Potential approaches for slowing down the progression of Parkinson's disease

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Potencijalni pristupi koji mogu usporiti napredovanje Parkinsonove bolesti

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Mentor: izv. prof. dr. sc. Rozi Andretić Waldowski

Ko-mentor: doc. dr. sc. Nicholas James Bradshaw

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SUMMARY

Parkinson's disease (PD) is the most common synucleinopathy and the second most common neurodegenerative disease worldwide. Its incidence usually increases with age and, due to extended lifespan and increased share of older population, it is estimated that almost 13 million people in the world will suffer from PD by 2040. These facts lead to a concern and need to find effective drugs that potentially cure the disease. PD is defined as progressive disorder in which comes to degeneration of dopaminergic neurons in substantia nigra pars compacta (SNpc) and afterwards is widespread in all parts of the brain. This neurodegeneration is recognized by stiffness, tremor and bradykinesia, but also by cognitive symptoms such as dementia, autonomic dysfunction, mood and sleep disorders. The biggest problem is that developing PD is not an outcome of a single factor. As for now, PD-linked missense mutations are identified on at least 20 genes. With genetic modifications, there are also environmental factors that were correlated with either increasing or decreasing the risk of PD. As synucleinopathy, PD is mainly characterised by the aggregation of α-synuclein (α-syn) that forms Lewy bodies (LB) and Lewy neurites (LN). Since it is localized in presynaptic terminals, it has been suggested that α-syn has a major role in regulating synaptic vesicle release. However, under pathophysiological conditions, it starts to aggregate into neurotoxic oligomers and fibrils that disrupt many functions on extracellular and intracellular level. Current therapy does not have possibility to modify the disease, but it manages symptoms and improves patient's quality of life. Levodopa is the most prescribed drug for PD patients because it converts to dopamine and increases its uptake. However, when its concentration in the plasma decreases, patient experiences a phenomenon called "wearing-off". Thereby, alternatives such as dopamine agonists, monoamine oxidase B (MAO-B) and catechol-O-methyltransferase (COMT) inhibitors were introduced to the market, but none of them showed better performance than

levodopa. There are also surgical procedures which improve levodopa phenomenon, although there is a greater risk of adverse side effects, complications during the procedure or even death. However, deep brain stimulation (DBS) proved to be successful in improving motor functions without severe outcomes and there is a great potential that in future it will be used for decreasing neurodegeneration. With DBS, there are several approaches that are currently in clinical trials and hopefully they will be introduced as disease-modifying treatment in the future.

Keywords: Parkinson’s disease, α-synuclein, neurodegeneration, dopaminergic neurons, substantia nigra

SAŽETAK

Parkinsonova bolest (PB) je najčešća sinukleinopatija te druga najčešća neurodegenerativna bolest u svijetu. Njena učestalost obično raste sa starošću te se, zbog produljenog životnog vijeka i povećanog udjela starijeg stanovništva, procjenjuje da će gotovo 13 milijuna ljudi bolovati od PB do 2040. godine. Ovakvi podaci izazivaju zabrinutost i potrebu za pronalaskom učinkovitih lijekova koji će potencijalno liječiti bolest. PB se definira kao progresivni poremećaj u kojem dolazi do degeneracije dopaminergičnih neurona u substantia nigra pars compacta (SNpc), a zatim se proširuje u sve dijelove mozga. Prepoznaje se po ukočenosti, tremoru i bradikineziji, ali i po kognitivnim promjenama poput demencije, autonomne disfunkcije, poremećaja raspoloženja i spavanja. Najveći problem je što razvoj PB nije posljedica samo jednog čimbenika. Zasada su *missense* mutacije koje su povezane uz PB identificirane na najmanje 20 gena. Uz genetske modifikacije, postoje i okolišni čimbenici koji mogu povećati ili smanjiti rizik obolijevanja. Kao sinukleinopatija, PB je uglavnom karakterizirana nakupljanjem α-sinukleina (α-syn) koje tvori Lewyjeva tjelešca (LB) i Lewyjeve neurite (LN). Budući da je lokaliziran na krajevima presinaptičkih neurona, pretpostavlja se da α-syn ima važnu ulogu u regulaciji otpuštanja sinaptičkih vezikula. Međutim, pod patofiziološkim uvjetima, počinje se nakupljati u neurotoksične oligomere i fibrile koji narušavaju brojne funkcije na izvanstaničnoj i unutarstaničnoj razini. Trenutna terapija nema mogućnost ublažavanja bolesti, ali kontrolira simptome i poboljšava kvalitetu života pacijenta. Levodopa je lijek koji se najčešće propisuje PB pacijentima jer se pretvara u dopamin i povećava njegovu razinu u mozgu. Međutim, kada se koncentracija novonastalog dopamina u plazmi ponovno smanji, pacijent doživljava fenomen koji se naziva engl. "*wearing-off*". Time su na tržištu uvedene druge opcije poput agonista dopamina, inhibitora monoamino oksidaze B i katehol-O-metiltransferaze, ali nijedan od njih ne pokazuje bolje rezultate od

levodope. Postoje i kirurški zahvati koji ublažavaju fenomen levodope, iako postoji veći rizik od neželjenih nuspojava, komplikacija tijekom zahvata, pa čak i smrti. Međutim, duboka mozgovna stimulacija (DMS) se pokazala uspješnom u poboljšanju motoričkih funkcija bez težih ishoda i postoji veliki potencijal da će se u budućnosti koristiti za smanjenje neurodegeneracije. Uz DMS, postoji nekoliko pristupa koji su trenutno u kliničkim ispitivanjima i nadamo se da će biti uvedeni kao terapija koja liječi PB u budućnosti.

Ključne riječi: Parkinsonova bolest, α-sinuklein, neurodegeneracija, dopaminergični neuroni, substantia nigra

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1. INTRODUCTION

In 1817, James Parkinson published his work “An Essay on the Shaking Palsy“. In that essay, he described a condition called paralysis agitans or shaking palsy. Today, the condition is well known as Parkinson's disease (PD).

PD is a progressive neurodegenerative disorder characterised by the loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc). SNpc is located in the basal ganglia which are responsible for the control of voluntary motor movements. However, the disease does not only cause dopamine deficiency, but also affects other neurotransmitters. Based on that, symptoms are divided into motor and non-motor. Three common motor symptoms are bradykinesia (slowness of movement), tremor and rigidity (1). Non-motor symptoms include autonomic dysfunction, cognitive abnormalities, sleep and mood disorders. Rapid eye movement (REM) sleep behaviour disorder is considered “predictor for the development of PD“ because it often precedes motor symptoms. Some non-motor symptoms can occur as a result of taking levodopa or other dopamine agonists for PD. Cognitive dysfunction usually includes bradyphrenia (slowness of thinking) and over the years, it becomes dementia (2). Unlike Alzheimer's, PD dementia does not affect language and memory, but PD patients can experience hallucinations and delusions. Interestingly, neurodegeneration does not start in the basal ganglia. It begins in the brain stem, where non-dopamine neurotransmitters are normally produced. As a result of that, their deficiency leads to some of the already mentioned non-motor symptoms (autonomic dysfunction, mood disorders). After that, neurodegeneration spreads in SNpc and cortex (3).

PD is a complex neurodegenerative disease caused by a combination of environmental and genetic factors. Even though it was described 200 years ago, some factors are still unknown. Exposure to pesticides (rotenone, paraquat) and chlorinated solvents has been related to increased PD risk. Most

of these chemicals are no longer in use. However, trichloroethylene can still be found in drinking water supplies, air, food and breast milk. Head injury is also one of the environmental factors, but the mechanism is still unknown. Interestingly, some lifestyle factors such as physical activity or consuming caffeine have been associated with decreased risk of PD (1).

Even though missense mutations in at least 20 genes have been linked to the risk of developing PD, they are the cause of the disease in only 5 to 10% of cases (1,4). Recently, genome-wide association studies (GWAS) identified 70 loci that are related to PD (5). *PARKIN* and *PINK1* are autosomal recessive genes whose mutations lead to mitophagy. Mitophagy is the autophagy of dysfunctional mitochondria in lysosomes. *PARKIN* also regulates the expression of peroxisome proliferator-activated receptor-gamma coactivator-1α (PGC-1α), a transcriptional regulator responsible for the expression of genes necessary for mitochondrial biogenesis and antioxidant effect (1). Another important gene is leucine-rich repeat kinase 2 (*LRRK2*) which phosphorylates several Rab proteins whose responsibility is to regulate crucial aspects of autophagy. Besides that, *LRRK2* is highly expressed in the immune system, especially in neutrophils, macrophages and monocytes. It has been suggested that LRRK2 promotes the secretion of proinflammatory cytokines such as IL-1β and helps in the defence against pathogens. However, due to PD mutations, hyperactivation of *LRRK2* leads to neuroinflammation and rapid cell death (6). The *SNCA* gene is a gene that is mainly brought to attention because it provides instructions for the synthesis of protein α-synuclein (α-syn). Due to *SNCA* mutation, the protein starts to misfold, but clearance pathways for abnormal proteins are inhibited. It continues to aggregate and becomes one of the major components of Lewy bodies (LB). LB are clusters in SNpc that cause neurodegeneration of dopaminergic neurons (1).

2. AIM

PD is the second most common neurodegenerative disorder after Alzheimer's disease. Out of 100 000, 5 to 35 people are diagnosed with PD annually and the incidence increases with age. Unfortunately, it is expected that the number of cases will double in the next two decades (1). Also, the real cause of developing PD has not yet been clarified. The main focus of this thesis is on *SNCA* and α-synuclein, but mutations are absent in the majority of PD patients. Since there is no single target, so far available therapies only treat symptoms of the disease. The purpose of this thesis is to provide a review of potential treatments whose aim is to decrease neurodegeneration.

3. α-SYNUCLEIN

Synucleins are a family of vertebrate-specific proteins that are enriched in presynaptic terminals. Even though these proteins are natively unfolded, they go through structural transition for lipid binding. There are three closely related members of the family: α-, β- and γ-synuclein. However, α-syn is the most studied protein for its correlation with PD and Lewy bodies (7). α-syn is a protein of 140 amino acids encoded by the *SNCA* gene. The *SNCA* gene consists of seven exons and it is located on chromosome 4. The majority of the protein is in the brain, but smaller amounts can be found in heart and muscles. Its structure can be divided into three regions. The first 60 amino acids make amphipathic α-helices with a conserved motif KTKEGV, important for membrane binding. Afterwards, the hydrophobic region extends from amino acid 61 to 95 that includes additional KTKEGV repeats and the non-amyloid β-component (NAC) region which is responsible for aggregation. At last, the C-terminal region contains acidic residues which are involved in the ligand binding, such as to proteins or small molecules (Figure 1). Because of its amino acid constitution, at the neutral pH α-syn is a negatively charged protein (pI = 4.7) (8).

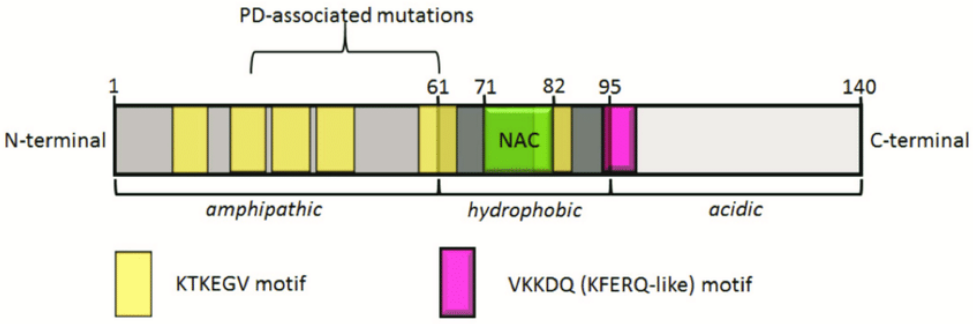

Figure 1: Regions of α-synuclein. Adapted from (9).

α-syn belongs to the family of natively unfolded proteins, which are characterised by low hydrophobicity and high net charge so it remains unclear how the protein becomes more compact (8). Uversky et al. found out that

mutations are not the only things that cause fibrillation (10). Fibrillation is a process that occurs in the conformational transformation of monomeric α-helix into large aggregates of the β-sheets followed by amyloid fibril formation (11). It appears that higher temperature increases hydrophobicity and acidic pH neutralizes negative charge leading to partially folded conformation (10).

Although the exact function of α-syn is still unknown, several potential roles have been proposed. Since it is mostly localized in presynaptic nerve terminals, the protein regulates certain enzymes, transporters, synaptic vesicles release and participates in fatty acid binding (8). Also, a study by Gretten-Harrison et al. showed that α-syn is important for neuronal survival. Knockout of all synucleins (α, β and γ) in mice leads to modification of axonal structures and age-dependent neuronal dysfunction (7).

In 1997, α-syn was identified as a major component in LB and Lewy neurites (LN) in PD (12). Since then, point mutations A53T, A30P, E46K and G51D and genomic multiplications in *SNCA* were correlated with autosomal dominant early-onset PD (8). For example, in families with A53T mutation, 85% of patients had clinical signs of the disease (13). Each of the aforementioned mutations leads to a different conformation of the protein. A30P mutation induces oligomer formation of α-syn, while A53T and E46K mutations fold the protein into fibrils (8). G51D is the most recent identified point mutation that causes PD combined with pyramidal symptoms (increased reflexes, spasticity). Studies by Lesage et al. showed that G51D α-syn oligomerizes slowly, but its fibrils are more toxic. It leads to rapid progression of the disease and death within a few years (14). In general, all of these mutations lead to aggregation of the protein.

The exact process of α-syn neurotoxicity is still unclear, but there are some suggested mechanisms. The common outcomes are disrupted function of mitochondria and endoplasmatic reticulum (ER), synaptic dysfunction and cell

membrane impairment. Consequently, this leads to impaired functioning of nerve cells and apoptosis.

3.1. POTENTIAL MECHANISMS OF α-SYN NEUROTOXICITY

Under physiological conditions, α-syn can be either in the cytosol as an unfolded soluble monomer or it can be folded and bound to the membrane. Due to mutations and environmental toxins, α-syn can undergo conformational changes. The NAC region starts the aggregation by creating a β-pleated sheet formation. After that, there are two pathways of formation. One of them is the conversion into toxic oligomers that do not develop into fibrils. In some cases, oligomers can transform into mature fibrils, resulting in accumulation into LB or LN (Figure 2) (15). Although there were several studies investigating which formation of the protein is more toxic, the answer remains unclear (13).

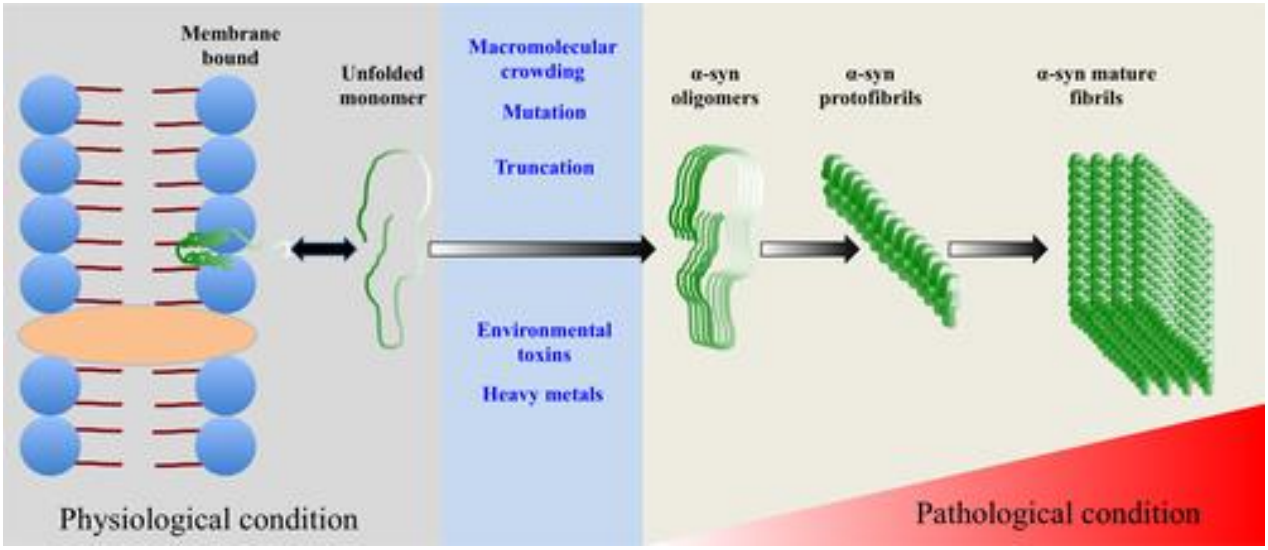

Figure 2: Forms of α-syn under physiological and pathological conditions. Adapted from (13).

α-syn prevails in presynaptic nerve terminals, but it is also present inside the cell body and neurites. Due to its location several potential mechanisms have

been proposed that cause neurotoxicity at either intracellular or extracellular level (16).

The cellular membrane is impermeable to ions and molecules so it can balance the electrochemical gradient. However, α-syn can disrupt its permeability by creating pores and channel-like structures (15). On the other hand, a study by Kayed et al. suggested another mechanism that affects membrane conductivity. α-syn oligomers increase the molecular volume of lipid acyl chains which leads to decreased hydrocarbon core (17). Either way, the protein increases membrane permeability which enhances the ability of ion movement through the membrane. This causes disturbance of electrochemical gradient that can be lethal for the cell (15).

Mitochondria are essential for ATP synthesis, neuronal survival and calcium uptake, but α-syn can disrupt their homeostasis. It promotes 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced degeneration of dopaminergic neurons (16). Its oxidative form MPP^+ binds to the mitochondrial complex I and blocks cellular respiration. Not only does the inhibition of complex I disrupt ATP synthesis, but it also increases the level of intracellular Ca^{2+}. Therefore, it activates the calmodulin-calcineurin cascade and Ca^{2+}-dependent enzymes (calpain I and II), leading to cellular damage and death (Figure 3) (16,18).

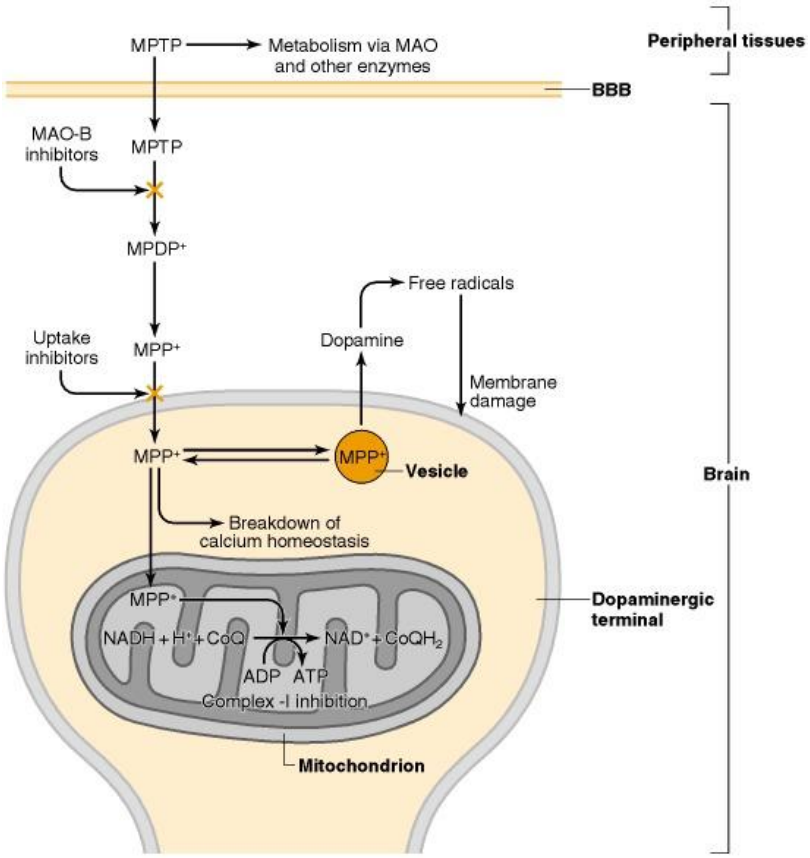

Figure 3: Pathway of MPTP-induced neurotoxicity. Adapted from (18).

Besides that, α-syn aggregates interrupt other intracellular pathways such as ER-to-Golgi trafficking and autophagy. ER-to-Golgi trafficking is important for folding synthesized proteins, but the presence of mutated α-syn leads to ER stress (16). In response to that, one of the ER proteins, inositol-requiring protein 1, promotes cell death by starting a cascade of signal transduction (19). Moreover, α-syn blocks the ER-to-Golgi transfer of the autophagic transmembrane protein ATG9 that is crucial for autophagosome biosynthesis. Also, α-syn disturbs chaperone-mediated autophagy (CMA), a pathway important for transporting damaged proteins to the lysosome through lysosomal-associated membrane protein-2A (LAMP2A). Specifically, A53T and A30P α-syn bind more tightly to the LAMP2A than the wild-type and prevent

their degradation, but also degradation of other misfolded proteins carried by the chaperone complex. Finally, α-syn reduces lysosomal activity by inhibiting multiple enzymes (glucocerebrosidase, β-galactosidase, cathepsin B).

Under physiological conditions, α-syn is localized in the presynaptic nerve terminal, regulating synaptic vesicle trafficking. After the vesicle is released from the presynaptic neuron, the C-terminal region of α-syn interacts with protein synaptobrevin-2 or vesicle-associated membrane protein-2 (VAMP2) that is bound to the membrane of the vesicle. The connection between α-syn and VAMP2 promotes formation of soluble N-ethylmaleimide–sensitive factor attachment protein receptor (SNARE) complex on the target membrane (Figure 4). SNARE complex helps vesicle fusion with the membrane and the release of its content. Due to mutations, multiple binding sites of α-syn oligomers can bind to VAMP2 and block SNARE complex formation leading to inhibition of dopamine release and decreased vesicle mobility (16).

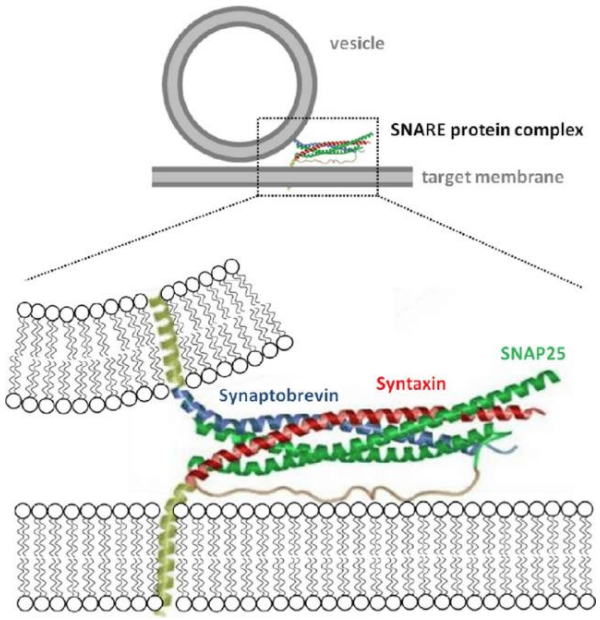

Figure 4: The SNARE complex. Adapted from (20).

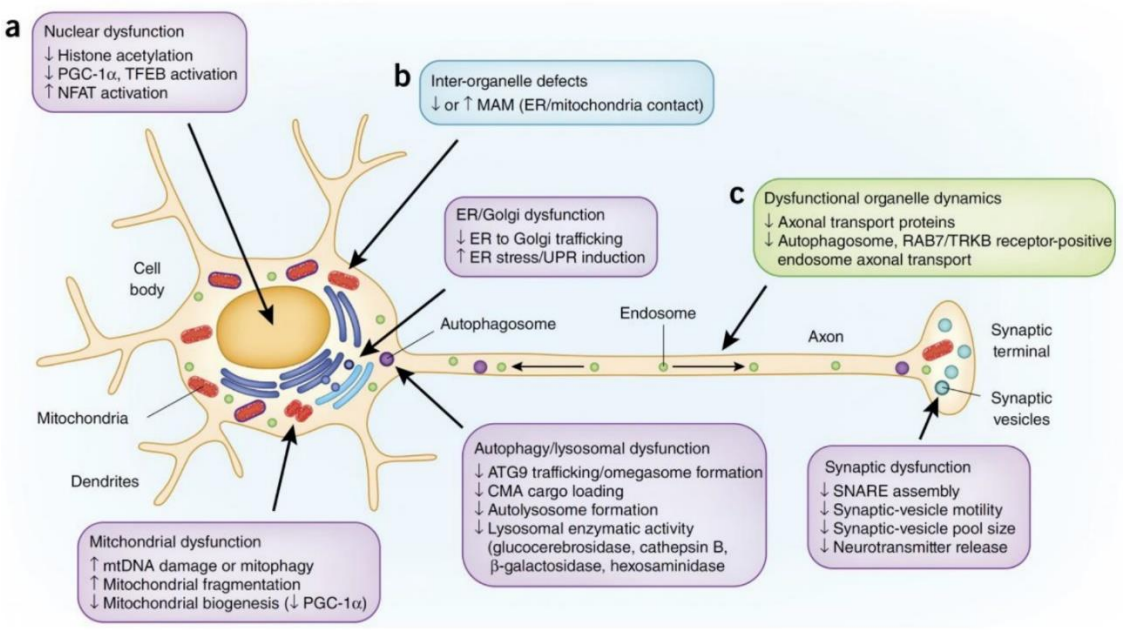

Figure 5: Schematic review of α-syn neurotoxicity. a – organelle dysfunction (purple boxes), b – defects in inter-organelle contacts (blue box), c – dysfunctional organelle dynamics (green box). TFEB – transcriptional factor EB, MAM - mitochondria-associated ER membrane, UPR – unfolded protein response, TRKB – tropomyosin receptor kinase B. Adapted from (16).

3.2. PHASES OF SYNUCLEINOPATHIES

Synucleinopathies are a group of neurodegenerative disorders characterised by the accumulation of oligomeric α-syn, leading to neurodegeneration, motor and cognitive symptoms. Besides PD, synucleinopathies include dementia with Lewy bodies (DLB) and multiple system atrophy (MSA). In each of these disorders, α-syn aggregation leads to death of different neuronal populations. It affects dopaminergic neurons in SNpc in PD; dopaminergic and cholinergic neurons in nucleus basalis of Meynert in DLB; and atrophy of cerebellum, putamen in basal ganglia and brain stem in MSA. These disorders are progressive, so it is important to react early and start with the treatment for preventing neurodegeneration while it has not been widespread yet (21).

There are three stages of synucleinopathies: presymptomatic, symptomatic and late stage. The presymptomatic stage is characterised by early α-syn aggregation and death of selected neuronal populations – in the case of PD, the death of dopaminergic neurons (21). According to Braak et al., α-syn oligomers are spread in the brain stem at the first two stages of PD-related pathology (3). Since diagnostic tests for PD are focused on SNpc, the disease is rarely identified at an early stage. Because severe neurodegeneration has not started yet, early diagnosis is crucial and increases the efficacy of the therapeutic treatment. Before it starts to propagate from one cell to another, there are several ways to stop α-syn accumulation. Firstly, small interfering RNA (siRNA) and micro RNA (miRNA) can reduce the expression of α-syn by silencing *SNCA*. The problem is that α-syn is still needed for synaptic transmission (21). The second therapeutic target is α-syn oligomerization and aggregation. Conformational stabilizers such as curcumin, epigallocatechin gallate, selegiline and polyphenol phthalocyanine tetrasulfonate (PcTS), can bind to α-syn and stop its pathogenic misfolding and aggregation. It has been suggested that the mechanism of α-syn aggregation depends on whether it is soluble or bound to a vesicle. Of these, PcTS is the only one that can inhibit the accumulation of the vesicle-bound α-syn (22). Since α-syn aggregates into other neurons and glial cells, it can inhibit autophagy and other protein clearance mechanisms (21). Thereby, the third aim for potential treatment is to increase α-syn degradation by autophagy and unfolded protein response inducers, chaperones and enzymes such as kallikrein-6, matrix metalloproteinase-3 and cathepsin D. Other promising approaches that could be effective at reducing propagation of α-syn aggregates are active and passive immunotherapy (21). Active immunization induces the immune system after exposure to the full antigen or just part of it. This leads to production of effective antibodies that will degrade α-syn aggregates and potentially reduce neurodegeneration. In contrast, passive immunization is an

administration of the antibodies produced outside the patient's immune system. This type of protection is good for patients whose immune system is unable to produce its own antibodies. A study by Games et al. showed that antibodies against C-terminal region of α-syn can reduce the oligomerization and C-terminal truncation, resulting in improvement of behavioural and motor functions. C-terminal deletion usually increases pI of the protein which leads to faster aggregation at physiological pH (23).

PD is usually diagnosed during the symptomatic stage when approximately 75% of dopaminergic neurons have died and PD symptoms are significant. Also, it is characterised by neuroinflammation including microglial and astroglial activation leading to oxidative stress and excessive secretion of proinflammatory cytokines such as TNF-α, IL-1β and IL-6. On this occasion, the main focus is on reducing neuroinflammation and restoring neurotransmitters. Even though several anti-inflammatory medications such as nonsteroidal anti-inflammatory drugs, TNF-α inhibitors, antidepressants, antioxidants and polyphenols have been tested, none of these significantly reduced cognitive and motor symptoms (21). However, only the antidepressant fluoxetine showed some potential in treating MSA. A study by Valera et al. detected the regulation of cytokine levels and significant reduction of astrogliosis in basal ganglia and hippocampus in transgenic model of MSA (24). Hopefully, fluoxetine and other antidepressants could be of interest as α-syn reducing agents for MSA and other synucleinopathies.

Lastly, the late stage causes severe neurodegeneration and more noticeable cognitive and motor symptoms. At this point, the therapeutic aim is to slow down neurodegeneration using neurotrophic factors or regenerative medicine. Stem cell therapy is a form of regenerative medicine that helps with repairing dysfunctional and damaged cells by using stem cells and modulating the immune system. Neurotrophic factors are a family of soluble proteins that play a role in promoting growth, survival and differentiation of neuronal and glial

cells. Some factors are brain-derived neurotrophic factor (BDNF), neurturin, glial cell line-derived neurotrophic factor (GDNF) and nerve growth factor. Even though at this phase the effectiveness of aforementioned therapies for earlier stages is reduced, it is important to consider that α-syn still aggregates. As a result, it is crucial to inhibit its accumulation so it does not hinder the efficacy of regenerative treatment (21).

4. CURRENT THERAPY FOR PD

Currently available therapies for PD have proven their efficacy in controlling PD symptoms and improving the quality of life of patients. Most of the treatment is based on dopamine replacement and stimulating dopamine receptors which lead to reduced motor symptoms. Since the disease affects other neurotransmitters, the therapy also involves non-dopaminergic medications that manage motor and non-motor symptoms. However, PD is a slowly progressive disease, so standard symptoms, with drug-related side effects such as dyskinesia, can change and evolve over time (25,26). There are many available medications for PD, but none of them showed such powerful antiparkinsonian effects as levodopa. Levodopa is a dopamine precursor that can cross the blood-brain barrier (BBB), unlike dopamine. Levodopa can be decarboxylated by the enzyme DOPA decarboxylase in both the central nervous system (CNS) and periphery. To increase its bioavailability in the CNS, it is usually taken in combination with carbidopa which inhibits peripheral DOPA decarboxylase. Once converted to dopamine, it recompenses decreased level of endogenous dopamine and stimulates dopamine receptors. Levodopa therapy starts with smaller doses and then increases over time, but the recommended dose is 300 to 1200 mg per day, divided into 3 to 12 portions (27). It is impressive how it is cheap because it is the only drug that can improve life expectancy. Regardless of its efficacy, levodopa can-not control all motor symptoms such as balance, posture and speech. In addition, non-motor symptoms, including hallucinations and cognitive damage, do not respond to levodopa therapy (25). Furthermore, patients, who receive higher doses of levodopa, are more likely to experience a phenomenon called "wearing-off" (26). "Wearing-off" or "end-of-dose effect" is a term of sudden changes in movement control that can occur after years of taking levodopa (28). The exact cause of motor complications is still unknown, but it is correlated with levodopa concentration in plasma and inability of dopamine

storage (26). Motor symptoms return or worsen when levodopa starts to "wear off" and improve after the next intake of medication ("on-time") (Figure 6). Since PD gets worse over time, the duration of "on-time" becomes progressively shorter and fluctuations become more noticeable (28). About 55% of PD patients rated "wearing-off" as their main complaint with the therapy. It is crucial to find alternatives for levodopa that could provide that kind of efficacy without motor complications, but it is difficult to achieve. One of the potential solutions is self-administered levodopa oral inhalation powder that allows rapid presentation of levodopa to absorptive membrane during "off-time". It avoids gastrointestinal absorption and has low metabolic activity, but it needs to be further investigated in future studies (26).

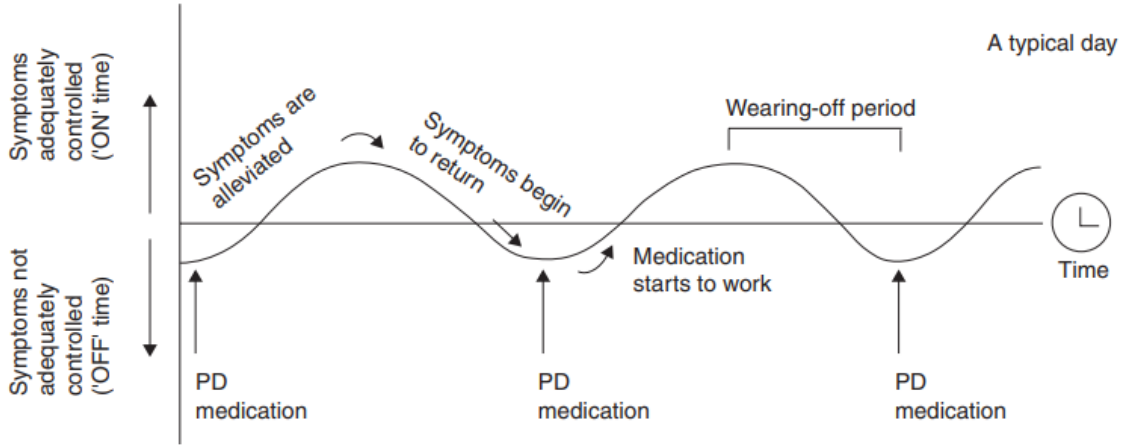

Figure 6: The "wearing-off" phenomenon. Adapted from (28).

Unlike levodopa, dopamine agonists (DA) prevent motor fluctuations and dyskinesia, but they are more expensive. DAs are designed to bind and activate certain postsynaptic dopaminergic receptors whose role is to control locomotor activity. They can be used as monotherapy at earlier stages of PD, but they are not as effective as levodopa. However, DAs prolong "on-time" and reduce "off-time", so they are usually prescribed as a supplement to

levodopa (25). There has been a hypothesis whether a longer half-life of DA provides continuous dopaminergic stimulation which would be the key to reduced risk of motor complications (29). DAs are divided into two classes: ergot and non-ergot derivatives. Ergot compounds were the first generation of DA which includes bromocriptine, cabergoline, dihydroergocriptine, lisuride and pergolide. However, they are no longer in use because of several cases of valvular and pleuropulmonary fibrosis that were reported in PD patients while taking these medications. Derivatives such as cabergoline and pergolide appeared to be agonists for 5-hydroxytryptamine 2B receptor, which is expressed on heart valve leaflets. Agonism induces thickening and stiffening of valves, leading to cardiac valvulopathy and heart failure. Because of that, it was crucial to synthesize non-ergot derivatives as the second generation of DA such as apomorphine, piribedil, pramipexole, ropinirole and rotigotine. This type of DA has equal effectiveness as ergot compounds, but they do not cause fibrotic reactions. However, other side effects might occur, such as nausea, hallucinations, peripheral oedema, daytime sleepiness and impulse control disorders (compulsive eating, hypersexuality, compulsive shopping, gambling) (29).

Catecholamine neurotransmitters are usually degraded by the enzyme catechol-O-methyltransferase (COMT). To extend its elimination half-life and prolong its bioavailability in the brain, levodopa is often administered in combination with COMT inhibitors such as entacapone and tolcapone. Tolcapone is the first COMT inhibitor that showed antiparkinsonian effects and reduced "off-time". Regardless of its efficacy, it has been suspended in several countries in European Union and the United States due to reported cases of liver toxicity (25). Unlike tolcapone, entacapone does not cause liver toxicity, but it has less pronounced effectiveness on reducing "off-time". In addition, it has been tested whether the combination of levodopa, carbidopa and entacapone could reduce the risk of motor fluctuations and provide more

stable plasma levodopa levels. However, patients treated with this combination consequently developed more dyskinesia than patients who administered levodopa and carbidopa alone. Besides dyskinesia, other side effects developed by COMT inhibitors were severe diarrhoea and urine discolouration. One of the newest COMT inhibitors in the market is opicapone which showed better performance than tolcapone and entacapone. It has a longer half-life and, comparing it with entacapone, it does not cause urine discolouration or diarrhoea. Opicapone has great advantages, but it still needs to be confirmed whether it reduces the risk of motor complications (26).

Dopamine and other monoamines can also be degraded via oxidative deamination, catalysed by monoamine oxidase (MAO). There are two isoenzymes: MAO-A, which mostly metabolizes serotonin, epinephrine and norepinephrine; and MAO-B, which is primarily located in the striatum and degrades dopamine (26). It has been suggested that MAO-A could also affect parkinsonism because it partly participates in dopamine degradation, but it still needs to be investigated (26,30). Moreover, PD therapy usually focuses on inhibiting MAO-B to prolong dopamine's half-life and improve motor symptoms. MAO-B inhibitors can be used as monotherapy at earlier stages of the disease or as a supplement to levodopa. It is important to mention that these groups of inhibitors are not completely specific, so they could also inhibit MAO-A, which could potentially improve non-motor symptoms such as mood disorders (26). Selegiline and rasagiline belong to the older generation of inhibitors and they irreversibly inhibit MAO-B. Not only does selegiline extend dopamine half-life, but also can slow down neurodegeneration by reducing oxidative stress in dopamine neurons. However, there were controversies about cardiac safety since one of its metabolites is methamphetamine, but this has never been confirmed (26). Recently, safinamide was introduced to the market and it is the only one that inhibits MAO-B reversibly. Also, it has a thousand times higher affinity for binding MAO-B than MAO-A. Besides

inhibition, safinamide also blocks voltage-dependent sodium channels, thereby reducing glutamate release and regulating the intracellular level of Ca^{2+}. By that, it could reduce dyskinesia and potentially prevent excitotoxic damage (26).

Anticholinergics were the first drugs prescribed for treating PD motor symptoms such as tremor. However, they are not recommended for older people anymore due to their cognitive side effects such as confusion or hallucinations (25). A study from 2019 reported increased risk of dementia in PD patients who were treated with anticholinergic drugs (31). Amantadine is an antiviral drug firstly used for treating symptoms associated with influenza A virus, but it is no longer approved because of drug resistance and side effects. However, it showed potential at decreasing dyskinesia and excitotoxicity in PD patients by inhibiting glutamate N-methyl-D-aspartate receptor (NMDA). Side effects that might occur are dry mouth, orthostatic hypotension, dizziness, hallucinations and peripheral oedema (26).

For patients who do not respond well to medications or suffer from severe motor fluctuations, there are surgical procedures such as thalamotomy, pallidotomy, deep brain stimulation (DBS) and focused ultrasound. However, none of them can prevent degeneration. Thalamotomy is a type of brain surgery in which thalamus is lesioned, leading to improved tremor and rigidity. Pallidotomy is another lesion surgery where the globus pallidus is removed. Globus pallidus is a part of the basal ganglia whose overactivity in PD causes tremor, rigidity and bradykinesia. Besides these symptoms, this procedure can also improve levodopa-induced dyskinesia and reduce "off-time" (32). These lesion surgeries are irreversible and can only be done on one side of the body because bilateral procedures result in adverse effects such as aphasia, cognitive disorders, dysarthria and dysphagia. Focused ultrasound is based on high intensity of ultrasound energy that degrades certain areas in the brain which are identified by magnet resonance. These targets are usually

correlated with tremor. The patient wears a helmet that contains transducers used for helping ultrasonic waves to cross through the skull and aim at labelled areas. Currently, it is only approved by Food and Drug Administration for unilateral thalamotomies, while focused ultrasound for pallidotomies is still under investigation. Even though production of effective lesions depends on skull thickness, this procedure enables immediate results and does not require physical brain penetration (33). Recently, it was reported that BBB can be opened by using focused ultrasound, which could facilitate the drug delivery to treat PD, other neurodegenerative disorders and brain tumour (34). DBS brought such an improvement in PD therapy since the development of levodopa. Unlike lesion surgeries, DBS offers safety and a reversible approach for controlling tremor and "wearing-off" dyskinesia. Electrodes are surgically inserted into the targeted area of the brain, usually SNpc and globus pallidus internus, which are connected to neurostimulator. The neurostimulator can be compared with a pacemaker – it emits electrical impulses to the target and alters neuronal functioning (26,33). Both targets lead to improved motor fluctuations, but SNpc DBS also includes significant medication reduction. However, it does not affect non-dopaminergic symptoms such as dementia and balance impairment (26). Even though there is still much to learn about its mechanisms of action, new DBS devices are expected to target different nuclei and thereby improve the treatment for PD, but also other neurodegenerative and behavioural disorders (33).

5. FUTURE POTENTIAL APPROACHES FOR PD THERAPY

5.1. IMMUNOTHERAPY

One of the characteristic outcomes of the PD is induced proinflammatory response that leads to oxidative stress and cell damage. Thereby, on-going neurodegeneration could be halted by modifying patient's immune system. There are four potential immunotherapeutic agents that showed neuroprotective effects and currently are on clinical trials: recombinant human granulocyte-macrophage colony stimulating factor (GM-CSF) sargramostim, active vaccines PD01A and PD03A, and passive vaccine PRX002 (35,36).

GM-CSF is a cytokine produced by many activated immune cell types such as T cells, B cells, macrophage and monocytes, and its function is to stimulate proliferation of effector granulocytes and macrophage for defence against pathogens. In contrast, it acts anti-inflammatory by differentiating monocytes into tolerogenic dendritic cells. Tolerogenic dendritic cells promote secretion of immunomodulatory cytokines such as IL-10 and TGF-β, and, unlike proinflammatory dendritic cells, they have low expression of costimulatory molecules. Costimulation is crucial for activating effector T cells, otherwise it leads to their anergy or activation of regulatory T cells. Consequently, GM-CSF-induced regulatory T cells suppress the immune response and, in the case of PD, preserve dopaminergic neurons (37). Sargramostim is a synthetic compound of GM-CSF produced by recombinant DNA technology in yeast. In the phase 1a clinical trial, it showed safe and well tolerated profile without severe side-effects among sargramostim-treated patients. Importantly, they had lower clinical ratings of disease severity compared with placebo-treated patients. The phase 1b is recruiting, but the problem with dosage might occur due to GM-CSF's short half-life. Larger doses might lead to adverse outcomes such as bone pain, injection site reactions and increased total white cell counts (35).

AFFITOPE PD01A is an active vaccine that mimics an epitope in the C-terminal region of the α-syn. During phase 1 clinical trial, 24 of 32 PD patients were immunized overall six times with either 15 μg or 75 μg of PD01A. Both doses were safe and well-tolerated with mild side effects such as local reactions. Produced PD01A-specific antibodies successfully promoted the immune response against α-syn and reactivated after booster immunization. In addition, it was reported that antibodies preferred to target α-syn aggregates rather than α-syn monomers leading to reduction of oligomeric and fibrillar α-syn. AFFITOPE PD03A showed similar results and the phase 2 is in preparation for both vaccines (36).

Prasinezumab (PRX002) is the first and only passive immunotherapeutic agent that is currently in the phase 2 clinical trial. It is humanized IgG monoclonal antibody directed against C-terminal region of α-syn (35). Phase 1 reported safety and good tolerability of PRX002, but also high affinity interactions between PRX002 and peripheral α-syn in cerebrospinal fluid (CSF). Phase 2 examines the efficacy of PRX002 at two different doses: high dose of 3500 mg or 4500 mg, depending on the body-weight of the patient, and low dose of 1500 mg. It is estimated that the phase will be completed in 2026 (36).

5.2. INHIBITORS FOR α-SYN AGGREGATION

Current and future approaches for PD therapy are mostly focused on suppressing α-syn oligomerization and fibrillation. Even though *SNCA* is not the only cause of the disease, the treatment could potentially improve neurodegeneration of PD, but also other synucleinopathies. Small compounds that target α-syn are potential candidates because of their BBB penetration and oral bioavailability. NPT200-11 is an α-syn misfolding inhibitor that is currently undergoing phase 2 clinical trial. Its binding to specific regions of α-syn prevents formation of neurodegenerative oligomers leading to improved

motor and cognitive functions, reduced neuroinflammation and normalized dopamine transport (38). Anle138b is a small molecule modulator that binds to already formed α-syn oligomers followed by inhibition of their neurotoxic impact and modulation into non-toxic oligomers or monomers. Single-centre, double-blind and randomised study was the first-in-human phase 1a clinical trial where 68 healthy participants were divided into three groups: single ascending dose (SAD), multiple ascending dose (MAD) and food effect study (FES). Volunteers from SAD and MAD received up to 300 mg of either anle138b or placebo, while participants from FES were administered 150 mg of the drug to examine the food effect on its pharmacokinetics. The study was completed in 2020 and reported good safety and tolerability among volunteers without severe side effects. Regardless of promising results, the drug was examined on healthy participants, so its performance on PD patients and risk profile remain unknown until further clinical trials (39).

5.3. SIGMA-1 RECEPTOR AGONISTS

Sigma-1 receptor (Sig-1R) is a transmembrane protein localized in the MAM and creates a complex with BiP protein. MAM is important for ER-mitochondria lipid and protein transport, ER stress response, calcium homeostasis and mitochondrial function, so its dysregulation has been associated with PD. When agonist binds to the Sig-1R, it dissociates from the BiP and translocates to bind other protein structures such as voltage-gated ion channels and hippocampal NMDA receptor. It modulates neuronal and synaptic excitability, but also it promotes BDNF secretion and other neurotrophic signalling. Thereby, it enables neuroprotection which is why it showed potential as a new target for PD therapy, but also for other neurodegenerative disorders (40). Blarcamesine (ANAVEX2-73) is an orally administered drug that activates Sig-1R leading to memory-preserving and neuroprotective effects. Phase 2 clinical trial for PD with dementia is completed and reported good safety and

tolerability (38). 132 patients received either placebo, 30 mg or 50 mg of ANAVEX2-73 daily for 14 weeks. Drug-treated patients showed improvement in cognitive functions and memory compared with placebo group (ClinicalTrial.gov Identifier NCT03774459). In addition, phase 2b/3 trial for Alzheimer's disease is ongoing and, with these promising results, Sig-1R agonists proved to be able to slow down the progression of neurodegenerative diseases (ClinicalTrial.gov Identifier NCT03790709).

5.4. THERAPY FOR OTHER DISEASES

This group represents drugs that could be potential therapy for PD and they are already introduced to the market, but for curing other diseases. However, it appeared that their mechanism of action affects some processes associated with PD and potentially modifies the disease. The advantage of this therapy is that it has already proven its safety and good pharmacokinetics in healthy volunteers and patients. Thereby, it can speed up clinical trials and examine whether it is effective on PD or other neurodegenerative diseases.

Ambroxol is an active ingredient in cough syrups that promotes mucus clearance. Besides that, it activates lysosomal enzyme β-glucocerebrosidase (β-GCase) that catalyses hydrolysis of glucocerebroside. β-GCase is encoded by *GBA1* gene, whose mutation is common genetic factor for developing PD. However, the correlation between PD and *GBA1* gene still is not entirely clear. It has been suggested that decreased *GBA1* expression leads to reduced autophagy and increased α-syn accumulation. By promoting β-GCase activity, ambroxol increases autophagy, resulting in decreased α-syn level in CSF of PD patients with and without *GBA1* mutations. In addition, it increases the level of PGC-1α which is important for mitochondrial biogenesis and neuroprotection (35). In the phase 2 clinical trial, they examined the safety, tolerability, pharmacokinetics and efficacy of ambroxol in ten *GBA1*+ PD

patients and ten *GBA1*- PD patients. Each patient administered the drug at 5 different doses (60, 120, 180, 300, 420 mg) over six months. The trial is completed, but there are yet no reported results (ClinicalTrial.gov Identifier NCT02941822).

Exenatide is a glucagon-like peptide-1 receptor (GLP-1R) agonist used for treating type 2 diabetes mellitus (T2DM). GLP-1 is an endogenous hormone that binds to its receptor on pancreas resulting in enhanced insulin secretion and decreased glucagon level. GLP-1R is not only found in the pancreas, but also in the brain stem and hypothalamus. GLP-1 binding activates PI3K/AKT signalling pathway, important for reducing neuroinflammation (35). T2DM has been linked to increased risk of developing PD because patients who are diagnosed with both conditions had experienced more severe symptoms than PD patients. However, the association between PD and T2DM has not yet been proven, but it has been suggested that T2DM drugs might be potential treatment for neurodegenerative diseases (35). Exenatide showed neuroprotective effects by reducing neuroinflammation, improved mitochondrial biogenesis and autophagy of misfolding proteins including α-syn. In addition, it resulted in major improvements in motor and cognitive symptoms. Currently, phase 3 clinical trial is ongoing to confirm its tolerability and it is expected to be completed in 2024. Moreover, there are three analogues of exenatide that are currently in phase 2 clinical trial: sustained-release exenatide (PT320), PEGylated exenatide NLY01 and liraglutide. All three compounds have better BBB penetration and longer half-life than exenatide (35).

Cooperation between mammalian target of rapamycin (mTOR) signalling and phosphorylated Akt kinase is important for regulating cell survival, autophagy, cell growth, autophagy, protein synthesis and proliferation. One of the stress-induced proteins is RTP801 which inactivates mTOR and Akt kinase. Even though its exact mechanism is still unknown, it has been confirmed that

RTP801 has dual role depending on a cell type. RTP801 impact leads to anti-apoptotic consequences for proliferating non-differentiated cells such as tumour cells, while it acts pro-apoptotic for non-dividing differentiated cells such as neurons (41). Increased level of RTP801 was reported in SNpc neurons of PD patients due to pesticide and MPTP exposure (41). Interestingly, α-syn aggregation inhibits autophagy by promoting mTOR activity (35). To increase autophagy and hinder RTP801 translation, rapamycin inhibits mTOR activity, but, unlike RTP801, it induces Akt phosphorylation leading to neuronal survival. Rapamycin is an antibiotic produced by bacterium *Streptomyces hygroscopicus* and it is usually used as immunosuppressant after organ transplantation. Currently, rapamycin is investigated as levodopa supplement because it also decreases "wearing-off" dyskinesia and protects dopaminergic neurons (35).

5.5. KINASE INHIBITORS

Along with α-syn, LRRK2 has been one of main targets for the treatment because its inhibition offers the neuroprotection and improved progression of PD. Currently, there are two LRRK2 inhibitors that achieved promising results in clinical trials: DNL-201 and DNL-151, both produced by the company Denali Therapeutics. Both of these completed the clinical trial 1 with good safety, oral bioavailability, BBB penetration and tolerability among healthy volunteers and PD patients. Interestingly, there were no adverse effects considering that LRRK2 is also localized in kidney, immune cells and lungs. However, Denali announced that they will continue with DNL-151 because it showed better pharmacokinetics on wider range of doses, but DNL-201 will serve as back-up. If both of them fail in further investigations, there are other potential LRRK2 inhibitors such as PFE-360 and MLi-2 which showed impressive outcomes in preclinical trials (42).

c-Abl is a tyrosine kinase and a cellular homologue of the Abelson murine virus oncogene localized in the nucleus and cytoplasm. Similar to mTOR signalling, it regulates many physiological processes such as cell survival, autophagy, cytoskeleton dynamics, receptor endocytosis and DNA repair. In CNS, c-Abl activation by oxidative and cellular stress leads to phosphorylation and inhibition of PARKIN E3 ubiquitin ligase. As a result, pathophysiologic α-syn oligomers are not ubiquitinated and degraded, and consequently they start to spread. In addition, c-Abl decreases PGC-1α level and causes mitochondrial dysfunction. It was confirmed that c-Abl is activated in PD patients and its inhibition could lead to disease modification (35). Three c-Abl inhibitors which were seen as potential PD treatments are imatinib, nilotinib and bafetinib. Even though they promote ubiquitination and α-syn autophagy, further studies are halted because of deficient BBB penetration, CSF exposure and poor effectiveness. Moreover, another problem is that they do not specifically inhibit c-Abl, but also other tyrosine kinases, leading to adverse side effects (35). Radotinib is another c-Abl inhibitor that showed better specificity, pharmacokinetics and BBB penetration compared to previous generation. Currently, phase 2 clinical trial is ongoing to determine its safety and tolerability and it is expected to be completed in 2025. Also, under investigation is the c-Abl inhibitor K0706 that confirmed its neuroprotective activity in preclinical trials. It is also currently in phase 2 clinical trial in which 504 patients in early stage of PD participate and it is estimated to be completed in 2023 (38).

5.6. HERBAL EXTRACTS

Ganoderma lucidum is an oriental fungus that has been known in Asian countries for its medicinal effects for more than thousand years. In China, it is called “lingzhi” which means “divine mushroom”, best known for its role of promoting longevity. Besides that, it also has other pharmaceutical benefits

such as lowering cholesterol, modulating immune system, antioxidative and antitumour effects, and lately it has been suggested in PD therapy because of potential neuroprotective effects (43). Since neuroinflammation is a part of the neurodegeneration, it has been tested whether lingzhi can decrease neurotoxicity by inhibiting microglia. Isolated and cultured microglia were activated after MPP^+ and lipopolysaccharide exposure, leading to released proinflammatory and oxidative factors such as TNF-α, IL-1β and nitric oxide. To examine if effects of lingzhi extract depend on the presence of reactive microglia, dopaminergic cell lines were cultured alone and co-cultured with microglia. These cell lines were treated with MPP^+ which resulted in significantly decreased dopamine uptake. When lingzhi extract was administered into cultures, decreased levels of neurotoxic factors were detected and interestingly, it increased dopamine uptake into dopaminergic cell lines no matter of the presence of microglia (Figure 7). This confirmed that not only lingzhi has anti-inflammatory effects, but also it promotes neuroprotection. An active compound of lingzhi responsible for this neuroprotective effect is still unknown, but active components that have been confirmed for other benefits are mostly polysaccharides and triterpenoids. Currently, phase 3 clinical trial is recruiting, but previous studies confirmed safety and good tolerability in early PD patients (43).

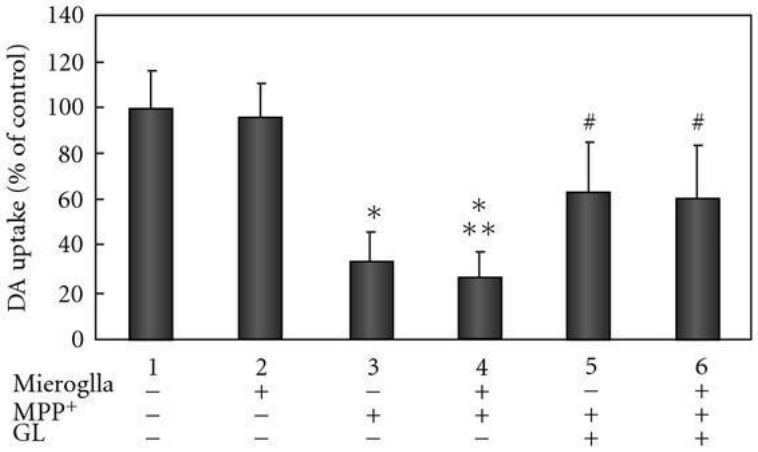

Figure 7: Lingzhi extract (GL) increases dopamine uptake. Adapted from (43).

WIN-1001X is a modified version of traditional Korean herbal formula "Chungsimyeolda-tang" usually used for treating cerebrovascular diseases. It is a 20% ethanol extract of three plant species: *Polygala tenuifolia*, *Angelica tenuissima*, and *Dimocarpus longan*. *Polygala tenuifolia* is well-known for improving memory, depression and neuroinflammation. *Angelica tenuissima* and *Dimocarpus longan* are responsible for modulating immune system, anti-inflammatory and neuroprotective impact (44). It was reported that WIN-1001X decreases MPP^+-induced neurotoxicity by promoting autophagy. It enhances the expression of autophagy-related proteins such as LC3 and beclin-1, leading to formation of an autophagosome. To confirm the improvement of autophagy, it was expected to report reverse correlation between p62 and autophagy. p62 is a scaffold protein that brings ubiquitinated proteins to the autophagosome and usually it is degraded with them through autophagy. Indeed, significantly decreased level of p62 was reported which means WIN-1001X successfully induces autophagy (44). In addition, it increases level of tyrosine hydroxylase in SNpc which is important in the dopamine pathway, where it induces dopamine synthesis and cell survival. Onjisaponin B and its metabolites tenuifolins are one of the major components responsible of autophagy induction (44). The aim of the currently recruiting phase 2 clinical trial is to determine an optimal dose of WIN-1001X between 400, 800 and 1200 mg and to test its safety comparing it to placebo (ClinicalTrial.gov Identifier NCT04220762).

Hypoestes rosea is indigenous plant from Nigeria that also showed neuroprotective effects. Its active compound hypoestoxide is a small diterpene that, besides antiparkinsonian, has anti-malarial, anti-cancer and anti-

inflammatory effects, and currently is undergoing phase 1/2 clinical trial. It penetrates the BBB and achieves its benefits by inhibiting NF-κB and IκB kinase, but also it activates peroxisome proliferator-activated receptors-γ (PPARγ). PPARγ plays a major role in regulating mitochondrial and proteosomal function, preventing neuroinflammation and oxidative stress and inducing autophagy. Preclinical trials reported impressive results of decreased α-syn aggregation in frontal cortex, hippocampus and striatum, which gives hope for future PD-modifying therapy (45).

5.7. REGENERATIVE MEDICINE

The aim of gene therapy is to regenerate dopaminergic neurons and compensate their loss in PD patients. Even though it claimed its efficacy in PD models, there have been difficulties due to complexity of human organism. This type of therapy still is confronted with many problems, especially when it comes to neurodegenerative diseases, but it is likely to become reality in the future. Gene therapy is divided into two approaches: *in vivo* and *ex vivo*.

ex vivo gene therapy is based on gene modification of isolated stem cells. After isolation, autologous or allogeneic cells are cultured and introduced to therapeutic genes that express neurotrophic growth factors or certain enzymes. Afterwards, modified stem cells are transplanted in the patient's brain (46). PD cell-based therapy started 40 years ago with transplantation of fetal ventral mesencephalic tissue and embryonic stem cells in blastocyst stage. Since they are not specialized, they differentiate into neural stem cells and then into functional dopaminergic neurons, leading to disease improvement. However, no matter of its benefits, this type of transplantation is halted because of ethical issues (46,47). After that, mesenchymal stem cells were investigated, but they did not appear effective because of their short lifetime and inability of BBB penetration (46). Recently, induced pluripotent

stem cells (iPSC) were brought to attention. Isolated mitotic cells, such as skin cells, are genetically engineered by transcription factors Oct4, Sox2, Klf4 and Myc that change DNA expression and revert cells into PSC. After autologous administration, iPSCs start to modify into dopaminergic neurons without immunologic reactions. Besides its advantages, genetic engineering of mitotic cells increases risk of cancer and other genetic diseases. Currently, phase 1/2 clinical trial is progress in Japan where efficacy and safety of human iPSC transplantation are being examined (35).

In the *in vivo* gene therapy, the therapeutic gene is packaged into vector that is injected in patient's brain. Whether it is viral or non-viral, vector must enable easy manipulation and long-term effect of therapeutic factor without activating immune responses. There are various serotypes of non-replicating viral vectors for targeting CNS, but in PD gene therapy, mostly used are adeno-associated virus (AAV) and lentivirus (46). There were several clinical trials for gene therapy, but three approaches continued to be investigated. AAV-GAD is a gene transfer of glutamic acid decarboxylase (GAD) via AAV2 in subthalamic nucleus (STN). Under pathophysiological conditions of PD, glutamate neurons in the STN are overactive and release glutamate that excites GABAnergic neurons in the globus pallidus resulting in inhibition of the thalamus. GAD has a major role in converting glutamic acid into GABA that inhibits glutamate neurons in the STN, thereby stimulates the thalamus to send movement signals to the cortex (46). Even though safety and significant improvement in motor symptoms are reported at phase 1 clinical trial, AAV-GAD did not show to be disease-modifying therapy. AAV2-GDNF is the next potential approach of PD therapy where the neurotrophic factor is induced by AAV2 into the putamen. Dopamine uptake and survival of dopaminergic neurons were detected at preclinical studies and the phase 1 clinical trial was finished in 2022. The aim of PR001 is to activate β-GCase by delivering functional copy of *GBA1* gene via AAV9. Activated β-GCase decreases α-syn

accumulation which was showed in PD models (48). Currently, phase 1/2a clinical trial for PR001 is ongoing and it is expected to be completed in 2028.

6. CONCLUSION

Regardless of the fact that PD is known for more than 200 years, there are still many unknowns that need to be explained. It is worrying that the rate of PD incidence is increasing, while there is still no therapy that will cure the disease. Another problem is late diagnosis because it is mostly confirmed by evident symptoms when most of dopaminergic neurons are already dead. Also, there is no specific test for diagnosing PD because of which patient starts with the treatment late resulting in its decreased efficacy. Levodopa is on the market since late 1960s and it has been considered "the gold standard" despite its side effects and "wearing-off" phenomenon. In fact, levodopa helped majority of patients with severe motor symptoms and improved their quality of life. However, it does not promote survival of dopaminergic neurons. Thereby, new potential approaches, which showed promising results in preclinical studies, are currently in clinical trials. Most of these are targeting α-syn aggregation, but *SNCA* mutation is not the only genetic factor of this disease. Along with genetic mutations, there are also environmental factors whose mechanisms that lead to PD are still unclear. Since the real cause of the PD is unknown, the aim for the future studies is to find multi-target therapies without severe side effects. Hopefully, these will slow down neurodegeneration, if not even cure the disease.

7. LITERATURE

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8. BIOGRAPHY

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Work experience

06/09/2021 – 17/09/2021 – Professional practice, The Galenic Laboratory of the Split-Dalmatia County Pharmacies

2021 – Receptionist, Split City Museum

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2019 – Student work at shop, Müller

Education

2019 – current – Biotechnology and Drug Research (Bachelor's Degree), Department of Biotechnology, University of Rijeka

2015 – 2019 – High School Diploma, IV. Gymnasium Marko Marulić, Split

2007 – 2015 – Elementary School Diploma, Elementary School "Mertojak", Split

Language skills

Mother tongue: Croatian

Other languages:

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LISTENING B2 READING B2 WRITING B2 SPOKEN PRODUCTION B2 SPOKEN INTERACTION B2

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Digital skills

Microsoft Office (Word, Powerpoint, Excel), Python (basics), PyMOL, Avogadro, ChemAxon Marvin, VMD, GAMESS, MacMolPlot

Honours and awards

STEM scholarship 2020/2021 and 2021/2022, Ministry of Science and Education

Volunteering

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