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Direct nose-to-brain transfer of a growth hormone releasing neuropeptide, hexarelin after intranasal administration to rabbits

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ABSTRACT

The purpose of this study was to investigate the olfactory transfer of a growth hormone releasing neuropeptide, hexarelin to the brain tissues by comparing brain uptake levels after intranasal administration with those after intravenous administration. The hexarelin nasal formulation was prepared using an aqueous cosolvent vehicle consisting of ethanol, propylene glycol, and *n*-tridecyl-β-D-maltoside as a permeation enhancer. Hexarelin was administered intravenously or intranasally to male rabbits at a dose of 1 mg/kg. Drug concentrations in the plasma, cerebrospinal fluid and six different regions of the brain, i.e., olfactory bulb (OB), olfactory tract (OT), anterior (CB1), middle (CB2), posterior (CB3) cerebrum, and cerebellum (CL) were analyzed by LC/MS method after solid phase extraction. The brain and cerebrospinal fluid levels achieved following intranasal administration were approximately 1.6 times greater than those attained after intravenous administration despite the intranasal plasma levels being significantly lower than the intravenous plasma levels. Intranasal administration resulted in significantly different spatial distribution patterns in various regions of brain with the rank order of $C_{OB} > C_{OT} > C_{CB1, CB2, CB3} > C_{CL}$ at 10, 20, and 40 min post-dosing, whereas intravenous administration yielded nearly similar distribution patterns in the brain. The intranasal administration into one nostril (left or right) exhibited markedly greater hexarelin concentrations in olfactory bulb and olfactory tract on the treated-side of brain tissues than those on the non-treated-side of the brain hemisphere. It was demonstrated that the hydrophilic neuropeptide hexarelin was transferred via olfactory pathway to the brain hemispheres and the drug transfer via this route significantly contributed to high brain concentrations after nasal administration to rabbits.

1. Introduction

Growth hormone (GH—22 kDa), which is secreted by the pituitary gland, stimulates the growth and cell reproduction in humans and other animals. In addition, studies have shown that GH possesses the following effects on metabolic processes: (a) increased rate of protein synthesis occurs in most cells; (b) decreased rate of carbohydrate metabolism in cells; and (c) increased metabolism of free fatty acids and use of fatty acids for energy (Russell-Jones et al., 1993; Bercu and Walker, 1994).

A deficiency in growth hormone production and/or secretion can result in various diseases or conditions, such as dwarfism, profound reduction in lean body mass and concomitant increase in total body fat, reduction in bone density, delayed wound healing, congestive heart failure, and insulin resistance. A lack of GH can also lead to a decrease in skeletal muscle, cardiac muscle mass, and strength that can result in significant decreases in exercise capacity and musculoskeletal frailty, which is typically associated with old age (Juul and Jørgensen, 1996).

Even though administration of recombinant GH is a current effective therapy for growth hormone deficiency, it is not ideal mainly due to its invasive parenteral delivery, adverse side effects, and high cost. Consequently, the potential application of small synthetic molecules in growth hormone replacement therapy is currently the subject of extensive investigation as GH secretagogues (GHS) suitable for nonparenteral administration have recently been synthesized. The growth hormone releasing peptides (GHRP) are a family of synthetic, five to seven amino acid peptides that selectively stimulate GH secretion. In clinical studies, the most potent GH secretagogue of the GHRP family to date is a hexapeptide, hexarelin (HEX), which has the following amino acid sequence: His-D-2-methyl-Trp-Ala-Trp-D-Phe-Lys-NH_2. HEX has been shown to possess a strong, long-lasting GH releasing activity both *in vitro* and *in vivo* in animals after intravenous (IV) and subcutaneous administration (Cella et al., 1995; Deghenghi et al., 1994).

In general, a common obstacle to routine use of peptide hormones is that they cannot be administered by oral route since they have a low permeability through the gastrointestinal mucosa because of their polar nature. They also undergo chemical and enzymatic inactivation in the gastrointestinal tract and have a significant first pass metabolism in the liver, although some small peptides

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have shown their pharmacological activities after oral administration.

An alternative administration of peptide hormone is by intranasal (IN) delivery since it has recently been shown to possess several advantages over other methods of administration. A major advantage of IN delivery is that the drug may be administered to achieve a systemic or localized effect, as required, because of the relatively high absorption efficiency of the drugs through the nasal mucosa with a high permeability and relatively lower enzymatic activity. In regard to the absorption efficiency of HEX in human, Ghigo et al. reported that the IN administration provided a significantly greater bioavailability ($4.8 \pm 0.9\%$) than that obtained after oral administration ($0.3 \pm 0.1\%$) (Ghigo et al., 1994).

Although IN administration produces a relatively high absorption efficiency for HEX as compared to that of the oral route, there is still a formidable problem associated with IN drug administration in that most drug molecules, in particular, hydrophilic peptides diffuse poorly and slowly through the nasal mucosal membrane and thus the desired levels of the therapeutic agent cannot be achieved by means of simple transnasal administration. The low oral and nasal absorption and systemic availability result in high intersubject variability of the GH-releasing effect. Thus, there continues to be a need for development of compositions and methods for convenient, efficient, and effective IN delivery of such substances to animals and humans.

In addition, recently a number of studies have shown the possibility of exploiting the nasal delivery route for direct transport of drugs from nose to brain through the olfactory pathway (Illum, 2000; Vyas et al., 2006; Westin et al., 2006; Nonaka et al., 2008). The extent of direct brain uptake of drugs is highly dependent on the molecular weight, the degree of lipophilicity, the degree of dissociation of the drugs and the type of formulation.

As part of the development studies for the HEX delivery system, the objective of the present study was to investigate simultaneously the plasma pharmacokinetics and brain distribution profiles of the hydrophilic growth hormone releasing peptide, HEX in rabbits after IV and IN administration and to assess whether there is a direct nose-to-brain transport pathway for neuropeptide molecules.

2. Materials and methods

2.1. Chemicals

HEX was purchased from GL Biochem Ltd. (Shanghai). Ethanol (EtOH), propylene glycol (PG), glycerol, acetonitrile (HPLC grade), acetic acid, formic acid, sodium ethylenediaminetetraacetate (EDTA-Na), diethylamine, heparin and benzalkonium chloride were purchased from Sigma–Aldrich (St. Louis, MO, USA). Methanol (HPLC grade) was purchased from J.B. Baker (Phillipsburg, NJ, USA). *n*-Tridecyl-β-D-maltoside (TDM) was obtained from Anatrace Inc. Deionized and distilled water was used.

2.2. Animals

Male New Zealand white rabbits (2.5–3.5 kg) purchased from Marland Breeding Farm, Inc. (Hewitt, NJ) were used in the *in vivo* pharmacokinetic studies. The experimental protocol was approved by the Animal Research Ethics Committee at St. John's University. Rabbits were housed in individual cages with free access to food and water in a room with an automatically controlled illumination (a 12 h light-dark cycle), temperature and relative humidity.

2.3. Preparations of HEX test formulations

The formulations for IN drug delivery were prepared by dissolving HEX in either cosolvent system consisting of 20% EtOH/10% PG/0.1% EDTA/0.5% TDM/20 mM acetate buffer or normal saline solution. HEX IV injection was prepared under aseptic conditions by dissolving drug into normal saline injection (Abbott Laboratory, IL) and filtering through a 0.22 μm filter.

2.4. Comparative nasal absorption studies

For comparative pharmacokinetic (PK) studies, IV and IN formulations were administered to conscious male New Zealand white rabbits ($n = 4$–5) weighing approximately 2.5–3.5 kg. Just prior to the experiment, the rabbits were weighed and restrained in a rabbit restrainer. In the IV treatment, the rabbits received HEX normal saline solution (0.5%) through a marginal ear vein at a dose level of 1 mg/kg. For IN dosing, each rabbit received 100 μl of IN formulation into each nostril by means of a Pfeiffer nasal spray device (Pfeiffer, Princeton, NJ) at the dose levels of 0.5 and 1 mg/kg. The blood samples (2 ml) were collected at 0 (pre-dosing), 5, 10, 15, 20, 30, 40, 60, 90, 120, 180, and 240 min after IV and IN administration via a 21-gauge butterfly infusion set inserted in the median artery of the rabbit ear. After vortexing with EDTA-2Na, blood samples were centrifuged at $3120 \times g$ for 15 min to obtain the plasma.

2.5. Nasal absorption and distribution studies

Forty conscious male New Zealand white rabbits weighing 2.5–3.5 kg were randomly divided into two groups for IN and IV administration, each consisting of 20 animals divided into five subgroups of four animals each. Just prior to the experiment, the rabbits were weighed and restrained in a rabbit restrainer. One group of rabbits received IV injection (1 mg/kg) via a marginal ear vein, and another group received 100 μl of IN formulation into each nostril via a Pfeiffer nasal spray device.

The blood, cerebrospinal fluid (CSF) and brain tissue samples were collected at 10, 20, 40, 60 and 120 min after IV and IN administration, respectively. The blood samples (1–2 ml/sampling) were collected through a catheter (21-gauge) inserted into median ear vein at each time point, and anticoagulated with EDTA-2Na and centrifuged at $3124 \times g$ for 15 min to obtain the plasma. After blood sampling, the rabbits were euthanized with an overdose of pentobarbital sodium (100 mg/kg). The CSF samples were collected by the technique of cisternal puncture. Briefly, the first layer of muscle was cut and the atlanto-occipital membrane was exposed. Thereafter, a 23-gauge 1-in. needle attached to a 1 ml syringe was inserted through the atlanto-occipital membrane into the skull and 1.0–1.5 ml of CSF was withdrawn.

After the CSF sampling, the skull was cut open by a bone saw and the whole brain was carefully removed from the brain cavity. The brain tissue was quickly rinsed with physiological saline and blotted up with Kimberly–Clarks wipes to remove blood-taint and macroscopic blood vessels as much as possible. All biological samples, i.e., aliquots of plasma, CSF, and brain tissues were stored at −40 °C until analysis by the developed LC/MS method via solid phase extraction. Two sides of the olfactory bulb (OB) were excised and combined before the solid phase extraction and LC/MS analysis. Same procedure was followed for olfactory tract (OT), anterior (CB1), middle (CB2), and posterior (CB3) parts of cerebrum and cerebellum (CL). Measurements were made using four rabbits at each time point.

2.6. Analytical procedures

2.6.1. Solid phase extraction

For extraction of HEX from various biological samples, the Oasis® HLB cartridges (Waters) were selected to obtain higher and reproducible recovery rates. The Oasis® SPE cartridges were preconditioned by sequentially adding 3 ml methanol and 3 ml water on a vacuum suction manifold (Supelco, Visiprep™ 24, Bellefonte,

PA, USA). Thereafter, 0.5 ml of acidified plasma or CSF samples spiked with different concentration of HEX containing 100 ng/ml of internal standard (3D-HEX) were loaded onto the preconditioned cartridges and allowed to drain. The cartridges were then washed by sequential addition of 1 ml acidified water, 0.5 ml of 5% methanol in water and 0.5 ml of 65% methanol in water. Finally HEX and the internal standard were eluted with 2 ml of 60% methanol in water containing 1% formic acid and evaporated to dryness under vacuum (less than 20 mmHg) at room temperature. The resultant residue was reconstituted in 0.2 ml of mobile phase and injected into a LC/MS system for analysis. The brain tissue sample with the internal standard was homogenized (VWR® VDI12 adaptable homogenizer, USA) in 1 ml of 1% diethylamine aqueous solution and centrifuged at 3124 × *g* for 60 min at 4 °C. After loading of the supernatant on the cartridge, the SPE procedure followed was exactly the same as that of plasma samples described above.

2.6.2. LC/MS method

The LC/MS analysis was performed using a PE Sciex API 150 EX mass spectrometer coupled with Shimadzu LC-10ADVP integrated HPLC system controlled by Analyst software 1.4 (Applied Biosystems, Toronto, Canada). Chromatographic separation was achieved at ambient temperature on an *Atlantis* dC_{18} column (150 mm × 1.0 mm I.D., 3 μm). The mobile phase consisting of 0.1% formic acid in 17% acetonitrile/83% water was delivered to MS at a flow rate of 50 μL/min. The retention time was 10 min for both HEX and internal standard. Mass spectroscopy data was collected in the positive ion mode under total ion chromatography (TIC) and extracted chromatography monitoring (XIC) with main parameters of MS set as following: nebulizer gas 15 L/min, curtain gas 15 L/min and a temperature of, 350 °C. Focusing potential, declustering potential and entrance potential optimized by the infusion method were 26, 164.8, and 4.0 V, respectively. The protonated molecules [M+2H] of HEX and internal standard were detected at the *m*/*z* ratio of 444.5 and 446 amu, respectively. Calibration curves of HEX were prepared with plasma and brain tissues spiked with known amounts of HEX utilizing its MS peak area ratio to the internal standard. The linear range of HEX in plasma and brain tissues was 5–500 ng/ml (or ng/g). The inter- and intra-day variations were less than 5% and 8% for plasma and brain samples, respectively. The extraction recoveries of HEX from plasma and brain tissue homogenates were more than 80% and 75%, respectively. The limit of quantification was 5 ng/ml (or ng/g) for plasma and brain tissue samples.

2.7. Data analysis

All concentration data were dose- and weight-normalized. The PK parameters were analyzed using the non-compartmental model of WinNonlin software (Pharsight Corporation, Cary, NC). The C_0 values following IV injections were calculated by extrapolating the initial plasma drug concentration–time curve to the *Y*-axis at time zero in the WinNonlin software. The absolute bioavailability of HEX after nasal administration of the cosolvent formulation was calculated by using the following Eq. (1):

$$F(\%) = \frac{\mathrm{AUC_{IN}}}{\mathrm{AUC_{IV}}} \times \frac{\mathrm{Dose_{IV}}}{\mathrm{Dose_{IN}}} \times 100 \quad (1)$$

The nasal bioavailabilities of HEX in the brain tissue and CSF were calculated as the ratio of $\mathrm{AUC_{CSF\ or\ brain}}$ after IN administration and $\mathrm{AUC_{CSF\ or\ brain}}$ after IV administration.

The mean drug concentration of HEX in the whole brain (ng/g) was calculated based on Eqs. (2) and (3):

$$C_{\mathrm{mean}} = \frac{Q_{\mathrm{b}}}{W_{\mathrm{B}}} \sum (C_{\mathrm{B}} F_{\mathrm{B}}) \quad (2)$$

$$Q_{\mathrm{b}} = (C_{\mathrm{OB}} \times F_{\mathrm{OB}} + C_{\mathrm{OT}} \times F_{\mathrm{OT}} + C_{\mathrm{CB}} \times F_{\mathrm{CB}} + C_{\mathrm{CL}} \times F_{\mathrm{CL}}) \times W_{\mathrm{B}} = \sum (C_{\mathrm{B}} F_{\mathrm{B}}) \times W_{\mathrm{B}} \quad (3)$$

where Q_{b} is the total drug amount in the brain; W_{B} is the whole brain weight; C_{OB}, C_{OT}, C_{CB}, C_{CL} are the HEX concentrations in OB, OT, CB, and CL; F_{OB}, F_{OT}, F_{CB}, and F_{CL} are the percentage weight fraction of OB, OT, CB and CL of brain tissue, respectively.

To evaluate the brain targeting efficiency, drug targeting efficiency (DTE) (Chow et al., 1999), which represents a time-average partitioning ratio, was calculated as follows:

$$\mathrm{DTE}(\%) = \frac{\mathrm{AUC_{brain}}}{\mathrm{AUC_{plasma}}} \times 100 \quad (4)$$

where $\mathrm{AUC_{brain}}$ and $\mathrm{AUC_{plasma}}$ is the area under the brain tissue concentration–time and plasma concentration–time curves, respectively.

In 2004, Zhang et al. reported the use of another term "brain drug direct transport percentage (DTP)" to determine the percentage of drug directly transported to the brain through olfactory pathway. The brain DTP can be estimated based on the following two Eqs. (5) and (6):

$$\frac{B_{\mathrm{iv}}}{P_{\mathrm{iv}}} = \frac{B_{\mathrm{x}}}{P_{\mathrm{in}}} \quad (5)$$

$$\mathrm{DTP}\% = \frac{B_{\mathrm{in}} - B_{\mathrm{x}}}{B_{\mathrm{in}}} \times 100 \quad (6)$$

where P_{iv}, B_{iv}, P_{in}, B_{in}, are the $\mathrm{AUC_{0–60\,min}}$ of HEX in plasma and brain tissue obtained after IV and IN administration. B_{x} is the brain AUC fraction contributed by systemic circulation through the blood–brain barrier (BBB) after IN delivery.

2.8. Statistical analysis

Student's *t*-test and ANOVA were used as appropriate for statistical analysis. Probability value $P < 0.05$ was considered as statistically significant.

3. Results and discussion

3.1. Intranasal absorption of HEX

The time courses of the plasma levels of HEX following IV injection and the IN administration of simple normal saline solution and hydroalcholic cosolvent formulation in rabbits at a dose of 1 mg/kg are presented in Fig. 1. The corresponding non-compartmental PK

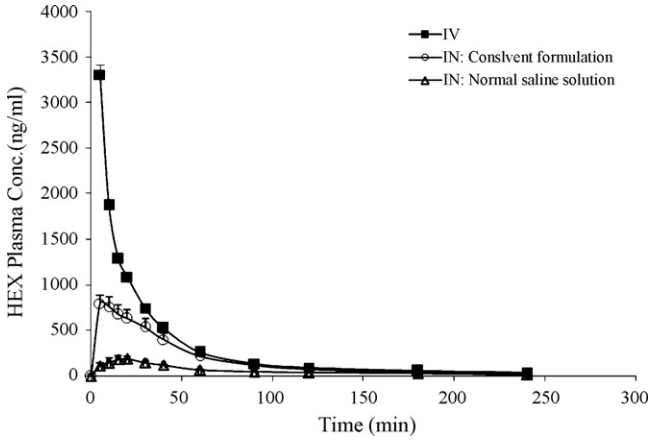

Fig. 1. Mean plasma concentration–time profiles (mean ± SEM) of HEX after IV and IN administration to rabbits (n = 4–5) at a dose of 1 mg/kg from plasma PK studies.

Table 1
Bioavailability and pharmacokinetic parameters of HEX after IV and IN administration of normal saline solution and cosolvent formulation to rabbits (n = 4–5).

Route/formulation	Dose (mg/kg)	C_{max} (ng/ml)	t_{max} (min)	$t_{1/2\beta}$ (min)	$AUC_{0-4\,h}$ (ng × h/ml)	AUC_{IN}/AUC_{IV} (%)
IV	1.0	5852.5 ± 353.3	0	85.1 ± 7.8	1499.2 ± 38.1	100.0
IN/normal saline	1.0	201.8 ± 42.9	20.0 ± 3.5	121.1 ± 30.0	234.1 ± 35.3	15.6 ± 2.4
IN/cosolvent	1.0	831.0 ± 87.6	7.0 ± 1.4	86.8 ± 19.5	724.3 ± 87.9	48.3 ± 5.9
IN/cosolvent	0.5	330.5 ± 57.4	11.3 ± 1.3	71.3 ± 4.6	393.9 ± 45.7	52.5 ± 6.1

Data are presented as mean ± SEM.

parameters determined utilizing WinNonlin software are summarized in Table 1. Table 1 also shows the PK parameters determined following IN administration of the cosolvent formation at a dose of 0.5 mg/kg in order to evaluate the dose-progression effect. The PK results demonstrate that the IN administration of HEX cosolvent formulation produced a fast absorption rate with the t_{max} of 7 min. The IN bioavailability of HEX from the cosolvent formulation containing *n*-tridecyl-β-D-maltoside as a permeation enhancer was found to be 48.3% on the basis of the $AUC_{0-4\,h}$ values whereas the simple normal saline solution achieved a 15.6% of bioavailability indicating that the former provided approximately 3 times greater absorption efficiency than that of normal saline solution. These comparative bioavailability data demonstrated that the cosolvent formulation containing 0.5% *n*-tridecyl-β-D-maltoside as a permeation enhancer was highly effective in enhancing the systemic nasal absorption of the hydrophilic hexapeptide, HEX. According to the results of *in vivo* absorption studies in human conducted by Ghigo et al. (1994), the IN administration of HEX solution in normal saline produced a 4.8% absolute bioavailability. Based on the results of the human PK study, a higher nasal systemic absorption of HEX is expected in human from the developed cosolvent type delivery system as compared to the normal saline solution.

The dose ranging studies were also conducted in rabbits after IN administration of cosolvent system at two different doses of 0.5 and 1 mg/kg. As shown in Table 1, the IN bioavailability of HEX from the cosolvent system was dose-independent as indicated by 52.5% and 48.3% of bioavailability for 0.5 and 1.0 mg/kg doses, respectively. When the IN dose level increased twice, the C_{max} and $AUC_{0-4\,h}$ values were nearly doubled indicating that the IN absorption of HEX from the hydroalcholic cosolvent system was dose-proportional over a dose range of 0.5–1 mg/kg.

3.2. In vivo absorption, CSF and brain distribution studies of HEX

These studies were undertaken to assess whether or not a hydrophilic peptide, HEX is transferred along the olfactory pathway to the CSF and brain after IN administration. In order to evaluate the effect of HEX cosolvent formulation on the nasal systemic absorption of HEX, and its CSF and brain distribution, the HEX concentrations in the plasma, CSF and various regions of brain tissues such as OB, OT, CB1, CB2, CB3 and CL of rabbits after IV and IN administration were determined simultaneously. In this study, four rabbits were used for each time point of the *in vivo* study. In order to determine the drug distribution patterns in the various regions of the brain, the weight fraction data for the different parts of rabbit brain are crucial. The mean weight percentages of the different regions of brain tissue reported by Ren (2007) are listed in Table 2. The whole brain weight was approximately 7–10 g for the rabbits weighing 2.5–3.0 kg. Thus, average percent weight of the brain was approximately 0.3% of total body weight. The mean brain concentrations were calculated based on Eqs. (2) and (3) described in Section 2.

Table 2
Weight fraction percentage of different regions of brain tissues of rabbits (n = 12).

Brain tissue	Olfactory bulb	Olfactory tract	Cerebrum	Cerebellum
Percentage (%)	2.68	4.67	72.36	20.29
SD	0.52	0.78	3.57	3.47

Figs. 2–4 represent the mean HEX concentration–time profiles in the plasma, brain, and CSF determined after IV and IN administration of HEX in rabbits at a dose of 1 mg/kg. The pharmacokinetic parameters obtained from the simultaneous PK studies in rabbits are listed in Table 3. The bioavailability of the IN administration of cosolvent formulation, calculated on the basis of the $AUC_{0-60\,min}$, was found to be 35.6%, which appeared to be somewhat lower than the bioavailability values of 40.3% (based on the $AUC_{0-60\,min}$) and 48.3% (based on the $AUC_{0-240\,min}$) found from the previous plasma PK studies as shown in Table 1.

As shown in Figs. 2–4, the maximum HEX levels attained in the brain and CSF after IV and IN administration were found to be substantially lower (38–353-fold) as compared to those achieved in the plasma. These results strongly suggest that a hydrophilic

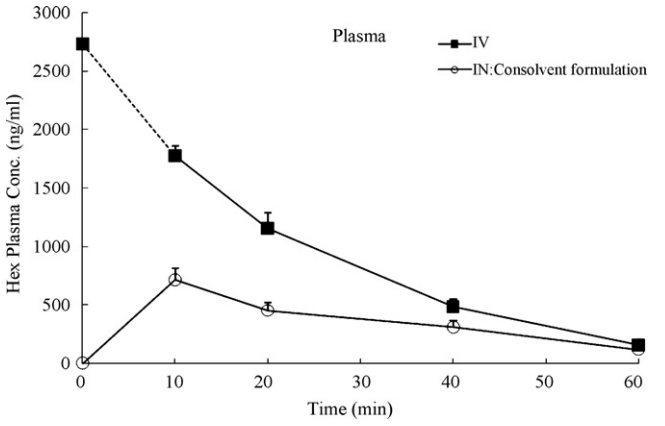

Fig. 2. Mean plasma concentration–time profiles (mean ± SEM) of HEX after IV and IN administration to rabbits at a dose of 1 mg/kg from simultaneous PK and distribution studies (n = 4 at each time point).

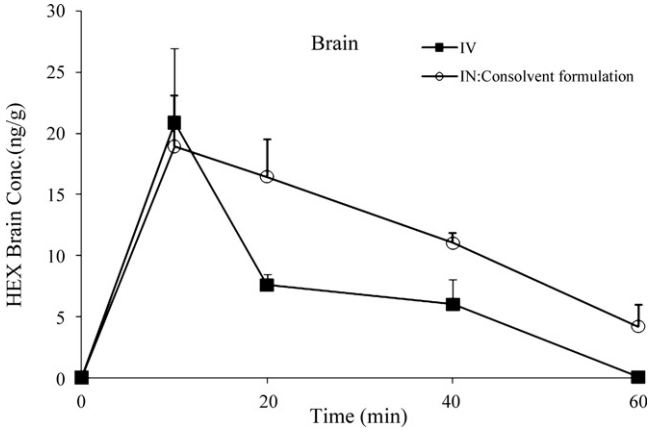

Fig. 3. Mean brain concentration–time profiles (mean ± SEM) of HEX after IV and IN administration to rabbits at a dose of 1 mg/kg from simultaneous PK and distribution studies (n = 4 at each time point).

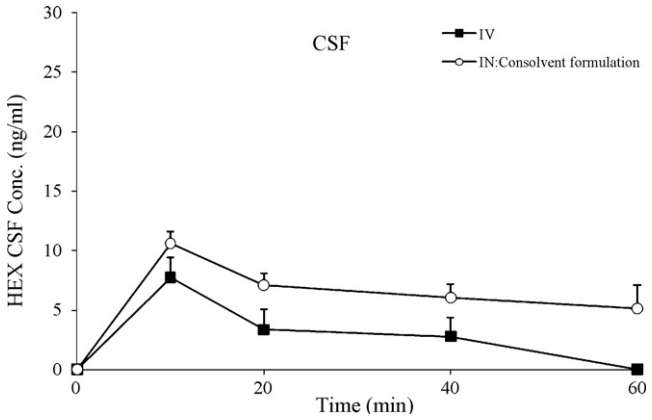

Fig. 4. Mean CSF concentration–time profiles (mean ± SEM) of HEX after IV and IN administration to rabbits at a dose of 1 mg/kg from simultaneous PK and distribution studies ($n = 4$ at each time point).

Table 3
Pharmacokinetic parameters of HEX after IV and IN administration of cosolvent formulation to rabbits at a dose of 1.0 mg/kg from simultaneous PK and distribution study ($n = 4$ at each time point).

Routes	C_{max} (ng/ml or g)	t_{max} (min)	$AUC_{0-60\,min}$ (ng × h/ml or g)	AUC_{IN}/AUC_{IV} (%)
Plasma-IV	2735.58 ± 137.17	0	999.07 ± 78.92	–
Plasma-IN	717.93 ± 95.69	10	355.99 ± 53.64	35.6 ± 3.6
CSF-IV	7.74 ± 1.69	10	3.70 ± 1.58	–
CSF-IN	10.59 ± 0.97	10	6.39 ± 1.05	172.7 ± 28.4
Brain-IV	20.87 ± 6.08	10	7.39 ± 1.94	–
Brain-IN	18.98 ± 4.13	10	11.66 ± 2.02	157.8 ± 27.3

Data are presented as mean ± SEM.

hexapeptide, HEX has very limited accessibility to the brain from the systemic circulation. Some of the reasons for the reduced transport of the HEX across the brain–blood barrier (BBB) include its hydrophilicity (log $P = -0.573$ at pH 7.4), the tightness of the BBB cell junctions, and the possible presence of efflux proteins in the BBB. For comparison, as shown in Figs. 2–4 and Table 3, HEX IN administration resulted in a significantly greater AUC_{brain} and AUC_{CSF} values than those of IV route in spite of its markedly lower plasma concentrations. Because the HEX access to the brain and CSF was limited after IV injections, there must be a direct pathway from the nasal cavity to the brain and CSF accounting for the high brain and CSF levels after IN administration. Also, as shown in Fig. 5, the spatial distribution of HEX in different brain regions differed appreciably between IV and IN administration. For this purpose, ANOVA has been performed for the drug distribution data obtained at the earlier time point of 10 min. Following IV administration, the HEX concentration levels were fairly similar and no significant region-to-region differences were observed at 10 min after IV administration as shown by analysis of variance as calculated F values of 1.705 against the tabulated F value of 9.000. On the other hand, following IN administration at the same time point, the HEX concentrations in separate brain regions varied significantly as indicated by ANOVA F value of 9.392 against the tabulated F value of 9.000. From the time points of 10, 20 and 40 min, the observed HEX distribution gradient in the brain from high to low

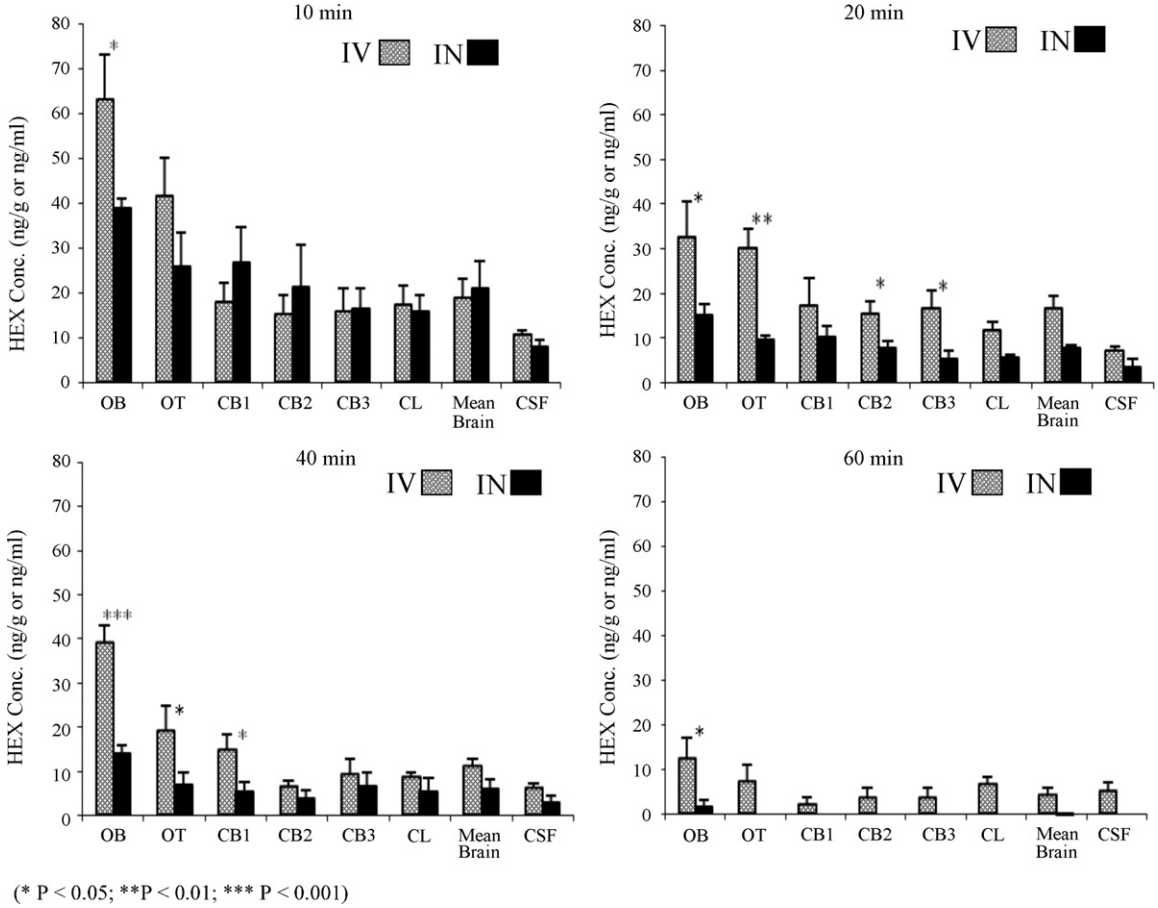

Fig. 5. HEX distribution (mean ± SEM) in various brain regions and CSF after IV and IN administration to rabbits at a dose of 1 mg/kg from simultaneous PK and distribution studies ($n = 4$ at each time point).

Table 4
Mean HEX concentrations (mean ± SEM) obtained in plasma, CSF and various regions of brain tissue at 10 min after IN administration of HEX cosolvent formulation into right or left side nostril of rabbits ($n = 3$) at a dose of 1 mg/kg.

Biologic sample	Right-side administration		Left-side administration	
	Right side (ng/g)	Left side (ng/g)	Right side (ng/g)	Left side (ng/g)
OB	141.9 ± 11.2^{a}	40.0 ± 15.0^{b}	28.3 ± 6.7^{g}	114.1 ± 8.0^{h}
OT	87.3 ± 19.4^{c}	18.9 ± 5.6^{d}	29.1 ± 2.0^{i}	49.4 ± 2.4^{j}
CB1	63.3 ± 10.1^{e}	19.7 ± 7.1^{f}	22.9 ± 4.6	27.0 ± 3.5
CB2	28.6 ± 13.0	12.4 ± 3.5	18.6 ± 4.6	20.7 ± 3.8
CB3	6.9 ± 2.5	7.7 ± 2.5	19.3 ± 2.8	18.8 ± 4.3
CL	25.6 ± 9.0		19.5 ± 3.0	
Mean brain	26.8 ± 3.1		23.0 ± 2.5	
CSF	40.4 ± 6.5		49.3 ± 2.1	
Plasma	706.2 ± 29.4		807.7 ± 33.1	

$P < 0.001$, a vs b; e vs f; g vs h; i vs j.
$P < 0.05$, c vs d.
Data are presented in mean ± SEM.

was: OB > OT > CB > CL > CSF. The HEX uptake levels in olfactory bulb and olfactory tract were significantly greater than those found in the cerebrum, cerebellum, and CSF. These distribution data strongly suggested that the HEX molecules reached the olfactory region first through the olfactory pathway and then distributed into deep brain tissues and CSF. These findings are in good agreement with the results of recent studies on the IN delivery of a hydrophilic compound; methotrexate ($\log P = -1.85$) showing that methotrexate can preferentially transfer into parts of central nervous system directly from the nasal cavity following the olfactory pathway (Wang et al., 2003). Another interesting point observed in the CSF and brain uptake levels seen in Figs. 2–4 is that the IN administration of HEX cosolvent system provided a slower and more extended drug excretion profiles from the CSF and brain tissues than the IV injection as indicated by the significant high drug levels detected in the CSF and brain after 60 min post-dosing as compared with a very low drug concentration detected at the same time period after IV administration. The extended and prolonged drug absorption profiles may be due to the possible accumulation of drug molecules in the local olfactory mucosal environment after IN administration and thus facilitate direct olfactory epithelial transport of HEX over the extended period of time.

To further investigate whether or not the direct nose-to-brain transport occurs for a hydrophilic peptide, HEX, one-sided nasal administration study was also conducted. In this study, HEX cosolvent solution was administered into either right- or left-sided nostril of the rabbits and the drug uptake profiles in the various regions of the right and left hemispheres of the brain were determined. The drug distribution data obtained 10 min after the nasal administration are summarized in Table 4 and depicted in Fig. 6. Overall, the concentrations of HEX in the OB and OT of the drug administered side of the brain hemisphere were significantly (3–4 times) higher than those of the other side of hemisphere, in particular, in the OB and OT. In addition, following right side IN administration, the levels of HEX into the right side of CB1 were also significantly higher than those in the left side of CB1. The higher levels of HEX attained in the OB and OT at the treated-side of brain hemisphere clearly indicate that HEX molecules were directly transported into the brain from the nasal cavity via olfactory pathway.

3.3. Evaluation of brain drug targeting efficiency

In order to quantify the direct olfactory transfer of HEX to the brain hemispheres, the drug targeting efficiency values (DTE%), defined as $[AUC_{brain}/AUC_{plasma}] \times 100$, were determined after IV and IN administration. As shown in Table 5, the DTE values deter-

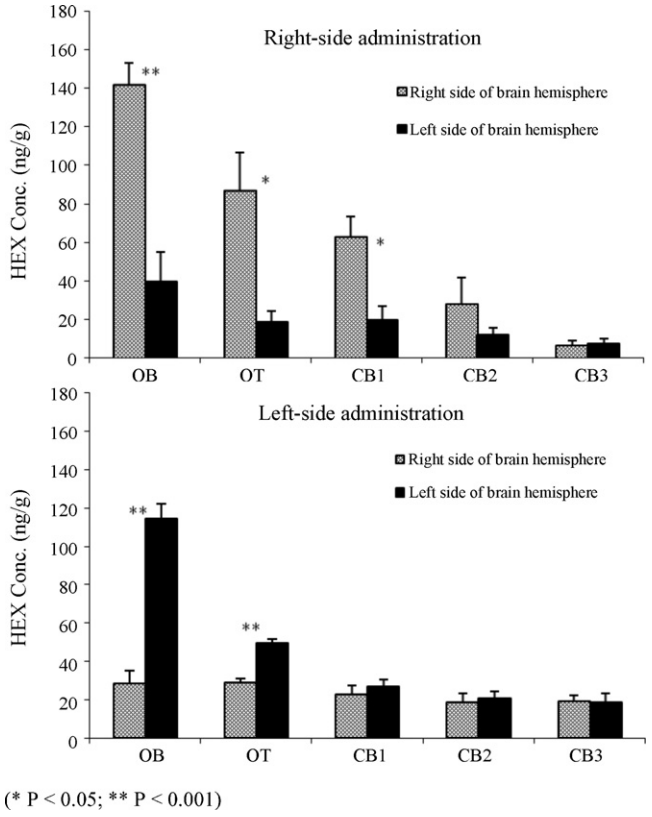

Fig. 6. HEX brain distribution profiles (mean ± SEM) obtained at 10 min after IN administration of HEX formulation into right or left side nostril of rabbits at a dose of 1 mg/kg ($n = 3$).

Table 5
Pharmacokinetic parameters of HEX after IV and IN administration of cosolvent formulation to rabbits at a dose of 1.0 mg/kg from simultaneous PK and distribution studies ($n = 4$ for each time point).

$AUC_{0-60\,min}$ ratio	IV	IN
AUC_{CSF} (ng × h/ml)	3.70 ± 1.58	6.39 ± 1.05
AUC_{plasma} (ng × h/ml)	999.07 ± 78.92	355.99 ± 53.64
$[AUC_{CSF}/AUC_{plasma}] \times 100$ (DTE_{CSF})	0.37 ± 0.16	1.80 ± 0.29
AUC_{brain} (ng × h/g)	7.39 ± 1.94	11.66 ± 2.02
AUC_{plasma} (ng × h/ml)	999.07 ± 78.92	355.99 ± 53.64
$[AUC_{brain}/AUC_{plasma}] \times 100$ (DTE_{brain})	0.74 ± 0.20	3.28 ± 0.57
$DTE_{CSF,IN}/DTE_{CSF,IV}$	–	4.86
$DTE_{brain,IN}/DTE_{brain,IV}$	–	4.43
DTP_{brain} (%)	–	77.43

Data are presented in mean ± SEM; DTE: drug targeting efficiency; DTP: drug direct transport percentage.

mined in the brain and CSF after IN administration were 3.28% and 1.80%, respectively. In contrast, after IV administration, the DTE (%) values obtained for the brain and CSF were 0.74% and 0.37%, respectively. A significant difference ($P < 0.01$) was observed when comparing the DTE (%) values between IV and IN administration, suggesting that IN administration of HEX cosolvent formulation provided 4.43 and 4.86 times greater HEX brain and CSF targeting efficiency than IV administration, respectively. These results indicate that IN administration of a hydrophilic neuropeptide, HEX from the hydroalcholic cosolvent vehicle provided a more than 4 times greater CNS targeting efficiency as compared to the IV administration. The brain drug direct transport percentage (DTP) values were also calculated using the formula proposed by Zhang et al. (2004). As shown in Table 5, following IN administration, approximately 77% of HEX content was directly transported to the brain from nasal cavity via the olfactory pathway. These results demonstrate that following IN administration the major portion of HEX molecules can enter into the brain directly from the nasal cavity possibly via the olfactory pathway, only partially transported into the brain via the BBB from the systemic circulation.

4. Conclusions

In vivo absorption and brain distribution studies in rabbits revealed that a hydrophilic neuropeptide, HEX could preferentially transfer into the CSF and brain tissues from the nasal cavity; the olfactory bulb and olfactory tract were the essential gateways for this direct pathway. A single administration of HEX solution formulated with a hydroalcholic cosolvent system containing 0.5% *n*-tridecyl-β-D-maltoside as a permeation enhancer may provide a promising and durable therapeutic option for the treatment of growth hormone deficiency.

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