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Non-invasive exosome diagnostics for Parkinson's disease

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Abstract

Parkinson's disease (PD) is a neurodegenerative disease, characterized by signs and symptoms such as festinating gait, bradykinesia, resting tremor, and cognitive delays.

Exosomes have emerged as biomarkers of clinical interest for the diagnosis and prevention of Parkinson's disease. These organelles between 30 nm and 200 nm in diameter play a crucial role in protein biogenesis, cellular homeostasis, and cell-to-cell signal transmission.

Exosomes with intraneuronal accumulation of α-synuclein protein are relevant in Parkinson's disease as a key pathological feature in disease progression and Lewy body formation.

Keywords: Exosome; a-Synuclein; Parkinson's disease; Lewy bodies; Extracellular vesicles; Basal ganglia

1. Introduction

Parkinson's disease is a complex neurodegenerative disease that appears mainly in adulthood and is currently recognized as the second most common neurodegenerative disease after Alzheimer's disease. It develops mainly between 55 and 65 years of age and between 1% and 2% in people over 60 years of age. It tends to increase with age, its peak is between 85 and 89 years with a percentage of 3.5%. The probability of suffering it is higher in men (1.4:1.0) than in women, and the overall incidence ranges between 5 and 35 cases per 100,000 people. Within all these statistical values, 80% of all cases are usually idiopathic and with risk factors (exposure to pesticides, herbicides and heavy metals such as manganese, mainly) [1].

Previous research shows that its anatomopathological basis is characterized by the progressive loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc) of the midbrain, in addition to the presence of Lewy bodies. Due to this, dopaminergic denervation of SNpc axons to the striatum occurs, which modifies the normal function of the basal ganglia leading to the characteristic clinical conditions of the disease [2].

2. Physiology of the basal ganglia

The physiological basis of Parkinson's disease is dysfunction of the basal ganglia (BG) system due to dopamine depletion. The BG are subcortical nuclei, including the striatum (caudate and putamen), the subthalamic nucleus (STN), the globus pallidus externus (Gpe) and globus pallidus internus (Gpi) with their connections to the substantia nigra pars compacta (SNpc) and substantia nigra pars reticulata (SNr) and the ventrolateral nucleus of the thalamus. The latter are interconnected by projections to the thalamus and brainstem, constituting a cortico-subcortical network. The BG are

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divided into 3 circuits: motor, limbic and associative/cognitive. The dorso-lateral portion constitutes the motor circuit that projects to the primary motor cortex and the supplementary motor area.

Dopaminergic depletion results in a potentiation of the so-called indirect or "inhibitory" pathway and decreases the direct or "facilitatory" pathway of movement. This manifests as hyperactivity of the STN and the Gpi/SNr complex and thus a thalamo-cortical inhibition [2].

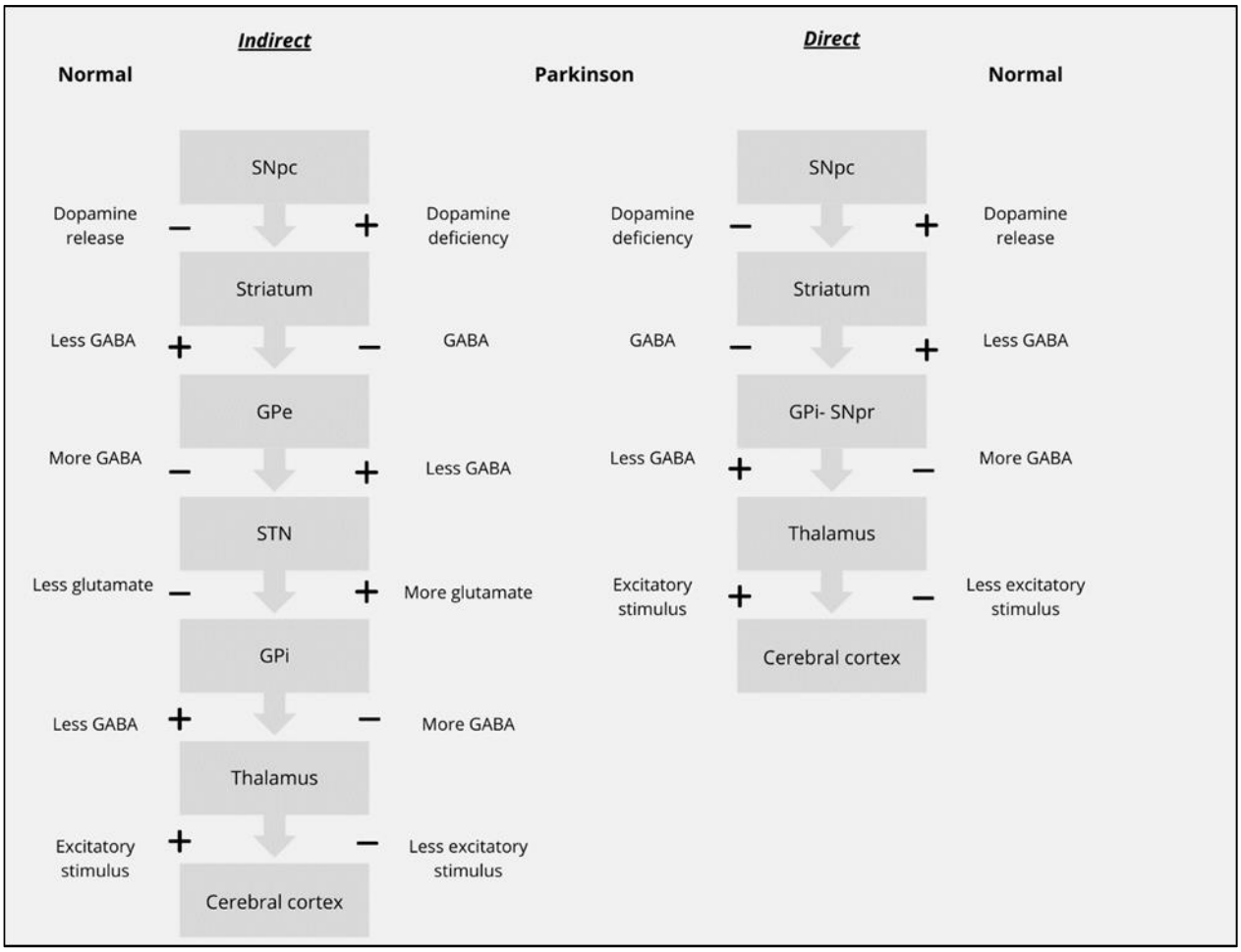

Figure 1 General scheme of the direct and indirect pathway in the basal ganglia. Modified from [3]

3. Clinical manifestations of Parkinson's disease

The loss of dopamine in Parkinson's disease generates neurophysiological alterations in the basal ganglia that are responsible for characteristic motor symptoms, such as tremor, rigidity, bradykinesia, postural instability, postural deformity, freezing, as well as sensory abnormalities (Table 1).

Table 1 Clinical manifestations of Parkinson's disease

Clinical manifestations	Description
Bradykinesia	Slowness of movement. This may include difficulties with tasks requiring fine motor control as well as loss of spontaneous movements and gesticulation, drooling, monotonic and hypophonic dysarthria, loss of facial expression, decreased blinking, and reduced arm swing while walking [4].
Tremor at rest	It has a frequency of 3-6 Hz, starts distally in an extremity, usually in a hand, and less frequently in the lips, jaw, tongue or a lower extremity. It disappears with movement [5].
Stiffness	The resistance to the efforts to obtain the passive joint, can affect any part of the body [6].
Postural instability	It is the inability to maintain balance due to loss of postural reflexes, balance, adoption of a flexed posture and trunk rotation [7].
Postural deformities	Can reduce dexterity and interfere with activities of daily living, alter gait, increase caudae and produce pain or discomfort [8].
Freezing (motor blockages)	A form of akinesia (loss of movement) and is one of the most disabling symptoms [4]. Is associated with the development of balance and speech problems [9].
Sensory anomalies	Such as olfactory dysfunction, pain, paresthesia, akathisia. One study found that olfactory dysfunction (hyposmia) may be an early marker of PD; it correlated with a 10% increased risk of the disease 2 years later. Olfactory dysfunction is related to neuronal loss in the corticomedial amygdala or decreased dopaminergic neurons in the olfactory bulb [4].

4. Physiopathology of Parkinson's disease

The exact cause of neuronal death in PD is not yet known. Genetic, environmental, mitochondrial, inflammatory, excitotoxic factors are included, although it is most likely that it is a combination of several of them that leads to the onset of the disease the origin of the degeneration process suffered by dopaminergic neurons could be mitochondrial dysfunction, endoplasmic reticulum (ER) stress, and α-synuclein aggregation [3].

The complex 1 of the electron transport chain in mitochondria is key for proper functioning in obtaining ATP; a decrease in this enzyme leads to a lower availability of energy in neurons, affecting their viability. Another cause associated with this dysfunction is oxidative stress with the accumulation of reactive oxygen species, molecules that cause damage to lipids, proteins and DNA; this damage is detrimental to dopaminergic neurons, which are metabolically very active as they require a lot of energy to function and when this energy is not provided, they become more vulnerable to damage, In addition, some environmental factors, such as exposure to certain toxins, may aggravate the problem. This suggests that PD occurs due to a combination of genetic predisposition and environmental exposure [10-14].

Endoplasmic reticulum (ER) stress occurs when this organelle accumulates misfolded or non-functional proteins in its lumen, activating the unfolded protein response (UPR). This response seeks to restore equilibrium, but when misfolded proteins overload the ER quality systems and damage persists. In addition, ER stress is closely linked to the dysfunction of autophagy, a process essential for eliminating defective proteins and damaged organelles. The interaction between the two generates a vicious circle: ER stress aggravates autophagy dysfunction, and autophagy, in turn, intensifies ER stress. As a consequence, the accumulation of α-synuclein, a key event in the progression of neurodegenerative diseases such as Parkinson's disease, is favored [15-18].

α-synuclein is a relevant protein in Parkinson's disease (PD) due to its involvement in the formation of Lewy bodies, intraneuronal inclusions composed of this protein Its accumulation is associated with the progressive loss of dopaminergic neurons in the substantia nigra, resulting in the motor symptoms of PD. In addition, it contributes to non-motor symptoms, such as sleep disturbances and autonomic dysfunctions, which precede motor symptoms and are useful for early diagnosis of the disease PD shares characteristics with other synucleinopathies, such as dementia with Lewy bodies and multiple system atrophy, which also present accumulation of α-synuclein and non-motor symptoms, such as autonomic dysfunction and cognitive impairment. From an anatomopathological point of view, Lewy bodies are localized in neurons of the nigrostriatal system, as well as in other regions of the central nervous system, including brainstem nuclei, diencephalon and prosencephalon. Strategies aimed at diagnosing the accumulation of α-synuclein

are being developed, it is being studied as a potential biomarker for early diagnosis and follow-up of PD. Detection of different forms of this protein in body fluids and peripheral tissues could be used for clinical management [19-22].

4.1. Exosomes

Small single-membrane secreted organelles between 30 and 200 nm in diameter that have the same topology as the cell and are enriched with selected proteins, lipids, nucleic acids and glycoconjugates that have been observed as potential disease markers, since their biogenesis they have diverse activities such as extracellular matrix remodeling, transmission of signals and molecules to other cells, some viruses co-opt pathways to both assemble infectious particles and establish host permissiveness, revealing the role of pseudomessengers and intermediates of some pathogens, as well as defects in protein quality lineage, making them viable for diagnosis and prevention of diseases such as cancer and parkinson's [23].

Disease progression is broadly correlated with the evolution of Lewy bodies and neuritic pathology, involving the accumulation and intraneuronal aggregation of α-synuclein, which, depending on its oligodendroglial or neuropathological origin, will be characteristic of a systemic muscle atrophy or PD respectively, it is necessary to translate the measurements of neuro-specific exosomal α-synuclein, so blood samples are used, which are subjected to a clinically useful test, which is the separation of exosome subpopulations in complex biological fluids from contaminants, including α-synuclein derived from peripheral sources so techniques for obtaining exosomes are important [24].

4.2. Sample collection for exasomal isolation

The procedure of choice is the collection of blood, tissue culture and fluid samples from an organic compartment. Samples with minimally invasive amounts of 30 μL of plasma are used in ~100 minutes with quite remarkable sensitivity. During this pre-analytical phrase, the characteristics of the source, the way the source material is handled and stored, along with the experimental conditions, can affect the recovery of extracellular vesicles [25].

4.3. Exosome isolation methodology

Exosomes are distributed throughout very complex body fluids, which makes high-throughput exosome isolation challenging Ultracentrifugation has been the “gold standard” for exosome separation due to its high throughput, high levels of protein aggregates and lipoprotein contamination in exosome samples prepared through this method greatly compromise their quantification and functional analysis [26,27].

Reproducible isolation and enrichment of exosomes is important to assess their biological functions. Exosomes are heterogeneous in size, content, function and source, which makes their isolation difficult In addition to this, most current isolation technologies cannot completely separate exosomes from lipoproteins with similar biophysical characteristics and extracellular vesicles derived from non-endosomal pathways, resulting in low exosomal purity for different purposes and applications, different isolation methods are selected, among which ultracentrifugation, size-based isolation techniques, polymer precipitation and immunoaffinity capture techniques are the most commonly used [28,29].

4.4. Ultracentrifugation techniques

Ultracentrifugation is currently the most widely used isolation technique for exosome separation. Ultracentrifugation mainly collects the required components based on the differences in size and density of each component in the overall solution, making it suitable for the separation of large dose sample components with significant differences in the sedimentation coefficient [30].

Johnstone et al, first applied this method to isolate exosomes in a reticulocyte tissue culture medium, and the method was optimized by Thery et al Exosome samples prepared by differential ultracentrifugation often exhibit low purity, potentially compromising many downstream applications, especially exosome-associated functional analysis [31,32].

4.5. Polymer precipitation

This method generally uses polyethylene glycol (PEG) as medium, and exosomes are collected in the centrifugation condition, which reduces the solubility of exosomes. This method was originally used to isolate viruses, because exosomes and viruses have similar biophysical characteristics, this method is often used in scientific research to isolate and purify exosomes. In this method, samples are co-incubated with PEG solution at 4°C overnight [33,34].

4.6. Size-based isolation techniques

This technique refers to ultrafiltration and size exclusion chromatography, which separates based on the size difference between exosomes and other components of biological samples. The separation principle of size exclusion chromatography is that macromolecules cannot enter the pores of the gel. The method typically uses ultrafiltration membranes with different molecular weight cut-off points to selectively separate samples. Isolated exosomes have a complete structure and uniform size, and their biological characteristics are not significantly affected, but they can be doped with other particles of similar size, resulting in reduced purity [35].

4.7. Immunoaffinity chromatography

It is a separation and purification technology based on specific binding of antibodies and ligands to separate desired substances from heterogeneous mixtures. Binding efficiency is closely related to biological affinity pairs, elution conditions and matrix carriers. This method is best suited for samples with smaller volumes. It can be used for qualitative and quantitative determination of exosomes. It has strong specificity, high sensitivity, high purity and high throughput, and does not set the upper limit of the initial sample volume when based on magnetic beads [36].

4.8. Other isolation techniques

Commercial kits based on the traditional isolation technology mentioned above are currently available on the market, such as the exoEasy Maxi kit (QIAGEN), the MagCapture™ PS exosome isolation kit (Wako) and the High Efficiency Minute (Invent)™ exosome precipitation reagent.

4.9. Feasibility of exosome diagnostics

Despite the great advances in recent decades regarding exosomes, there are still many complications in isolating exosomes; this is due to the complexity of biological fluids, the consideration of overlapping physicochemical and biochemical properties between exosomes, viruses, lipoproteins and other extracellular vesicles. The complexity of isolating exosomes has resulted in the lack of acceptance of any specific separation technique, and even the centrifugation method often suffers from protein and lipoprotein contamination. The combined use of isolation is more efficient and feasible when purifying exosomes, but this implies a significant increase in cost, and a greater complexity in the procedure. All this leads us to analyze this methodology for diagnostic practice, mainly because therapeutic applications are limited by the lack of an efficient method to isolate high quality exosomes in masses. Therefore, it is not currently considered a useful method, but it is believed that in the future with technological innovations this practice will reach high levels of utility in different pathologies, including Parkinson's disease [37].

Unfortunately, currently there is still no specific test to aid in this diagnosis, however, cell-specific proteins and genetic materials in exosomes are able to reflect their cellular origin and physiological state, which could be explored as a preclinical biomarker in many types of cancer, used in the clinic because of their molecular specificity, such as lung cancer, hepatocellular carcinoma, pancreatic cancer, etc, Cancer cells need to communicate with each other, with normal cells and with the immune system to survive, proliferate and metastasize. Exosomes have been shown to interfere with communication between tumor and normal cells leading to TME modifications that favor tumor growth, survival, immune escape and invasion, these tumor-acting macrophage-derived exosomes induce immune suppression of tumor progression by mediating cell-to-cell communication with other immune cells, such as T cells and NK lymphocytes that play an important role in innate host defense and tumor cell destruction such as tumor necrosis factor alpha (TNF-α) and interleukin 6 (IL-6) thus favoring the basis for current diagnostic and therapeutic use with exosomes seeking to transpolate this knowledge into Parkinson's diagnosis in which they can help in cases of uncertainty or to discern between systemic muscular atrophy and PD, evaluating the exasomal origin [38-41]

5. Conclusion

It is firmly believed that exosomes are going to have an evolution hand in hand with technology that will help us to purify the way to obtain samples in a pure way where lipoproteins do not interfere and allow us to obtain the origin in a better way, since as we mentioned the origin of exosomes according to studies can define whether Parkinson's disease or a systemic muscular atrophy depending on its origin, for this and for economic reasons is that even today it is not the preferred method of diagnosis because it is not entirely reliable, however, it builds the basis for a new form of diagnosis in the future that will also help to predict the possibility of developing Parkinson's disease and therefore be able to adopt a lifestyle that can restrict the possibility of developing it and achieve a future with more years of activity for people with this risk.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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