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Drug repositioning in neurodegeneration: An overview of the use of ambroxol in neurodegenerative diseases

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ABSTRACT

Amyotrophic lateral sclerosis (ALS) is the most common motor neuron disease in adults. While it is primarily characterized by the death of upper and lower motor neurons, there is a significant metabolic component involved in the progression of the disease. Two-thirds of ALS patients have metabolic alterations that are associated with the severity of symptoms. In ALS, as in other neurodegenerative diseases, the metabolism of glycosphingolipids, a class of complex lipids, is strongly dysregulated. We therefore assume that this pathway constitutes an interesting avenue for therapeutic approaches. We have shown that the glucosylceramide degrading enzyme, glucocerebrosidase (GBA) 2 is abnormally increased in the spinal cord of the SOD1^{G86R} mouse model of ALS. Ambroxol, a chaperone molecule that inhibits GBA2, has been shown to have beneficial effects by slowing the development of the disease in SOD1^{G86R} mice. Currently used in clinical trials for Parkinson's and Gaucher disease, ambroxol could be considered as a promising therapeutic treatment for ALS.

1. Sphingolipids in Amyotrophic Lateral Sclerosis

Amyotrophic Lateral Sclerosis (ALS) is the most common motor neuron disease in adults, affecting approximately 30,000 people worldwide (Petrov et al., 2017). It is characterized by the selective death of cortical motor neurons and bulbar and spinal motor neurons, causing progressive paralysis and the death within 2–5 years after diagnosis (van Es et al., 2017). To date, no curative treatment is available and symptomatic treatments have limited effectiveness. Two drugs are currently indicated for the treatment of ALS, Rilutek® and Edaravone. These molecules increase the life expectancy of patients by a few months (Abe et al., 2017; Dorst et al., 2018).

Historically, ALS has been classified as a strict motor neuron disease. However, in recent years, several studies have demonstrated the contribution of metabolic alterations in the development of pathology in ALS patients. Indeed, a high incidence of dyslipidemia and hypermetabolism have been identified in ALS patients (Desport et al., 2001; Dupuis et al., 2011; Pradat et al., 2010). These metabolic presentations are clinically associated with the severity of symptoms (Jésus et al., 2018; Steyn et al., 2018). Therefore, ALS is no longer considered as just a motor neuron disease, but rather, a systemic disease. The identification of characteristic markers allowing for early diagnosis is therefore essential in ALS. Metabolomic studies have focused on the metabolism of sphingolipids. Sphingolipids are complex lipids, involved in several vital functions of neuronal cells, including their maturation, the formation of myelin sheaths or even the transmission of neuronal signals (Olsen and Færgeman, 2017).

In 2002, a seminal study reported that an accumulation of sphingolipids triggers the death of motor neurons in a transgenic mouse model of ALS, as well as in ALS patients (Cutler et al., 2002). This study led the scientific community to focus on possible metabolic signatures in ALS patients. In 2015, Dodge and collaborators showed abnormal levels

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of glycosphingolipids in the spinal cord of patients with ALS and in the SOD1^{G93A} mouse model of ALS (Dodge et al., 2015). In line with this, combined lipidomic and transcriptomic studies have demonstrated that there is an alteration in the levels of sphingolipids in the spinal cord of the SOD1^{G86R} mouse model of ALS (Henriques et al., 2018, 2015). These data suggest that lipid screening of the cerebrospinal fluid of ALS patients could serve as a means to identify characteristic signatures of the disease, with the view to provide information on its evolution (Blasco et al., 2017). Of interest, the disturbances in lipid metabolism appear to more specifically involve the degradation pathways of glucosylceramide (GlcCer), which are also involved in the pathophysiology of other diseases such as Parkinson's and Gaucher disease (Mullin et al., 2019). As such, deregulation of GlcCer could play a key role in the pathophysiology of ALS, and therefore represent a therapeutic target.

Henriques et al. (2017) showed an improvement in functional recovery in a model of nerve compression and delayed disease onset in SOD1^{G86R} mice, after the administration of Conduritol B Epoxide (CBE), an irreversible inhibitor of the GBA1 and GBA2, two beta-glucocerebrosidase enzymes (GCases). These data suggest that GlcCer strongly contributes to the stability of the motor unit. However, the use of CBE should be avoided because of its long-term toxicity. Thus, to study the contribution of GlcCer disturbances to the neurodegenera- tive process in ALS, we used pharmacological approaches to regulate the levels of GlcCer by targeting its degradation via GBA1 and GBA2.

2. Ambroxol: a promising drug candidate for therapeutic repositioning

Ambroxol is a generic expectorant and mucolytic drug used in the treatment of respiratory tract diseases (cough, bronchitis, etc.). Ambroxol is one of the synthetic derivatives of vasicine, the active substance in the plant Adhatoda Vasica, a plant used for respiratory indications in ancient India (Malerba and Ragnoli, 2008). Ambroxol is approved for use in 74 countries for the treatment of respiratory diseases, and in particular, for its fluidizing properties of bronchial secretions. Its favorable safety profile, even at high doses, and the low risk of side effects allow for ambroxol to be sold over the counter in most of the European Union. Its safety and tolerance have made it possible to determine the various properties of ambroxol.

Ambroxol limits the production of inflammatory cytokines (Jang et al., 2003; Ottonello et al., 2003), protects against oxidative stress by degrading free radicals which are toxic to cells (Felix et al., 1996; Lee et al., 2002), and has anesthetic properties for the treatment of sore throats (Schutz et al., 2002). The anesthetic and analgesic effects of ambroxol are explained by its action on the voltage-gated sodium channels, and more specifically on the Nav1.8 and Nav1.2 channels (Gaida et al., 2005; Weiser and Wilson, 2002). In addition, ambroxol is also described as being capable of modulating GCase activity (Shanmuganathan and Britz-McKibbin, 2011). Thus, due to the activity of ambroxol on GCase, its possible neuroprotective effects in neurodegenerative or lysosomal overload diseases deserve to be studied.

To counteract an enzymatic defect found in incurable diseases, it is possible to implement *Pharmacological Chaperone Therapy*. This innovative approach uses small molecules capable of entering cells and improving or restoring the activity of a failed enzyme. Limiting lysosomal overload or the formation of toxic aggregates responsible for the death of neurons are favorable arguments for using this therapy (Arakawa et al., 2006; Brooks, 2007; Fan, 2003; Parenti et al., 2015). Ambroxol crosses the blood-brain barrier, with a volume of distribution of 7 L/kg; this ensures widespread tissue distribution (Narita et al., 2016; Yang et al., 2015), thereby allowing for it to be considered in this new therapeutic strategy with the aim of modifying GlcCer and ceramide levels in the central nervous system.

Parkinson's and Gaucher diseases are two pathologies in which a toxic accumulation of lysosomal GlcCer is observed in the nervous system (Table 1). This accumulation occurs due to a deficiency in GBA enzyme activity, which may be due to the multiple mutations described for GBA1 (Migdalska-Richards et al., 2016). Thus, chaperone molecules such as isofagomine, which may bind to the mutant enzyme, were developed to facilitate transport to lysosomes and to restore activity in the acidic environment (Lieberman et al., 2009). The goal of current burgeoning therapeutic strategies is to use ambroxol as a chaperone molecule to restore the enzymatic activity of GBA1 by improving its presentation to the lysosome (Maegawa et al., 2009). This approach has demonstrated benefits in Gaucher disease: ambroxol has been shown to increase the enzymatic activity of GBA1, reduce the myoclonus observed in affected patients, and improve their motor functions (Narita et al., 2016). Ambroxol was recently approved to progress to a phase 2 clinical trial in patients with Parkinson's disease. This trial, once again, highlights the safety of the molecule, even at high doses, and confirms that ambroxol has potential for targeting the sphingolipid pathway, and in particular the activity of GCases in the nervous system (Mullin et al., 2020).

In addition to its chaperone activity on GBA1, ambroxol has the capacity to inhibit the enzymatic activity of a closely related enzyme: GBA2, which is located at the endoplasmic reticulum and the plasma membrane. In the SOD1^{G86R} mouse model of ALS, we have detected an abnormal increase in the GBA2 enzyme activity in the spinal cord (Bouscary et al., 2019). To offset this increase, we used ambroxol to inhibit GBA2 activity in SOD1^{G86R} mice. We have shown that ambroxol slows the time to onset of the first motor symptoms of the disease, and significantly extends the lifespan of transgenic mice. In this model of ALS, ambroxol limits the destruction of the integrity of neuromuscular junctions (NMJs) and also prevents muscular denervation. In addition, we have shown that ambroxol stimulates axonal plasticity and motor

Table 1
Summary of major clinical studies using ambroxol in given neurodegenerative diseases.

Pathology	Impairment	Patients/ mice	Effects of AMB	References
Gaucher disease	Accumulation of GlcCer due to a deficit in GBA1 activity	Patients	Safe; effective; no side effects	Zimran et al. (2013)
		Patients	Improves motor and neurologic function; increases GBA1 activity	Narita et al. (2016)
Parkinson's disease	Accumulation of α-synuclein due to deficit in GBA1 activity	Patients	Reduces alpha-synuclein levels	Mullin et al. (2020)
Amyotrophic Lateral Sclerosis	Dysregulation of sphingolipid metabolism in spinal cord and CSF	Patients	N/A	(Dodge et al., 2015; Henriques et al., 2017, 2015)
	Dysregulation of sphingolipid metabolism in spinal cord	Mice	Slows the onset of motor symptoms; improves motor functions; extends survival; promotes axonal growth and neuritic network	Bouscary et al. (2019)

This table lists some clinical trials carried out in patients with Gaucher or Par - kinson's disease and pre-clinical investigations in ALS. The pathophysiological damages of the metabolism of sphingolipids are presented. The effects of ambroxol are shown. Currently, there is no clinical trial assessing the therapeutic benefit of ambroxol in patients with ALS.
AMB: Ambroxol; GBA1: Glucocerebrosidase; CSF: Cerebrospinal fluid; ALS: Amyotrophic Lateral Sclerosis.

recovery in a model of nerve compression. In support of these *in vivo* results, we have also shown that ambroxol promotes the elongation of the neurite network and the formation of NMJs in an *in vitro* model of motor units (Bouscary et al., 2019). Thus, in ALS, we hypothesize that ambroxol can interact indirectly, via gangliosides, with neurotrophic receptors responsible for the growth and survival of neurons. Indeed, studies suggest that certain sphingolipids such as GM1 gangliosides, (an oligosugar GlcCer derivative), have a high affinity for neurotrophin receptors (Ledeen and Wu, 2018) by interacting with Tropomyosin receptor kinase A (TrkA) (Chiricozzi et al., 2019). TrkA is also known as neurotrophic kinase receptor A which is a kinase that binds nerve growth factor and triggers a signalling pathway that leads to cell differentiation (Deinhardt and Chao, 2014). The interaction between GM1 and TrkA improves neuronal migration, dendritic arborisation, and axonal growth (Di Biase et al., 2020).

Interestingly patients producing IgM antibodies to GM1 develop multifocal motor neuropathy, an immunological disease resembling ALS (Harschnitz et al., 2014). We have shown that GM1, as labelled by cholera toxin, is lost at the NMJ of SOD1^{G86R} mice at very early stage of the disease (Henriques et al., 2017). It is therefore possible that the beneficial effects of ambroxol observed in neurodegenerative diseases could be explained by its ability to modify gangliosides critical to neurotrophic/neuroprotective processes.

Overall, our work to date has highlighted the previously unknown neuroprotective properties of ambroxol in a mouse model of ALS. Combined with data from the literature, these promising results make it possible to consider drug repositioning of ambroxol for the treatment of ALS. Ambroxol has obtained orphan drug designation for ALS from the European Medicines Agency. Thus, the rapid establishment of the clinical trials in the field of ALS could be facilitated by the availability of ambroxol, although the key question of human posology must be addressed.

Contribution authors

AB and CQ conducted the literature search and wrote the manuscript. All authors critically reviewed the manuscript.

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