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RESEARCH ARTICLE

Future Advances In Early Phase Clinical Development for Disease Modifying Therapies for Neurodegenerative Diseases

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ABSTRACT
With aging populations in many countries, the prevalence of neurodegenerative diseases is expected to increase in the upcoming decades. Currently, no disease modifying therapies for these conditions exist. Advances in genetics and proteomics have identified novel druggable targets for neurodegenerative diseases. Compounds modulating these targets have recently entered clinical trials. These compounds can be orally administered small drug molecules, intravenously dosed antibodies, intrathecally injected antisense oligonucleotides (ASOs), gene therapies, stem cells or viral vectors. For the development of these compounds to be successful, multiple challenges have to be overcome. In this review we discuss advances in drug development for each of the major neurodegenerative diseases, which, when applied to early phase drug studies, increase the chance of successful clinical development. Here we will limit ourselves to: 1) the use of biomarkers for understanding target and pathway engagement at an early stage of development, 2) novel approaches for increasing blood-brain barrier penetration and 3) advances in understanding cerebrospinal fluid flow dynamics in relation to neurodegeneration and target site distribution for intrathecally administered compounds.

Introduction

Neurodegenerative disorders are increasingly becoming a major source of morbidity worldwide.[1] Very few disease modifying therapies currently exist for these conditions. With aging populations in many countries, the burden of these diseases is expected to rise significantly.

Neurodegenerative diseases such as Alzheimer's disease (AD), Parkinson's disease (PD), and amyotrophic lateral sclerosis (ALS) have common cellular and molecular mechanisms with protein misfolding and aggregation, being long recognized. These protein aggregations probably represent the end stages of a molecular cascade, of which earlier steps are more directly linked to the pathogenic process. An exact mechanistic understanding remains incomplete. Current advances in genetics, proteomics and immunology, among others, have allowed us to gain insight into perturbed pathophysiological processes shared by distinct neurodegenerative disease entities. These pathways include, but are not limited to, the endolysosomal system, mitochondrial function and neuroinflammation.

A disease modifying treatment for a neurodegenerative disease is defined as an intervention that produces an enduring change in clinical progression by interfering with the underlying pathophysiology.[2] The increase in potential central nervous system (CNS) targets has spurred efforts in drug development for neurodegenerative diseases, many of which classify as disease modifying treatments. Many compounds modulating these targets have recently entered clinical trials. Currently, there are more than 100 compounds in clinical development for diseases such as Alzheimer's disease and Parkinson's disease.[3,4] These compounds can be orally administered small drug molecules, intravenously dosed antibodies, intrathecally injected antisense oligonucleotides (ASOs), gene therapies, stem cells or viral vectors. Each of these therapies requires a specific approach to ensure sufficient central nervous system distribution.

For the development of these compounds to be successful, multiple challenges have to be overcome. In this review we discuss three major advances, which, when applied to early phase drug studies for neurodegenerative diseases, increase the possibility of successful clinical development. These advances are 1) the use of biomarkers for understanding target and pathway engagement for common pathophysiological processes at an early stage of development, 2) novel approaches for increasing blood-brain barrier penetration and 3) advances in understanding cerebrospinal fluid (CSF) flow dynamics in relation to neurodegeneration and target site distribution for intrathecally administered compounds.

Pathophysiology for neurodegenerative diseases have overlapping molecular mechanisms.

Lysosomes are at the intersection between many degradative pathways, such as endocytosis (phagocytosis) and autophagy.[5] Lysosomes are involved in innate and adaptive immune function and inflammation because of their capabilities to engulf foreign material, activate pattern recognition receptors (Toll-like receptors (TLRs) and nucleotide oligomerization domain-like receptors) and antigen processing and presentation.[6] Together with mitochondria, lysosomes coordinate metabolic processes, autophagy, proliferation and cell death.[7] Defects in vesicular trafficking to the lysosome are one of the most prominent mechanisms of pathogenesis that link to Parkinson's disease-associated genetic variants.[8–10] Variants in the gene for leucine-rich-repeat kinase-2 (*LRRK2*) result in defects to lysosomal vesicular trafficking through aberrant Rab signaling.[11–13] *GBA* mutations also link lysosomal function to Parkinson's disease risk, probably by potentiating α-synuclein toxicity.[14] Common genetic variants for autoimmune disorders are also associated with risk of Parkinson's disease.[15] Neurohistological and neuroimaging studies support a neuroinflammatory process and α-synuclein aggregates have been reported to activate microglia.[16,17] In Alzheimer's disease, genome-wide association studies suggest a role for trafficking factors and lysosomal dysfunction in the pathology[18] and have revealed multiple genes implicated in neuroinflammation to be associated with Alzheimer's disease [19], such as triggering receptor expressed on myeloid cells 2 (TREM2) and APOe4, implicating microglia in this process as well.[20–22] Mitochondrial biogenesis is impaired in AD patients as indicated by reduced levels of the transcriptional regulator of mitochondrial biogenesis peroxisome proliferator-activated receptor-γ coactivator 1α (PGC1-α).[23] Genes related to both ALS and frontotemporal dementia in genome-wide association studies most notably cluster in lysosomal or autophagy pathways.[24,25] The contribution of neuroinflammation in ALS has been characterized by PET studies showing activated microglia in the brains of living patients and through autopsy studies revealing activated astrocytes, microglia and T-cells at sites of motor

neuron injury.[26–28] This overlap in pathophysiological molecular mechanisms has allowed the use of biomarkers in the clinical development programs to translate across neurodegenerative diseases.

Biomarkers aimed at detecting molecular mechanisms translate across diseases

For the purpose of this review, we refer to biomarkers as measures of a pharmacological change in response to exposure of an investigational medicinal product. There are many levels at which this response can be measured. For early phase clinical trials, biomarkers are most informative when incorporated to establish that the investigational medicinal product 1) occupies the molecular target (target engagement) and 2) activates the intended molecular pathway (pathway engagement). More distal biomarkers, such as efficacy response markers, oftentimes are not likely to change in the relatively short timeframe and limited number of patients in these trials. A model for target and pathway engagement in relation to exposure can be derived and this information serves as a valuable tool to guide clinical development. More extensive reviews on the importance of including pharmacological and mechanistic biomarkers in early phase clinical trials have been published elsewhere.[29–31] With the central nervous system as the target site for the pharmacological response, measurements are complicated by the presence of a blood-brain barrier, and the unavailability of direct tissue sampling. CSF collection can serve as an approximation, but the measurements that can be done are limited and this does not fully reflect the biological processes in the tissue and extracellular fluid surrounding the brain and spinal cord. There have been considerable advances in biomarker development for measuring the processes of endolysosomal function, mitochondrial maintenance and neuroinflammation as common pathways perturbed in multiple neurodegenerative diseases.

Blood based biomarkers

To overcome the challenges of measuring pharmacological responses in central nervous system target sites, blood-based biomarkers can serve as indirect measures. In healthy volunteer studies, for targets that are accessible in the plasma, measuring changes to the plasma concentrations serve as a first read-out of the pharmacology of the drug. Examples are T-cell proliferation and T-cell subsets after mTORC1 inhibitors and the ratio of monounsaturated to saturated C16 and C18 fatty acids after stearoyl-CoA-desaturase (SCD) inhibition.[32,33] When, for example, the target site is also expressed on peripheral blood mononuclear cells, activity can be measured more robustly following cell sorting by flow cytometry. Examples are glycosphingolipid levels following glucocerebrosidase (GCase) activators [34] and reduced phosphorylation at serine-935 (pS935) or Rab10 at threonine-73 (pT73 Rab10) following LRRK2 inhibition.[35] When the target is expressed only in disease conditions, the cell type under investigation might require stimulation before target expression is quantifiable. This can be done with an immune challenge such as lipopolysaccharide (LPS) or the pan-caspase inhibitor zVAD-FMK.[36,37] Examples are reductions of pS166 RIPK1 (receptor interacting serine/threonine -protein kinase 1) following RIPK1 inhibition [38] and reductions in IL-12/IL-23 after phosphatidylinositol-3-phosphate 5-kinase (PIKfyve) inhibition.[39] Alternatively, a patient population can be enrolled. These techniques allow the assessment of the pharmacological responses into the endolysosomal system, intracellular trafficking, and neuroinflammatory target pathways.

Cerebrospinal fluid based biomarkers

The CSF surrounds the cells and tissues of the central nervous system.[40] It is a clear fluid containing proteins, lipids, hormones, ions, microRNA's, neurotransmitters and glucose.[41,42] There are few to no cells in the CSF under physiological conditions. CSF has a pivotal role in homeostasis of cerebral interstitial fluid and the neuronal environment by regulating electrolyte balance, circulation of active molecules and elimination of catabolites.[43] Changes in the intracellular level of the molecules can sometimes result in changes in CSF concentrations. Examples are total and phosphorylated tau (anti-tau and anti-amyloid therapies), amyloid-β (γ- and β-secretase inhibitors, anti-amyloid antibodies), mHTT (antisense oligonucleotides that inhibit huntingtin messenger RNA) and colony stimulating factor 1 receptor (CSF1R) as a measure of microglial activation.[44] Neurofilament light chain has received considerable attention as a general marker of neurodegeneration over multiple neurodegenerative diseases and lysosomal storage disorders.[45] More experimental techniques include lipidomics and proteomics of the CSF.[46,47]

Imaging biomarkers

Imaging techniques allow a compound interpretation of cellular function, and this can serve as a measure of pharmacological response. Positron emission tomography (PET) of the brain with an 18F-

fluoro-2-deoxy-D-glucose (FDG) tracer allows the assessment of cerebral glucose metabolism. While it is suggested that this signal is driven, in part, by neuronal synaptic activity [48], loss-of function mutations in TREM2, a microglial gene involved in metabolism and activation, strongly impair cerebral glucose uptake as measured by FDG-PET.[49] Conversely, increases in microglial glucose uptake and activity substantially increase the FDG-PET signal.[50] A correlation between microglial activity and glucose uptake was confirmed in patients with different neurodegenerative diseases.[50] PET scanning with amyloid-β tracers ([^{18}F]-florbetapir) or tau tracers (flortaucipir) has received wide utility in demonstrating effects of amyloid clearing therapies for Alzheimer's disease.[51–53] Other PET tracers allow the assessment of cholinergic signaling, by determining receptor occupancy for different subtypes of cholinergic receptors to a level of detail that allows the differentiation between brain regions.[54] For dopamine imaging with single photon emission computed tomography (SPECT), tracers specific for the presynaptic dopamine transporter (DaT) allow pharmacological effects to be assessed at the level of the presynaptic dopaminergic terminal and for tracers specific for the D_2-dopamine receptor, the postsynaptic dopamine terminal.[55] Similar results can be obtained with PET scanning. While radiopharmacological tracers specific for aggregated protein can quantify levels of these aggregates in the brain before and after investigational product administration, this is a burdensome technique that is complicated by radiation exposure. Hyperspectral imaging has the potential to more directly visualize changes to the retina as a result of protein aggregation, as these cells form an embryological outpouching of the central nervous system.[56] It remains to be determined whether this technique is sensitive enough to capture changes in other neurodegenerative diseases, and whether observed changes are responsive to pharmacological intervention. Mitochondrial function can be measured by phosphorous magnetic resonance spectroscopy (31P-MRS) after activation of the occipital cortex with checkerboard flashes.[57] This can be used to study pharmacologic effects for compounds enhancing brain metabolism in neurodegenerative diseases. In an open-label study, administration of anaplerotic therapy during 1 month improved the inorganic phosphate to phosphocreatine ratio compared to no treatment in a study involving 10 Huntington disease patients.[58] In a placebo controlled study involving 24 patients with Huntinton's disease, no change in inorganic phosphate to phosphocreatine ratio was seen after administration of a compound improving mitochondrial respiration by binding to cardiolipin, compared to placebo.[59]

Novel approaches for increasing blood-brain barrier penetration

Drug distribution into the CSF is not a surrogate for drug distribution into the brain parenchyma. Epithelial plasma membranes in the choroid plexus differ in permeability compared to endothelial plasma membranes in cerebral capillaries.[60,61] Drug CSF concentrations are a function of transport across the choroid plexus, while drug concentrations in the brain parenchyma are the composite of transport across the capillary endothelium.[62] Plasma proteins and large molecules, including antibodies, cross the choroid plexus, at a rate inversely related to their molecular weight.[61,63] These large molecules do not consistently cross the blood-brain barrier unless they have affinity for a specific blood-brain barrier transport system via receptor mediated transporters.[64] Therefore, to adequately reach the site of action for many large molecules with central nervous system targets, novel approaches to traverse the blood-brain barrier need to be developed.

Blood-brain barrier transport vehicles

Small molecule delivery into the brain can benefit from dual affinity for carrier mediated transporters in parallel to the CNS target. Examples of carrier mediated transporters are GLUT-1 glucose transporter, LAT-1 L-type amino acid transporter, OATP-B organic anion-transporting peptide-B, and CAT1 cationic amino acid transporter.[65] Advances in high-throughput drug affinity screens allow for the dual affinity properties to be selected on. Large molecules, including recombinant proteins, require re-engineering to bind receptor mediated transporters to allow receptor mediated transcytosis into the brain. Examples of these receptors are the insulin receptor [66], transferrin receptor [67], insulin-like growth factor receptor [68], low density lipoprotein receptor-related protein 1 (LRP1)[69] and the leptin receptor [70]. A principal efflux receptor for IgG, from brain to blood, is the neonatal Fc receptor. Decreasing the affinity for this receptor can increase the half-life of antibodies in the CNS compartment.[71] Molecules engineered to cross the blood-brain barrier by binding to one of these receptor mediated transporters are at various stages of clinical development. Iduronate-2 sulfatase, the enzyme dysfunctional in mucopolysaccharidosis type II, is fused to a monoclonal antibody against the human insulin

receptor and has been tested in preclinical stages.[72] The same enzyme was appended to the C-terminus of an antibody targeting the human transferrin receptor with its Fab domains by a peptide linker and was tested in a human transferrin receptor knock-in model in mice and monkeys.[73,74] This confirmed the brain uptake of the enzyme and reduction in substrate concentrations in the brains of these animals.[73,74] This enzyme-antibody fusion molecule was later tested in patients with mucopolysaccharidosis II in phase 2/3 clinical trials, and found to reduce substrate concentrations in CSF and improve neurocognitive development.[75,76] An alternative approach is to re-engineer the Fc domain of human immunoglobulin G1 (IgG1) to bind the human transferrin receptor and fuse the lysosomal enzyme with this modified domain.[77] This complex has been tested head-to-head with the enzyme-antibody fusion complex in preclinical models.[78] Peptide-drug conjugates have entered clinical stages of development primarily as ways to increase chemotherapy delivery to the brain.[79] Examples are the linkage of paclitaxel to a 19 amino acid peptide targeting LRP1.[80,81] Through this 'transport vehicle' principle, many different proteins can be shuttled over the blood-brain barrier. For neurodegenerative diseases, these techniques can increase the exposure to compounds which have been in clinical trials without blood-brain barrier shuttle strategies, such as β-secretase (BACE1) inhibitors [82], or open up targets previously not accessible. Other strategies for shuttling drugs over the blood-brain barrier, such as nano-delivery vehicles including micelles [83], are in the early preclinical stages of development.

Modulation of blood-brain barrier permeability

Osmotic manipulation of blood-brain barrier permeability has been achieved in animal models by intracarotid arterial infusion of hypertonic solutions such as mannitol.[84] Intracarotid arterial infusion of vasoactive substances such as bradykinin analogs, to increase delivery of chemotherapy, has been studied extensively in animal models of primary and metastatic brain tumors.[85,86] While these techniques are interesting from a basic science perspective, their invasiveness does not allow them to translate well to the clinic. Ultrasonography-guided liposomes carrying a neuronal growth factor transiently disrupted the blood-brain barrier and was used in a rat model of Parkinson's disease.[87] In phase I safety studies in patients with Alzheimer's dementia and Parkinson's disease dementia, magnetic resonance guided focused ultrasound in combination with intravenous microbubbles allowed safe, reversible and repeatable focal opening of the blood-brain barrier as evidenced by gadolinium enhancement.[88,89] However, long term repeated or chronic opening of the blood-brain barrier might be harmful and hinder the application of this approach in neurodegenerative diseases.[90]

Prodrugs

Prodrugs are inactive drug derivatives that are metabolized *in vivo* into pharmacologically active drug following chemical or enzymatic modification.[91] In neurodegenerative diseases, the most widely used prodrug is levodopa, the carboxylic acid of dopamine. Levodopa enters the brain through its affinity for the LAT-1 carrier mediated transporter, and is decarboxylated to dopamine by aromatic amino-acid decarboxylase.[91] Galantamine benzoate is an inactive lipophilic prodrug of galantamine. The active drug is liberated on cleavage by carboxyesterases in the CNS. In Alzheimer's disease patients, this resulted in increased performance as measured by a neurocognitive test battery, and fewer gastrointestinal side effects compared to oral galantamine.[92] A valine-conjugated prodrug of tramiprosate (homotaurine) offers improved tolerability and bioavailability over the parent compound when tested in a phase 1 clinical trial in Alzheimer's disease patients.[93] Finally, an amide prodrug derivative of sobetirome, a selective thyroid receptor β agonist, increased brain penetration and lowered CNS very-long chain fatty acids (VLCFA) compared to the parent compound in an ABCD1 knock-out mouse model for the human disease adrenoleukodystrophy.[94] Adding moieties to centrally acting molecules has proved a successful strategy for increasing brain penetration of molecules not otherwise sufficiently brain penetrant in the past and will continue to do so in the future.

Invasive routes of administration

For antisense oligonucleotides, gene therapies, stem cells or viral vectors, biochemical modifications will not always be feasible approaches to increase brain penetration. In those cases, invasive routes allowing direct CNS distribution can be considered. There are various invasive routes by which drugs can be deposited locally or more widespread throughout the central nervous system, bypassing the blood-brain barrier. A prior clinical trial of intraputamenal delivery of glial cell-line derived neurotrophic factor failed to meet its primary endpoints in patients with PD [95], but limitations in diffusion of the drug from the catheter tip to the surrounding brain tissue might have contributed to the failure.[96] Newer trials have changed the infusion

techniques and protocols, but have not achieved an objective response on the primary endpoint.[97] Intraventricular administration presents an option to deliver a drug more uniformly throughout the brain. Sustained intraventricular infusion during 6 weeks of a human IgG1 antibody targeting β-secretase (BACE-1) in non-human primates led to widespread distribution throughout the brain parenchyma, albeit with large differences in drug concentrations between sites in close proximity to the CSF surface and deep parenchymal structures.[98] Intraventricular infusion over hours has been unable to show significant drug penetration in deeper brain areas at a distance from the CSF compartment.[99] These results suggest diffusion as the primary mechanism of distribution following intraventricular infusion.[62] Intrathecal injection of drug into the lumbar subarachnoid space presents an invasive, yet relatively easy method of delivering drug into the central nervous system. More so than intraventricular infusion, distribution from the lumbar subarachnoid region to the cerebrum is complicated by the distance and direction of CSF flow. Currently, intrathecal injection into the lumbar subarachnoid space has only been proven beneficial when the drug target is on the surface of the brain or spinal cord, such as intrathecal chemotherapy to treat leptomeningeal metastases [100] or SMN2 splice site modulatory antisense oligonucleotide therapy to treat motor neuron disease in spinal muscular atrophy.[101,102] Procedures to improve this method of administration are discussed in the next section.

CSF flow dynamics in relation to target site distribution for intrathecally administered compounds

The highly selective permeability of the blood-brain barrier has hampered drug development for central nervous system disorders. The blood-brain barrier isolates the interstitial fluid space (surrounding neurons and glial cells) from the plasma fluid space.[103] The perivascular spaces around intracerebral arteries (Virchow-Robin spaces) are in direct continuity with the perivascular spaces around subarachnoid arteries, connecting the interstitial fluid space to the CSF space.[104,105] Pathological changes to the blood-brain barrier further complicate drug delivery to the brain in neurodegenerative diseases, due to concomitant vascular changes such as endothelial degeneration, disrupted BBB transport systems and perivascular inflammation.[106] This has made intrathecal dosing an attractive method to overcome blood-brain barrier selectivity when targeting the central nervous system in neurodegenerative diseases. However, there are several limitations to this approach.

Enhancing brain distribution following lumbar intrathecal injection

There is significant variability in CSF concentrations between individuals after intrathecal drug administration.[107,108] The variability appears to depend on the size and type of drug compound administered. This is supported by rodent studies, where half-lives of compounds with various biochemical properties differed greatly.[109] Clearance of CSF, its flow dynamics, mode of delivery and binding to proteins such as albumin, are other important factors influencing the interindividual variability.[109,110] All these variables should be taken into consideration when targeting pharmacologically active concentrations of intrathecally administered drugs in the central nervous system. To optimize intrathecal dosing and enhance neuraxial spread, two parameters that can be modified are the volume of the bolus, and adjusting the dose to the protein binding of the compound.[109,111] In a computational fluid dynamics model of neuraxial distribution after lumbar intrathecal administration, an increase in bolus volume enhanced the intracranial distribution.[112] Neuraxial CSF movement can also be enhanced by respiration and cardiac driven convective forces, and this finding led to studies on the effects of thoracic percussive treatment on the rostral distribution of intrathecal injection volumes, an intervention that could be applied in clinical studies.[113] From the above, it can be concluded that for many compounds, there is no clear understanding of the CSF distribution after intrathecal administration. More research into this area is required to improve our mechanistic understanding of cerebrospinal flow dynamics and achieve enhanced brain distribution of intrathecally administered compounds.

Conclusion

Future disease modifying therapeutics for neurodegenerative diseases will not only be novel in their mechanism of action and central nervous system target site, but also in their clinical development trajectories. Rational biomarker development together with advanced techniques for blood-brain barrier penetration and brain delivery, will turn out to be decisive in advancing only the most promising molecules to the next stages of development.

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