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Ambroxol – a leading candidate for treatment of neurodegenerative disorders

The pathophysiology of Alzheimer's Disease (AD) begins with disruptions to neuronal proteostasis. Removal of proteins such as beta amyloid and tau from the intracellular compartments of neurons become dysregulated, driving the formation of cytotoxic protein aggregates (Nixon, 2013). Although the pathology begins inside neurons, current pharmacological efforts have been directed primarily at the removal of extracellular aggregates of beta amyloid and tau that are the most obvious markers of the disease. This mismatch presents opportunities for development of drugs that improve neuronal handling of intracellular cytotoxic proteins.

Preclinical evidence suggests that ambroxol may aid neurons in clearing intracellular waste, and its favorable safety and pharmacokinetic profile supports its use in treating AD. Originally developed as a mucolytic for treatment of respiratory disease (Malerba and Ragnoli, 2008), more recent evidence has shown that ambroxol impacts the autophagic cellular waste disposal system in multiple beneficial ways (Maegawa et al., 2009; McNeill et al., 2014; Magalhaes et al., 2018; Mullin et al., 2020). Early realizations of its potential included successful treatment of patients of the lysosomal storage disorder, Gaucher's disease (Zimran et al., 2013; Narita et al., 2016; Istaiti et al., 2021). Over the history of its clinical use, Ambroxol has proven to be an exceptionally safe drug that is well-tolerated at high doses (Mullin et al., 2020; Istaiti et al., 2021) even by medically fragile patient populations (lung in young neonate study). Ambroxol also has exceptionally high brain penetrance when taken orally (Mullin et al., 2020), simplifying administration of clinical trials.

Because of these findings, Ambroxol has been selected as a prime candidate for repurposing against multiple neurodegenerative disorders. Most famously, the International Linked Clinical Trials program selected Ambroxol as one of a small selection of Federal Drug Administration-approved drug candidates for repurposing in the treatment of Parkinson's disease (PD)(Stott et al., 2021). Following promising phase 2 trial results (Mullin et al., 2020), the program partnered with the Van Andel Institute and the John Black Charitable Foundation to fund a phase 3 trial Ambroxol for the treatment of PD. Only 5 other candidate drugs have ever reached this stage of clinical investigation in PD. Ambroxol was further selected by a panel of experts for the RENEWAL program as the most promising candidate for clinical trials in Lewy Body Dementia (O'Brien et al., 2022). Finally, Ambroxol is under evaluation as a treatment for amyotrophic lateral sclerosis based on strong preclinical study results (Bouscary et al., 2019).

Ambroxol's effects on the autophagy-lysosomal pathway

In all of these cases, a panel of experts has determined that ambroxol shows superior promise in the treatment of conditions characterized by an inability to break down cytotoxic proteins. Much of the evidence supporting this notion indicates that Ambroxol exerts profound effects on the cellular system specialized for waste disposal – the autophagy-lysosomal pathway (Maegawa et al., 2009; McNeill et al., 2014; Fois et al., 2015; Magalhaes et al., 2018; Mullin et al., 2020)

The first evidence of Ambroxol's unexpected effects on the autophagy-lysosomal pathway came from screens demonstrating its ability to regulate enzymes that process sphingolipids. Sphingolipids are

molecules that are largely processed by enzymes resident to lysosomes that become highly dysregulated in AD patients as well as those in the prodromal stages of AD(Byeon et al., 2021; Baloni et al., 2022). Ambroxol was identified as the top hit in a library screen as a molecular chaperone of glucocerebrosidase (GCase)(Maegawa et al., 2009). Increased GCase activity has been shown to increase the capacity of lysosomes to degrade protein aggregates such as those formed by alpha-synuclein(Mazzulli et al., 2016). Ambroxol has been shown to increase the activity of GCase in human patients of Gaucher's disease (Narita et al., 2016) as well as idiopathic PD (Mullin et al., 2020) with positive effects on reported quality of life in each case. Ambroxol has also been shown to inhibit sphingomyelinase (Carpinteiro et al., 2021) an enzyme that becomes over-active in patients with AD(Baloni et al., 2022). Increased sphingomyelinase activity is associated with enhanced amyloidogenic processing of the amyloid precursor protein by gamma secretases (Grimm et al., 2005). Conversely, inhibition of sphingomyelinase has been proven to rescue synaptic deficits in mouse models of AD (Baloni et al., 2022).

Ambroxol also exerts a direct effect on autophagy by increasing Transcription Factor EB activity. Ambroxol is known to accumulate in lysosomes, the primary degradative organelle of the cell. In low pH environments like the interior of lysosomes, Ambroxol acts as a charged buffer of protons, causing a transient rise in pH (Fois et al., 2015). This process mobilizes intraorganellar calcium stores which results in the activation of lysomome-associated transcription factor EB (TFEB). Activated TFEB relocalizes to the nucleus where it initializes transcription of a gene network with master regulatory control over lysosomal and mitochondrial biogenesis (Medina et al., 2015). Upregulation of this gene network results in increased expression of lysosomal enzymes such as cathepsin D (Sardiello et al., 2009) known to degrade lysosomal substrates including alpha synuclein, beta amyloid, and hyperphosphorylated tau (Martini-Stoica et al., 2018; Suire and Leissring, 2021). Ambroxol-driven TFEB activity has been shown to enhance expression of these degradative enzymes in the lysosomes of multiple model systems (McNeill et al., 2014; Choi et al., 2018; Magalhaes et al., 2018). as well as markers of mitochondrial turnover (Magalhaes et al., 2018). In terms of AD pathology, bromhexine, the prodrug of ambroxol, enhances clearance of hyperphosphorylated tau from a neuroblastoma cell culture altered to express mutant human tau (Chauhan et al., 2015).

Increased TFEB activity also enhances regulated secretion of cytotoxic proteins using the cellular mechanisms of autophagy. This regulated release serves as a mechanism by which neurons remove hard to dispose of substances from their cytoplasm (Ponpuak et al., 2015). For instance, increased TFEB activity reduces intraneuronal hyperphosphorylated tau in a mouse model of AD via lysosomal exocytosis. Conversely, blocking TFEB in this model leads to reduced tau secretion but increased intracellular hyperphosphorylated tau deposits (Xu et al., 2020). Ambroxol has been shown to increase lysosomal exocytosis (Fois et al., 2015) and increases TFEB activity (McNeill et al., 2014; Migdalska-Richards et al., 2016; Magalhaes et al., 2018) and secretion of membrane-bound alpha synuclein in primary neural cultures (Magalhaes et al., 2018). Concordantly, human PD patients on high dose ambroxol regimen had increased levels of alpha synuclein in their cerebrospinal fluid and improvements to their motor function (Mullin et al., 2020).

Finally, ambroxol acts as a potent regulator of immune response in a manner that is beneficial for AD. Increases in pro-inflammatory cytokines such as interleukin 1β, 6, 8, and tissue necrosis factor alpha in

longitudinally sampled AD patient sera are predictive of poor clinical trajectory (Heneka et al., 2015). In contrast, heightened levels of anti-inflammatory cytokines such as interleukin 10 and adaptive immune-associated interferon-γ are predictive of slower cognitive decline in AD patients (Yang et al., 2022).

Studies across multiple organ systems, particularly the brain (Jiang et al., 2020), lungs (Takeda et al., 2016; Zhang et al., 2016; Kókai et al., 2021), and gut (Schneider et al., 2021; Cavalu et al., 2022) have confirmed that ambroxol reshapes inflammation and the immune response. For example, ambroxol has been shown to decrease expression of pro-inflammatory cytokines such as interleukins 1β, 6, 8, and tissue necrosis factor-α in lung tissue (Bianchi et al., 1990; Jang et al., 2003; Wang et al., 2011) as well as reductions in pathways upstream of inflammasome activation such as NF-κB in the gut (Cavalu et al., 2022). This suppression of inflammation is due at least in part to ambroxol's ability to scavenge free radicals (Peroni et al., 2013) and may also depend on ambroxol's effects on autophagy as activation of TFEB drives degradation of key mediators of inflammation including components of the inflammasome (Shi et al., 2012; Deretic, 2021). In addition to its suppressive effect on pro-inflammatory signaling pathways, ambroxol has been shown to drive upregulation of interleukin 10, 12, and interferon-γ in lung tissue in response to pathogen and ovalbumin challenge (Takeda et al., 2016; Kókai et al., 2021) indicating enhanced anti-inflammatory and adaptive immune response. Together, these effects on immune response may account for preclinical observations of reduced inflammation in models of ulcerative colitis (Schneider et al., 2021) and reduced microlglial activation in a model of intracerebral hemorrhage (Jiang et al., 2020).

A unique drug leads to unique possibilities

Many of the cellular mechanisms addressed by ambroxol are targets for current or future candidate compounds for the treatment of AD. A recent survey of current clinical trials of AD modifying drugs fall into target categories including metabolism/bioenergetics, proteostasis, oxidative stress, and inflammation (Cummings et al., 2022). Several startups with GCase activators in their intellectual property portfolio have shown preclinical data indicating an interest in positioning their compound for the treatment of neurodegenerative disorders (Gain Therapeutics, 2023; Vanqua Bio, 2023). Given the complexity of AD pathophysiology, it is unsurprising that such approaches targeted to diverse aspects of underlying cell biology are being tested.

Ambroxol has demonstrated activity in many of the cellular processes that become dysregulated in AD. As discussed above, ambroxol works predominantly to regulate the intracellular response to cytotoxic protein species. As such, ambroxol serves a complimentary and potentially synergistic function to the therapeutic approaches like those exemplified by lecanemab, which is a monoclonal antibody that attaches to and removes aggregated beta amyloid from the brain parenchyma (van Dyck et al., 2023). It has an excellent safety profile with gram-level dosage well-tolerated by patients and can be taken with many standard-of-care drugs with minimal risk of adverse interactions. It has been approved for decades for use in the treatment of bronchopulmonary disease, further mitigating the risk of unexpected long-term side effects arising in chronic use cases. Ambroxol is therefore a string candidate compound for the treatment of AD.

Additionally, because the cellular mechanisms targeted by ambroxol are broad, there are many disease states for which it may be indicated. In addition to the neurodegenerative and lysosomal storage disorders previously mentioned, ambroxol is under investigation for the treatment of neuropathic pain (Russo et al., 2022), ulcerative colitis (Schneider et al., 2021), as adjuvant in tuberculosis treatment regimens (Choi et al., 2018), and infantile neuroaxonal dystrophy (Lin et al., 2023) to name just a few. When considered alongside the application of ambroxol in AD, future clinical indications add considerably to the potential value of this unique compound.

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