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Can Nasal Drug Delivery Bypass the Blood-Brain Barrier?

Questioning the Direct Transport Theory

Frans W.H.M. Merkus[1] and *Mascha P. van den Berg*[2]

1 Leiden/Amsterdam Centre for Drug Research, Leiden University, Leiden, The Netherlands
2 Pfizer Global Research and Development, Sandwich, UK

Abstract

The connection between the nasal cavity and the CNS by the olfactory neurones has been investigated extensively during the last decades with regard to its feasibility to serve as a direct drug transport route to the CSF and brain. This drug transport route has gained much interest as it may circumvent the blood-brain barrier (BBB), which prevents some drugs from entering the brain. Approximately 100 published papers mainly reporting animal experiments were reviewed to evaluate whether the experimental design used and the results generated provided adequate pharmacokinetic information to assess whether the investigated drug was transported directly from the olfactory area to the CNS. In the analysis the large anatomical differences between the olfactory areas of animals and humans and the experimental conditions used were evaluated. The aim of this paper was to establish the actual evidence for the feasibility of this direct transport route in humans.

Twelve papers presented a sound experimental design to study direct nose to CNS transport of drugs based on the authors' criteria. Of these, only two studies in rats were able to provide results that can be seen as an indication for direct transport from the nose to the CNS. No pharmacokinetic evidence could be found to support a claim that nasal administration of drugs in humans will result in an enhanced delivery to their target sites in the brain compared with intravenous administration of the same drug under similar dosage conditions.

The connection between the nasal cavity and the CNS by the olfactory neurones has been investigated extensively during the last decades with regard to its feasibility to serve as a direct drug transport route to the brain. This drug transport route has gained much interest as it may circumvent the blood-brain barrier (BBB), which prevents drugs from entering the brain. More than 100 research papers have described studies on the nose to CNS route (mainly in animals), with ambiguous results. Careful review of these papers reveals that the methods used do not often utilise the necessary design to determine the actual drug transport route. In most cases the animal experiments cannot be translated to the human situation. It must not be forgotten that the ultimate goal of the exploration of this direct nose to CNS pathway is to be able to apply this delivery route to humans, facilitating CNS drug concentrations that can elicit a central pharmacological effect. All papers were reviewed to evaluate whether the experi-

mental design used and the results generated provided adequate information to assess whether the investigated drug was transported directly from the nasal cavity to the CNS.

1. The *in Vivo* Fate of Intranasal Drugs

Before defining criteria that should be included in an experimental design when investigating drug transport from the nasal cavity into the cerebrospinal fluid (CSF) and/or brain tissue, the *in vivo* fate of intranasal drugs will be described (see figure 1). This will serve as a basis for the theoretical experimental design discussed in the next section.

Firstly, drugs reach the respiratory epithelium, from where they are absorbed into the systemic circulation or cleared by mucociliary clearance and swallowed. Drugs that are absorbed into the systemic circulation may enter the CNS after passing through the BBB. When a nasal drug formulation is deposited directly on the olfactory epithelium it is possible that drug transport via the olfactory neurones occurs. Two possible routes exist by which molecules can be transported from the olfactory epithelium into the brain and/or CSF. The first is the *epithelial pathway*, where compounds pass paracellularly across the olfactory epithelium into the perineural spaces, crossing the cribriform plate and entering the subarachnoid space filled with CSF. From here the molecules can diffuse into the brain tissue or will be cleared by the CSF flow into the lymphatic vessels and subsequently into the systemic circulation. The second possibility is the *olfactory nerve pathway*, where compounds may be internalised into the olfactory neurones and pass inside the neuron through the cribriform plate into the olfactory bulb. It is possible that further transport into the brain can occur by bridging the synapses between the neurons. After reaching the brain tissue, drugs are cleared either via the CSF flow or via efflux pumps such as p-glycoprotein at the BBB[1] into the systemic circulation. Furthermore, the trigeminal nerve[2] and, in animals, the vomeronasal organ[3] also connect the nasal cavity with the brain tissue.

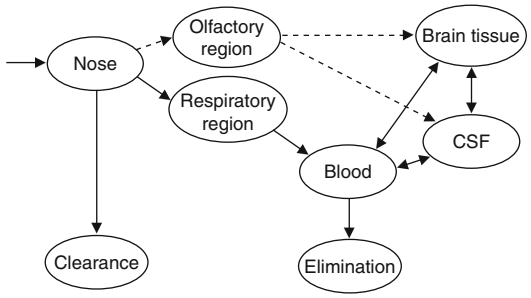

Fig. 1. Schematic overview of the *in vivo* fate of drugs following nasal administration. **CSF** = cerebrospinal fluid.

2. Experimental Models to Study Nose to CNS Drug Transport

2.1 Are the Experiments Realistic for the Human Situation?

In most animal studies the investigators suggest that nose to CSF/brain transport is also feasible in humans. However, there are large anatomical differences between animals and man. For instance, in rats 50% of the nasal cavity is occupied by olfactory epithelium. In humans this is only 3%[4] and, more importantly, the olfactory area, located in the roof of the nasal cavity, is difficult to reach using nasal drops or a nasal spray. Secondly, in most mammalian species (including rodents) the nose contains a vomeronasal organ with nerves also connecting directly to the brain. However, the existence and functioning of this organ in humans is still under debate.[3] Furthermore, many formulations used in animal studies contain mucosa-damaging permeation enhancers (e.g. propylene glycol, ethanol or other organic solvents in concentrations up to 40%),[5-11] while some nasal formulations were used in an extremely aggressive way (e.g. spraying a formulation over 1 minute using an atomiser connected to a respiratory pump with high pressure)[5-8] or in a large volume.[12-32] When olfactory epithelial cells are in contact with these solutions for a long time, severe cell structure damage occurs. Aggressive spraying methods are very painful and severely damage the olfactory area.

Some researchers,[9,33-40] for instance, have applied surgical modifications to rats[41] in which the trachea of the animal is canulated to maintain respi-

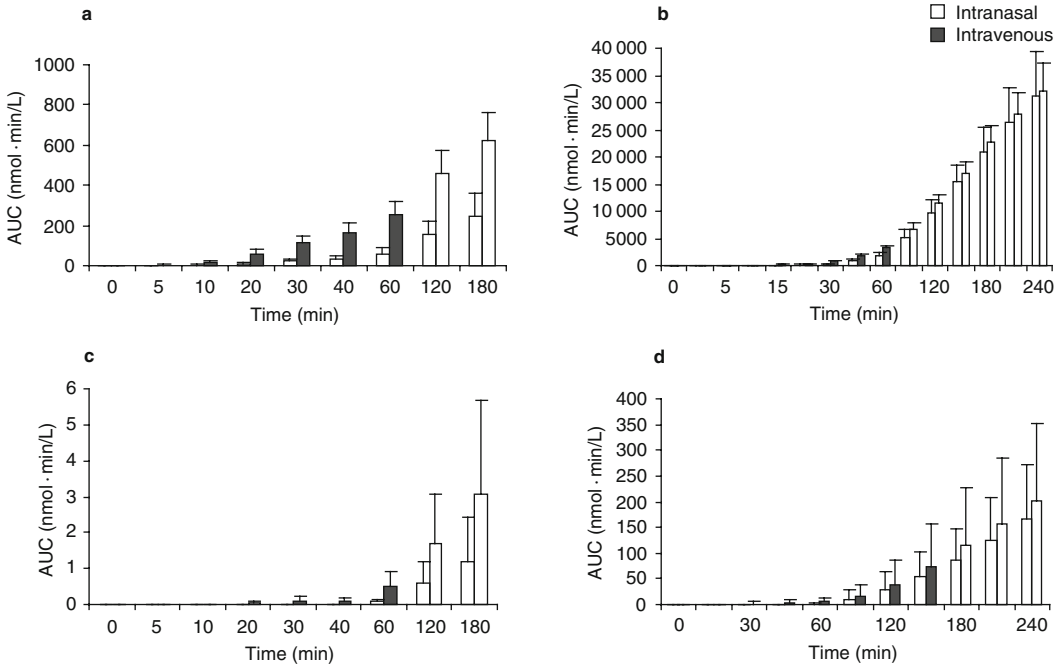

Fig. 2. Hydroxocobalamin area under the concentration-time curve (AUC) for plasma (**a, b**) and cerebrospinal fluid (**c,d**) following intranasal administration and intravenous infusion in humans (**a** and **c**) [n = 5] and in rats (**b** and **d**) [n = 8].

ration, the oesophagus tied off to prevent swallowing of the nasal drug formulation, and the nasopalatine duct sealed to prevent any nasal formulation from being cleared to the mouth. In addition, the nasal perfusion method[42] has been used to deliver the formulation.[9,34,36,37,39] The use of such a treatment would be unrealistic, even unthinkable, in humans. Therefore one should exercise caution in the interpretation of such animal results.

A realistic comparison of human and rat data was made recently in studies where similar methods were used in patients and in rats.[43-45] By administering identical intranasal and intravenous drug formulations of the lipophilic drug melatonin and the hydrophilic drug hydroxocobalamin and using similar sampling times, analogous results were obtained (figure 2). The drugs investigated were transported to the CSF by the systemic pathway: after first being absorbed in the systemic circulation, the drug reached the CSF via the blood-CSF barrier. The fact that only a few human studies report drug concentrations in the CSF[14,45] or brain (using a positron emission tomography [PET] scan[46]) is because of the difficulties and risks associated with CSF collection in humans and the costly and specialised technique of PET scanning.

When investigating the direct transport route a distinction must be made between drugs transported from the nasal cavity via (i) the systemic circulation after crossing the respective BBB and blood-CSF barrier, or (ii) via the olfactory epithelium directly into the CSF and/or brain tissue. In addition, a nasal delivery method appropriate for the species must be chosen. Features such as delivery volume, dose frequency and safety of the formulation need to be considered. Delivery volumes that do not overload the nasal cavity must be used, and should not exceed 20μL[47] in rats and 100μL[45] in humans. Investigating drug transport requires a pharmacokinetic approach, determining and comparing drug concentrations in the relevant biocompartments after intranasal drug administration and after a slow intravenous infusion, giving plasma concentrations similar to those expected after intranasal applica-

tion. Ideally the drug plasma profiles achieved should be similar. This ensures that the rate of passive diffusion from the systemic circulation into the CNS is the same after both intranasal and intravenous administration. The actual route of drug transport can be determined by calculating the relative distribution of a drug to the CNS (CSF or brain) and plasma after both methods of administration according to equation 1:

$$\text{CNS/plasma ratio} = \frac{AUC_{CNS}}{AUC_{plasma}} \quad \text{(Eq. 1)}$$

The CNS/plasma ratio of a compound is expressed by the area under the CNS concentration-time curve (AUC_{CNS}) divided by the area under the plasma concentration-time curve (AUC_{plasma}) following the same administration route.[48] In case of direct drug transport from the nasal cavity, it is expected that the CNS/plasma ratio following intranasal delivery will be significantly higher than that after intravenous administration. When this ratio is equal to or lower than the intravenous one, the observed drug transport can be considered to be systemic and not via the olfactory neurones.

All the above-mentioned features necessary for a realistic experimental design to study nose to CNS drug transport are summarised in table I.

Table I. Criteria for a realistic experimental design when investigating nose to CNS drug transport

The method of nasal administration should be appropriate for the animal species used and realistic when compared with the human situation. In other words the volume/dose should be realistic and the excipients and/or permeation enhancer used in the formulation and the administration method used should be safe for human application
In order to compare exactly the transport of the drug via the blood-brain barrier and via the olfactory route, the drug should be administered both intranasally and intravenously, aiming at comparable plasma drug concentrations from both routes
Drug concentrations in plasma and CNS (CSF and/or brain) should be measured and preferably AUC values for plasma and CNS (CSF and/or brain) should be calculated
Drug distribution over the CNS compartment and the systemic compartment should be compared following intranasal and intravenous delivery

AUC = area under the concentration-time curve; **CSF** = cerebrospinal fluid.

2.2 Study Designs Used in the Literature

The literature investigated for this review can be divided into four categories: nose to brain, nose to CSF, nose to brain and CSF, and pharmacodynamic research (table II).

For each category the following aspects are discussed: (i) species used; (ii) the delivery route with which the intranasal administration is compared, also referred to as the reference route; (iii) the type of samples taken and sampling techniques used; and (iv) general remarks about the disadvantages and advantages of the study design. As the main focus of this paper is on transport of drugs, the literature on dyes, metals, micro-organisms and tracers like WGA-HRP were not reviewed.

In the majority of the studies drugs were formulated in an aqueous solution such as saline or buffered solutions. In 15 papers[14,22,25,49-51,69,70,94-99,114] the composition of the formulation was not mentioned, and in seven articles the compound was dissolved in a mixture of water, ethanol and propylene glycol,[5-8] diluted ethanol[9,10] or a 40% isopropyl alcohol solution containing 10% sefsol (a skin permeation enhancer).[11] Administration of drugs into the nose is most often achieved using a piece of tubing attached to a microsyringe or micropipette in animals and a spray in humans. Typical delivery volumes are 5–10 µL/nostril in mice, 10–25 µL/nostril in rats and 75–100 µL/nostril in humans. These volumes were used as criteria in the eventual test described below. In a substantial number of investigations in animals[12,15,16,19,20,22,25,26,30-32] and humans[13,14,17,18,21,23,24,27-29] (21 of 104), a relatively large dose (and in total a large volume) was administered. These studies involved the instillation of multiple aliquots of the nasal formulation, taking up to 30 minutes to administer the complete dose.[12-32]

2.2.1 Nose to Brain Research

Drug transport from the nasal cavity specifically into brain tissue has been studied mainly in rats and mice. In most cases the intranasal route of administration was compared with an intravenous bolus injection (16 of 26 papers). Brain tissue was obtained as a whole,[50,52-54] in dissected brain re-

Table II. References for the identified publications per research category

Category	References	No. of papers
Nose to brain	12,16,30,31,33,34,46,49-68	27
Nose to CSF	6-8,14,25,36-38,40,43-45,69-88	32
Nose to brain and CSF	5,35,39,89-93	8
Pharmacodynamics	9-11,13,15,17-19,21-24,26-29,32,94-113	37
Total		**104**

CSF = cerebrospinal fluid.

gions[16,30,33,49,51,52,55-59] or in slices.[30,51,52,55-57,59] About half of the studies (13 of 26) investigated the drug absorption in blood[30,33,46,49-51,53,54,57-59] and/or drug uptake into other tissues as well.[30,51,52,55,57]

In nine papers the drug uptake into the brain following nasal delivery was studied qualitatively and quantitatively.[30,46,51,52,54-57,59] By using a radiolabelled compound and autoradiographic analysis, the drug uptake and distribution throughout the brain can be visualised. The drug concentration in brain tissue was often determined in several brain areas at a single[51,57] or at three to six time points.[30,52,54-56,59] The technique of PET allowed Wall et al.[46] to take up to 18 'brain samples' from each volunteer during a 90-minute post-dose period to detect drug uptake into the brain following nasal administration of (^{11}C) zolmitriptan. High uptake values were found for the extracranial area in contrast to the inside of the cranium, and hence it was concluded that zolmitriptan entered the brain after passing through the BBB, so no direct transport was found.

Data analysis using the brain/plasma ratios as described in equation 1 was used in five papers,[33,49,50,53,58] whereas three other papers only looked at the brain/plasma ratio at a certain time point.[12,60,61] Vyas et al.[54] used the so-called drug targeting efficiency (DTE) quotient to determine the drug transport route into the brain following nasal delivery (see equation 2):

$$\text{DTE quotient} = \frac{(AUC_{CNS}/AUC_{plasma})_{IN}}{(AUC_{CNS}/AUC_{plasma})_{IV}} \quad \text{(Eq. 2)}$$

However, this calculation method does not consider whether or not the two CNS/plasma ratios for intranasal (IN) and intravenous (IV) administration are significantly different. Finally, this transport route has also been investigated on the cellular level using microscopy techniques.[62-66]

The disadvantages of nose to brain research are: (i) the need for many experimental animals; (ii) it is not applicable in humans, except when using scanning techniques like PET; and (iii) analysing whole brain tissue dilutes the actual drug concentration at the target site and is therefore less informative than results obtained from specific brain regions. The advantages of this approach are that: (i) drug transport can be studied in a quantitative and qualitative way; and (ii) drug concentrations can be measured at the expected target site.

2.2.2 Nose to CSF Research

The nose to CSF pathway has been investigated in a quantitative way only and was mainly studied in rats (23 of 34 papers). All studies compared the intranasal route with intravenous administration (mainly by bolus injection), except for Chou and Donovan,[71-73] who looked at intra-arterial delivery in rats, Anand Kumar et al.,[6] who investigated the intramuscular route in monkeys, and Born et al.,[14] who compared nasal drug delivery with placebo treatment in human volunteers. With the exception of one article,[72] all papers report serial blood sampling per subject, resulting in drug concentration-time profiles in plasma or serum. The CSF was sampled mainly in a serial manner. However, in 11 papers, single CSF samples were taken per animal; in most cases only one sample was collected at the end of the experiment.[25,36-38,40,70,74,75] Only a few studies generated CSF concentration-time profiles by taking a single CSF sample per animal and using several animals per profile.[76-78] Serial CSF sampling is performed by microdialysis[72,73,79-81] or cis-

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