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Biological sources and Pro-Drug properties of L-DOPA against Parkinson's Disease: A Review

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ABSTRACT

Parkinson's disease was first formally identified by British physician James Parkinson in 1817 as "The Shaking Palsy". L-DOPA (3,4-dihydroxy- phenyl-L-alanine) has been considered as a gold-standard treatment for Parkinson's disease. Parkinson's disease (PD) is a neurodegenerative disorder influenced by both genetic and environmental factors. The mechanisms leading to neuro degeneration in PD are still under investigation, with several mechanistic models currently proposed. A number of microorganisms have been associated with increased risk of PD in humans, and recent research using newly developed models has begun to elucidate how these microbes may factor into disease development.

Keywords: Parkinson's Disease; L-DOPA; Biological Sources, Pro-drug

INRODUCTION

Parkinson's disease (PD) is the second most frequent neurodegenerative disease, affecting more than 1% of sindividuals older than 65 years. The pathological hallmark of PD is degeneration and death of dopa minergic neurons in the substantia nigra pars compacta, a central nervous system (CNS) region playing a key role in motor control. PD is diagnosed based on the classic triad of motor symptoms: bradykinesia, rigidity, and resting tremor.

Parkinson's disease (PD) has been a constant challenge to public health around the world. 3,4 dihy- droxy phenyl-L-alanine (L-DOPA) is an amino acid analogue and drug of choice in the PD-a degenerative neu- rological disorder. In the United States alone, about 1 million people are affected by PD and worldwide about 5 million. PD occurs among 1% of individuals aged 60 years while 4% of those 80 years old. The disease (PD) is characterized by progressive death of dopamine producing neurons in the basal ganglia of the brain Loss of the neurotransmitter dopamine can cause a number of effects, including rigidity (muscles resistant to movement), akinesia (inability to initiate movement), bradykinesia (slowness of movement), and rest tremor. Many brain cells of PD patients contain Lewy bodies which are unusual deposits or clumps of the protein al-phasynuclein, along with other proteins. Researchers are unable to find out formation of Lewy bodies or their role in development of the disease. For many decades, there were no effective treatments for PD, and it was thought to be a terminal illness. But in the 1940s and 1950s, neurosurgeons began to perform surgery on the basal ganglia of brain which gave little cure in PD symptoms. Though surgery was effective, it was risky, with about 12 percent of patients dying as a result of operation. The biggest advancement in Parkinson's treatment came in the 1960s, when researchers identified low levels of dopamine in brain among diseased persons. This research revolutionized the treatment of PD, led to the development of levodopa, also called L-DOPA (Larodopa, Dopar), which is been used as medication. L-DOPA can easily cross the blood brain barrier and gets converted into dopamine, it can be administered orally to relieve the symptoms of PD. Levodopa is still the cornerstone of Parkinson's treatment today. L-DOPA is a non-proteinogenic amino acid.

The time consuming process and the use of harsh conditions for production of L-DOPA led to the eco-friendly biotransformation of L-tyrosine to L-DOPA, which is a one-step oxidation reaction by submerged fermentation. Tyrosinase, a copper containing enzyme found in microbes, plants and animals, is the main key enzyme responsible for biosynthesis of L-DOPA and other enzymes like tyrosine hydroxylas and tyrosine phenollyase. Till date, the stoichiometric formulation of L-DOPA from L-tyrosine has not yet been achieved. *Mucuna pruriens* (velvet bean) is known worldwide for its biological activity against various disease, thus causing less or no side effects. These are mainly indigenous to tropical regions like Africa, India and West Indies. The seeds are rich in L-DOPA along with alkaloids, trypsin, histidine,

glutathione and many other amino acids. Methanolic extract of *Mucuna* seeds was found to contain L-DOPA with 92% purity. Among all the various plant sources, *Mucuna* genus seeds were found to contain highest levels of L-DOPA. *Mucuna* is an herbaceous twinning annual plant belonging to the Fabaceae family which contains bioactive compounds like free phenolics, tannins, phytic acid and protein content of 26-29%. L-DOPA from *Mucuna* has also been reported to be effective against hypertension, renal failure and cirrhosis. Velvet bean has very good nutritional properties and is cultivated as a green manure and cover crop in some parts of Asia. These legumes can also tolerate adverse environmental stress and a wide range of climatic conditions. The leaves of this plant are pinnately trifoliate with purple or white colour flowers and are self-pollinated. Often the toxicity of LDOPA has some significant deleterious effects in animals that are characterized by complex interactions with the nutritionally important amino acids. Extraction of bioactive materials from herbal world is a very important step to standardize the herbal formulations reducing the tendency of indiscriminate use of herbal medicines. Therefore, optimization of extraction of LDOPA is very crucial and many researches are primarily based upon these particular aspects. Although the first microbial synthesis of L-DOPA was detected from a fungal source in 1969, it has been found that L-DOPA is abundantly found in various biological resources, preferably plant sources, extraction of which can be economically favorable under mild process conditions.

Within the past three years, L-DOPA therapy has been considered as the subject of several extensive reviews by investigators in various parts of the world. The preliminary studies in both animals and humans date back to 1957 while first clinical trials to 1960 and 1961 . This review summarizes the biological sources for L-DOPA production which can be used as an alternative for chemically synthesized drugs, surgery and multidisciplinary management against the disease

BIOCHEMISTRY OF DOPAMINE

Interest in L-DOPA therapy for Parkinson's disease has been considerably enhanced since the recent release of this drug to all medical practitioners. Both experimental and clinical studies have suggested that the depletion of dopamine can be corrected by the administration of L-DOPA either orally or intravenously. A level of homovanillic acid (the main breakdown product of dopamine) in the cerebrospinal fluid of patients before and after the administration of oral L-DOPA proves its applicability.

Parkinson's disease affects a part of brain called the "basal ganglia", which controls movement. Cells in the basal ganglia begin to degenerate as a result of the condition, and lose of their ability to produce a neurotransmitter (a chemical that carries messages between brain and nerve cells) called dopamine. As dopamine levels drop, the production of another neurotransmitter, called acetylcholine, increases. The balance between these two is critical, because they have opposite effects; acetylcholine stimulating muscle contraction, and dopamine damping it down. When the balance shifts in favour of acetylcholine, muscles become rigid with increasing jerky movements which are difficult to control this is often accompanied by tremors in hands. Whilst conventional drugs can be administered to control symptoms, they have a range of unpleasant side effects and cannot limit its progression. During the early stages of the disease, conventional drugs, called anticholinergics, can reduce symptoms of muscle rigidity and excess salivation by blocking the action of acetylcholine. However, they can cause dry mouth, constipation, anxiety, drowsiness and blurred vision. Whereas, direct administration of L-DOPA also has dangerous side-effects, including nausea, internal bleeding, palpitations, dizziness and depression because of it is being converted to dopamine before it reaches to brain.

L-DOPA TOXICITY

A number of PD patients treated with L-DOPA. Patients have to suffer a variety of side effects; most commonly are nausea, vomiting, low blood pressure and restlessness. The drug can also cause drowsiness or sudden sleep onset, which can make driving and other activities dangerous. The repeated pulsatile stimulation of striatal dopamine receptors with chronic oral L-DOPA treatment induces plastic changes in basal ganglia circuits that can lead to the development of motor response complications (MRC). A more pressing concern regarding L-DOPA is it causes hallucinations and psychosis after long-term use. Some patients exhibit severe dyskinesias soon after starting low doses of L-DOPA. There are controversies in the treatment, whether it causes the motor complications or it is toxic to dopaminergic neurons, but it has not yet been proven and clinical trials have not clarified this situation.

There were number of therapies have been developed to improve PD management, such as dopaminergic agonists, inhibitors of catechols-O-methyltransferase (COMT) and monoamine oxidase (MAO-B). In combination therapy usually, patients are given levodopa combinedwith another substance called carbidopa (decarboxylase inhibitor). Addition of carbidopa lowers the amount of levodopa that is required and may reduce some of its side effects such as nausea and vomiting by reducing the sup- ply of "free" dopamine outside the brain. Carbidopa de- lays the conversion of levodopa into

dopamine until it reaches the brain, preventing or diminishing some of the side effects that often accompany levodopa therapy. It also reduces the amount of needed levodopa.

L-DOPA is very useful drug for reducing the tremors and other symptoms of PD during the early stages of the disease. It allows the majority of PD patients to extend their period of time of normal and productive lives. The dramatic improvement can be seen in PD patients after starting levodopa therapy. However, in order to get maximum benefit there is need to increase the dose gradually. A highprotein diet can interfere with the absorption of levodopa, so physicians recommend that patients shouldn't take protein-rich meals during their early stages of the treatment. L-DOPA therapy is necessary for PD patients as there is no other therapy provides more powerful antiparkinsonian effect.

SYNTHESIS OF L-DOPA

Chemical Synthesis

Chemical synthesis of L-DOPA involves use of extensive chemicals, catalyst under harsh production conditions. Resultant L-DOPA by the process is racemic DL mixture, which is inactive and to separate enanti- ometrically pure L-DOPA is very difficult. The recemic mixture was easy to obtain but difficult to separate by the time it has been resolved, the projected costs doubled. Monsanto's position in vanillin, which provided the L-DOPA moiety, found that they were custom manufacturing a recemic intermediate, which was done by de- blocking to L-DOPA according to Hoffman LoRche. The synthesis, which followed closely the Erlenmeyer azl actone procedure described in organic synthesis, went by way of a pyrochiral enamide, which was hydrogenated to block DL-DOPA . Currently in the market the available tablets for L-DOPA are under various brand names Sinemet®, Atamet®, Parcopa® and Stalevo®. More over L-DOPA obtained from mentioned chemical methods was found to be with 90% recovery.

Sources of L- DOPA

L-DOPA (l-3,4-dihydroxyphenylalanine) was first isolated from seedlings of *Vicia faba* by Marcus Guggenheim in 1913. The growth of *Mucuna pruriens* is a leguminous plant commonly found in humid, tropical climates of Caribbean and parts of China, India and Africa. The velvet bean (seed) variety of *M. pruriens* is found to contain 4-7% of L-DOPA. Other food sources of Dopamine are bananas, blackberries, broccoli, cauliflower and some herbs like rosemary, basil and cilantro. Recently a wild plant Mucuna monosperma and a vegetable Antheum graveolens have been targeted for extracting sufficient amount of L- DOPA. A new species of Mucuna sanjapee containing high amount of L-DOPA is still under research. Baptisia, Lupinus and latex of Euphorbia are found to contain less amount of L- DOPA which requires advanced extraction process. Kurt et al., reported folded higher dopamine levels in recombinant bacteria (*Citrobacter freundii* and *Erwinia herbicola*) bearing the Vitreoscilla hemoglobin gene. A mutant strain of Aspergillus oryzae gave 3.72 folds of LDOPA production than the parental strain by the conversion of L-tyrosine to L-DOPA using the mould mycelium. Other leguminous seed found to contain L-DOPA are *Mucuna gigantean, Mucuna atropurpurea, Canavalia ensiformis, Canavalia gladiate* and *Cassia floribunda*. *Astragalus cicer* is also reported to contain high amount of L- DOPA. Biotransformation of L tyrosine to L-DOPA has been carried out by Neurospora crassa and Agaricus bisporus. Out of all the reported varieties, *Mucuna pruriens* var utilis has been found to contain 8.05% of L- DOPA.

Dopamine and its Neurotransmission

Dopamine is a neurotransmitter discovered by Arvid Carlsson, about 50 years ago45 that controls motor and nonmotor functions of the CNS. Besides, dopamine also plays other roles in our body including circulatory, renal, digestive and immune system. Dopamine belongs to the class of catecholamines, having a catechol ring and an amide side chain. The action of dopamine is exerted upon binding to five DA receptors in brain D1-514.

The synthesis of dopamine starts with the hydroxylation of phenylalanine to tyrosine and then to levodopa and finally to dopamine via decarboxylation. Tyrosine hydroxylase is the rate limiting enzyme in the synthesis pathway. Localization of tyrosine hydroxylase occurs in human at chromosome. The biosynthesis of dopamine from L-DOPA requires pyridoxal phosphate as a coenzyme and tetrahydrofolic acid for conversion of L-tyrosine to L-DOPA. NADH is required for the formation of the above-mentioned coenzymes.

Dopamine is a cytosolic protein transferred into synaptic vesicles by the vesicular monoamine transporter (VMAT2) from where the release of dopamine in a quantal manner occurs whenever a nerve impulse is generated and travels down the axon reaching the nerve terminal. Upon reaching the synaptic cleft, dopamine can act both on postsynaptic and presynaptic receptors. The metabolism of dopamine to HVA and DOPAC is done via MAO (intraneuronal enzyme) and that to 3-MT via COMT (extra neuronal enzyme). Reversible COMT inhibitors such as Entacapone and Tolcapone are used as adjuvants to L-DOPA, approved for clinical use in PD patients, thus improving the clinical benefits of L-DOPA therapy. The end of dopamine signal is marked by the spontaneous amine diffusion, amine reuptake and local enzymatic degradation in

presynaptic neurons by dopamine transporter. The first description of dopaminergic neurons and pathways was introduced using histofluorescence techniques in the 1960s and 1970s. The dopamine receptors were characterized biochemically into 2 families in the brain- D1 and D5 that could stimulate adenylyl cyclase and improve cAMP production and D2, D3 and D4, negatively coupled to adenylyl cyclase activity thus inhibiting cAMP production. The crossing of BBB of L-DOPA and decarboxylating into dopamine occurs in the nigrostriatal pathway by brain enzymes.

Inactivation and degradation of dopamine

The inactivation of dopamine is done by two processes: Reuptake of dopamine is done by dopamine transporter (DAT) and VMAT. DAT helps to transport dopamine from extracellular to intracellular space and VMAT reloads dopamine into the vesicles. This whole process is energy dependent and uses Na-K ase from ATP hydrolysis to create a concentration gradient of ions across the presynaptic membrane. This drive opens the transporter and co-transport Na and Cl ions and dopamine from the synaptic cleft. The released K ions in the synaptic cleft help in the equilibration of ionic gradient across the presynaptic membrane. Metabolism of dopamine by MAO and COMT is one of the effective mechanisms for dopamine inactivation. This includes several pathways like oxidative deamination by MAO, conjugation by glucoronidases or sulfotransferases and O-methylation by COMT. MAO acts intracellularly and is located at external membrane of mitochondria whereas COMT acts extracellularly and is located within the external cell membrane.

Storage and exocytosis of dopamine

Dopamine is transported (translocated) to storage vesicles from the cytoplasm at a high concentration nearing its limit to solubility by the help of VMAT. Dopamine is stored in classical vesicles and SER at the dendrites. These vesicles protect dopamine from enzymatic degradation, facilitate regulated release and rapid replenishment of depleted stores.

The vesicles are composed of lipid bilayer membranes embedded with integral protein that takes part in vesicular trafficking, fusion and docking. To maintain the proton gradient, the vesicles contain H+ATPase.

Mode of action of L-DOPA

Dopamine is a chemical messenger which helps the brain to regulate and control all the processes in our body. The deficiency of dopamine affects both the motor and nonmotor function in human and is dominant in males. The dopamine relayed messages fail to pass through one neuron to the other and in turn affect the Central Nervous System (CNS). This is a slow and progressive disorder which may be found with aging.

Levodopa (L-DOPA/ 3, 4 - L - dihydroxyphenylalanine) is an amino acid and a precursor of dopamine can easily cross the blood brain barrier and convert to dopamine by Dopa Carboxylase by a single enzymatic step, thus increasing the store of dopamine in the brain. Unlike dopamine, L- DOPA can be taken orally or intravenously. It is rapidly taken up by dopaminergic neurons and converted to dopamine. The conversion of L- DOPA to dopamine mainly occurs in the periphery as well as in CNS. Earlier in clinical trials, the conversion was expected to occur in the basal ganglia of the patients. In Parkinson's, the basal ganglia are degenerated, thus hampering the production of dopamine which controls movement. Another mneurotransmitter acetylcholine levels up with the decrease in dopamine levels, resulting in opposite effects in which muscles become rigid. The patients undergoing oral treatment of L- DOPA were found to had 9-15-fold higher levels of dopamine and homovanillic acid. The biotransformation of L- DOPA in the brain was marked by several observations. In 1950 Hornykiewicz studied the reversal of reserpine induced state of tranquilization by LDOPA and the preferential location of occurrence of dopamine was confirmed mostly in basal ganglia. The combination of L-DOPA with carbidopa or benserazide, peripheral DOPA decarboxylase inhibitors ensures a higher percentage allowance of dose to cross the BBB, thus preventing peripheral metabolism of L-DOPA. None of the other recently discovered dopamine agonists could surpass the clinical benefits of L-DOPA therapy. The long duration response of L-DOPA helps in adequate symptomatic control with dosage schedules due to the ability of nigrostriatal system to convert L-DOPA to dopamine, thereby storing it in the presynaptic vesicles and release in response to any kind of physiological stimuli. It was found that the L- DOPA decarboxylating activity was largely confined in the Parkinsonian striatum along with a widespread distribution of monoamine oxidase and catechol-0-methyl transferase (COMT). The dopamine activity (striatal dopaminergic neurotransmission) can be restored in a Parkinsonian patient by external supplementation of L- DOPA to stimulate auxiliary electrode and a glassy carbon working electrode. The use of the enzyme glucan sucrase and sucrose helped in the glycosylation of L-DOPA thus reducing the oxidation of phenolic hydroxyl groups and their methylation.

Biological Sources

Until the middle of the 20th century, the amino acid 3,4-dihydroxyphenylalanine (L-DOPA) was just seen as an intermediate in the biological synthesis of melanin and epinephrine. In the earliest 1960s, it was proved that, L-DOPA is a neurotransmitter precursor has wide thera- peutic applications. There were many biological sour- es reported for enantiometrically pure L-DOPA

The production of L-DOPA from biological sources involves the oxidation of L-tyrosine by enzyme tyrosinase (E.C.1.14.18.1), which is copper containing enzyme widely distributed in plants, animals and microorganisms. Oxidation product of tyrosinase subsequently con-verted in to melanin; as it functions like alternative sub- strate for tyrosinase ultimately stimulate catalytic efficiency. This cresolase and catecholase mechanisms oc- curs in melanin synthesis pathway in microorganisms, while in brain naturally the L-tyrosine is converted in the epinephrine finally by the series of enzymatic reactions initiated with tyrosine hydroxylase. The process for the synthesis of L-DOPA form different species is carried out using various methods.

Enzymatic Synthesis

Mushroom tyrosinase has been commercially used in the enzymatic synthesis of L-DOPA by enzyme immobilization. It lowers the production cost due to the reusability of the enzymes. The various techniques used for enzyme immobilization include entrapment in poly- meric gels, adsorption onto insoluble materials, encapsu- lation in membranes, cross-linking with bifunctional or multifunctional reagents and linking to an insoluble carrier . The substrate used for the synthesis of L-DOPA were catechol, sodium pyruvate and ammonium acetate.

Fungal Sources

Mostly, L-DOPA from fungal species was obtained in the reaction mixture containing substrate L-tyrosine and mycelia in the buffer. Briefly, the mycelia were sus- pended in reaction mixture containing L-tyrosine, L- ascorbic acid and intact mycelia under optimized condi-tion. In addition, specific additives were used as elicitors for enhanced yield of L-DOPA. L-DOPA produced by the method was enantiometrically pure as well as it is cost effective. Similarly, biotransformation of L-DOPA from L-tyrosine was carried out using *Acremonium reticulum* by submerged fermentation process yields more amount of L-DOPA in the broth. Production of L-DOPA also reported from yeast species.

Bacterial Sources

L-DOPA produced from different bacterial species in- cludes both in broth as well as in buffer with substrate and acclimatized cells. Initially, the medium optimization was performed in order to maximum production of L-DOPA. To overcome the tedious downstream process- ing after production of L-DOPA by this process, use of acclimatized cells with buffer gave the best results within less time and experimentation. In case of L-DOPA production using enzymatic process of recom- binant *E. herbicola* cells carrying a mutant transcripttional regulator TyrR yield obtained was near about 15g·l−1·h−1 . This mechanism of synthesis is now ac-cepted commercially by the industry named as Agino-moto CO.LTD.

Biologicalsources of L –DOPA Source L- DOPA Producers

Plant source
Mucunapruriens
Viciafaba (Fava bean)
Mucunamonosperma
Tissue cultures of Banana
Mucunamutisiana (seed)
Vigna vexillata (seed)
Vicianarbonensis (green plant with pod)
Viciafaba var minor (green flowering plant)
Mucunapruriens var. utilis (white whole seed)
Mucuna pruriens (endocarp)
Mucuna pruriens (endocarp)
Bacterial source
Recombinant *Erwinia herbicola*cells strain AJ2985
Bacillus sp. JPJ
Pseudomonas melanogenum
Vibrio tyrosinaticus
Brevundimonas sp. SGJ
Fungal source
Aspergillus oryzae
Acremonium retilum
Aspergillus niger
Actinomycetes

CONCLUSION

Most of the L-DOPA isolated is either from natural sources or synthesized chemically, but biological sources could be used as an alternative source for L-DOPA pro- duction. The alternative sources of L-DOPA and a further clinical trial will open the subject of extensive research. Chemical synthesis methods were time consuming, re- quired costly and toxic chemicals like catechol, pyro- catechol and also resulted in recemic mixture of L-DOPA. Biological synthesis of L-DOPA will be the most prom- ising approach to overcome ill-effects of chemical drugs. Different sources like enzymatic, fungal, bacterial and plant, yield maximum amount of enantiometrically pure L-DOPA under optimized cultural conditions. Most of the plant sources belong to legumes which can be used for the consumption, along with their L-DOPA content; role of plant phenolics (antioxidants, antimutagens and scavengers of free radicals) becomes vital as far as total health is concerned. L-DOPA from natural sources re- duces the secondary complications also helps to delay the progression of the disease. Taken together, most of the research on synthesis of L-DOPA has been done us- ing biological sources but now it is necessary to find ex- act mechanism of action and chances to cure the Parkin- son's disease in future.

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