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Nasal administration of a physostigmine analogue (NXX-066) for Alzheimer's disease to rats

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Abstract

The nasal route has been receiving attention for the administration of systemically active drugs because delivery is convenient, reliable and rapid. The aims of this study were to investigate the systemic absorption of nasally administered (3aS)-*cis*-1, 2, 3, 3a, 8, 8a-hexahydro-1, 3a, 8-trimethyl-pyrrolo-[2,3b]-indol-5-yl 3, 4 dihydro-2-isoquinolincarboxylate (NXX-066), a physostigmine analogue, in rats and to compare the uptake of the drug into the cerebrospinal fluid (CSF) after nasal and intravenous administration. NXX-066 (3 μmol/kg) was administered to both nostrils or into the vena jugularis of male Sprague–Dawley rats. Blood and CSF samples were obtained at regular intervals from the arteria carotis and by cisternal puncture, respectively. The concentrations of NXX-066 in the blood and CSF samples were measured using HPLC with fluorescence detection. NXX-066 was absorbed rapidly after nasal administration with the peak concentration occurring within 1.5 min. The nasal bioavailability of NXX-066 was $100 \pm 30\%$ and the elimination from plasma was as rapid as that following intravenous administration. Low concentrations of NXX-066 were detected in the CSF after both intravenous and nasal administration. In conclusion, NXX-066 was rapidly and totally absorbed into the systemic circulation and uptake into the CSF was not enhanced by nasal administration in rats.

Keywords: Nasal administration; Acetylcholinesterase (AChE) inhibitor; Brain delivery; Olfactory pathway; Rat; Cerebrospinal fluid (CSF)

1. Introduction

The administration of drugs by the nasal route is a promising method of achieving rapid systemic delivery. The anatomy and physiology of the nasal passage make it a practical route for the introduction of therapeutic drugs into the systemic circulation. The mucosa is highly vascularised, resulting in rapid absorption and administration of drugs by the nasal route also avoids degradation in the gastrointestinal tract and first-pass metabolism in the liver. The concentration-time profiles achieved after nasal administration are often similar to those after intravenous

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administration, with resultant rapid onset of pharmacological activity (Hussain, 1998). Another attractive feature is the facilitated administration routine that nasal administration offers the patient compared to the more invasive alternatives.

Rapid absorption into the systemic circulation after nasal administration has been demonstrated with several drugs that are active in the central nervous system (CNS). These include (S)-UH-301, a seretonin-1a receptor antagonist, (Dahlin and Björk, 2000), apomorphine (Neef et al., 1994 Sam et al., 1995), physostigmine and arecoline (Hussain and Mollica, 1991), propiomazine (Bjerre et al., 1996) and dextromethorphan (Char et al., 1992).

Targeting the brain via the nasal administration of drugs has been studied frequently in recent years. The olfactory receptor cells are in direct contact with both the environment and the CNS and they provide a route of entry to the brain that circumvents the blood–brain barrier (BBB) (Uraih and Maronpot, 1990). Some 35–40 substances have been reported to reach the CNS after nasal administration in experimental animals. In recent studies, dopamine (Dahlin et al., 2000), neurotoxic metals (Henriksson and Tjälve, 1998), local anaesthetics (Chou and Donovan, 1998a), carboxylic acids (Eriksson et al., 1999) and nerve growth factor (NGF) (Frey et al., 1997) have been reported to enter the CNS by this route.

Alzheimer's disease (AD), the most common cause of dementia, affects millions of people over the age of 65 in the Western world and an increase in the occurrence of AD is expected in the future, as the proportion of older people in the population grows. AD is a chronic neurodegenerative disorder accompanied by the gradual and progressive loss of functional and psychomotor abilities (Daly, 1999). Both environmental and genetic factors have long been thought to cause AD. Genetic mutations, degeneration of cholinergic neurons and structurally altered amyloid β-protein leading to senile plaques have been demonstrated in affected patients. All these are possible pharmacological targets for the prevention and/or cure of AD (Schorderet, 1995). However, despite the apparent progress in research, successful treatment of neurodegeneration associated with AD remains elusive. The goal of caring for patients with AD is to enhance function, maintain quality of life and preserve autonomy (Daly, 1999).

Inhibition of acetylcholinesterase (AChE) is a promising approach and the most common method under investigation for the treatment of AD (Giacobini, 1993). Physostigmine appears to improve memory function in patients with AD and nasal administration of this cognition enhancer to rats was shown to be a feasible alternative to parenteral administration (Hussain and Mollica, 1991). Previous experience with the nasal delivery of neuropeptides (Gozes et al., 1996) and neurotropic factors (Frey et al., 1997) to rats has shown that the nose could be a possible administration route for these potential drugs in treating AD. The latter study also indicated that ^{125}I-NGF was transferred directly into the brain along the olfactory pathway.

The substance used in this study, (3aS)-*cis*-1, 2, 3, 3a, 8, 8a-hexahydro-1, 3a, 8-trimethyl-pyrrolo (2,3b) indol-5-yl 3,4 dihydro-2-isoquinolincarboxylate (NXX-066, Fig. 1), is a physostigmine analogue which acts as a potent inhibitor of AChE and could therefore be a potential drug for treating AD. NXX-066 is well absorbed from the gastrointestinal tract, but the oral bioavailability is poor to moderate in rats and dogs because of pre-systemic metabolism. Thus, nasal administration could provide an alternative route for the systemic delivery of NXX-066.

Various techniques have been used to study the uptake of substances into the CNS after nasal

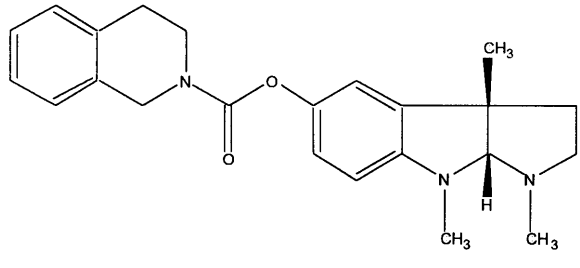

Fig. 1. Chemical structure of the physostigmine analogue and acetylcholinesterase inhibitor, (3aS)-*cis*-1, 2, 3, 3a, 8, 8a-hexahydro-1, 3a, 8-trimethyl-pyrrolo-[2,3b]-indol-5-yl 3, 4 dihydro-2-isoquinolincarboxylate (NXX-066).

administration. The most common involve sampling the cerebrospinal fluid (CSF) (Chou and Donovan, 1998a; Yajima et al., 1998; Dahlin and Björk, 2000) or brain tissue (Dluzen and Kefalas, 1996 Frey et al., 1997; Dahlin et al., 2000). Autoradiography (Henriksson and Tjälve, 1998; Eriksson et al., 1999; Dahlin et al., 2000) and microdialysis (Chou and Donovan, 1998b) are also useful methods for exploring the olfactory pathways into the brain.

Nasal administration of NXX-066 has potential and this study was undertaken to determine the extent of systemic absorption of NXX-066 after nasal absorption in male Sprague–Dawley rats. The uptake of NXX-066 into the CSF after nasal and intravenous administration was also compared. It was assumed that, if the concentrations of NXX-066 were higher in the CSF after nasal drug delivery than after an intravenous bolus dose, a direct pathway into the target organ, from the nasal olfactory area, must exist for this physostigmine analogue.

2. Material and methods

2.1. Drugs and reagents

(3aS)-*cis*-1, 2, 3, 3a, 8, 8a-hexahydro-1, 3a, 8-trimethyl-pyrrolo-[2,3b]-indol-5-yl 3, 4 dihydra-2-isoquinolincarboxylate (NXX-066, 377.5 g/mol) and (3aS)-*cis*-1, 2, 3, 3a, 8, 8a-hexahydro-1, 3a, 8-trimethyl-pyrrolo [2,3b] indol-5-ol, (1-methyl-1, 2,3,4-tetrahydroisoquinolinyl) carbamate (NXX-453) were donated by AstraZeneca R&D Södertälje (Sweden). Heparin (500 IU/ml) was acquired from Løvens, Denmark and thiobutabarbital sodium (Inactin) was obtained from Research Biochemical Internationals (USA). Ultrapure deionised water (Milli-Q UF Plus Millipore, France) was used for preparation of solutions. Solvents were of HPLC grade and all other chemicals were of analytical grade and commercially available.

2.2. Animals

The study was carried out in compliance with approval numbers C 84/94, issued by the Animal Research Ethics Committee in Uppsala.

Male Sprague–Dawley rats, weighing between 265 and 354 g, were used in this study. The animals were obtained from B&K Universal AB (Sweden), housed at 22°C with a 12:12 h light:dark cycle and given a standard pellet diet (Lactim R36) with free access to water.

2.3. Animal experiments

The animal experiments were performed as reported earlier (Björk and Edman, 1988). During the experiments, care was taken to maintain the normal functions of the nasal cavity by minimising disturbance of the mucosa with mechanical manipulation. The rats were anaesthetised with an intraperitoneal injection of thiobutabarbital sodium (150 mg/kg), and then placed on a heated plate to maintain the body temperature. The trachea and carotid artery were cannulated with polyethylene tubes, PE 200 and PE 50 respectively. NXX-066, which has a solubility in water of 0.094 mg/ml, was dissolved in an acetate buffer (pH 5), in which the solubility was 6.5 mg/ml. The concentrations of the solutions used for nasal and intravenous administration were 6.4 and 3 mg/ml, respectively. After the operation (30 min), NXX-066 (3 μmol/kg) was applied to each nostril (25–30 μl), using polyethylene tubes (PE 90) attached to a micropipette or given intravenously as a bolus dose through a catheter (PE 50) into the external jugular vein.

Blood samples of 200 μl were withdrawn from the carotid artery prior to and 1.5, 3, 7, 15, 30, 60, 90 and 120 min after administration in six rats who had received intravenous NXX-066 and seven who had received the drug intranasally. The plasma was separated, after the addition of one drop of heparin (24-gauge needle), by centrifugation at 7000 rpm for 10 min.

Puncture of the cisternal magna was performed after fixing the head of the rat (Waynforth and Flecknell, 1980; Dahlin and Björk, 2000). The CSF (100–150 μl) was withdrawn before and 3, 7,

15, 30 and 60 min after administration of NXX-066, by gentle suction through a 30-gauge needle attached to a polyethylene tubing (PE 50) connected to a syringe. Collection of CSF was terminated as soon as blood appeared and the blood-tainted portion of CSF was prevented from reaching the collecting tube. CSF was collected from three rats at each time point. Blood samples were withdrawn from the arteria carotis about 2 min after each CSF sampling, for correlation with the absorption data.

After the experiments, the animals were killed with an overdose of pentobarbital (100 mg/ml) and all samples, both plasma and CSF, were stored at −80°C until analysis.

2.4. Analysis

In the analysis of the plasma and CSF samples, (3aS)-*cis*-1, 2, 3, 3a, 8, 8a-hexahydro-1, 3a, 8-trimethyl-pyrrolo [2,3b] indol-5-ol, (1-methyl-1, 2, 3, 4-tetrahydroisoquinolinyl) carbamate (NXX-453) was used as an internal standard. In order to extract NXX-066 and NXX-453, 100 μl of the plasma sample, 100 μl of NXX-453 (313.3 nM), 200 μl of sodium hydrogen carbonate solution (0.5 M) and 2 ml of organic solvent mixture (diethylether/*n*-heptane 30/70 v/v%) were added to test tubes. After extraction for 2 min in a tumble mixer, the phases were separated by freezing in an ethanol-dry ice bath. The supernatant was transferred to a new tube and evaporated to dryness under a gentle stream of nitrogen at 40°C. The extraction was repeated and the residue was redissolved in 200 μl phosphate buffer (0.01 M; pH 2). Standard and control samples were prepared as above.

The plasma samples (170 μl) were injected (Kontron 460, Kontron, Switzerland) into the chromatographic system and separation was achieved using a reversed phase column (Zorbax Sb-CN, 75 × 4.5, 3.5 μm) and a precolumn (CN 12.5 × 4, 5 μm) at 35°C (Jones 7950, Jones Chromatography, UK). The mobile phase, which consisted of 23 v/v% acetonitrile and 0.5 mM dimethyloctylamine in ammonium formate buffer pH 4.4, was delivered at a flow rate of 1.3 ml/min (Shimadzu LC 9A, Shimadzu, Japan). NXX-066 and NXX-453 were detected using a fluorescence detector (Waters 470, Waters Division of Millipore) at wavelengths of 245 for excitation and 346 nm for emission.

The CSF samples were assayed directly without any pre-treatment. Before injection (80 μl) into the chromatography system, 50 μl NXX-453 was added to 50 μl CSF. Standard and control samples were made from artificial CSF (Elliot's B solution) according to Martindale (30th edition). The peak heights were used for quantification and the ratio NXX-066/NXX-453 was used for calculation of the concentrations of NXX-066 in the plasma and CSF samples.

2.5. Calculations and statistics

The area under the plasma concentration–time curve (AUC) was calculated by the standard trapezoidal method without extrapolation to infinity. The initial intravenous concentrations, however, were obtained by extrapolating, using the two sampling points. The absolute nasal bioavailability was calculated by dividing the mean AUC value from the nasal group by the mean from the intravenous group. Noncompartmental analysis was used for calculating the individual terminal half-life and distribution volume for NXX-066 in plasma.

A computer program (C log *P*, Biobyte) was used to calculate the log *P* value (C log *P*) for NXX-066.

Results are generally presented as mean values ± S.D. The Student's *t*-test (two-tailed) was used to test the significance between two means. A value of $P < 0.05$ was considered statistically significant.

3. Results and discussion

Nasal administration of NXX-066 resulted in extremely rapid and complete absorption into the systemic circulation followed by a rapid decline in the plasma concentrations (Fig. 2a). The intravenous and nasal concentration–time profiles of NXX-066 were similar, coinciding after ≈15 min and the elimination could be described biexponentially.

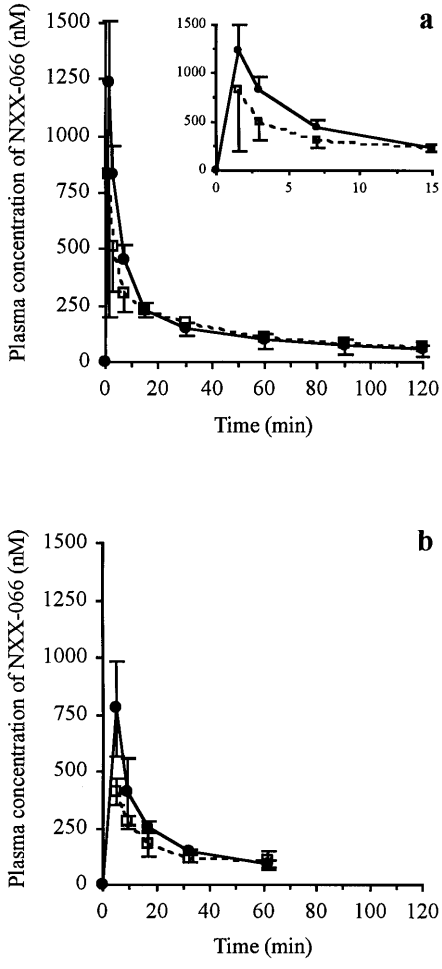

Fig. 2. (a) The concentration-time profiles of NXX-066 in plasma after (- -□- -) intravenous ($n = 6$) or (–●–) nasal ($n = 7$) administration (3 μmol/kg) to rats. The initial intravenous concentrations were obtained by extrapolation. The inserted figure is a magnification of the first four sampling points. (b) The concentration-time profiles of NXX-066 from the corresponding plasma samples ($n = 3$–4 at each time point) obtained 2 min after CSF sampling. All data are expressed as means ± S.D.

The pharmacokinetic parameters for NXX-066 in plasma after intravenous and nasal administration are presented in Table 1. The mean values of each parameter did not differ significantly between the two administration routes ($P < 0.05$). The average half-life of elimination from plasma for the intravenous and nasal routes was 70 ± 14 and 75 ± 22 min, respectively. The volume of distribution was comparatively large: 13.2 ± 4.1 and 13.0 ± 2.7 l/kg for the intravenous and nasal routes, respectively.

The peak plasma concentrations had already been reached at the first sampling point (within 1.5 min) in all animals receiving NXX-066 nasally, confirming the rapid absorption of small lipophilic compounds after nasal administration found by other workers (Corbo et al., 1989; Fisher et al., 1992). The molecular weight of NXX-066 is 377.5 g/mol and C log P was calculated as 4.35. A fairly short time to peak plasma concentration (≈ 7 min after nasal administration) and 100% bioavailability were also found in a study of a seretonin-1a receptor antagonist (Dahlin and Björk, 2000), (S)-UH-301 (MW 265.4 g/mol; log $P \approx 4$).

A rapid onset of action is desirable for sedatives. Bjerre et al. (1996) showed that propiomazine was absorbed within 5 min following nasal administration to rats. The neuroprotective agent [^{14}C]-dextromethorphan hydrochloride was also rapidly absorbed after nasal administration (Char et al., 1992); peak radioactivity levels were recorded 2 min after administration to rats. NXX-066 and nicotine (Jung et al., 2000) appear to be absorbed into the systemic circulation after nasal administration more quickly than other centrally active substances, according to the available literature. Centrally acting substances which are absorbed comparatively less rapidly after nasal administration include the antimigraine drug sumatriptan, also available as an intranasal formulation (time to peak plasma concentration 30 min) (Ayres et al., 1996) and the narcotic analgesic oxymorphone (time to peak plasma concentration 24 min) (Hussain and Aungst, 1997).

The mean AUC values after intravenous and nasal administration were 18800 ± 5500 and 19000 ± 2000 nM min, respectively and the absolute nasal bioavailability of NXX-066 was consequently calculated as $100 \pm 30\%$. The variation (S.D.) is mainly due to deviations in the intravenous AUC values as a result of small differences in real time between administration and the first sampling point during the rapid decline in plasma concentrations. The bioavailability of NXX-066 is similar to that of nasally adminis-

Table 1
Pharmacokinetic parameters of NXX-066 in rat plasma calculated according to non-compartmental analysis after administration of 3 μmol/kg as an intravenous bolus dose or nasally to both nostrils[a]

Administration route	Rat	T_{max} (min)	AUC (nM min)	$t_{1/2}$ (min)	V_d (l/kg)
Intravenous	A	–	28637	79	8.3
	B	–	19866	56	10.4
	C	–	14506	67	15.5
	D	–	15423	54	13.1
	E	–	20109	79	11.9
	F	–	14058	88	20.0
	Average	–	18800	70	13.2
	S.D.	–	5500	14	4.1
Nasal	1	1.5	20001	46	8.9
	2	1.5	22058	73	10.0
	3	1.5	16983	66	14.1
	4	1.5	17155	57	12.1
	5	1.5	16949	87	15.8
	6	1.5	20217	92	14.7
	7	1.5	19705	108	15.1
	Average	1.5	19000	75	13.0
	S.D.	–	2000	22	12.7

[a] There were no significant differences between the administration routes ($P<0.05$). AUC, area under the plasma concentration-time curve; $t_{1/2}$, terminal half-life; T_{max}, time to reach maximum plasma concentrations; V_d, volume of distribution.

tered physostigmine in rats ($100 \pm 20\%$) (Hussain and Mollica, 1991). However, the time to the peak plasma concentration was 6.5 ± 1.7 min for physostigmine, i.e. slower than for NXX-066. The molecular weight of physostigmine is 275.3 g/mol and the log P value is 1.58 (Hansch and Leo, 1995), so the slower rate of absorption for physostigmine could perhaps reflect the difference in the log P values of the two compounds. Bearing in mind that oral administration is not practical because of low bioavailability due to presystemic metabolism, the rapid and complete systemic absorption of NXX-066 after nasal administration suggests that this would be a potential alternative route to parenteral administration.

Only low concentrations of NXX-066 were detected in the CSF and the drug was not detected at all in the last two samples obtained 30 and 60 min after both intravenous and nasal administrations (Fig. 3). The concentration of NXX-066 in the CSF was significantly higher ($P < 0.05$) 3 min after intravenous administration than after nasal administration. The plasma concentration–time profile obtained from blood samples taken 2 min after the collection of CSF (Fig. 2b) correlated well with the data from systemic delivery of NXX-066 (Fig. 2a), indicating that absorption through the nasal membrane was not influenced by turning the animal before CSF sampling.

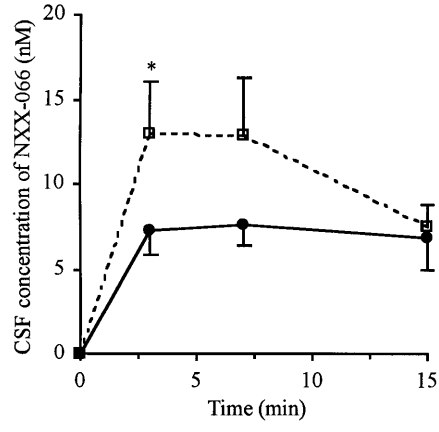

Fig. 3. Concentration-time profiles of NXX-066 in the CSF after (- -□- -) intravenous or (–●–) nasal administration (3 μmol/kg) to rats ($n = 3$–4). The data are expressed as means ± S.D.; *$P < 0.05$ by Student's t-test.

Nasal administration of [^{14}C]-dextromethorphan hydrochloride (Char et al., 1992) resulted in a bioavailability of $\approx 80\%$ and a plasma concentration peak after only 2 min. However, despite the rapid systemic absorption of the drug, an unexplained initial absorption phase into the brain occurred after nasal administration, and the total brain uptake was lower than seen with intravenous administration. A similar initial absorption phase into the CSF occurred with NXX-066 in this study. Although NXX-066 was totally absorbed into the systemic circulation after nasal administration, uptake into the CSF was significantly higher after intravenous administration at the first sampling time 3 min after administration (Fig. 3). The reason for this is not known, but it is possible that the high initial plasma concentration of NXX-066 after intravenous administration may have caused rapid transport of the drug through the BBB by passive diffusion.

Since the uptake of NXX-066 into the CSF was no higher after nasal administration than after intravenous administration, there was no evidence that NXX-066 was transported from the nasal cavity along the olfactory neurons into the brain. Similar results were shown with a seretonin-1a receptor antagonist (Dahlin and Björk, 2000), [^{14}C]-dextromethorphan (Char et al., 1992) and a cognition enhancing drug (Hussain et al., 1990). Hussain et al. suggested that transport along the olfactory neurons into the CNS may only be significant for poorly absorbed substances and that it would be insignificant for compounds that are absorbed rapidly and mainly into the systemic circulation. A good correlation exists between the lipid solubility of a drug and its ability to penetrate or diffuse across the BBB (Oldendorf, 1974). However, degradation and active transport mechanisms in the BBB could be an obstacle to substances entering the brain, even for those that are well absorbed from the nasal cavity. Sakane et al. (1991) observed a significant correlation between the drug concentrations of sulfonamides in the CSF and the partition coefficient (Pc). With regards to these sulfonamides, with comparatively low lipophilicity, an increasing Pc resulted in higher drug concentration in the CSF after nasal administration.

NXX-066 is a lipophilic compound. The large distribution volume appears to be consistent with the lipophilic character of the molecule and this suggests that there is significant tissue binding of the compound, which could explain the low concentrations of NXX-066 in the CSF. Since there are no apparent barriers to the penetration of the BBB for NXX-066, the drug presumably diffuses into the more lipophilic environments of the brain tissue rather than remaining in the CSF. Thus, it could be useful to include a complementary method of sampling highly lipophilic compounds such as NXX-066 along with CSF sampling. Although the plasma concentrations may not be correlatable with the effect because of AChE inhibition, brain tissue sampling or microdialysis could be alternative methods of measuring the concentration of the drug at the target site.

4. Conclusion

The physostigmine analogue NXX-066, an AChE inhibitor, was rapidly and totally absorbed into the systemic circulation after nasal administration in rats. Since the oral bioavailability is poor to moderate due to pre-systemic metabolism, nasal delivery could be an alternative administration route for NXX-066 to parenteral routes. Enhanced uptake of NXX-066 into the CSF was not apparent after nasal administration and there is, therefore, no evidence for a direct pathway from the nasal cavity along the olfactory neurons into the CNS.

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