REVIEW ARTICLE

Bypassing the blood−brian barrier using established skull base reconstruction techniques

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Abstract *Background:* Neurological disorders represent a profound healthcare problem accounting for 6.3% of the global disease burden. Alzheimer's disease alone is expected to impact over 115 million people worldwide by 2050 with a cost of over $1 trillion per year to the U.S. economy. Despite considerable advances in our understanding of the pathogenesis and natural history of neurological disorders, the development of disease modifying therapies have failed to keep pace. This lack of effective treatments is directly attributable to the presence of the blood−brain and blood−cerebrospinal fluid barriers (BBB and BCSFB) which prevent up to 98% of all potential neuropharmaceutical agents from reaching the central nervous system (CNS). These obstacles have thereby severely limited research and development into novel therapeutic strategies for neurological disease. Current experimental methods to bypass the BBB, including pharmacologic modification and direct transcranial catheter implantation, are expensive, are associated with significant complications, and cannot be feasibly scaled up to meet the chronic needs of a large, aging patient population.
Transmucosal drug delivery: An innovative method of direct CNS drug delivery using heterotopic mucosal grafts was described. This method is based on established endoscopic skull base nasoseptal flap reconstruction techniques. The model has successfully demonstrated CNS delivery of chromophore-tagged molecules 1000 times larger than those typically permitted by the BBB.

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Conclusions: This innovative technique represents the first described method of permanently bypassing the blood—brain barrier using purely autologous tissues. This has the potential to dramatically improve the current treatment of neurological disease by providing a safe and chronic transnasaldelivery pathway for high molecular weight neuropharmaceuticals.

Introduction

The spectrum of neurological disorders represents a profound healthcare problem accounting for up to 6.3% of the global disease burden according to the World Health Organization.[1] By the year 2050, Alzheimer's disease (AD) alone is expected to afflict 115.4 million people with a cost of $1.1 trillion to the US healthcare system.[2] Similarly, the US National Institute for Neurological Disorders and Stroke estimated the total number of cases of Parkinson's disease (PD) in the US at 500,000 with 50,000 new cases diagnosed annually resulting in a cost to the US economy of $23 billion per year.[3,4] While precise numbers are difficult to obtain, the prevalence of PD in industrialized countries is estimated at 1% for people over 60.[5] The scale of this chronic disease burden suggests that therapies capable of delaying or reversing these disorders will translate into enormous cost savings to the global healthcare system. Although researchers have made considerable advances in our understanding of the pathogenesis and natural history of neurological disease, the development of effective therapeutic options have failed to keep pace. For example levodopa remains the most effective agent available for the symptomatic treatment of PD despite the fact that it was first introduced in 1961.[6] The current paucity of effective treatments for neurological disease is directly attributable to the presence of the blood—brain barrier (BBB) which effectively prevents up to 98% of all potential neuropharmaceutical agents from reaching the central nervous system (CNS) thereby severely limiting the development and implementation of novel disease modifying therapies.[7]

Blood—brain and blood—cerebrospinal fluid barrier physiology

The BBB represents a dynamic interface between the CNS and the periphery, which functions to protect the brain from xenobiotic agents and toxins while selectively permitting molecules essential for maintaining metabolic homeostasis.[7] The BBB is classically described within the microvascular endothelial cells of the cerebrovasculature. However an additional component, known as the blood—cerebrospinal fluid barrier (BCSFB), is also present within the arachnoid membrane surrounding the brain and spinal cord. The BCSFB has been recognized since 1913 when Edwin Goldman demonstrated that trypan blue failed to diffuse into the extra axial tissues following injection into the cerebrospinal fluid within the subarachnoid space.[8,9] As the BBB and BCSFB share similar morphologic and functional characteristics, the

terms will be used interchangeably throughout this manuscript. The BBB utilizes several mechanisms to restrict drug delivery to the CNS. The first is a physical barrier comprised of a thick basement membrane and densely packed cells bonded through tight junctions which represent a complex of transmembrane (junctional adhesion molecule-1, occludin, and claudins) and cytoplasmic (zonula occludens-1 and -2, cingulin, AF-6, and 7H6) proteins linked to the actin cytoskeleton.[10] The BBB also functions as an enzymatic barrier by degrading pharmaceuticals using a host of enzymes including monoamine oxidase types A and B, L-amino acid decarboxylase, several cytochrome P450 dependent mono-oxygenases, NADPH-cytochrome P450 reductases, UDP-glucuronosyltransferases, alkaline phosphatases, glutathione peroxidases, and epoxide hydrolases.[11] Finally, the BBB employs a host of specific transport mechanisms, such as the glucose transporter-1, which simultaneously enable the uptake of large molecules and peptides necessary for CNS function while actively excluding most exogenous drugs.[7] The combination of these three barrier mechanisms restricts simple diffusion only to small lipophilic drugs that have a cross sectional area of less than 70 Å[2,9] or a molecular weight of less than 500 Daltons (Da).[11]

Current obstacles in neuropharmaceutical delivery

The restrictions on drug uptake imposed by the BBB have dramatically limited clinical progress in both symptomatic and disease modifying therapies for neurological diseases. Promising experimental therapies such as glucocerebrosidase (60 kDa),[12] IL13-*Pseudomonas* Toxin (66 kDa),[13] IDUAe1 (73 kDa),[14] CTP-MPB (85 kDa),[15] and β-Galactosidase (116 kDa)[16] have been developed to treat several devastating neurological disorders such as Gaucher's Disease, Diffuse Pontine Glioma, Mucopolysaccharidosis I, Alzheimer's disease, and lysosomal storage disorders, respectively. However, as these compounds exceed 500 Da, their molecular weight precludes meaningful uptake by the CNS. Glial derived neurotrophic factor (GDNF, 35 kDa) represents the most intensely studied of these high molecular weight neuropharmaceuticals as it has been shown to promote mesencephalic dopaminergic neuronal survival thereby delaying or even reversing disease progression in Parkinson's Disease.[17] The progressive global burden of neurological disease coupled with the obstacles posed by the presence of the BBB have catalyzed an enormous body of research targeted at bypassing the blood—brain barrier. Current strategies can be broadly categorized into non-

invasive and invasive methods, each of which is associated with certain limitations. Non-invasive methods include strategies such as 1) local permeabilization of the BBB via osmotic[18] or ultrasound disruption[19] techniques, 2) pharmacological approaches using fatty acid[20] and lipid carriers,[21] and 3)physiological approaches that exploit pre-existing receptor mediated transport mechanisms such as the transferrin[16] and insulin[22] uptake pathways. While these methods have demonstrated promise in limited studies, they are short lived, require extensive drug manipulation, and would be extremely expensive to scale up to meet the chronic needs of the growing population of patients with neurological disease. Invasive methods rely on physically breaching the BBB using intra-cerebroventricular (ICV) infusion,[23] convection enhanced delivery,[24] or simple direct intracerebral injection.[25] As noninvasive methods obviate the need for extensive drug reformulation with its attendant regulatory considerations and costs, these methods have been more extensively tested in clinical trials. Gill et al[26] reported on their experience on direct intraputamenal GDNF injection in a phase 1 safety trial in Parkinson's disease. Though clinically effective, all patients developed vasogenic edema at the catheter tip and 40% required additional catheter manipulation or explantation. These safety issues are emblematic of the inherent complications associated with inducing direct trauma to the brain for the purposes of drug delivery as well as the implantation of catheters which are prone to infection and malposition. These findings underscore the fact that there remains a profound unmet need for a safe method of chronically bypassing the blood—brain barrier without requiring direct intracerebral injections or the placement of indwelling foreign bodies.

Potential of Direct Transnasal Drug Delivery: The evident potential of high molecular weight neuropharmaceutical delivery to the brain has driven considerable investigation into the direct transnasal pathway. This method postulates the retrograde axonal transport of drugs by neurons within the olfactory mucosa and offers the additional advantage of direct uptake into the CNS while avoiding the side effects associated with systemic drug exposure.[27] Using a modified "Hirai" model, Fisher et al[28] confirmed that transmucosal absorption of molecules 70 kDa and larger is possible. DeRosa et al[29] reported that intranasal nerve growth factor delivery (27.5 kDa) was capable of reversing cognitive defects in AD mice through olfactory mucosa uptake. In a clinical study, Reger et al[30] exploited the presence of the native BBB insulin receptor demonstrating that intranasal insulin (5.8 kDa) was capable of improving cognition in AD mice without altering plasma insulin levels. Of note, the authors conceded that the insulin levels measured in the CSF were up to 10 times lower than physiologic concentrations thereby calling into question the concentration of insulin delivered and the mechanism underlying their clinical endpoint. While these studies appear to support the potential of simple transnasal drug delivery to the CNS, it has been suggested that this pathway may not be sufficient to provide a reliable platform for the delivery of a broad range of neuropharmaceutical agents for three principle reasons. First, the majority of preclinical studies demonstrating the efficacy of this pathway rely on retrograde uptake into the CSF through the olfactory mucosa in a rodent model.[31–33] While the olfactory epithelium occupies up to 50% of the nose in a rodent, it comprises only 3% of human nasal mucosa with a total surface area of 1—2 cm.[2–4] This small region of olfactory tissue is then even further reduced by progressive replacement with respiratory mucosa as human age thereby accounting for the normal decline in smell function throughout life.[34] This implies that studies in rodents vastly overestimate the surface area available for drug uptake clinically, particularly when applied in an elderly population. Second, human olfactory mucosa is located in a narrow recess in the roof of the nasal cavity known as the olfactory cleft. As a result of this unfavorable location, drug deposition in this region is minimal regardless of the delivery method used.[35] Third, once the drug contacts the nasal epithelium it has an effective mucosal residence time of only 15—20 min before it is rapidly cleared out of the nose by mucociliary action. This, coupled with the poor distribution to the olfactory cleft, even further reduces the amount of agent available for mucosal absorption.[36] These factors serve to explain why even when applying a relatively low molecular weight molecule in the presence of a selective receptor mediated insulin uptake pathway, the amount of insulin capable of reaching the CNS in the Reger et al[30] study still fell below physiologic concentrations. These limitations effectively rule out this pathway for other drugs requiring higher therapeutic concentrations and lacking a similar native uptake mechanism. Merkus et al[37] summarized these concerns regarding the clinical utility of transnasal drug delivery noting that among 100 preclinical and clinical papers studying this pathway, only 2 provided convincing evidence of direct CNS uptake, both of which were in rat models. While this evidence demonstrates the limitations of the clinical potential for direct transnasal CNS neuropharmaceutical delivery, the considerable permeability demonstrated by the sinonasal mucosa to high molecular weight agents does suggest the feasibility of alternative transmucosal pathways.

Endoscopic skull base reconstruction

Endoscopic access to lesions that involve the brain parenchyma requires the removal of the intervening tissues including nasal mucosa, bone, dura, and arachnoid membrane to facilitate complete resection. These approaches thereby necessitate creating a large window in the skull base which allows for direct communication between the CSF space, brain, and the interior of the nasal cavity. Consequently, the ability to safely and reliably reconstruct these defects is an absolute requirement in the successful performance these surgeries.[38] Over the past decade, a variety of mucosal grafts[39–42] have been developed for the specific purpose of skull base reconstruction, providing for reliable, water-tight, and immunocompetent repairs which safely and permanently separate the intranasal and intracranial compartments for defects up to 20 cm.[2,43,44] These repairs are extraordinarily safe with rates of long term infection, CSF leak, and mucocele formation estimated at approximately <1%, 5%,[45] and 3.6%,[43] respectively. The proven long term safety profile of mucosal graft reconstruction of large skull base defects has catalyzed a

dramatic expansion in the use of these techniques with endoscopic surgeons currently performing this procedure routinely throughout the world. As nasal mucosa is known to be over 1000 times more permeable than the native BBB,[46] an unanticipated side effect of this technique is the creation of a semipermeable conduit for high molecular weight drug delivery directly to the CSF and brain.

Transmucosal drug delivery using mucosal graft techniques

In order to explore this potential, our team embarked on a research program to develop a mouse model of mucosal graft reconstruction in order to test the feasibility of using nasal mucosal grafts for high molecular weight drug delivery to the brain.[46] This model recapitulated the anatomy and graft morphology encountered at the anterior skull base. Briefly, the nasal septum was harvested *en bloc* from a donor mouse and was used to repair a craniotomy defect following arachnoid resection.

In our findings we demonstrated that the mucosal graft is capable of transporting molecules 1000 larger than those excluded by the BBB directly into the brain. When examining dextran conjugated fluorophores ranging from 20 to 500 kDa, we found that transmucosal uptake and spatial distribution were directly correlated with duration of exposure and inversely correlated with molecular weight. The detailed results of these findings have been previously reported.[46]

Discussion

The BBB continues to be the greatest obstacle to the elaboration and implementation of effective pharmaceutical therapies for a variety of neurologic disorders. The development of a safe and reliable method to bypass the BBB represents a "holy grail" of neuropharmacology. Our data demonstrate that the application of nasal mucosal grafts over arachnoid defects may represent one such method by creating a permanent semipermeable conduit directly from the nose to the CNS. Utilization of nasal mucosal grafting offers several advantages over previously described methods: 1) Compared to ICV catheter placement, the mucosal graft allows for drug diffusion directly into the CSF within the subarachnoid space adjacent to the basolateral side of the graft. As the CSF continually circulates with a turnover rate of 10–20 mL per hour,[47] a counter-current exchange phenomenon occurs continually maximizing the diffusion gradient across the graft. However, the graft utilizes only autologous tissues and avoids penetration of the brain parenchyma thereby obviating the complications, such as vasogenic edema and catheter malposition, seen with traditional ICV studies.[26] Furthermore the long track record of safety associated with endoscopic skull base repairs[43,45] ensures that the graft remains stable over the lifetime of the patient without incurring significant risk of infection, CSF leak, or ascending meningitis. 2) Unlike non-invasive pharmacologic[20,21] and physiologic[16,22] methods of BBB disruption, the mucosal graft is inherently permeable to a broad range of high

molecular weight compounds enabling the delivery of both experimental and currently available agents without the need for expensive drug manipulation. 3) Pharmaceutical distribution and mucosal residence time at the surface of the graft is dramatically enhanced relative to simple transnasal olfactory epithelium delivery. This results from the position of the graft within the sphenoid which creates a natural reservoir to retain a higher volume of the drug despite the clearance action associated with mucociliary transport.[36] Furthermore, the graft provides 10–20 times the surface area of healthy olfactory epithelium and does not diminish with age.[44]

Conclusion

Drug permeability across the BBB is highly limited and none delivery technique have proved to be sufficiently effective in reaching the brain. The heterotopic mucosal grafting technique represents a completely novel method of permanently bypassing the BBB using purely autologous tissues. This approach has the potential to dramatically improve the current treatment of neurological disease by enabling the delivery of high molecular weight neuropharmaceuticals directly to the CNS.

Financial disclosures/conflicts of interest

The first author (BSB) is inventor on a patent for drug delivery to the brain assigned to the Massachusetts Eye and Ear Infirmary.

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