detecting haloperidol, electrospray ionization (ESI) was

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used to ionize the analyte, and haloperidol ion was detected as $[M + H]^+$ ion in positive mode at m/z value 376.2^+.

2.2.8. Statistical Analyses. GraphPad Prism software and Microsoft Excel were used for statistical analyses. Behavioral parameters and tissue concentrations across different groups were analyzed using one-way ANOVA with Tukey's post hoc test. For receptor binding, $\log[IC_{50}]$ values were compared between assays using an *F*-test. The threshold of significance was $p < 0.05$.

3. RESULTS

3.1. Solubility Enhancement of Haloperidol with Amine Terminated, G-5 PAMAM Dendrimers. Haloperidol concentrations in different formulations are presented in Table 1. Solubility enhancement using dendrimers even at 0.25% (w/v) concentrations was 10-fold more than that achieved by 20% ethanol and 7-fold more than that achieved by the combination of 20% ethanol and 2% Tween 20. Combination of 1% dendrimers, 20% ethanol, and 2% Tween 20 yielded a solution with a haloperidol concentration of 1223 μg/mL, which was a 100-fold enhancement as compared to solubility in water alone. The number of haloperidol molecules complexed with each dendrimer molecule can be calculated as the ratio of molar concentration of haloperidol to molar concentration of dendrimers; as such the average number of haloperidol molecules complexed with each dendrimer molecule is 9.4.

Table 1. Haloperidol Concentration in Different Formulations

	composition				
formulations	water (% v/v)	dendrimers (% w/v)	ethanol (% v/v)	Tween-20 (% v/v)	haloperidol concn[a] (μg/mL)
water	100				11.5 ± 1.6
HPE	80		20		36.7 ± 2.0
HPE-T	78		20	2	56.8 ± 1.8
HPD 0.25	100	0.25			340.6 ± 11.5
HPD 0.5	100	0.5			374.3 ± 5.4
HPD 1.0	100	1.0			421.3 ± 8.6
HPED	80	1.0	20		1018.7 ± 20.7
D-HP	78	1.0	20	2	1223.4 ± 27.6

[a]Mean values with standard deviation ($n = 3$).

3.2. Effect of Haloperidol Entrapment on the Size Distribution and Zeta Potential of Dendrimers. Size distribution and zeta potential of formulation D-Veh and D-HP are given in Table 2. It was observed that the size distribution and zeta potential were not altered significantly as a result of haloperidol incorporation in the dendrimers. Dendrimers in final formulation, D-HP, were in the range 10–20 nm.

3.3. In Vitro Release of Haloperidol from Dendrimer–Haloperidol Formulations. Haloperidol was released more rapidly from the dendrimer–haloperidol formulation in 0.1 N HCl than that in 20 mM PBS pH 7.4 (Figure 2). 60% of the drug content was released from the formulation in 20 mM PBS within 3 h, while in 0.1 N HCl, more than 70% of the drug was released within the first hour. However, the amount released at the end of the 24 h period was similar in both release media (approximately 90%).

Table 2. Size Distribution and Zeta Potential of (1) Formulation Prepared with Blank Dendrimers (D-Veh) and (2) Dendrimer–Haloperidol Formulation (D-HP)

sample no.	formulations	size ± SD (*d*, nm)	zeta potential ± SD (mV)
1	blank dendrimers	14.09 ± 4.4	13.4 ± 1.02
2	D-HP	15.10 ± 5.4	10.7 ± 1.75

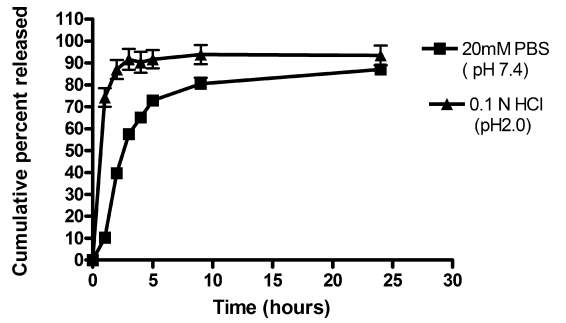

Figure 2. In vitro release profile of haloperidol from dendrimer–haloperidol formulation in 20 mM PBS (pH 7.4) and 0.1 N HCl. Cumulative percent released with standard error of mean has been plotted against time.

3.4. Evaluation of Receptor Binding of Dendrimer–Haloperidol Formulation. Competitive receptor binding (Figure 3) showed that the D-Veh formulation did not change maximum binding of $[^3H]$NPA to dopamine D_2 receptors, whereas HP-A and D-HP decreased maximum $[^3H]$NPA binding to nearly 25% ($R^2 = 0.9724$ and 0.9448, respectively). An *F*-test revealed no significant difference between the two $\log[IC_{50}]$ values of D-HP and HP-A ($F_{1,121} = 1.074$, $p = 0.3021$).

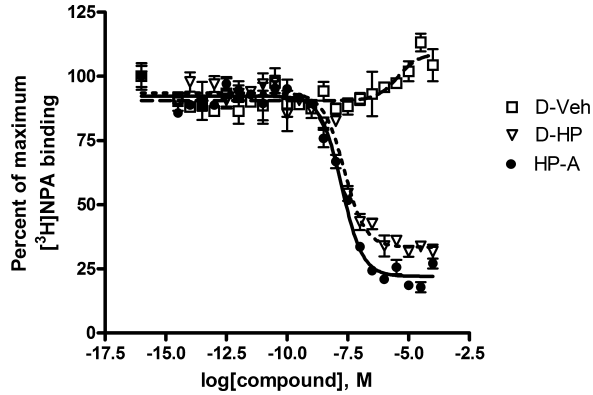

Figure 3. Effect of HP-A, D-Veh, and D-HP on specific $[^3H]$NPA binding to dopamine D_2 receptors in bovine striatal membranes. Error bars represent standard error of mean ($n = 3$).

3.5. In Vivo Behavioral Response of the Developed Formulation. Evaluation of the cataleptic response revealed that the IN administration of D-HP exhibited a cataleptic response (Figures 4A and 4B), which was similar to that achieved by IP administration of 6.7 times larger doses of the same formulation or HP-A during 30 or 60 min postadministration. Oral administration of D-HP formulations at doses similar to those given by the IN route did not cause any catalepsy until 60 min postadministration.

Groups of rats that were administered D-HP IP, HP-A IP, and D-HP IN exhibited significant motor suppression as

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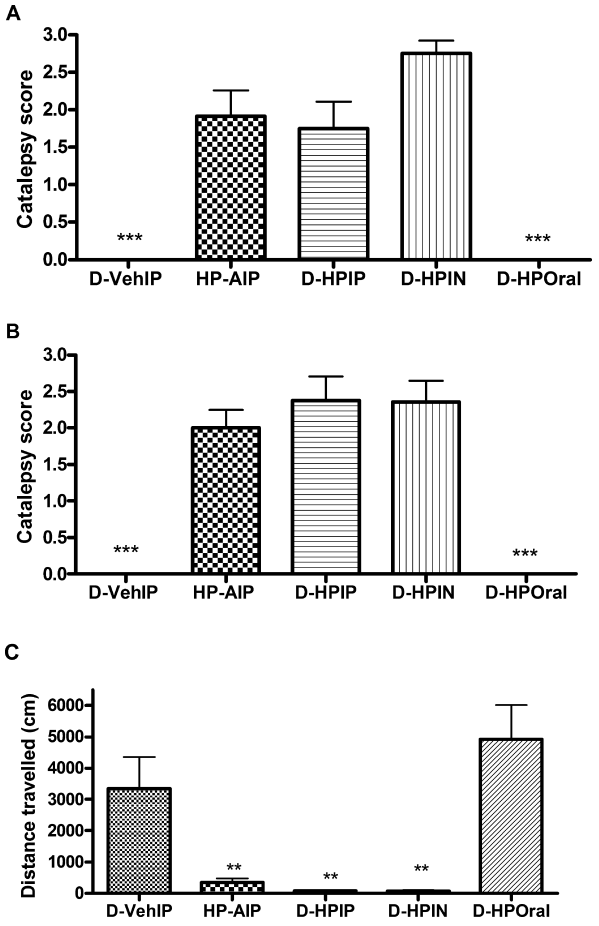

Figure 4. Effect of administration of dendrimer−haloperidol formulation through IP (D-HP IP), IN (D-HP IN), and oral (D-HP Oral) routes, control haloperidol formulation through the IP route (HP-A IP), and blank dendrimer formulation through the IP route (D-Veh IP) on catalepsy in adult rats (n = 3) at 30 min (A) and 60 min (B) postadministration and on locomotion during 2 h postadministration (C). Average values with error bars representing standard error of mean have been plotted against different formulations.

compared to controls (D-Veh IP) (p < 0.05) (Figure 4C). However, motor suppression in the group of rats treated with D-HP IN was similar to that exhibited by the rats which were treated with a 6.7 times larger dose of haloperidol in the form of D-HP IP or HP-A IP. Motor suppression caused by D-HP IN administration was significantly higher than that produced following D-HP oral administration of a similar dose by gavage (p < 0.05). Motor suppression caused following oral administration was not significantly different from that caused by IP administration of D-Veh (p > 0.05).

3.6. Haloperidol Content in the Brain Tissues and Plasma. Haloperidol was estimated in the brain tissues and plasma of rats administered different formulations using LC−ESI-TOF-MS. The method developed for estimating haloperidol in brain tissue exhibited good linearity (correlation coefficient, R^2 = 0.993) over a dynamic range of 3 orders of magnitude; this corresponded to 8 ng to 1000 ng per gram of brain tissues. The intra-assay variation was 1.23%, and the interassay variation was 4.06%. The method for estimating haloperidol in plasma had a correlation coefficient of 0.966 over a haloperidol standard concentration range of 25−200 ng/mL (in plasma). The intra-assay variation and interassay variation were 1.89% and 4.86% respectively.

Concentration of haloperidol in brain tissues and plasma are presented in Table 3. The highest concentrations of haloperidol in brain and plasma were obtained following the IP administration of D-HP. Compared to the concentrations obtained following the IP administration of the same dose of formulation HP-A, these were about four times greater in the olfactory bulb and cerebellum and more than twice the concentration in the striatum and plasma. Though the dose of D-HP administered by the IN route was 6.7 times lower, the concentration in the striatum was 2−3 times lower than those obtained following the IP administration of formulation HP-A, while the concentration in the olfactory bulb and cerebellum was 3−4 times lower. Haloperidol was not detected in brain tissues of rats that were orally administered doses of D-HP equal to that administered to the IN administered group.

The efficiency of different modalities of administration on targeting haloperidol to brain tissues were determined by calculating percent of administered doses present in per unit mass of brain tissues, which have been presented in Figure 5. It shows that the percent of administered haloperidol dose present in the olfactory bulb and cerebellum after 1 h of IP administration of D-HP were significantly higher than those obtained following the IP administration of an equivalent dose of HP-A, but not from those obtained following IN administration of D-HP. Mean value of the percent of administered doses present in the striatum were highest in the rats administered with D-HP by the IN route, which was significantly higher than those obtained following IP administration of the control formulation.

The mean value of the percent of administered dose present in the plasma was highest in the rats administered D-HP by the IP route, which was significantly higher than those obtained following IP administration of the control formulation.

Table 3. Tissue Concentration per Gram of Tissue at 60 min Following Administration of Different Haloperidol Formulations and Different Routes[a]

	av tissue concns (ng/g or ng/mL) ± SD (n = 3)			
tissue	D-HP IP	D-HP IN	D-HP oral	HP-A IP
olfactory bulb	3294.6 ± 770.4[b]	224.9 ± 128.6	nd	787.7 ± 510.1
striatum	2817.6 ± 423.9[b]	588.9 ± 193.1[c]	nd	1370.4 ± 115.3
cerebellum	3194.0 ± 705.9[b]	232.6 ± 180.6	nd	813.0 ± 530.0
plasma	385.3 ± 41.1[b]	nd	nd	127.0 ± 74.7

[a]D-HP IP: Dendrimer−haloperidol formulation administered intraperitoneally, dose-2 mg/kg. D-HP IN: Dendrimer−haloperidol formulation administered intranasally, dose 0.3 mg/kg. D-HP Oral: Dendrimer−haloperidol formulation administered orally using gavage, dose 0.3 mg/kg. HP-A IP: haloperidol in acetic acid solution administered intraperitoneally, dose 2 mg/kg; nd, nondetectable. [b]Significantly different (p < 0.01) from HP-A IP and D-HP IN (one way ANOVA, Tukey post hoc test). [c]Significantly lower than HP-A IP (p < 0.05; one way ANOVA, Tukey post hoc test).

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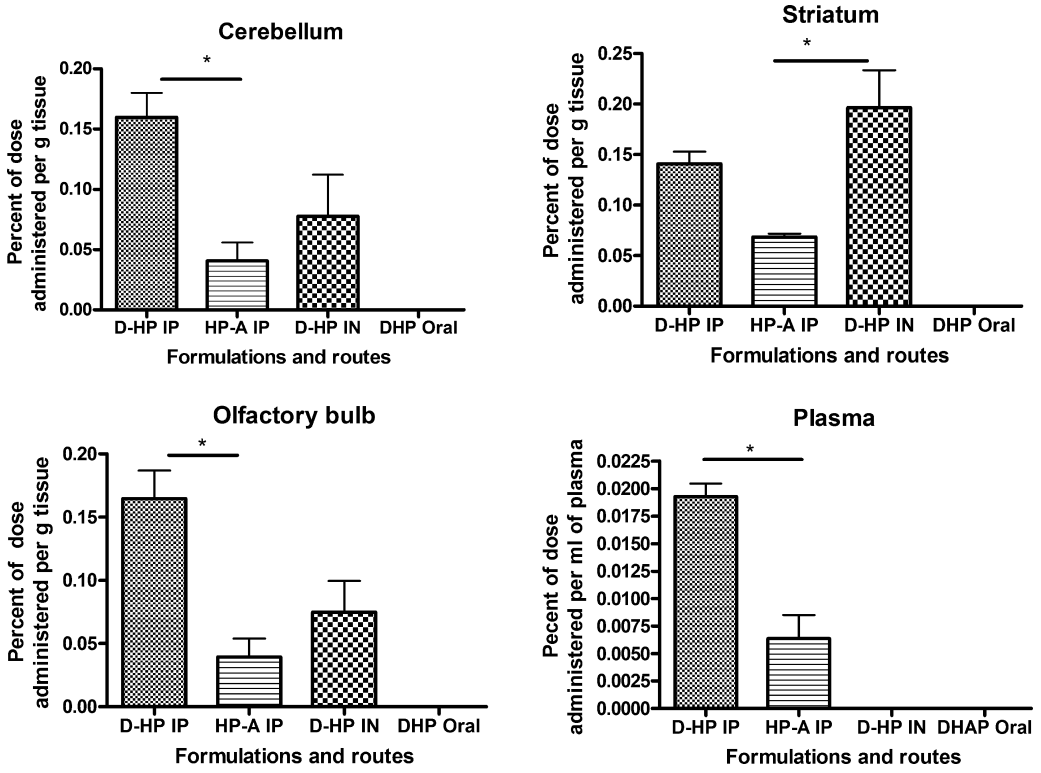

Figure 5. Distribution of haloperidol in the olfactory bulb, striatum, cerebellum, and plasma of adult rats (n = 3) at 60 min following administration of dendrimer−haloperidol formulation through the IP (D-HP IP), IN (D-HP IN), and oral (D-HP Oral) routes and control haloperidol formulation through the IP route (HP-A IP). Average values of percent of total dose administered, which was present in 1 g of tissue, have been plotted against respective modality of administration. Error bars represent standard error of mean.

Haloperidol was not detected in the plasma of the rats which were administered with a 6.7 times lower dose of D-HP by the IN or oral route.

The potential of IN delivery of D-HP in targeting entrapped drugs to the striatum was calculated by comparing the ratio of percent dose in brain tissue to that in plasma (Figure 6). The results show, in the case of formulation D-HP, that the IN route was seven times more efficient in targeting haloperidol to the striatum than the IP route. Targeting potential of formulation D-HP through the IN route was 4-fold higher than that of the control formulation HP-A administered through the IP route.

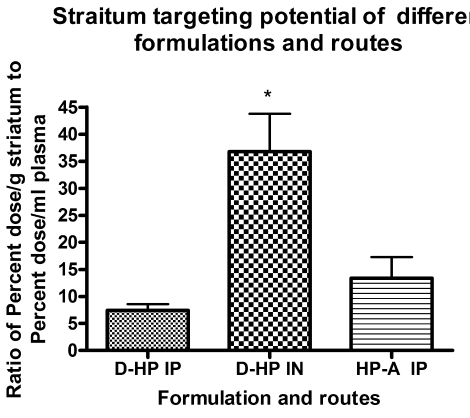

Figure 6. Striatal targeting potential of dendrimer−haloperidol formulation administered through the IP route, the same formulation through the IN route, and control haloperidol formulation through IP route. Ratio of percent of dose per gram of striatum to percent dose per mL of plasma has been plotted against different formulations. Error bars represent standard error of mean.

4. DISCUSSION

The efficacy of a drug can be improved by modifying the formulation and in vivo delivery method. Dendrimers work as a multifunctional excipient by enhancing the aqueous solubility of a drug in the formulation stage and enhancing dissolution, stability, permeation, and bioavailability in the delivery stage. In the present study, we have used dendrimer nanotechnology for brain targeting of a water-insoluble drug, haloperidol, via the IN, IP, and oral routes. We compared their efficacy in terms of the behavioral effects as well as tissue distribution following these delivery strategies. These modalities of haloperidol administration were also compared to the behavioral effects and tissue distributions obtained following IP administration of a control formulation.

Enhanced solubility of haloperidol in the presence of dendrimers is consistent with other reports where such an effect of dendrimers has been observed in improving the solubility of a wide variety of drugs, including quercetin,[20] beclometasone dipropionate,[21] daidzein,[22] 5-fluorouracil,[23] risperidone,[24] simvastatin,[13] silybin,[25] niclosamide,[26] indomethacin,[27,28] and furosemide.[29] It has been suggested that small organic and hydrophobic molecules are entrapped in the interior hydrophobic core of the dendrimer and display enhanced solubility.[12] Yang et al.[30] studied the solubilization of phenylbutazone utilizing two-dimensional nuclear Overhauser effect spectroscopy (2D-NOESY) and isothermal titration calorimetry (ITC) and suggested that both entrapment and electrostatic interaction together are responsible for

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solubility enhancement. We found that the solubility of haloperidol was increased more than 100-fold in the presence of dendrimers, Tween-20, and ethanol. This observation is in corroboration with a previous report on the role of the coexcipient to drastically increase the aqueous solubility propensity of dendrimers.[12]

Dendrimer size in the blank formulation and the formulation containing haloperidol was within the range 10−20 nm, although PAMAM G 5.0 dendrimers are approximately 5 nm in diameter. This suggests that there is some aggregation, possibly due to presence of surfactant (Tween 20), but these aggregates were soluble in the formulation. Such aggregation of dendrimers in the presence of the surfactants has been reported previously by Cheng et al.[31] However, the size of these aggregates was below 20 nm, thus the advantage in terms of size requirement for nose to brain transport was retained.

Though the mechanism of nose to brain transport of drug carriers is incompletely understood, the available literature suggests that such small size carriers would be transported to the brain more easily than the nanoparticles in the size range 100−200 nm.[7,32]

The zeta potentials of dendrimer formulations are in the positive range, though the values are lower than 30 mV, which indicates incipient instability for colloids. However, we did not observe the formation of insoluble aggregates in the case of dendrimers. The probable reason for this is that dendrimers, being individual molecules, might not undergo aggregation as readily as larger sized colloids, and the association of dendrimers with Tween 20 in the formulations could have further discouraged the aggregation of large sized insoluble aggregates. Lower values of positive zeta potential are desirable because lower values are associated with lower toxicity.[33]

In vitro release of more than 60% of the haloperidol content within 3 h in 20 mM PBS and within 1 h in 0.1 N HCl suggests that the release of haloperidol was limited by the dissolution rate and solubility in the release media. Under in vivo conditions, where sink conditions are encountered, we expect faster drug release. Formulation D-HP displays similar binding potency to the dopamine D_2 receptor as control haloperidol, which means either that haloperidol was readily released from the formulation or that dendrimer bound drug was free to interact with the receptors.

The intensity of motor suppression and catalepsy observed in rats administered D-HP via the IN route was comparable to that induced by IP administration of a 6.7-fold larger dose of formulations: D-HP, as well as HP-A. These observations demonstrate the effectiveness of dendrimers in transporting drugs to the brain following IN administration. Behavioral effects of D-HP following IN administration can be explained by the higher percentage of dose reaching the striatum following administration via IN routes than that obtained following IP administration of the control formulation and comparable to those obtained following IP administration of D-HP. Although the concentrations of haloperidol in the striatum were 4−5-fold higher in the animals which were administered formulation D-HP by IP routes and 2-fold higher in the animals which were administered HP-A by the IP route than those which received intranasal administration (since IP doses were 6.7-times higher than IN doses), similar behavioral effects were observed suggesting that this difference in striatal haloperidol concentration was not sufficient to induce quantitatively different cataleptic behavior and locomotion suppression in the designed tests. Failure of orally administered D-HP to induce behavioral effects and to yield detectable haloperidol concentrations in the plasma or brain tissue rules out the possibility that oral ingestion (swallowing) of an IN administered dose caused the effects observed following IN administration of D-HP. The absence of detectable haloperidol in the plasma of rats that were administered D-HP by the IN route also suggests that the drug was transported to the brain directly from the nasal cavity and not via absorption into systemic circulation. Furthermore, the doses administered through the IN route were lower, and therefore it is not surprising that we did not detect haloperidol in the plasma of rats administered with formulation D-HP by the IN route. These lower plasma concentrations are desirable for the prevention of systemic side effects of the drug, such as cardiac and extrapyramidal side effects following intravenous administration of haloperidol reported by Meyer-Massetti et al., 2010.[34]

For the calculation of targeting potential (ratio of percentage dose present in striatum to that present in plasma), it was assumed that the plasma of IN administered rats contained concentrations equal to the lowest limit of detection for haloperidol, which were higher than the actual plasma concentrations. In spite of this assumption, we observed that the targeting potential of the formulation D-HP by IN route was about 8-fold higher than that observed by IP administration of the same formulation, which shows the advantage of intranasal delivery in targeting haloperidol exclusively to the brain, especially the striatum.

A significantly higher percentage of the dose in the plasma, cerebellum, and olfactory bulb following IP administration of D-HP than those obtained following IP administration of the HP-A propose the role of dendrimers in prolonging circulation times of haloperidol and thus making it available for transport across the blood brain barrier. Improved bioavailability of haloperidol to the brain following IP administration of the dendrimer based formulation as compared to IP administration of the control haloperidol formulation shows the efficiency of dendrimers in improving drug transport across the BBB. These results thus suggest that though dendrimer-based solubilization might be an attractive option for delivering drugs to the brain by their administration into the systemic circulation, delivery through the nasal route is a better option for targeting drugs to the brain. The IN route also has the potential to eliminate extra-CNS side effects by reducing the drug concentration in the plasma.

Investigation of mucosal toxicity of the developed formulation is in progress. Mucosal toxicity would be dependent on the concentration of dendrimers in the formulation and the total amount of delivered dendrimers. Earlier Perez et al. have found that 60 μL of formulation containing G-7 dendrimers at a concentration 0.033 mg/mL did not exhibit any significant toxicity to rat mucosa.[14] Although the concentration and dose used by Perez et al. were lower than those used in the present study, the G-5 dendrimer used in this study is considerably less toxic than G-7 dendrimers. In another study, Dong et al., 2010, did not find any toxicity of 10 mg/mL dendrimers for G-3 dendrimers, when administered intranasally.[35] Looking at intraperitoneal toxicity, Chauhan et al. administered G4 dendrimers to Swiss albino mice at doses up to 9.5 mg/kg body weight for 15 days and did not find any toxicity in terms of general behavior, food intake, body weight, carbohydrate, lipid and protein metabo-

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lism, hematological parameters, histopathology, and cell viability.[36]

Dendrimer-based solubilization of haloperidol is an important prerequisite for the IN administration of haloperidol. Due to the lack of solubility of haloperidol at neutral pH, it is not possible to administer simple aqueous solutions of haloperidol by the IN route, and the acidic pH at which haloperidol is solubilized is considerably irritating and toxic to the nasal mucosa. There are reports in the literature showing the transport of nanoparticles lower than 200 nm to the brain from the nasal cavity,[7] and it is likely that the dendrimers present in the formulation with their lower size and slight positive charge can take the entrapped drugs to the brain from the nasal cavity. Alternatively, it is possible that the drug is released in the nasal cavity in the olfactory mucosa and is subsequently transported to the brain. In both these scenarios, the dendrimers provide a fascinating alternative for brain targeting of drugs, which cannot be normally solubilized at neutral pH, through the IN route.

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS USED

D-HP, dendrimer−haloperidol formulation; HP-A, haloperidol in water containing 0.2% acetic acid; IN, intranasal/intranasally; IP, intraperitoneal/intraperitoneally; BBB, blood brain barrier; PAMAM, poly(amido amine); LC−ESI-TOF-MS, liquid chromatography electrospray time-of-flight mass spectrometry

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