Ambroxol clinical trials and Experimental Models of Parkinson Disease; Review Article

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Abstract

A clear understanding of Parkinson's disease (PD) pathogenesis would aid in the development of therapies that may be able to slow or prevent the progression of this neurodegenerative disorder. Ambroxol (AMB), an FDA-approved drug for the treatment of respiratory diseases is currently under investigation in PD patients. AMB acts as a chaperone to convert glucocerebrosidase (GCase) to its full-length form and facilitates trafficking of GCase through the endoplasmic reticulum (ER). AMB is reported to increase GCase activity in brainstem, midbrain and cortex of alpha synuclein (α-syn) transgenic mice, to improve lysosomal biochemistry and to rescue defective GCase in GBA1 mutation-linked PD, GBA1 is the gene encoding glucocerebrosidase. Moreover, AMB not only increased GCase activity in wild type (WT) mice but also reduced α-syn levels and restored GCase activity in mice overexpressing human α-syn. Additionally, AMB treatment has been shown to improve the translocation of mutant GCase to the lysosome, increasing GCase activity in the lysosomes of fibroblast and lymphoblasts carrying GBA1 mutations. Also, in control fibroblasts treated with AMB, the activity of GCase was increased. Furthermore, AMB increased GBA1 mRNA, protein levels and activity, as well as, several lysosomal proteins such as cathepsin D, lysosomal marker (LAMP1), the GCase transporter lysosomal integral membrane protein type-2 (LIMP2) and GCase endogenous activator saposin C. Also, nuclear translocation of transcription factor EB (TFEB), a master regulator of lysosomal biogenesis was increased upon treatment with AMB. That is associated with activation of macroautophagy. Experimental models can be categorized into two main flavors: toxic and genetic (and sometimes, both approaches are combined)

Keywords: Ambroxol clinical trials, Parkinson Disease

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Introduction

Parkinson's disease (PD) is the second most common progressive neurodegenerative disorder affecting millions of elderly worldwide **(1).**

This disease is marked by a selective and progressive degeneration of dopaminergic neurons and the presence of proteinacious cytoplasmic inclusions known as Lewy bodies (LB) in the neurons of the affected brain region **(2).**

There are two types of symptoms associated with PD, premotor symptoms such as hyposmia, constipation, Rapid eye movement (REM) sleep behavior disorder and depression that may antecede motor symptoms for years and motor symptoms such as tremor at rest, rigidity, bradykinesia and postural instability. Diagnosis of PD during early premotor phase symptoms may help in effective treatment by applying measures to delay the overall progression of disease **(3).**

Levodopa preparations, dopamine agonists, and monoamine oxidase-B (MAO-B) inhibitors are useful initial therapies for PD patients. For young individuals with prominent tremor, anticholinergic agents are also useful. Catechol-O-methyl transferase inhibitors and MAO-B inhibitors block enzymes that degrade dopamine, prolonging the benefits of levodopa. Dyskinesias are treated by reducing dopaminergic medications or adding amantadine **(4).**

Additionally, effective exercise interventions for PD include gait and balance training, progressive resistance training, treadmill exercise, strength training, aerobic exercise, physiotherapy, occupational therapy, and speech therapy are useful **(5).**

Moreover, deep brain stimulation involves surgical placement of unilateral or bilateral leads transcranially in the subthalamic nucleus or the globus pallidus interna. These leads are attached to a battery in the chest, similar to a pacemaker battery. Following surgical recovery, individuals with deep brain stimulation attend programming visits to optimize stimulation parameters and medications. It is used to treat the effects of wearing off that involve motor symptoms, tremor, and dyskinesia **(4).**

In addition to selective serotonin and norepinephrine reuptake inhibitors, tricyclic antidepressants may all be useful for treating depression in PD. But, if psychosis persists and requires treatment, there are 3 main options: pimavanserin, clozapine, and quetiapine **(6).**

Furthermore, multiple randomized clinical trials show that clozapine improves PD psychosis via serotonergic and dopaminergic pathways. Sildenafil is useful for treating sexual dysfunction and botulinum toxin injections have the most evidence for treating sialorrhea in PD, but glycopyrrolate and sublingual atropine are also prescribed **(7).**

Ambroxol clinical trials

Ambroxol (AMB), an FDA-approved drug for the treatment of respiratory diseases is currently under investigation in PD patients. It acts as a chaperone to convert GCase to its full-length form and facilitates trafficking of GCase through the ER. AMB is reported to increase GCase activity in brainstem, midbrain and cortex of α-syn transgenic mice, to improve lysosomal biochemistry and to rescue defective GCase in GBA1 mutation-linked PD cells**.** Moreover, AMB not only increased GCase activity in WT mice but also reduced α-syn levels and restored GCase activity in mice overexpressing human α-syn **(8).**

Besides being a GCase chaperone, AMB, also acts on other pathways, such as mitochondria, lysosomal biogenesis, secretory pathway being antioxidant and anti-inflammatory. Additionally, AMB treatment has been shown to improve the translocation of mutant GCase to the lysosome, increasing GCase activity in the lysosomes of fibroblast and lymphoblasts carrying GBA1 mutations. Also, in control fibroblasts treated with AMB, the activity of GCase was increased **(9).**

Furthermore, AMB increased GBA1 mRNA, protein levels and activity, as well as, several lysosomal proteins such as cathepsin D, LAMP1, the GCase transporter LIMP2 and GCase endogenous activator saposin C. Also, nuclear translocation of TFEB, a master regulator of lysosomal biogenesis was increased upon treatment with AMB. That is associated with activation of macroautophagy **(10).**

Moreovre, **Maor et al. (11)** reported that AMB treatment in fly models that carried GBA1 mutations and presented a PD phenotype, showed that AMB can reverse mutant GBA1 PD like phenotype.

Additionally**, Migdalska-Richards et al. (8)** showed that the mice that expressing WT, mutant GBA1, or overexpressing human α-syn had increased GCase activity levels in brain upon treatment with AMB. Also, α-syn protein levels are decreased in mices upon AMB treatment. Interestingly, this observation in α-syn mice coupled with decreased GCase activity observed in aged and sporadic PD brains support the hypothesis that with AMB is a potential disease modifying therapy for the treatment of not only PD with GBA1 mutations and gaucher disease (GD), but also sporadic forms of PD **(12).**

Also, AMB in concentrations greater than 1μM accumulates in lamellar bodies, which are the acidic Ca^{2+} stores in pneumocytes, much like lysosomes, leading to the release of Ca^{2+} and increased exocytosis. Notably release of lysosomal Ca^{2+} was reported to activate calcineurin, which can then dephosphorylate and thus activate TFEB. TFEB activation has been implicated in regulating lysosomal exocytosis, by increasing the pool of lysosomes in proximity to the plasma membrane and promoting the fusion of lysosomes with the plasma membrane. In lysosomal

storage diseases, lysosomal exocytosis has been described as a beneficial event that relieves the cells from storage material and degradation products **(13)** .

Furthermore, lysosomal exocytosis has also been linked to plasma membrane repair, neurite outgrowth, improved phagocytosis and the release of signalling molecules and digestive enzymes. Lysosomal exocytosis has also been reported to generate specific plasma membrane domains containing LC3-II. If this occurs in neurons, it might contribute to the higher LC3-II levels observed following AMB treatment that were not increased further by bafilomycin A1 treatment **(12)**.

Moreover, clinical trials for AMB are under process to establish it as a novel disease-modifying agent for the treatment of dementia and cognitive impairment in PD due to its role in increasing GCase activity. In this regard, **Mishra & Krishnamurthy, (14)** found that ambroxol attenuated motor deficits and normalized GCase enzymatic activity, striatal dopamine(DA) concentration, and Nissl bodies in rats. Also, it reduced the aggregation of α-syn toxic oligomers, mitochondrial dysfunction, and cytochrome-C release from mitochondria and led to inactivation of intrinsic pathway of apoptosis.

Experimental Models of Parkinson's disease

Experimental models can be categorized into two main flavors: toxic and genetic (and sometimes, both approaches are combined)

I- Toxic Models of Parkinson's disease

a) 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)

The most popular species, besides primates, is the mouse, as rats were found to be resistant to this toxin. A number of intoxication regimens or administration methods have been used over the years in mouse and in primates. In both species, MPTP primarily causes damage to the nigrostriatal DA pathway with a profound loss of DA in the striatum and Substantia nigra (SN). Like in PD, MPTP causes greater loss of DA neurons in SN than in ventral tegmental area (VTA) or retrorubral field and, at least in monkeys treated with low doses of MPTP, greater degeneration of DA nerve terminals in the putamen than in the caudate nucleus. An often raised weakness with this model is the lack of LB **(15)**.

b) 6-hydroxydopamine (6-OHDA)

Like MPTP, 6-OHDA is a selective catecholaminergic neurotoxin that is used, mainly, to generate lesions in the nigrostriatal DA neurons in rats. Since 6-OHDA cannot cross the BBB, systemic administration fails to induce PD. So, this induction model requires that 6-OHDA be injected (typically as a unilateral injection) into the SN, medial forebrain bundle or striatum. The effects resemble those in the acute MPTP model, causing neuronal death over a brief time course (12 h to 2–3 days). The intrastriatal injection of 6-OHDA causes progressive retrograde neuronal

degeneration in the SN and VTA. However, like in the MPTP model, 6-OHDA does not produce LB-like inclusions in the nigrostriatal pathway. Also, drug-free sensorimotor behavioral tests have been developed in both rat and mice that may be helpful for the preclinical testing of new symptomatic strategies **(16).**

c) Rotenone

Rotenone is a potent lipophilic inhibitor of complex I of the mitochondrial electron transport chain. Chronic rotenone exposure has been demonstrated to induce mitochondrial respiratory chain inhibition and dopaminergic neuronal loss. At low doses, rotenone was found to alter calcium signaling and induce oxidative stress, apoptosis and α-syn aggregation ("Lewy neurites"), which is also a characteristic of early stages of PD **(17).**

Furthermore, chronic administration of low doses of rotenone was reported to induce motor anomalies even in animals that do not develop histological signs of PD. Also, chronic exposure to rotenone, which causes dopaminergic damage, may also lead to peripheral motor neuropathy reflected by decreased the motor nerve conduction velocity (MCV) which might be considered a viable biomarker for central dopaminergic neuronal damage **(17).**

Moreover, chronic rotenone intoxication, that causes enhanced oxidative and nitrosative stress and induces mitochondrial dysfunction and ultrastructural damage, also resulted in apoptotic cell death in the striatum via a cytochromec/caspase-3 signaling cascade **(18).**

Additionally, striatal tyrosine hydroxylase immunoreactivity (TH-IR) was found to be markedly decreased in rats exposed to high doses of rotenone. However, loss of striatal TH-IR was not correlated with motor behavior in individual rat neurotoxic damage **(17).**

Also, rotenone increased microglial activation in both the SN and striatum in rats, activated microglia via the nuclear factor kappa B (NF-κB) signaling pathway and induced neuronal death by the microglial phagocytosis of neurons **(19).**

d) Paraquat/Maneb

Although the idea that the herbicide paraquat, may cause PD in humans has attracted some interest, at this time, as pointed out by Berry and collaborators, epidemiological and clinical evidence that paraquat may cause PD is inconclusive and, the same view seems to apply to the fungicide Maneb **(20).**

However, some researchers reported that, following the systemic application of paraquat, mice exhibited reduced motor activity and a dose-dependent loss of striatal TH fibers and SN neurons with relative sparing of the VTA **(21).**

Additionally, Maneb has been shown to decrease locomotor activity and produce SN neurons loss and potentiate both the MPTP and the paraquat effects. However, as with rotenone, this

model showed contradictory results, variable cell death and loss of striatal dopamine content (22).

e) Amphetamine-Type Psychostimulants

Some amphetamine derivatives such as methamphetamine (METH) and 3, 4-methylenedioxymethamphetamine (MDMA) also have neurotoxic effects on the nervous system causing not only functional deficits but also structural alterations. The first study to show DA depletion in rats following repeated, high-dose exposure to METH was conducted by **Kogan et al., (23)**. Moreover, **Sonsalla et al. (24)** showed that high-dose treatment with METH in mice resulted in a loss of DA cells in the SN. Since then, several studies have reported selective DA or serotonergic nerve terminal as well as SN neuronal loss in rodents, primates or even guinea pig following the administration of very high doses of METH. Additionally, 3, 4-MDMA can also elicit significant neurobehavioral adverse effects. In mice, repeated administration of MDMA produces degeneration of DA terminals in the striatum and TH+ neuronal loss in the SN **(25).**

II- Genetic Models of PD

A number of cellular and molecular dysfunctions have been shown to result from these gene defects like fragmented and dysfunctional mitochondria**,** altered mitophagy**,** ubiquitin–proteasome dysfunction, and altered Reactive oxygen species (ROS) production and calcium handling. Some studies have reported alterations in motor function and behavior in mice. However, almost all of the studies evaluating the integrity of the nigrostriatal DA system in these genetic models failed to find significant loss of DA neurons thus, recapitulation of the genetic alterations in mice is insufficient to reproduce the final neuropathological feature of PD **(26)**.

1. α -Synuclein

α-syn was the first gene linked to a dominant-type, familial PD, called Park1, and is the main component of LB which are observed in the PD brain. Three missense mutations of α-syn, encoding the substitutions A30P, A53T, and E46K, have been identified in familial PD so far. Furthermore, the duplication or triplication of α-syn is sufficient to cause PD, suggesting that the level of α-syn expression is a critical determinant of PD progression **(27).**

Although a lot of behavioral alterations have been described in both the A30P and A53T mice**,** the mouse prion protein promoter failed to reproduce the cell loss in the SN or locus coeruleus **(28)**.

2. leucine rich repeat kinase (LRRK2)

Mutations in LRRK2 are known to cause a late-onset autosomal dominant inherited form of PD. Several mutations have been identified in LRRK2, the most frequent being the G2019S mutation, a point mutation in the kinase domain, whereas R1441C, a mutation in the guanosine triphosphatase domain, is the second most common **(29)**.

Additionally, LRRK2 knock out (KO) mice are viable and have an intact nigrostriatal DA pathway up to 2 years of age. Neuropathological features associated with altered neuronal structure or neurodegeneration were absent, but α-syn or ubiquitin accumulation has been reported in these mice **(26)**.

3. PTEN-induced putative kinase 1 (PINK1)

Mutations in the gene PINK1 cause another form of PD called PARK6. PINK1 KO mice have an age-dependent, moderate reduction in striatal DA levels accompanied by low locomotor activity, but do not exhibit major abnormalities in the DA neurons or striatal DA levels. These mice showed no LB formation or nigrostriatal degeneration for up to 18 months of age **(28)**.

However, in PINK1 KO mice, overexpression of α-syn in the SN resulted in enhanced dopaminergic neuron degeneration as well as significantly higher levels of α-syn phosphorylation at serine 129 at 4 weeks post-injection. Recently, a PINK1 null mouse with an exon 4-5 deletion displayed a progressive loss of DA in the striatum, but there was no degeneration in the SN **(30).**

4. Parkin (E3 ubiquitin ligase)

Mutations in Parkin are a cause of familial PD and are also seen in some young-onset sporadic PD cases. Several Parkin KO mice have been generated, typically produced by deletion at exon 3, exon 7, or exon 2 in the PRKN gene. However, they show no substantial DA-related behavioral abnormalities. Some of these KO mice exhibit slightly impaired DA release and reduced norepinephrine levels in the olfactory bulb and spinal cord with an abnormal nigrostriatal region but without loss of SNc neurons **(31)**.

5. Protein deglycase DJ-1

DJ-1 mutations are linked to an autosomal recessive, early onset PD. KO mice models of DJ-1 mice with a targeted deletion of exon 2 or insertion of a premature stop codon in exon 1 showed decreased locomotor activity, a reduction in the release of evoked DA in the striatum but no loss of SN DA neurons and no change of the DA levels. However, one line of DJ-1 KO mice showed loss of DA neurons in the VTA **(32).**

6. ATPase Cation Transporting 13A2 (ATP13A2)

Mutations in ATP13A2 (PARK9), encoding a lysosomal P-type ATPase, are associated with both Kufor–Rakeb syndrome (KRS) and neuronal ceroid lipofuscinosis. KRS has recently been classified as a rare genetic form of PD. Despite the accumulation of lipofuscin deposits in the SN and late-onset sensorimotor deficits, there was no change in the number of DA neurons in the SN or in striatal DA levels in aged Atp13a2 KO mice **(33)**.

7. Mitochondrial transcription factor A in DA neurons (MitoPark)

Conditional disruption of the gene for mitochondrial transcription factor A in DA neurons (MitoPark) resulted in a PD phenotype in mice that includes an adult-onset, slowly progressive impairment of motor function, DA neuron death, degeneration of nigrostriatal pathways and intraneuronal inclusions **(34).**

8. Autophagy-related genes (Atg7)

Cell-specific deletion of the essential autophagy gene Atg7 in midbrain DA neurons caused DA neuron loss in the SN at 9 months, accompanied by late-onset locomotor deficits. Atg7-deficient DA neurons in the midbrain also exhibited early dendritic and axonal dystrophy, reduced striatal DA content, and the formation of somatic and dendritic ubiquitinated inclusions **(35).**

9. Vesicular monoamine transporter 2 (VMAT2)

It has been suggested that VMAT2 defect may be an early abnormality promoting mechanisms leading to nigrostriatal DA neuron death in PD. VMAT2-deficient mice displayed a progressive loss of nigral DA and locus coeruleus cells, loss of striatal DA and α-syn accumulation **(36).**

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