Review

Nose-to-Brain delivery of insulin for Alzheimer’s disease

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Abstract

The transport of small molecules, peptides and proteins via the olfactory epithelium and along olfactory and trigeminal nerve pathways from the nasal cavity to the brain is very well known and clinically established for central nervous system (CNS) active drugs like oxytocin, sumatriptan or insulin. Insulin is a clinically well-established biopharmaceutical with a validated function in cognition. Central supply with insulin via intranasal administration improves cognition in animal models and in human, making insulin a so-called cognitive enhancer. Furthermore, dysregulation of insulin is implicated in the pathogenesis of Alzheimer’s disease, which is associated with lower levels of insulin in the cerebrospinal fluid and is involved in amyloid-beta (Aβ) regulation. Clinical trials with intranasal insulin implicate positive effects on learning and memory, but a massive lack of pharmacokinetic and efficacy data hamper a pharmacokinetic – pharmacodynamic relation and a possible clinical development as cognition enhancer. A lack of such data also prevents resolving the mechanisms involved in directing insulin to the central or to the peripheral compartment. Here we discuss the basic mechanism of Nose-to-Brain delivery, evidences for intranasal insulin as cognition enhancer, medical devices for intranasal delivery and safety aspects.

Keywords

intranasal; insulin; blood-brain-barrier; medical device; efficacy; cognition; metabolism.

CNS delivery – an unmet medical need

The World Health Organization (WHO) estimates that more than a billion people worldwide are suffering from diseases of the central nervous system (CNS; [1,2]). Alzheimer’s disease (AD) is the most common neurodegenerative dementia in the industrialized world, with prevalence rates well over 30 % in the over 80-years-old population [1,2]. AD causes enormous costs to the social healthcare systems, as well as personal tragedies for the patients, families and caregivers. Like most neurodegenerative diseases, AD has a poor prognosis and only symptomatic therapy is currently available. Efficient treatment strategies are still limited and an aging society in demographic change presents an enormous challenge to the health systems of industrialized nations. Despite the extensive research and effort to uncover the mechanism of AD pathogenesis, more or less all drug candidates failed to demonstrate significant effects on cognition in clinical trials [3,4].

A highly critical point in that context is the low central availability of drugs. The passage of most CNS-active drugs and in particular of biopharmaceuticals is massively hampered by the blood–brain barrier...
The BBB is located at the level of the cerebral microvasculature and is important for maintaining CNS homeostasis. Though, the BBB restricts the access of potentially neurotoxic substances into the brain, it impedes massively the delivery of therapeutic drugs to the CNS. Tight junctions between the endothelial cells in the CNS block the natural transport and hence seal the blood compartment from the brain compartment [5]. A limited number of essential nutrients like glucose and amino acids, co-factors like iron, and peptide hormones like insulin are actively transported across the BBB [6-9]. Molecules that do not possess a specific transport mechanism have only a chance to pass the BBB via passive diffusion. While there are at least some good examples of chemical modifications for small molecule drugs to enhance their central bioavailability, nearly all of the larger molecules such as peptides and proteins fail to cross the BBB [10]. Currently, all biopharmaceuticals that are used or being evaluated in the clinics for CNS disease act predominantly via peripheral mechanism: e.g. anti-amyloid-β (Aβ) capturing monoclonal/polyclonal antibodies in AD [3,4] or immune cell regulating biopharmaceuticals in multiple sclerosis [11]. However, candidate biopharmaceuticals reveal promising results in cellular models or in animal models when delivered intracerebroventricular. The current state-of-the-art to deliver drugs with a low central bioavailability is intrathecal, intracerebroventricular or intraparenchymal injections that deliver directly to the cerebrospinal fluid (CSF) of the CNS, some of them are given chronically via an implanted intrathecal micropump (e.g. SynchroMed®; [12,13]). Although, such delivery systems are commonly used for baclofen for the treatment of spasticity or analgetics for the treatment of cancer pain [14], these routes of administration are invasive and provide a long list of adverse events and contraindications [15,16]. Hence, a safe and efficient drug delivery platform technology for CNS active molecules is needed [17].

**Nose to Brain (N2B) intranasal delivery**

Intranasal nose to brain (N2B) delivery to the upper third of the nasal cavity bypasses the blood-brain barrier to rapidly target therapeutics to the CNS along the olfactory and trigeminal neural pathways (for excellent summary see [18]). The N2B route of administration provides a non- to minimal-invasive method of bypassing the BBB.

**Anatomy and histology of the nasal cavity**

The nasal cavity is divided longitudinally by the nasal septum and extends from the nostrils to the nasopharynx (roughly 12–14 cm), but has an impressively large mucosa surface area (about 160 cm²) [18,19]. The frontal and lateral views are shown in Figure 1. Three turbinates (also called conchae) are the cause for the large surface area and their biological function is to humidify, warm and filter the inspired air. Nasal secretions and inhaled particles are transported to the nasopharynx via mucociliar clearance, where they are swallowed or expectorated. Importantly, the nasal mucosa provides very important immune function since countless inhaled pathogens are filtered here and transported to the nasopharynx-associated lymphoid tissue [20]. Therefore, intranasal vaccinations like FluMist® replace more and more injection-associated vaccinations.

The nasal mucosa consists of four different epithelia: respiratory, olfactory, squamous and transitional epithelium [21]. The squamous epithelium covers the nasal vestibule from the nostrils to the anterior part of the turbinates and harbours hairs and glands. The transitional epithelium is located at the transitions between the other three types of epithelium and appears not to have a relevant role in intranasal delivery.

The nasal respiratory epithelium is a pseudostratified columnar secretory epithelium that is formed by ciliated cells, goblet cells, intermediate cells and basal cells. The tissue shares high similarity to the
respiratory epithelium of the lung and covers up to 90% of the nasal cavity in humans and roughly 50% in rodents [18]. Through close contact with inspired air, it warms and humidifies the air and air-borne particles and pathogens are removed. Different serous glands produce the nasal mucus and nasal secretion that are propelled from the ciliated cells to the nasopharynx. The mucus is well characterized and creates a mild acidic and antibacterial milieu with a pH of 5.5 - 6.5 [19]. The nasal respiratory epithelium is innervated by the trigeminal nerve, the fifth of the cranial nerves. Numerous intraepithelial trigeminal fibres are located near the basal region of the epithelium; some of them extending outward to nearly reach the epithelial surface stopping at the line of tight junctions about 1 µm from the surface [22]. The largely branched trigeminal nerve projects mainly but not exclusively to the brainstem and is highly implicated into N2B transport [22,23]. The respiratory epithelium has a large surface and is highly perfused; hence well suitable for the systemic absorption of drugs [24].

Figure 1. The anatomy of the human nasal cavity. The olfactory region permits the transport of APIs to the CSF and the olfactory bulb. (A) frontal view with inferior, middle and superior turbinate. (B) lateral view with cribriform plate, olfactory bulb and CSF in close vicinity to the olfactory region. The nasopharynx-associated lymphatic tissue (NALT) has immunological functions and is located in lower part of the nasal cavity close to the nasopharynx.

The olfactory cleft at the roof of the nasal cavity up to the superior parts of the turbinates is covered with olfactory epithelium. In humans the olfactory region comprises up to 10% of the surface area of the nasal epithelium while accounting for about 30% of the surface area in rodents [18]. The olfactory epithelium is formed by columnar epithelial cells, olfactory neurons, supporting cells, basal cells and Bowman’s glands [19]. Olfactory neurons are the only neurons having their cell bodies located in a distal epithelium and their non-motile cilia processes extend into the mucus hence being in direct contact to the environment (Figure 2). Turbulences at the olfactory cleft increase the residence time of the inspired air to increase the interaction of the olfactory receptors with odorants. Hence, small number of odorant substances can be detected [19]. The unmyelinated axons of olfactory neurons spread through the basal lamina and form the fila olfactoria nerve bundles enclosed by olfactory ensheathing cells and olfactory nerve fibroblasts [25]. The ensheathed nerve bundles travel through the cribiform plate of the ethmoid bone into the CNS and terminate at the olfactory bulb that project directly to the piriform cortex, amygdala, entorhinal cortex and olfactory nuclei [26]. Compared to the respiratory epithelium the olfactory region is less perfused, but still well vasculated. The serous mucus produced by the Bowman’s
glands is not well characterized in the literature. The lamina propria underneath the olfactory epithelium harbours blood and lymphatic vessels in addition to immune cells connected to the deep cervical lymph.

![Figure 2. Postulated N2B transport mechanism: dispersed drug particles (coloured in green) are inspired with the aid of a nasal pump spray or an aerosol generator. Drug molecules depositing at the olfactory region diffuse through extracellular pathways (green arrows) to the CSF (see left side) or being transported via intracellular pathways to the olfactory bulb (see right side) and from here to other parts of the CNS.](image)

**Absorption and transport mechanism of the N2B route**

Although, numerous studies have demonstrated central effects of N2B delivered drugs in rodents, most of these studies did not show pharmacokinetic (PK) data or evidence for brain uptake. Hence, the PK and transport mechanism is still far from being elucidated. It appears that intranasal delivery is a combination of different pathways [18]. It was found that crossing of the epithelial barrier can include intracellular or extracellular pathways. Intracellular pathways across the olfactory epithelium include endocytosis into olfactory neurons shown for several viruses and some proteins like peroxidase or albumin [27-32] or transcytosis across supporting cells to the lamina propria. In addition, the intracellular uptake by endocytosis and transcytosis across the respiratory epithelium into trigeminal nerve processes or basal lamina, respectively, has been observed [33,34]. Paracellular diffusion through epithelial tight junctions to the underlying basal lamina is the dominant extracellular transport pathways across either the olfactory or respiratory epithelia (Figure 2) [18]. The permeability of the nasal epithelium is comparable to the intestinal epithelium [35]. The use of absorption enhancers like the natural polymer chitosan or lipophilic
additives can increase the paracellular passage [36].

After uptake to or through the epithelium, different transport pathways have been implicated. N2B delivery of radioactive labelled IGF-1 and interferon-β in rodents and monkeys seemed to occur along trigeminal and olfactory nerves and to reach first the brainstem and olfactory bulb before the proteins are distributed over the CNS [37,38]. Molecules being taken-up via intracellular pathways continue their passage via anterograde axonal transport. Peroxidase is likely to be purely transported with axonal transport and kinetic studies match very well with mathematical predictions taking into account the transport rate [18]. Drugs that have been taken-up by transcellular diffusion and convection can be adsorbed by the lymphatic or vascular system (and thereby having a low probability of entering the brain) or diffuse to perineural or perivascular spaces and thereby enter the cranial compartment. The perineural spaces of the olfactory and trigeminal nerves seem to allow transport to the CSF of the subarachnoid space [39]. However, some studies suggest that N2B transported substances may be present in the brain without being detectable in the CSF [37]. Mathematical predictions, however, strongly suggest that convection/bulk flow along olfactory and trigeminal nerves is the most plausible mechanism [18].

Is N2B insulin appropriate as therapy for neurodegenerative diseases? Evidences from in vivo and clinical studies

Insulin has a molecular weight of 5.8 kDa and is one of the oldest recombinant biopharmaceuticals. Insulin has well-known peripheral metabolic effects and lowers the serum glucose concentration, but it also plays an important central role in cognition, learning and memory [40-43]. Furthermore, dysregulation of insulin is involved in the pathogenesis of AD [44]. AD is associated with lower levels of insulin in the CSF and is involved in amyloid-beta (Aβ) regulation. In cellular and animal AD models, insulin reduces Aβ-oligomer formation and protects against Aβ-toxicity [45]. In vitro studies demonstrated that insulin stimulates the formation of the insulin degrading enzyme (IDE), which is capable of degrading Aβ [46]. Moreover, the activity of glycogen-synthase kinase-3-beta, a tau kinase associated with the formation of neurofibrillary tangles, has been reported to be down-regulated in response to insulin [47]. Post mortem analyses of human AD brains have documented progressive disruption in central insulin regulation.

Radioactive-labelled insulin was distributed widely throughout the mouse brain 1 h following intranasal administration, with the highest levels detected in the trigeminal nerve and the olfactory bulb [43]. Though insulin undergoes transcytosis at the BBB, intranasal administration reached significantly higher CNS levels when comparing with subcutaneous administration at the same time. Intranasal insulin also slowed development of cognitive decline in different disease models [43] and improved learning and memory in wild type mice [48].

Very interestingly, incretins – a group of metabolic hormones – favour insulin release in the periphery and likewise they have a comparable central activity as insulin. Exendin – an agonist at the GLP-1 (glucagon-like peptide-1) receptor – improved learning [49] when delivered via N2B. Higher brain levels of exendin were found after intranasal administration when compared to intravenous injection [50].

Clinical trials with intranasal insulin have provided positive effects in small cohorts of AD patients and mild cognitive impairment in verbal, visuospatial and episodic memory [40,45,51]. Unfortunately, only one study in humans gives detailed PK data (plasma and CSF) after N2B delivery of peptides including insulin [52]. Interestingly, the plasma levels of insulin have not been altered by intranasal administration of 10 IU insulin while a clear peak in CSF levels was detectable after 30 and 80 minutes. This study has been
conducted with a conventional nasal spray atomizer filled with insulin formulated for subcutaneous delivery. The same group observed that insulin delivered intranasally improved memory and mood [40,53]. In addition, N2B delivered insulin decreased food intake and decreased postprandial serum insulin [54,55]. In patients with amnesic mild cognitive impairment the treatment modulated plasma levels of Aβ and improved memory, concluding intranasal delivery of insulin as a possible treatment for AD [56]. In AD patients a chronic treatment over 21 days with intranasal insulin (20 or 40 IU Novolin R) improved memory, attention and functioning [51]. In this study, patients were placed in a supine position with the head tilted back and insulin or placebo saline was administered with a needle-less syringe into alternating nostrils with a total administration volume of 400 µL. A validation of this administration technique like the group of Mori et al. did [57] was not presented. Moreover, no determination or estimation of the volume that reached the olfactory cleft was published. In addition, the volume that was swallowed or aspirated during the procedure was not determined. Hence, a calculation of the central bioavailability e.g. insulin levels in CSF samples is due to the lack of data not possible. The serum levels of insulin and glucose at baseline and 45 minutes after administration are not altered, though, the insulin levels in all three arms were rather high for fasting non-diabetic subjects. However, a PK study focusing on the administration of intranasal insulin preparations for the treatment of diabetes mellitus showed that the serum insulin levels peaked rapidly after 15 minutes with 25 IU insulin and returned to the baseline after 45 minutes [58]. This data were meanwhile confirmed without adsorption enhancer and 160 IU intranasal insulin with diabetic and control subjects [59]. In addition, the authors specified in the procedure that the patients were fasting at administration, but did not specify this for blood collection [60]. Hence, the published samples collected in the latter study are insufficient for PK-PD estimation.

A four-month pilot study from the same group around Craft et al. was designed with three arms delivering a daily dose of 20 IU or 40 IU intranasal insulin (Novolin R) or placebo twice a day with the nasal drug delivery device ViaNase™ [61]. The 20 IU insulin group benefited from the treatment with improved delayed memory and both groups profited from improved daily function and cerebral metabolism determined by FDG-PET. No changes in insulin CSF levels were observed. Again this study had several limitations that were discussed by the authors. The CSF and FDG-PET data were collected for only a subset of participants. Insulin levels in CSF were not collected directly after insulin administration, though it is known that they drop to baseline within 1 hour after administration [52]. Compared to placebo, more patients suffered from nose bleeds in the verum group.

A recently published study used the long-acting insulin (Detemir, Levemir®) delivered intranasally via the ViaNase™ device twice daily over 21 days in AD patients [62]. Detemir binds to albumin resulting in a prolonged release and greater parenchymal penetration. However, peripheral administered Detemir is not transported across the BBB to the brain [63]. Here, the 40 IU group had the largest benefit for the memory composite compared with placebo. The effect was significantly modulated by APOE4 carriage - a genetic risk factor for AD, but also metabolic diseases - and baseline insulin resistance, both being associated with higher baseline insulin AUC (area under the curve). APOE4 negative patients showed an increased insulin resistance after 21 days treatment with 40 IU insulin Detemir. No effects were reported for daily or executive functioning.

Two recent proof-of-concept studies evaluated the acute effects on cerebral vasoreactivity and cognition of a single 40 IU dose of intranasal insulin (Novolin®) via a ViaNase™ device compared with placebo in type 2 Diabetes (T2DM; [64,65]). Across all subjects, intranasal insulin administered improved visuospatial memory and increased resting-state functional connectivity in older adults with T2DM.
Furthermore, intranasal insulin administration was well tolerated. Systemic glucose levels were not significantly altered, though a tendency towards decreased levels was evident in diabetic individuals. Heni et al. reported improved peripheral insulin sensitivity via hypothalamus and parasympathetic outputs after 160 IU insulin dosed with a nasal pump spray without absorption enhancer [66]. The investigators used a hyperinsulinemic-euglycemic glucose clamp with an intravenous bolus injection of 6.25 mU/kg insulin 90 minutes prior to intranasal administration and a continuous intravenous infusion of 0.25 mU/kg/min over 210 minutes. Even under this conditions plasma insulin peaked after 15 minutes compared to placebo. The glucose consumption needed to keep the serum glucose euglycaemic was significantly higher in the intranasal insulin group. The authors discuss the use of N2B insulin for central insulin resistance in obesity; AD is also associated with impaired central insulin resistance.

One study with 20 IU could not resolve any significant effect either on cerebral glutamate concentration and on memory [67]. Several other unpublished studies are summarized in Table 1. According to the published studies, intranasal insulin administration generally neither causes nasal irritation nor destroys the olfactory function and projections [48,68]. Though, no statistically significant effects on lipid metabolism were observed in the 3-week Detemir-study, the supplementary data of the study implicates a dose-dependent tendency associating insulin treatment with higher total cholesterol, LDL and lower HDL levels. No critical discussion was found in any study, asking about the long-term mutagenic effect of the growth factor insulin that is highly associated with increased risk of cancer.

**Table 1.** A selection of unpublished completed clinical trials using intranasal insulin for either central or peripheral metabolic activity (source: [www.clinicaltrials.gov](http://www.clinicaltrials.gov)).

<table>
<thead>
<tr>
<th>study/sponsor</th>
<th>dose</th>
<th>status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Safety and Effectiveness Study of Intranasal Insulin Glulisine on Cognitive and Memory in Mild-Mod AD Patients. (NCT01436045) / HealthPartners Institute for Education and Research</td>
<td>20 IU</td>
<td>completed (2013)</td>
</tr>
<tr>
<td>Memory and Insulin in Early Alzheimer's Disease (NCT00581867) / University of Kansas</td>
<td>40 IU</td>
<td>completed (2013)</td>
</tr>
<tr>
<td>Safety Study of Intranasal Insulin in Type 1 Diabetes and Diabetic Peripheral Neuropathy (NCT01469559) / University of Calgary</td>
<td>20 IU</td>
<td>completed (2012)</td>
</tr>
<tr>
<td>A Study to Evaluate the Effect of Nasal Insulin on Postprandial Glycemic Control in Type 2 Diabetic Patients (NCT00624767) / Nastech Pharmaceutical Company, Inc.</td>
<td>30 IU</td>
<td>completed (2008)</td>
</tr>
</tbody>
</table>

**Medical devices and dosage forms for intranasal delivery**

One of the first studies by Born et al. delivered insulin by using a conventional manual nasal pump spray atomizer [52] that generates droplets of roughly 50 to 100 μm dependent from many different factors like pressure and distance to the nozzle [69,70]. Hence, the handling of device by different patients or caregivers may vary easily and result in deposition at different sites of the nasal mucosa. Moreover, aerosols delivered by nasal pump sprays cleared rather quickly: about 50 % was cleared after 15 minutes, and after 6 hours, less than 5 % of activity was retained in the nose [69].

The above-mentioned clinical trials at Washington University administered insulin via aerosols
generated with the electronic atomizer ViaNase™ (Kurve Technology Inc.), a vortex-propelled nebulizer system. The ViaNase device generates nebulized particles between 9 and 11 μm in size to be inhaled with an occlusive nosepiece over 2 minutes [69,71]. According to the manufacturer, this electric device covers a higher content of the nasal mucosa with drug-containing droplets compared to nasal pump sprays without targeting the lungs [71,72].

A bidirectional breath-powered nasal delivery platform is OptiMist™ by OptiNose. The novel technology overcomes undesired pulmonary delivery by a breath-actuation mechanism. The mechanism is extraordinarily simple: The device has a nosepiece and a mouthpiece, which is inserted into one nostril and the patient blows into the mouthpiece. Exhalation pressure releases drug as (solid or fluid) particles through the nosepiece into the nasal cavity. Exhalation closes the soft palate and the air flow can exit through the other nostril as bidirectional flow reaching both parts of the nasal septum [73]. Through the closed soft palate drug particles cannot enter the lungs [74]. OptiMist™ generates droplets of 43 μm diameter and targets larger initial and cumulative deposition in the upper posterior sector of the nasal cavities and significantly lower deposition in the anterior segment. The device is currently evaluated in a clinical study for the use with the peptide oxytocin.

Pressure operated devices aim to work as Precision Olfactory Delivery (POD®, Impel Neuropharma) devices to deliver drugs to the upper nasal cavity for direct N2B transport. According to the manufacturer the POD device technology results in over 50 % deposition at the olfactory region and enhanced uptake into the CNS in comparison to other nasal delivery devices, which are quickly cleared by respiratory epithelium and absorbed into systemic circulation [75].

In addition, standard medical nebulizers can be used for aerosol generation and delivery into the nasal cavity. However, most of them have been developed to target the lower airways, but newer models engineered for the delivery of the paranasal sinuses (e. g. PARI Sinus™) might be suitable for N2B delivery. The influence of liposomes, nanoparticle and gels as dosage forms for intranasal delivery is summarized in Table 2.

**Intranasal insulin for metabolic diseases**

N2B insulin delivery appears to be a clinically safe application method for the dissociation of central and peripheral insulin effects [76]. An intranasal insulin spray containing the adsorption enhancer cyclopentadecalactone (CPE-215) was developed as Nasulin™ for the needle-free regimen of diabetes. The programme was stopped after disappointing results of a Phase 2a proof-of-concept clinical trial [77]. The primary objective to demonstrate that subjects receiving Nasulin™ would achieve a larger increase from baseline in the mean proportion of time spent in euglycemia than those receiving placebo failed statistical significance. Like in the latter AD trials, no critical safety signals were detected with Nasulin™. The most common adverse events were those attributable to administration site reactions associated with the nasal route of delivery, the majority of which were mild. The percentage of subjects reporting hypoglycemia was similar between both the Nasulin™ and placebo groups. Nevertheless, it seems that intranasal insulin is yet not dead. The FDA gave a Black Box Warning to the pulmonary administered insulin Exubera® since clinical studies reported a four-fold increase in the incidence of lung cancer among patients with a history of smoking. In 2007 Exubera® was discontinued and shifted back the interest to nasal insulin administration with very recent studies on beneficial metabolic effects of central insulin action [59,66,78]. Several companies continue their nasal insulin development programmes for the regimen of diabetes, all of them...
using different absorption enhancers like dodecyl-β-D-maltoside (Aegis therapeutics Inc.; [79]), microcrystalline cellulose (SNBL; [80]) and CriticalSorb™ (Critical Pharmaceuticals; [81]).

**Table 2. Dosage forms used in intranasal delivery.**

<table>
<thead>
<tr>
<th>Liposomes</th>
<th>Study</th>
<th>Compound</th>
<th>Formulation</th>
<th>Particle size</th>
<th>Outcome</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preclinical/rodents</td>
<td>Galantamine HBr</td>
<td>Soy phosphatidylcholine, cholesterol, propylene glycol</td>
<td>112 ± 8 nm</td>
<td>2.8-fold increase of AUC compared to free drug via N2B; 3.4-fold increase compared to oral delivery</td>
<td>[82]</td>
<td></td>
</tr>
<tr>
<td>Preclinical/rodents</td>
<td>Rivastigmine</td>
<td>Cholesterol, soy lecithin</td>
<td>10 ± 2.8 µm</td>
<td>3-fold increase of AUC compared to free drug via N2B; 5.5-fold increase compared to oral delivery</td>
<td>[83]</td>
<td></td>
</tr>
<tr>
<td>Preclinical/rodents</td>
<td>Ovalbumin as surrogate for protein therapeutics</td>
<td>Dioleoylphosphatidylcholine, cholesterol, stearylamine</td>
<td>299 ± 26.4 nm</td>
<td>&gt;4-fold higher AUCbrain/AUCblood for the liposomal formulation compared to the PBS preparation</td>
<td>[84]</td>
<td></td>
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<tr>
<th>Nanoparticles</th>
<th>Study</th>
<th>Compound</th>
<th>Formulation</th>
<th>Particle size</th>
<th>Outcome</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preclinical/rodents</td>
<td>Insulin (for blood glucose management)</td>
<td>Glycopolymer poly(2-lactobionamidooethylmethacrylate-3-acrylamidophenylboronic acid) p(LAMA-r-AAPBA) 2:1</td>
<td>289.4 ± 2.5 nm</td>
<td>80% decrease of peripheral blood glucose levels with nanoparticles, 4-fold increase compared to PBS formulation</td>
<td>[85]</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Gels</th>
<th>Study</th>
<th>Compound</th>
<th>Formulation</th>
<th>Outcome</th>
<th>Source</th>
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<tbody>
<tr>
<td>Preclinical (rodents) and clinical (humans) study</td>
<td>Insulin (for blood glucose management)</td>
<td>Carbopol 934P and hydroxypropyl methylcellulose</td>
<td>Prolonged effect on blood glucose levels in rodents; decrease of blood glucose levels by 20% with nasal gel, no control group for comparison.</td>
<td>[86]</td>
<td></td>
</tr>
<tr>
<td>Preclinical/rodents</td>
<td>Insulin (for blood glucose management)</td>
<td>Chitosan and polyvinyl alcohol (PVA)</td>
<td>Prolonged 60% decrease of peripheral blood glucose levels with gel formulation</td>
<td>[87]</td>
<td></td>
</tr>
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</table>

**Conclusive remarks about the lack of valuable PK-PD studies**

Nasal insulin delivery using such above mentioned absorption enhancers shows rapid delivery of insulin to the serum [58,88]. In addition, comparative studies are needed to resolve the mechanisms needed to target insulin or other biopharmaceuticals either to the periphery or to the brain. For intranasal insulin that includes insulin and glucose levels in serum and CSF to calculate PK-PD relations. The bioavailability and steady-state kinetics of intranasal insulin administered over 3 weeks to 4 months has hardly been evaluated. For a convincing safety profile this data should be provided. In addition, safety aspects and adverse events like hypoglycaemia or dyslipidaemia should be adequately discussed in the light of a chronic treatment. An excess of insulin that is distributed to the vasculature might mediate such adverse events.
Though, any AD study that does not fail causes euphoria, the use and the transport mechanism of intranasal insulin with and without additives needs to be analysed very tightly and discussed more critically in relation to its safety profile.

The number of AD cases increases with the demographic change and we need to act now. Future AD patients are dependent on our analytical knowledge, pharmaceutical expertise and scientific creativity to develop candidates like N2B delivered insulin to a safe, suitable and validated drug therapy.

A recent empirical analysis uncovered the massive lack of quantitative data in published N2B scientific publications: only 3% of the studies determine and publish quantitative data like bioavailability or similar [89]. However, the development of a successful N2B technique requires quantitative PK-PD data to engineer a safe and efficient delivering strategy.

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Intranasal Insulin as Hope for AD


[81] CriticalSorb is a proprietary absorption promoter that has the potential to provide a viable alternative to injection for a wide variety of biological and challenging small molecule drugs., [http://www.criticalpharmaceuticals.com/technology/criticalsorb](http://www.criticalpharmaceuticals.com/technology/criticalsorb) (04/16/2015).


