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Review Article

PEPTIDE DELIVERY VIA NASAL ROUTE: EXPLORING RECENT DEVELOPMENTS AND APPROACHES

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ABSTRACT

There has been a significant increase in interest in using the nasal route to administer peptides. This is mainly due to its advantages, including less invasiveness, rapid absorption, and the ability to bypass initial metabolism in the liver. The incorporation of nanotechnology has emerged as a prominent strategy, with nanocarriers such as nanoparticles and liposomes being employed to augment stability and bioavailability of peptides, as extensively discussed in this review. These carriers serve the crucial function of safeguarding peptides against enzymatic degradation while also enabling a sustained release, thus extending the therapeutic impact. Additionally, this review delves into mucoadhesive polymers and permeation enhancers, which have undergone extensive exploration to enhance nasal retention and augment the transportation of peptides across the nasal mucosa. Recent breakthroughs in nasal peptide delivery have heralded a new era in peptide-based therapies. These advancements encompass innovative formulation technologies, the utilization of nanocarriers, permeation enhancers, and the integration of intelligent materials and nasal drug delivery devices, all of which are geared towards enhancing the efficiency and efficacy of nasal peptide delivery.

Keywords: Peptide, Nasal drug delivery, Nanocarriers, Mucoadhesive, Insulin, Intravail®

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INTRODUCTION

Natural and synthetic peptides have become increasingly recognized as innovative treatment choices for a wide range of medical conditions and indications. These peptides offer a unique advantage in deciphering protein-receptor interactions, a challenge often encountered when dealing with conventional small molecules.

Peptides occupy a unique position, straddling the boundary between small molecules and proteins exhibiting distinctive biochemical and therapeutic attributes. Unlike small molecules, peptides undergo well-defined and predictable metabolism but are susceptible to enzymatic degradation. Consequently, their suitability for oral administration is limited, often resulting in poor bioavailability and restricted membrane permeability. Medical practitioners tend to favour intravenous and subcutaneous administration for better control over dosage, but these methods can be uncomfortable for patients and pose challenges for self-administration [1].

The advent of synthetic peptide synthesis techniques marked a significant breakthrough in the field. Notably, the use of insulin in the 1960s played a pivotal role in establishing synthetic peptides as a viable therapeutic option. Currently, the (Food and Drug Administration) FDA has approved 80 peptides as therapeutic agents [2], with many more synthetic peptides having been successfully synthesized. Few of which are summarized in table 1. Remarkably, in 2022, out of the 37 newly approved drugs, four were peptides, and one was an oligonucleotide [2].

Nasal route is a non-invasive means of drug delivery. It has several advantages, including rapid onset of action due to the extensive surface area and blood flow in the nasal mucosa. It offers a non-invasive alternative to injections or oral ingestion, enhancing patient comfort and compliance. By bypassing first-pass metabolism in the liver, it often results in higher drug bioavailability. Nasal delivery is particularly suitable for drugs like peptides and proteins that would be degraded in the gastrointestinal tract. Additionally, nasal sprays and inhalers are user-friendly, making them convenient for self-administration.

However, nasal drug delivery has its drawbacks. The limited capacity of nasal passages restricts the volume of medication that can be administered. It may also cause irritation or local side effects like sneezing, nosebleeds, or a runny nose. The duration of action can be shorter compared to other routes, necessitating more frequent dosing. Absorption can vary among individuals due to factors such as nasal congestion and mucosal health, leading to inconsistent drug levels. Some drugs may face challenges crossing the nasal mucosal barrier, limiting their effectiveness through this route. Peptides and proteins, especially, encounter obstacles concerning nasal absorption, resulting in reduced bioavailability upon nasal administration due to their high molecular weight and increased amino acid count. Among the prevailing strategies, integrating enhancers into formulations and developing nano-or micro-particle systems using diverse polymers have emerged as prominent techniques [1].

Borrajo and Alonso had done extensive research on literature across PubMed and Scopus database to deduce the number of publications on the nasal drug delivery of peptides, which depicts that there is a substantial increase in the researchers being inclined towards the peptides as therapeutics from the year 2004. Between 2014-2019 the greatest number of research papers had been published as depicted in fig. 1 [3].

Nasal physiology

The human nasal cavity is partitioned into two parts by the cartilaginous bony nasal septum and is approximately 12 cm long, with a volume capacity of around 15 ml and a large surface area exceeding 150 cm^2. These two sections consist of the nasal vestibule, which is a skin extension not used for drug delivery, and the respiratory and olfactory regions. The nasal vestibule, located at the entrance of the nose, contains coarse hairs and sebaceous glands that assist in filtering out larger airborne particles. The olfactory region, situated high in the nasal cavity, houses the olfactory epithelium, responsible for detecting odors. Specialized nerve cells in this region transmit sensory signals to the brain for the sense of smell. Additionally, the respiratory region, the largest part of the nasal cavity, is lined with a mucous membrane containing cilia, facilitating the filtration of smaller particles and microorganisms from inhaled air. The nasal cavity has three bony projections called nasal conchae or turbinates: the superior, middle, and inferior conchae as shown in fig. 2. These conchae increase the surface area within the nasal passages, promoting efficient air filtration, humidification, and warming [21].

Paranasal sinuses are air-filled spaces surrounding the nasal cavity. Two important sinuses are the ethmoid sinuses and the sphenoid sinuses, each with distinct locations and functions.

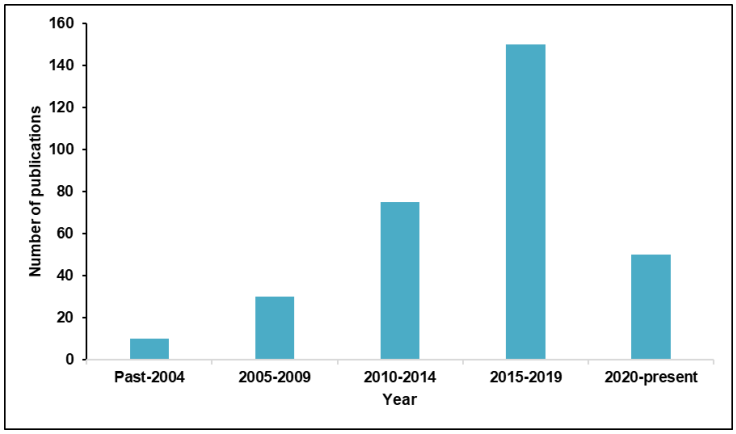

Fig. 1: Publications on nose-to-brain delivery of peptides therapeutics [3]

Table 1: Summary of peptides transported intranasally

Peptide drug	Molecular weight (Da)	Disease	References	Current approval status	Brand name
Insulin	5800	Alzheimer's Disease	[4, 5]	Marketed	ViaNase®
Oxytocin	1000	Autism spectrum disorder, sexual dysfunction, schizophrenia	[6, 7]	Marketed	Empower pharmacy® pharma labs global®
Erythropoietin	30,400	Alzheimer's Disease, Cerebral Ischemia, Epilepsy	[8, 9]	Research ongoing	NA
Human Nerve Growth Factor	26,500	Alzheimer's Disease	[10, 11]	Marketed	Stimulator NGF®
Insulin-like growth factor I	7650	Alzheimer's Disease, Huntington's disease	[8, 12]	Marketed	Now Sports® Pharma Grade®
Glucagon-like peptide I	4100	Obesity	[13, 14]	Research ongoing	NA
Hexarelin	887	Growth Hormone Deficiency	[15]	Marketed	Pharma labs Global® Direct Peptides®
Brain derived neurotrophic factor	26,900	Alzheimer's Disease,Autism Spectrum Disorder	[9]	Research ongoing	NA
Exendin (9–3 9)	3400	Congenital Hyperinsulinism	[14, 16]	Research ongoing	NA
Glucagon	3766	Hypoglycemia	[17]	Marketed	Baqismi®
Neurotrophin-4	22,400	Multiple sclerosis	[9]	Research ongoing	NA
Growth differentiation factor 5	27,400	Parkinson's disease	[18]	Research ongoing	NA
Exendin-4	4186	Cerebral ischemia	[19]	Research ongoing	NA
Neuropeptide Y	4253	Post-traumatic Stress Disorder, Depression	[20]	Research ongoing	NA

Ethmoid sinuses

The ethmoid sinuses, situated within the ethmoid bone between the eyes and behind the nasal bridge, serve a pivotal role in air filtration, humidification, and supporting the structure of the nasal cavity. Their proximity to the olfactory region means that issues in the ethmoid sinuses can potentially affect a person's sense of smell. Infections or inflammation in these sinuses can lead to discomfort and a diminished sense of smell [22].

Sphenoid sinuses

The sphenoid sinuses are deeply embedded within the sphenoid bone, positioned behind the eyes and above the nasopharynx. These sinuses feature a butterfly-shaped configuration and contribute to reducing the skull's weight and enhancing vocal resonance. However, due to their deep location, infections or inflammation in the sphenoid sinuses can be challenging to diagnose and treat. Issues in the sphenoid sinuses may lead to discomfort and headaches, often requiring specialized medical attention [21-23].

The respiratory region within the nasal cavity is particularly suitable for drug absorption. This suitability arises from its extensive surface area, which is facilitated by numerous microvilli and cilia on the nasal epithelium. These micro projections, measuring 4–6 µm and moving at a speed of 1000 strokes per minute, actively move the mucus towards the back of the nasal cavity, thus enhancing the transfer and absorption of drugs. Moreover, this area is rich in capillary blood vessels and intricately connected with fibres from the trigeminal nerve. The combination of an extensive capillary network and a significant surface area designates this region as the primary site for systemic drug absorption [23].

Within the nasal respiratory mucosa, there exists a layer of mucus, which is approximately 5 µm thick. This mucus consists of two distinct parts: a viscous gel on the upper portion and an aqueous sol layer on the lower part [24]. The composition of mucosal secretion includes approximately 95% water, 2% mucin, 1% salts, 1% albumin, immunoglobulins along with 1% lipids and has a of pH 5.5 to 6.5. Importantly, the nasal epithelia undergo a renewal process, generating a fresh mucus layer roughly every 10 minutes [24].

The region responsible for the sense of smell, located towards the uppermost part of the nasal cavity and connected to endpoints of olfactory nerve, possesses a unique characteristic of exposing the olfactory nerve to the external environment. This region's mucosa contains supporting epithelial cells, basal cells, and olfactory neurons that originate in the brain's olfactory bulb [23].

In the nasal cavity, olfactory and respiratory epithelia are firmly connected through intercellular junctions that encircle the epithelial cells. These junctions can be classified into three primary complexes as shown in fig. 2.

Tight Junctions (Zona Occludens): Positioned closest to the cells' apical surface, tight junctions, or zona occludens, are primarily responsible for regulating the passage of molecules and ions between cells, forming a secure seal.

Adherens Junctions (Zona Adherence): Just below the zona occludens, adherens junctions, or zona adherence, facilitate cell-to-cell adhesion, ensuring that cells remain connected and maintaining the structural integrity of the epithelial layer.

Desmosomes (Macula Adherence): Situated beneath the zona adherence, desmosomes, also known as macula adherence, are

specialized junctions that provide robust adhesion between neighbouring cells, essential for tissue stability and resistance to mechanical stresses.

Together, these intercellular junctions play a crucial role in safeguarding and regulating substance exchange within the nasal cavity [25].

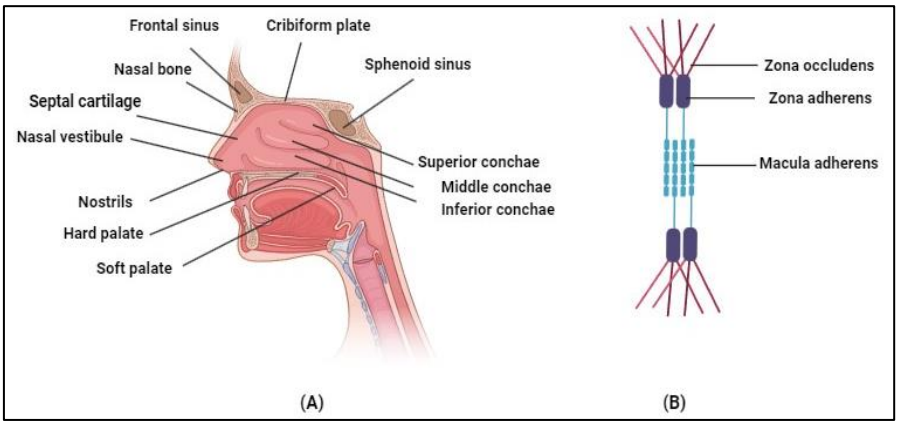

Fig. 2: Schematic representation of nasal physiology containing (A) Nasal anatomy and (B) Intercellular junctions in nasal cavity. (Drafted by CorelDRAW® version X7)

Studies have shown that the width of tight junctions between nasal epithelial cells ranges from 3.9 to 8.4 angstroms (Å). Even when absorption enhancers are employed, the maximum diameter achieved when these junctions open is approximately 15 nm [24].

When drugs are administered nasally, they target the respiratory epithelium. Specialized devices can distribute drugs to various nasal areas, including the olfactory region. The drug's fate depends on factors like molecular weight and lipophilicity; it may be absorbed into the bloodstream or cleared by the mucociliary system. Enzymes within the nasal cavity can also potentially degrade the drug. Notably, the olfactory region has higher cytochrome P450 enzyme activity compared to the liver, impacting drug metabolism.

The nasal cavity's highly vascularized sub-epithelial layer enables rapid drug absorption by allowing direct entry of blood flow into the systemic circulation, bypassing first-pass metabolism. Utilizing their patented Intravail® technology, Aegis Therapeutics recently created an innovative exenatide formulation. This formulation achieved comparable blood glucose levels with a straightforward metered nasal spray, eliminating the necessity for twice-daily injections and encourages strong patient adherence [21, 26].

Nose to brain Central Nervous System (CNS) absorption pathways

When it comes to reaching the brain and cerebrospinal fluid (CSF) from the nasal cavity, drugs can follow three primary routes:

Olfactory neural pathway

The olfactory pathway plays a crucial role in nasal drug delivery. When a drug is administered intranasally, it can travel through the nasal passages and reach the olfactory region paracellular or transcellular transport which contains specialized olfactory receptor neurons. These neurons have cilia that extend into the nasal mucus. When a drug molecule reaches these cilia, it can bind to olfactory receptors, triggering sensory signals that are transmitted to the olfactory bulb in the brain through the cribriform plate. From there, the drug can potentially access the central nervous system, including the brain, providing a direct and rapid route for drug delivery.

Trigeminal pathway

The trigeminal pathway, also known as the trigeminal nerve pathway, is another important route for nasal drug delivery. Unlike the olfactory pathway that primarily targets the brain, the trigeminal pathway involves the trigeminal nerve, a cranial nerve responsible for sensation in the face, including the nasal passages. When drugs are administered intranasally, they can activate the trigeminal nerve's sensory receptors. This pathway is useful for drugs intended for local effects in the nasal cavity or for systemic effects by absorption into the bloodstream through the rich vascular network in the nasal mucosa [26, 27].

Systemic pathway

Drugs absorbed into systemic circulation may also have the potential to reach CNS if they possess sufficient lipophilicity. This characteristic allows them to cross the blood-brain barrier (BBB) and gain direct access to the CNS. The effectiveness of this pathway depends on the drug's specific properties and its ability to penetrate the BBB.

These three pathways offer various mechanisms for drugs to reach the CNS from the nasal cavity, as shown in fig. 3, presenting a range of options for targeted drug delivery to the brain and cerebrospinal fluid.

Compounds administered intranasally take advantage of anatomical openings in the skull located within the nasal olfactory epithelium to bypass the BBB. These openings enable neural connections to the olfactory bulb, where peptides gain access to the brain by passing through olfactory epithelial cells. Subsequently, they follow the olfactory and trigeminal nerve pathways, facilitating their non-invasive delivery to more distant regions of the brain [26, 27].

The transport of drugs through the nasal mucosa primarily occurs through three distinct pathways: paracellular, transcellular, and intracellular, as shown in fig. 3. [28]. In the paracellular pathway, drugs pass between adjacent cells in the nasal epithelium. This route relies on the permeability of tight junctions that connect nasal epithelial cells. Small and lipophilic drugs like proteins and peptides can traverse this pathway more easily than larger or hydrophilic ones. The transcellular pathway involves drugs moving directly through individual nasal epithelial cells. This route typically includes drug absorption through the lipid-rich cell membrane. It's suitable for lipophilic drugs that can penetrate cell membranes effectively. Passive and active transport mechanisms may also play a role in this pathway. The intracellular pathway involves the uptake of drugs by nasal epithelial cells, transport through the cell, and then release on the opposite side. Vesicular transport processes like endocytosis and exocytosis facilitate this pathway. larger molecules like peptides or proteins often utilize transcytosis to cross the nasal epithelium [29].

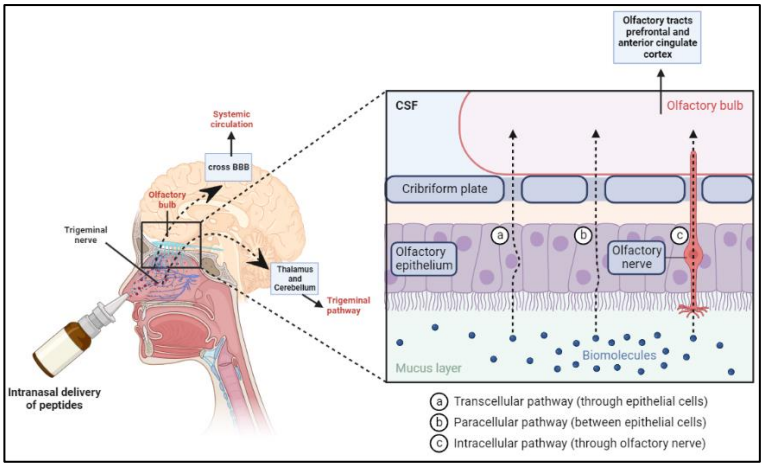

Fig. 3: Representation of drug delivery pathways through the nasal route showing the transport of drugs to through the olfactory bulb, systemic circulation and the trigeminal pathway and (a), (b), (c) showing different pathways drug transport through the nasal mucosa. (Drafted by CorelDRAW® version X7)

Factors affecting the design of peptide formulation for nasal administration

The attractive characteristics of peptides and proteins are overshadowed by their susceptibility to denaturation, hydrolysis, and poor absorption in the Gastro-Intestinal Tract (GIT), making their oral administration difficult. Consequently, alternative administration methods, such as injection, are often necessary, which presents a significant drawback. Unlike small-molecule drugs, peptides exhibit notably less stability, necessitating meticulous attention to formulation and storage to prevent undesired degradation. Even when administered parenterally, small peptides frequently undergo rapid clearance, although efforts are increasingly being made to address this issue through modifications to the peptide structure. Additionally, challenges related to synthesis costs and solubility may arise, though these limitations often vary from one peptide to another [30].

The primary challenges to intranasal delivery of peptides and proteins have historically centered on concerns of low bioavailability and nasal irritation. Bioavailability tends to decrease with increasing molecular weight. For example, salmon calcitonin which has a molecular weight of 4000 Da and displays a mere 3% bioavailability. These therapeutic agents, often exceeding 1k Da, typically have low bioavailability, ranging from 0.5% to 5%. The challenge with nasal delivery of high molecular weight protein and peptide drugs is their poor membrane permeability [31, 32]. However, the advent of highly effective and non-irritating absorption or permeation enhancement agents has provided a reliable solution to these issues. The physiological and biological characteristics of nasal cavity, encompassing factors such as mucociliary clearance, pH, enzymatic activity, affect the absorption of foreign substances [30].

One of the challenges in drug development is improving the physico-chemical stability of pharmaceutical substances. An example of a drug that addresses this challenge is Desmopressin (DDAVP®). It has been formulated to have an improved half-life and stability, which helps maintain its effectiveness over a longer period [33]. Bypassing biological barriers, such as the gastrointestinal tract is another challenge. Semaglutide is designed specifically for this purpose as Rybelsus® and is formulated with (sodium N-[8-(2-hydroxybenzoyl) amino] caprylate) SNAC, which enhances its absorption in the stomach. This formulation enables effective drug delivery despite the presence of natural barriers [34]. Controlling pharmacokinetic parameters is also an important factor in drug development. leuprolide Depot 3 M is an example of a drug developed to address this challenge. This drug is created using the W/O/W (water-in-oil-in-water) or solvent evaporation method and is based on polylactic acid microspheres. This formulation allows for controlled release and absorption of the active substance [35]. Improving target selectivity is also crucial for therapeutic efficacy. A fusion protein of Belatacept (Nulojix®) exemplifies this challenge. It is formulated using specific amino acid substitutions, enhancing its selectivity for CD86 and CD80 receptors. This allows for more targeted modulation of immune responses [36]. Reducing immunogenicity, which refers to the immune response triggered by therapeutic substances, is another important challenge in drug development. Pegademase bovine (Adagen®) addresses this challenge by utilizing PEGylated protein therapy. This approach extends the half-life of the drug and reduces its immunogenicity, ultimately improving its efficacy [37]. Non-invasive administration of drugs is desirable to enhance patient convenience and compliance. Insulin inhalation powder (Afrezza®) is an example of a drug formulated for non-invasive administration. It contains microparticles that are formulated with fumaryl diketopiperazine, which enables effective inhalation delivery of insulin [38].

Advantages, disadvantages and limitations of nasal route

Advantages

Nasal drug delivery systems present a range of advantages that make them a favorable choice for administering medications. To begin with, they offer simplicity and convenience, enabling patients to self-administer their medication with minimal effort. Furthermore, these systems ensure a swift onset of drug action, ensuring that the therapeutic effects are quickly realized. This enhances patient convenience and adherence, as individuals tend to find this non-invasive approach more manageable. Additionally, nasal delivery circumvents hepatic first-pass metabolism and avoids potential irritation of the gastrointestinal membrane, resulting in enhanced drug absorption through highly vascularized nasal mucosa. The extensive surface area of the nasal mucosa further contributes to efficient drug absorption. Importantly, this delivery route allows drugs to bypass the BBB, which can be advantageous for specific therapeutic purposes. Furthermore, nasal delivery guards against drug degradation in the GIT and facilitates increased bioavailability of large drug molecules through absorption enhancers. It also provides an alternative to the oral route, especially for drugs unsuitable for ingestion, such as proteins and peptides. This positions it as a valuable option for long-term therapy, ultimately enhancing the overall patient experience [39-41].

Disadvantages

Nasal drug delivery systems have certain limitations that warrant consideration. Firstly, they are unsuitable for high molecular weight compounds since the nasal route typically has a mass cutoff of approximately 1 kDa. This restriction limits the drugs that can be administered by this method. Mucociliary clearance and ciliary beating can also impact drug permeability, potentially reducing the efficiency of nasal delivery. Furthermore, the volume delivered into

the nasal cavity is constrained to within a range of 25–200 µl, which may pose challenges for drugs requiring larger doses.

Enzymatic barriers present within the nasal cavity can hinder drug permeability, affecting the bioavailability of certain medications. Additionally, the effectiveness of nose-to-brain drug delivery can be negatively influenced by pathological conditions within the nasal passages. In comparison to the GIT, the nasal mucosa provides a smaller absorption surface, potentially affecting drug absorption. Moreover, there is a potential for nasal irritation, which may be less tolerable and less convenient for some individuals when compared to the oral route [39, 40].

The use of high concentrations of added surfactants as absorption enhancers may have the unintended consequence of disrupting or even dissolving the mucosal membrane. Furthermore, incorrect administration techniques may result in drugs being unintentionally delivered to other parts of the respiratory tract, such as the lungs, leading to the loss of the intended dosage form. Additionally, the efficacy of the nasal route can be significantly influenced by pathological conditions like coughs and colds, which can interfere with drug delivery and absorption. Furthermore, there is notable interpatient variability in this route, resulting in differences in drug absorption and individual responses [40, 41].

Limitations to nasal drug delivery

Mucociliary clearance

Mucociliary clearance, a crucial defence mechanism in the respiratory system, poses a significant limitation for nasal drug delivery. In this, a layer of mucus lines the respiratory tract and entraps the drug particles, making it challenging for them to reach their intended target tissues or be absorbed into the bloodstream, resulting in reduced drug efficacy. Tiny hair-like structures called cilia then sweep this mucus along, effectively removing the trapped substances. When drugs are administered through the nasal route, they encounter this mucus layer and the mucociliary clearance system, which can hinder drug delivery. To overcome this limitation, various strategies, including specialized formulations, mucoadhesive agents, optimizing particle size, and tailored delivery techniques, are employed to enhance drug retention and absorption in the nasal cavity, ultimately improving the effectiveness of nasal drug delivery [39-41].

Low bioavailability

The availability of polar drugs, including those with low molecular weight, is typically limited, usually around 10%. In the case of peptides like calcitonin and insulin, their bioavailability is even more restricted, often not exceeding 1%. In the context of nasal drug absorption, especially for large molecular-weight substances like peptides and proteins, the primary obstacle is their low membrane permeability. Although larger peptides and proteins can traverse the nasal membrane through an endocytotic transport process, the amount that successfully makes its way through is constrained. To significantly improve the nasal absorption of such polar drugs, particularly those with low permeability, co-administration with absorption-enhancing agents is a commonly employed strategy, as will be explored further in subsequent sections [41].

Enzymatic degradation

Enzymatic degradation is another significant limitation when it comes to nasal drug delivery, particularly for peptides and proteins. This often-overlooked factor plays a crucial role in reducing the bioavailability of these molecules. Within the nasal cavity and as they cross the nasal epithelial barrier, there are enzymes like exopeptidases (including mono-and diaminopeptidases) that have the capacity to cleave peptides at their ends (N and C termini). Additionally, there are endopeptidases like serine and cysteine enzymes that can break internal peptide bonds. This means that when peptides and proteins are administered nasally, they are susceptible to enzymatic breakdown before they can reach their target sites or be absorbed into the bloodstream. Consequently, strategies to protect these molecules from enzymatic degradation, such as enzyme inhibitors or modified formulations, are essential for enhancing the effectiveness of nasal drug delivery for peptides and proteins [39-42].

Approaches employed to prevent enzymatic degradation, including the use of inhibitors for proteases and peptidases. For instance, aminopeptidase inhibitors like comostate amylase, as well as trypsin inhibitors like leupeptin and aprotinin, have been utilized to hinder the degradation of substances such as calcitonin. Certain absorption enhancers like bile salts and fusidic acid can also be used. The use of inhibitors possessing trypsin-inhibiting properties has proven effective in enhancing the nasal absorption of salmon calcitonin [32].

Formulation of nasal drug delivery systems

Peptides, which can be relatively short, typically composed of 2 to 50 amino acids, and proteins, which are longer and consist of 50 or more amino acids, have naturally developed in the human body to exhibit a remarkable ability to precisely target specific proteins. Their considerable size and three-dimensional configurations create numerous interaction points with specific protein sites, thereby enhancing the efficacy of peptides and proteins while reducing their potential for harm compared to smaller molecules. Nevertheless, as the clinical utilization of peptides and proteins continues to expand, new challenges have arisen [43, 44]. Despite their complex structures that boost potency and specificity when contrasted with small molecules, they are vulnerable to poor stability. They tend to degrade readily under standard storage conditions and are sensitive to factors like the presence of common proteases, variations in temperature, and changes in pH when administered in vivo [44]. Moreover, the rapid dispersal of stabilizing agents in bodily fluids further complicates their stability [45].

Mucoadhesive agents

Bioadhesive polymers, known for their ability to engage with biological materials through interfacial forces and remain affixed to them for extended periods, are commonly referred to as mucoadhesive polymers. The molecular underpinning of mucoadhesion involves attractive forces like hydrogen bonding, electrostatic attractions, van der Waals interactions and hydrophobic affinities. The strength of a polymer's bioadhesive effect is contingent upon factors such as the type of polymer used, environmental pH, swelling characteristics, and physiological variables like mucin turnover and underlying health conditions. Biodegradable polymers, including hydroxypropyl methylcellulose (HPMC), chitosan, carbopol, alginate, poly-vinyl alcohol, hydroxypropyl cellulose, starch, and gellan gum, have been harnessed to create mucoadhesive systems [39, 46].

Absorption enhancers

Penetration enhancers assume a pivotal role in the formulation of proteins and peptides by disrupting mucosal barriers and facilitating the permeation of large macromolecules across membranes. A variety of compounds, such as chelating agents (e. g., Ethylenediamine tetra acetate (EDTA), mucoadhesive polymeric systems (e. g., Thiomers, Cellulose Derivatives), surfactants (e. g., Polysorbate, SLS, Pluronic F-68), fatty acids (e. g., Sodium Caprate), and phospholipids (e. g., phosphatidyl choline), are frequently employed as permeation enhancers. These enhancers function as detergents and surfactants, encouraging the transcellular transport of drugs by disrupting lipid membrane bilayers or via calcium chelation, which facilitates the paracellular transport of hydrophilic drug substances [47].

Solubilizers

Solubilizers work by increasing the drug's ability to dissolve in the liquid formulation, which is essential for efficient absorption and bioavailability. For instance, glycols and alcohols can improve solubility by breaking down the intermolecular forces that prevent the drug from dissolving. Transcutol is a specific glycol ether known for its solubilizing properties. Medium-chain glycerides can enhance drug solubility in lipid-based formulations, and labrasol, a polyglycolyzed glyceride, can improve drug solubility while also aiding in drug penetration across mucosal barriers [39].

Microenvironment modulation

Microenvironment modulation is a strategy employed in the context of nasal drug delivery of peptides to enhance their therapeutic

effectiveness. Peptides and proteins often face challenges in nasal drug delivery due to their substantial size and susceptibility to degradation in bodily fluids under normal physiological conditions. To address these limitations, one approach involves altering the microenvironment, and this can be achieved through various means, including the introduction of protease inhibitors and the use of penetration enhancers. Penetration enhancers, such as SNAC, play a crucial role in modifying the microenvironment for nasal drug delivery. These enhancers can adjust the local pH within the nasal cavity or actively enhance the transcellular absorption of peptides and proteins by altering the microenvironment. This alteration facilitates the passage of these large molecules through biological barriers, increasing their chances of effective absorption and therapeutic action [34, 45].

PEGylation technique

Polyethylene glycol (PEG) possesses unique osmotic characteristics and excellent solubility in both aqueous and organic solvents. Consequently, it finds extensive applications across various industrial processes and serves as an inert polymer and laxative in early studies related to intestinal transit. PEG has the ability to bind numerous water molecules and is generally considered safe for human use. The PEGylation technique enhances the half-life of many proteins in circulation and reduces the likelihood of antibodies being generated against them [45, 48].

Chemical modification

Chemical modification of drug delivery systems for protein and peptide drugs is a critical strategy aimed at enhancing enzymatic stability, improving membrane permeation, and reducing immunogenicity. This involves alterations to amino acids and the introduction of hydrophobic properties.

Amino acid modification

Amino acid modification plays a crucial role in the realm of nasal drug delivery for peptides, involving alterations in the amino acid composition of protein and peptide drug molecules. One significant modification strategy is the substitution of D-amino acids for their naturally occurring l-amino acid counterparts. This modification holds immense promise due to its potential to impact various physiological properties of peptide drug delivery systems. A notable example in this context is the case of vasopressin analogs, exemplified by desmopressin and deaminovasopressin. Desmopressin, an artificial version of vasopressin, undergoes amino acid modification with the deamination of its first amino acid and the replacement of l-arginine with its D-enantiomer, D-arginine. This transformation enhances the stability of the drug within the nasal cavity, making it more resistant to enzymatic degradation, thus facilitating better absorption and therapeutic effectiveness. Deaminovasopressin, another vasopressin derivative, illustrates how such modifications can alter receptor binding and improve drug stability [47].

Hydrophobization

The introduction of hydrophobic moieties is indeed a critical approach in drug delivery, and a prime example of this is the palmitoylation of Nobex INSULIN. This process involves chemically conjugating insulin with a hydrophobic compound called 1,3-dipalmitoylglycerol, which contains free amino acid groups like glycine, phenylalanine, and lysine. This modification is designed to enhance the stability of insulin against enzymatic degradation, particularly when administered through non-invasive routes like nasal delivery. Palmitoylation effectively shields insulin from enzymatic attacks by encapsulating it within a hydrophobic layer, making it less susceptible to rapid breakdown in the body's aqueous environments. This hydrophobic modification not only improves the stability of insulin but also extends its pharmacokinetics, enabling a more sustained and controlled release of the drug [47].

Different drug delivery systems for nasal route

Various drug delivery systems tailored for nasal administration have been developed to address specific challenges and enhance the efficacy of therapeutic agents. Different drug delivery systems are discussed in this section and a summary of the marketed peptide formulations administered through nasal route is tabulated in table 2.

Nasal solutions

Nasal solutions are predominantly aqueous-based formulations with the advantage of providing moisture to counteract dryness in mucous membranes, a common issue in allergic and chronic diseases. There are several examples in which solutions are delivered intranasally, like insulin (Nasulin®), caclitonin (Miacilin®), H102 peptide [49]. However, they come with challenges like microbiological stability, potential irritation, and the risk of inducing allergic rhinitis. The use of preservatives in these solutions may affect mucociliary function. liquid formulations also face issues related to the limited chemical stability of dissolved drugs and their short residence time in the nasal cavity [39, 40, 50].

Nasal drops

Nasal drops are a simple and user-friendly way to administer medication through the nose, but they come with a drawback of imprecise dosing. Research has indicated that when it comes to substances like human serum albumin, nasal drops are more efficient at delivering them to the nostrils, making it easier for absorption, especially when compared to nasal sprays. This suggests that while nasal drops are straightforward, they may offer certain advantages in terms of efficient drug delivery for specific compounds [39, 41, 50]. Desmopressin (DDAVP) intranasal drops were prepared by Gerbutaviciene *et al.* using phosphate buffer (pH 4.5-5.5). DDAVP is a vasopressin analogue used in the treatment of Diabetes Insipidus [51].

Nasal gels

Nasal gels are thick solutions or suspensions that stay in the nasal cavity for a longer period. They offer several advantages, including reducing the risk of post-nasal drip, minimizing any unpleasant taste, preventing leakage from the front of the nasal passage, and enabling precise drug delivery to the nasal mucosa. This extended residence time and targeted application enhance the absorption of medications through the nasal route, making nasal gels a practical and effective option for certain treatments [39, 40]. Peptides like insulin, calcitonin, desmopressin, cyanocobalamine can be administered in the form of nasal gels [49]. *In situ* gel of peptide drug PAOPA, dopamine D2 receptor modulator is an example where formulation of gel increases the nasal retention time and allows controlled release of the peptide drug [52].

Dry Powder Inhalers (DPIs)

DPIs are the formulations with dried powder to be inhaled by the patient with forced inhalation. They offer benefits like the absence of preservatives and enhanced drug stability. DPIs are devices designed to deliver a dry powder formulation through inhalation. They are commonly used for respiratory conditions and have applications in diabetes treatment. DPIs typically house medication in capsules or proprietary forms and are inhaled deeply, delivering small doses [39, 40].

Research has explored dry-powder formulations with mucoadhesive polymers for nasal peptide and protein delivery. These powders absorb moisture upon contact, forming a gel for extended residence time. Examples include insulin with cellulose derivatives and Carbopol®, somatostatin analogs with cross-linked dextran and microcrystalline cellulose, glucagon with microcrystalline cellulose, and leuprolide and calcitonin with microcrystalline cellulose and hydroxypropyl cellulose [46, 53].

Nasal sprays

Nasal sprays are versatile, as they can be made with both liquid solutions and suspended particles, enabling accurate dosing. They are often favoured over powder sprays to prevent potential irritation to the sensitive nasal lining (mucosa). This adaptability and reduced risk of irritation make nasal sprays a preferred choice for many when it comes to delivering medications through the nose. Several marketed formulations for peptides are available as nasal sprays like Desmopressin acetate, Salmon calcitonin, Buserlin acetate, Nafarelin acetate, oxytocin, cyanocobalamine [34, 35, 50]. In a randomized controlled trial carried out by Matsumoto *et al.* nasal spray formula of parathyroid peptide [hPTH(1-34)] was administered to patients with osteoporosis. The study demonstrated that the spray was safe and rapidly increased the lumbar bone mineral density [54].

Metered dose pumps

Most pharmaceutical nasal products, including solutions, emulsions, and suspensions, use metered-dose pump sprays. These sprays release a fine mist into the nostrils via a hand-operated pump mechanism for local or systemic relief of nasal congestion. The precision of dosing depends on the formulation's properties [39, 40].

Table 2: Marketed peptide formulations administered through nasal route

Drugs	Formulation	Commercial name	Company	Indication/Application	FDA approval year	References
Desmopres -sin acetate	Solution, spray,	Concentraid®	Ferring pharmace-uticals	To assess the degree of renal impairment in pediatric patients with urinary tract abnormalities.	1990	[55]
	Solution, spray, metered	Stimate®	Ferring pharmace-uticals	Hemophilia A, von Willebrand's Disease (Type I)	1994	[55]
	Solution, Spray	Minirin®	Ferring pharmace-uticals	Primary nocturnal enuresis, central cranial diabetes insipidus	2002	[55, 56]
	Oil-in-water emulsion, spray, metered	Noctiva®	Serenity pharmaceuticals	Nocturia	2017	[55, 57]
Salmon calcitonin	Solution, Spray	Miacalcin®	Novartis	Postmenopausal osteoporosis	1995	[55, 58]
Buserelin acetate*	Solution, Spray	Suprefact®	Sanofi-Aventis	Prostate cancer	1998	[55, 58, 59]
Nafarelin acetate	Solution, Spray	Synarel®	Pfizer	Endometriosis, central precocious puberty (CPP)	1990	[55, 56]
Oxytocin	Solution, Spray	Syntocinon®	Novartis	Induce or augment labor	1995	[56, 59]
Cyanocoba -amine	Gel	Nascobal®	Par Pharm Co.	Treat low blood levels of vitamin B12	1996	[55]
Cyanocobal-amine	Solution, Spray	Nascobal®	Par Pharm Co.		2005	[55, 60]

Approaches

Liposomes

Researchers have been looking at using liposomes to improve how medications are delivered through the nose. liposomes can help peptides like insulin and calcitonin get absorbed better, at least in laboratory tests. This happens because the liposomes help the peptides stay in the nose for a longer time. Especially, liposomes with a positive charge (cationic liposomes) have shown a good effect in carrying calcitonin. They stick to the surface inside the nose and help the drug get into the body. This has also worked well with desmopressin-loaded cationic liposomes, which led to stronger antidiuretic effects in tests with rats. In simple terms, liposomes seem to be a promising way to make nasal drug delivery more effective, especially for certain medications [32]. Moreover, liposomal encapsulation of H102, a novel β-sheet breaker peptide utilized in Alzheimer's disease treatment, serves the purpose of diminishing peptide degradation while augmenting its ability to traverse the BBB. upon intranasal administration [61-63].

Microspheres

The widespread application of microsphere technology in crafting nasal drug delivery systems is noteworthy. These microspheres frequently employ mucoadhesive polymers such as chitosan (ex-insulin chitosan microspheres) [64] and alginate, offering substantial advantages. They not only shield drugs against enzymatic degradation but also ensure a controlled release, thereby extending the drug's duration of action within the nasal cavity. Incorporation of insulin into degradable starch microspheres (DSM) is an example wherein the DSMs, adhere to the mucous membrane when administered in the dry from, swell and draws water from the mucus and epithelial cells and cause widening of the tight junctions and aids in the transport of insulin [65]. Furthermore, when an enhancer like lysophosphatidylcholine is introduced in combination with insulin and starch microspheres, the AUC experiences an astonishing boost of 1657%. [29, 32, 61, 62].

Nanoparticles

Nanoparticles, which are solid colloidal particles typically measuring between 1 and 1000 nm, are created from large molecular materials and serve practical purposes in fields like enhancing vaccine effectiveness or transporting drugs within the body [61, 62]. These nanoparticles offer advantages due to their diminutive size. However, it's imperative to recognize that only the smallest nanoparticles can traverse mucosal membranes via the paracellular route, albeit in limited quantities due to the narrowness of mucosal tight junctions, which typically measure between 3.9 to 8.4 Å. Researchers have been working to formulate nanoparticles of various peptides like insulin, calcitonin, Human Growth Hormone, leuprolide to be administered by the intranasal route [39, 49, 66]. An intranasal *in situ* gel of peptide drug l-prolyl-l-leucyl-glycinamide (PLG), (3(R)-[(2(S)-pyrrolidinylcarbonyl)amino]-2-oxo-1-pyrrolidineacetamide (PAOPA), dopamine D2 receptor modulator has been formulated as nanoparticles of oxidized starch and carboxymethyl chitosan that allows retention of the peptide drug at the nasal and controlled release of PAOPA [52].

Microparticles

Microparticles have emerged as a valuable strategy for nasal drug delivery, particularly for peptides, since their introduction in 1987. They serve to increase drug residence time within the nasal cavity, enhancing therapeutic efficacy and patient convenience. A prominent example of their success is the formulation of leuprolide in microparticles, as seen in the case of lupron Depot [67]. This approach has significantly reduced the need for frequent subcutaneous injections of the peptide hormone leuprolide, leading to improved patient compliance and minimized side effects. lupron Depot's long-term market success highlights the effectiveness of microparticle-based nasal drug delivery for peptides. Another noteworthy example is Afrezza, [45], which utilizes microparticles formulated with fumaryl diketopiperazine to deliver insulin via inhalation. This innovative approach offers a non-invasive alternative to traditional insulin injections, improving patient comfort and adherence. Dry powder formulation of Calcitonin gene-related peptide (CGRP) receptor antagonist peptide is also an example [68]. These examples underscore the potential of microparticles in revolutionizing peptide drug delivery, offering extended release, reduced invasiveness, and improved therapeutic outcomes, making them a valuable option in the field of pharmaceuticals and medical treatments [45].

Micellar formulation

Micelles prove effective in delivering peptide-based drugs and proteins through intra-nasal route. Bile salts, commonly utilized as enhancers, are employed in the form of micellar solutions. The use of micelles composed of sodium glycocholate, both individually and in combination with linoleic acid, to increase absorption for a model dipeptide ((D-Arg2)-kyotorphin) and insulin in rats is an example of a micelle-based approach [69]. Phospholipid micelles formulated using PEGylated lipids like DSPE-PEG2000 (distearoylphosphatidylethanolamine chemically conjugated to PEG2000) can be used to deliver peptides like Vasoactive Intestinal Peptide (VIP), Neuropeptide Y (NPY), Glucagon-like Peptide-1 (GLP-1) through the intranasal route. Micelles formed using this method are also called as sterically stabilized micelles [70, 71].

Prodrug approaches

One promising approach to boost the effectiveness of nasal peptide delivery is by using prodrugs. These prodrugs are designed to

protect peptide medications from being broken down by enzymes in the nasal cavity and, at the same time, enhance their ability to dissolve in fluids. By doing so, prodrugs can help improve the bioavailability of peptides administered through the nasal route, making them more effective and reliable in their therapeutic action [72]. Example includes, Avizafone, peptide prodrug of diazepam was delivered with Aspergillus oryzae (A. O.) protease, an enzyme that converts avizafone to diazepam in the nasal spray [73].

***In situ* gels**

In situ gel-forming polymeric formulations start off as liquid solutions before they are introduced into the body, but they transform into a gel-like state once administered. This transformation into a gel is triggered by factors like temperature changes, pH shifts, or the presence of specific ions. This gel then serves as a delivery system for drugs, allowing for their gradual and controlled release over time, offering a more sustained and predictable drug delivery method. Tengamnuay *et al.* had prepared an *in situ* nasal perfusion that was administered to the rats using chitosan as an absorption enhancer and was proved to be safe [46, 49, 69]. *In situ* gel of peptide drug PAOPA, dopamine D2 receptor modulator is an example where formulation of gel increases the nasal retention time and allows controlled release of the peptide drug [52].

Devices for intranasal drug delivery

Peptide hormones (PHs) have traditionally been administered through parenteral routes such as intramuscular (IM), intravenous (IV), and subcutaneous injection (SC). However, an alternative means of delivering peptide hormones has emerged through intranasal (IN) administration. Researchers have investigated the use of nanoparticulate (NP) systems in conjunction with nasal delivery to enhance drug effectiveness [74].

In recent times, many devices have been developed to facilitate drug administration to the nasal cavity, which are mentioned in table 3. Optinose A/S, for instance, manufactures and tests a device that allows drug administration to an expanded portion of the nasal cavity while preventing pulmonary deposition [75]. Application of Optinose includes ow-dose intranasal oxytocin [76-78]. Kurve Technology, Inc. has also created a device ViaNase™ capable of delivering drugs into the paranasal sinuses by Controlled Particle Dispersion™ [79]. These devices, despite potentially having higher manufacturing costs compared to metered spray pumps [80]. Pharma labs Global®has also marketed many nasal formulations of peptides like oxytocin, hexarelin, AICA 95-Aminoimidazole-4-carboxamide) ribonucleotide.

A family of molecules known as Intravail™ (Aegis Therapeutics) absorption enhancement agents has been patented. Tetradecylmaltoside is the most extensively studied molecule in this class, as it facilitates intranasal delivery and transmucosal delivery of various therapeutic compounds, including peptides, proteins, and non-protein macromolecules with molecular weights exceeding 20,000 Da. Achieving bioavailabilities greater than 50% when compared to subcutaneous injection, these chemically synthesized molecules undergo metabolism to CO2 and H2O, enabling controlled transient permeation of the nasal mucosal barrier [80].

For peptides and proteins with molecular weights up to 20 kDa, intranasal bioavailabilities exceeding 50% when compared to subcutaneous injection can be achieved. Smaller peptides like calcitonin can exhibit bioavailabilities exceeding 95% [80].

Table 3: Patents on intranasal drug delivery devices

Delivery devices	Patent number	Patent holder	References
Pfeiffer/Aptar single-dose device	US9211253B2. (2015)	Roger Crystal, Michael Brenner Weiss.	[81, 82]
Nasal breathing device	US5727543A (1998)	Luigi Corsaro.	[83]
Nasal dilator	US20170027736A1 (2019)	Martin O'Connell, Keith Yeager.	[84, 85]
Nasal inserts	US8517026B2 (2013)	Adva Beck Arnon.	[86]
Ergonomic nasal cannula	US10322252B2 (2019)	Darin BA	[87]

Systemic delivery

The intranasal route for systemic drug delivery seems to be a viable approach when compared to oral and intravascular routes, as research suggests. Research has highlighted the potential of intranasal (IN) administration as an alternative method for delivering peptide hormones in the treatment of severe hypoglycemia. This approach offers the advantage of rapid action without the hindrance of poor hepatic first-pass metabolism [74, 88, 89].

Currently, an ongoing clinical investigation is underway for a new IN glucagon product called AMG504-1, developed by locemia Solutions in Montreal, Canada. This product consists of a dry powder glucagon housed in a single-use nasal powder dosing device, enabling straightforward administration in a single step [90, 91].

Furthermore, IN vaccination offers the advantage of not requiring a sterile dosing technique or product [92]. The nasal mucosa serves as an excellent site for vaccine administration, rich in organized lymphatic tissues and specialized cells [93]. In humans, immune responses originating from the nasal mucosal surface are primarily mediated through interactions with nasopharyngeal-associated lymphoid tissues (NALT), also known as Waldeyer's ring, which are responsible for the initial defense against airborne microorganisms. Most invading pathogens enter the body through mucosal surfaces, with mucosa acting as the body's first line of defense against infection [94].

In the realm of nasal drug delivery, Dr. Djupesl and of OptiNose AS in Norway has introduced an innovative approach using a device called OptiMist. This device employs two nozzles inserted into the nostrils, offering a significant advantage. Notably, it greatly reduces the deposition of medication in the lungs, with less than 1% of the total dose being collected in the lungs during bidirectional delivery. Moreover, OptiMist maximizes the distribution of aerosolized droplets within the nasal cavity [95, 96]. This pioneering concept is known as Breath Powered Bi Directional™ and has been developed to address specific treatment needs, allowing for discrete or concurrent delivery depending on the desired therapeutic approach [97, 98].

Impel Neuro Pharma's Pressurized Olfactory Delivery (POD) device serves as a notable example currently undergoing clinical evaluation. This device has demonstrated its effectiveness in delivering aerosolized drugs to the nasal cavity [24, 99, 100].

CONCLUSION

The nasal route offers several advantages for the delivery of peptides as a non-invasive and effective method. The incorporation of nanotechnology, such as nanoparticles and liposomes, as well as the use of mucoadhesive polymers and permeation enhancers, has demonstrated significant potential in improving the stability, bioavailability, and therapeutic impact of nasal peptide delivery. Recent advancements in formulation technologies, nanocarrier utilization, and integration of intelligent materials and nasal drug delivery devices have further enhanced the efficiency and efficacy of nasal peptide delivery. Despite certain limitations and challenges associated with nasal drug delivery, ongoing research and breakthroughs continue to expand the understanding and utilization of this route for peptide-based therapies. Overall, the development of innovative strategies and formulations, coupled with a deeper insight into nasal physiology and drug absorption pathways, opens up new possibilities for nasal route delivery of peptides in various disease conditions.

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AUTHORS OF CONTRIBUTIONS

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CONFLICT OF INTERESTS

Declared none

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