3.1 Review on Huntington’s disease

HD is an autosomal dominant inherited disease caused by the repetition of elongated Cytosine-Adenosine-Guanine (CAG) on the short arm of chromosome 4 at 4p16.3 in the Htt gene (Bates GP et al., 2005). As long as the CAG repeat increases, the onset of the disease is earlier. HD can be defined as a movement disorder with a heterogeneous phenotype which is characterized by involuntary motor impairment with cognitive, psychiatric deficits and bio energetic inadequacy (Montoya A, Lepage M., 2006)

Illustrating the part of the brain affected by the Huntington’s disease

3.1.1 History

In 1872 Huntington’s disease was first identified and described in a paper by George Huntington, granting a model for the disease’s phenotypes. His patients had a common lineage – all had family members which had emigrated from Suffolk, England in the mid-1600s. Before him, his father and grandfather also studied the same group of patients. It is believed that the occurrence of Huntington’s disease was seen in 1600s, but it was misunderstood as a “dancing disorder” and was viewed as witchcraft. Although it is believed that previous characterizations of people with Huntington’s disease were recorded, the credit for the development of the disease characterization is still granted to George Huntington.

Even after his paper was published, it was over 100 years ago the gene associated with Huntington’s disease was discovered. In order to isolate this unknown gene, researchers used the DNA samples of families in Venezuela, where Huntington’s disease and consanguinity are highly prevalent. Finally in 1993, the
researchers discovered a trinucleotide repeated chain which was unstable when expanded and which they believed was strongly linked to Huntington’s disease.

3.1.2 Epidemiology

Huntington's disease is a rare neuropsychiatric disorder with a prevalence of 5-10/100,000 in the population. Genetic confirmation of the CAG repetition is the hallmark of epidemiological measure of HD. Prevalence studies incorporating both genetic and clinical diagnostic standards. Germ line instability of intermediate alleles increases with CAG repeat length, indicating that longer CAG repeats in the general population results in higher CAG expansion rate and higher prevalence of HD.

3.1.3 Pathophysiology

The most neuropathology in HD shows within the neostriatum, in which gross atrophy of the caudate nucleus and putamen is accompanied by selective neuronal loss and astrogliosis. Marked neuronal loss also is seen in deep layers of the cerebral cortex. Other regions, including the globus pallidus, thalamus, subthalamic nucleus, substantia nigra, and cerebellum, show varying degrees of atrophy depending on the pathologic grade.

Huntington’s disease affects both cognitive and motor abilities. Patients experience chorea - over-the-top jerky movements which are uncontrolled. Due to these erotic movements, many see increased muscle tone, also called dystonia. Often, the uncontrolled muscles begin with those farthest along the limbs from the trunk, i.e. fingers and toes, and those muscles in the face and tongue. Memory, especially working memory, becomes severely limited. A loss of this type of memory is due to damage of the caudate nucleus and other subcortical areas. Nonetheless, damage to basal ganglia is reflected in the inability to follow procedural memory. Implicit memories are also lost, culminating in difficulty chewing and swallowing. However, long-term memory is still available, and episodic memories, with prompting, can still be accessed. It is harder for Huntington’s disease patients to initiate behaviors, yet once started, they become fixated on these behaviors, losing sight of other activities.
Mitochondria enzymes

In biochemical studies defects in respiratory chain is found in HD individuals. The activity of complex II/III are greatly decreased in comparison of complex IV in HD patients but pre symptomatic patients has shown no changes in the activity of complex II, III and IV. Minor changes were observed in respiratory chain enzymes of cerebral cortex but no changes were observed in blood cells. The other enzymes of oxidative metabolism were also reported with reduced activity in the striatum. The levels of aconitase and pyruvate dehydrogenase complex were also significantly decreased in HD individuals. These decreased enzymes levels were observed in symptomatic patients having atrophy of striatum.

Molecular understandings

The huntingtin gene is present on the short arm of chromosome four. The huntingtin gene is believed to have a role in cell signalling as well as adenosine monophosphate as a binding protein and to help the body prevention of cell toxicity and cell death. The wild type of gene is generally seen in the nervous system. The protein has presence in the cytoplasm and vesicles of neuronal cells in the brain.
This specific gene codes for three CAG cycles that are repeated up to 27 times in a normal, wild type genome. If an individual has between 36-40 repetitions, he/she has a chance of developing Huntington’s disease. The mutation that occurs in Huntington’s disease involves this trinucleotide cycle continuing to repeat unchecked 40 or more times which forms the mutant huntingtin protein found in exon one of the gene. The repeat occurs on the 5’ end of the chromosome and the repetitive sequence is then translated into a polyglutamine (polyQ) region.

Htt gene is located at chromosome 4 at 4p16.3 that encodes the protein huntingtin, the normal function of which is unknown. Htt gene is abundantly expressed in the brain and testes with moderate expression observed in other organs such as liver, heart, and lungs.

Huntington’s disease gene

The length of the CAG trinucleotide repeat that encodes Poly Q segment of varying length can be determined in any individual (normal, at risk or clinically diagnosed with HD). This repeated sequence of CAG is polymorphic in the normal population in the range of 6–35 units; when expanded to ≥40 units, the mutation occurs at higher rate, which initiates the disease process that leads to the onset of motor symptoms that can be diagnosed. Repeats of 36–39 CAG units show reduced penetration rate, as some individuals with these CAG lengths have HD, whereas others live a normal lifespan without being clinically diagnosed. The CAG repeat shows instability through meiotic transmission that can be seen in the intermediate CAG repeat range 27–35 units; this instability increases with increasing in CAG length. CAG repeat typically increases or decreases in length by one or few CAGs.
The length of the CAG repeat in Htt gene determines whether an individual will develop HD or not; it is also the primary determinant of the rate of pathogenesis leading to the characteristic motor signs that underlie the clinical diagnosis. Importantly, with respect to these motor signs, the timing of onset is determined by the allele with the longer CAG repeat in a completely dominant manner; the second Htt allele, regardless of its length (normal or otherwise), does not alter the rate of the process that leads to a clinical diagnosis.

The precise nature of the pathogenic trigger that conforms to these genetically defined criteria (CAG length dependence and allele dose in dependence) is not known, but the demonstration that the length of the CAG repeat, even in the normal range, correlates with measures in some cellular assays (for example, cellular energy charge suggests that it might involve a gain of function that acts through augmentation or deregulation of one or more normal functions of huntingtin. Although several genes — including ADORA2A, ATG7, CNR1, GRIK2, GRIN2A, GRIN2B, HAP1, PPARC1A, MAP2K6, MAP3K5, NPY, NPY2R, OGG1, PEX7, TP53 and UCHL1 — have been proposed as genetic modifiers of HD.

**HTT mutation that causes Huntington’s disease**
Basal ganglia, is constitute by the major portion of striatum which integrates signals from the cerebral cortex, and is the centre for appropriate selection of behavioural action. Its dysfunction has been associated with classical motor disorder such as HD, dystonia and depression. The main barrier for such interrogation has been its apparent homogeneity, which obscures its inner compartments. The identification of two intermingled populations of medium spiny neurons (MSNs), expressing either dopamine receptor D1 or D2 is an important step in understanding of striatal subdivisions. The efferent projections of these molecularly defined populations constitute two separate pathways in the basal ganglia; a direct pathway (D1) projecting to the substantia nigra pars reticulata (SNr) and the internal globus pallidus (Gpi), and an indirect pathway (D2) projecting to the external globus pallidus (Gpe). The mechanism of selective degeneration of striatal neurons suggests that reduced trophic support renders striatal neurons more vulnerable to the toxic actions of mHtt gene. Numerous in vitro and in vivo studies have shown that striatal neurons require brain-derived neurotrophic factor (BDNF) for their survival and functioning. A deficiency in BDNF-mediated signaling alone is sufficient to cause dendritic abnormalities and neuronal loss in the cerebral cortex and striatum. Moreover, reduced levels of striatal BDNF were detected in both HD animal model and HD patients.

3.1.4 General Mitochondrial Defects in HD Patients

★ Mitochondrial enzymes

In biochemical studies defects in respiratory chain is found in HD individuals. The activity of complex II/III are greatly decreased in comparison to complex IV in HD patients but pre symptomatic patients showed no changes in activity of complex II, III and IV. Minor changes were observed in respiratory chain enzymes of cerebral cortex but no changes were observed in blood cells. The other enzymes of oxidative metabolism were also reported with reduced activity in the striatum. The levels of aconitase and pyruvate dehydrogenase complex were also significantly decreased in HD individuals. These decreased enzymes levels were observed in symptomatic patients having atrophy of striatum.
**Mitochondrial membrane potential**

Mitochondria which is isolated from mHtt gene expressing cells, showed decreased membrane potential. The mitochondrial membrane depolarization was increased in lymphoblast of HD patients as compared to control lymphocyte, when subjected to apoptotic stress. This loss of potential was correlated with CAG repeat expansion. The neural cells showed high sensitivity to Ca\(^{2+}\) induced permeability transition. The loss in mitochondrial membrane potential and permeability transition was demonstrated by directly interaction of recombinant mHtt gene with the outer mitochondrial membrane.

**Mitochondrial Ca\(^{2+}\) buffering capacity**

Increased cytoplasmic Ca\(^{2+}\) levels are toxic to neurons. The Ca\(^{2+}\) buffering capacity of cells expressing mHtt gene can be reduced.

3.1.5 Inheritance of HD

Everybody has two copies of the Htt gene, but only one changed copy of the gene can develop the disorder. The normal copy cannot compensate for the effects of the copy that is mutated. When people who have Htt gene with mutation have offspring, they can pass on either their normal copy of the gene or the copy with the mutation to their offspring. This means there is a 1 in 2, or 50% chance of their child inheriting the gene with mutation. There is also a 1 in 2 or 50% chance of their child inheriting the normal copy of the gene. A person who inherits the HD gene, and survives long enough, will sooner or later develop the disease. In some families, all the children may inherit the Htt gene; in others, none do. If one child inherits the gene has no bearing on whether others will or will not share the same fate. A small number of cases of HD are sporadic, that is, they occur even though there is no family history of the disorder. These cases are thought to be caused by a new genetic mutation or alteration in the gene that occurs during sperm development and that brings the number of CAG repeats into the range that causes disease.

3.1.6 Mechanism of cortical and striatal nerve cells death in HD patients

The exact function of normal huntingtin protein is not yet known by the scientists but it is very important for development and remains active in the whole
body. The symptoms of HD individuals might be due to regular interaction of huntingtin protein with protein found only in the brain and this changed form of huntingtin protein disrupts this interaction leading to nerve cell death. Various studies showed that huntingtin protein interacts with two proteins; huntingtin-interacting protein (HIP-1) and huntingtin-associated protein (HAP-1) present only in the brain and this is the reason that HD affects only the brain. The interaction of HIP & HAP with huntingtin protein depends on the number of CAG repeats in Htt gene. The increase number of CAG repeat leads to binding of huntingtin protein more to HAP-1 than HIP-1.

3.1.7 Genetics of HD

The mutation responsible for HD constitutes an unstable expansion of cytosine-adenine-guanine (CAG) repeats within the coding region of the IT15 gene which is located on the short arm of chromosome 4 (4p63) and is composed of 67 exons and encodes a protein of 3,144 amino acids, called huntingtin (Htt). Exon 1 contains a CAG trinucleotide repeat that encodes the amino acid glutamine, followed by another repeat that encodes proline. Consequently, mutant huntingtin bears a tract of consecutive glutamine stretch polyQ>36 residues in its NH2-terminal, 17 amino acids downstream of the initiator methionine. In unaffected individuals, there are 10–34 CAG repeats. In those affected by HD, there are more than 40 repeats. In those with 35–39 a repeat, the disease is variably penetrant. The age of onset of the disease varies inversely with the number of CAG repeats. Individuals with juvenile onset usually have over 55 repeats, and they usually inherit the gene from their father. Men occasionally have expanded repeats in their sperm. The expansion is thought to occur via slippage during the DNA replication process.

The normal allele is transmitted from generation to generation in a Mendelian fashion. The mutant allele is unstable during meiosis, changing in length in the majority of intergenerational transmissions, with either slight increases of 1–4 units or decreases of 1–2 units. In rare occasions, larger-sized increases occur in parental transmissions, reflecting a particularly high mutation rate during spermatogenesis.
3.1.8 Huntingtin Protein

Huntingtin is a protein composed of >3100 amino acids and with a molecular mass of 349 kDa, depending on the exact number of glutamine residues. A polymorphic proline-rich segment follows the polyglutamine tail. Huntingtin does not show structural homology with any other known protein.

a. Wild-Type Huntingtin: The wild-type protein is widely expressed throughout the body, in both neuronal and non-neuronal cells but the function of this protein remains unknown. Huntingtin is expressed in the cytoplasm of most cells in the body. In the brain, expression is found predominantly in neurons. Within the cell, wild-type huntingtin is mainly structural homology with any other known protein localized in the cytoplasm associated with organelles such as mitochondria, the Golgi apparatus, the endoplasmic reticulum, synaptic vesicles and several components of the cytoskeleton. Wild-type huntingtin is also present inside the nucleus, although to a lesser extent. Wild-type huntingtin binds to numerous proteins in which 234 high-confidence huntingtin associated proteins were identified.

b. Mutant Huntingtin (mHtt): The expanded polyglutamine is believed to confer a new function to huntingtin that is toxic to the cell (toxic gain of function). Indeed, the mutant protein (in either its soluble or its insoluble aggregate form) has been shown to disrupt several intracellular pathways by abnormally interacting and/or sequestering key components of these multiple pathways into the aggregates. On the other hand, several lines of evidence also suggest that a loss of function of wild-type huntingtin (due to its decreased expression and/or sequestration into the aggregates by interacting with the mutant protein) also contributes to the disruption of intracellular homeostasis, culminating in neuronal dysfunction and death. The contribution of both the toxic gain of function of mutant huntingtin and the loss of function of wild-type huntingtin for the deregulation of relevant intracellular pathways that ultimately lead to cell loss in HD.

3.1.9 Neuropathology

HD is primarily characterized by neuronal loss in the striatum (caudate nucleus and putamen) and cortex. Gross striatal atrophy is prominent, but thinning of
the cortical mantle and low brain weights and volumes are documented well. However, many other nuclei including the globus pallidus (GP), thalamus, hypothalamus, subthalamic nucleus, substantia nigra (SN), and cerebellum also are affected. There is also diffuse loss of cerebral white matter.

Based on the pattern of striatal degeneration in post-mortem tissue HD is classified into five different severity grades (0-4). Grade 0 appears indistinguishable from normal brains after gross examination. However, 30-40% neuronal loss can be detected in the head of the caudate nucleus upon histological examination.

**Grade 1** shows atrophy in the tail and in some cases the body, of the caudate nucleus. Neuronal loss and astrogliosis are evident in the head (50% loss), tail and, to a lesser extent, in the body of the caudate nucleus.

**Grade 2** is associated with gross striatal atrophy that is more pronounced than that detected in grade 1 brain.

**Grade 3** displays severe gross striatal atrophy.

**Grade 4** includes HD cases with severe atrophy of the striatum and up to 95% neuronal loss. Grades 1 and 2 non striatal structures are generally spared or only show a slight atrophy, whereas in grades 3 and 4 the cerebral cortex (particularly layers III, V and VI), globus pallidus, thalamus, subthalamic nucleus, substantia nigra, white matter and cerebellum can be markedly affected.

Hypothalamus can be significantly atrophied in HD patients, which is in agreement with findings of loss of somatostatin-positive neurons in the lateral tuberal nucleus and of orexin (hypocretin)-secreting neurons in the lateral hypothalamus. Because of this generalized cerebral atrophy observed with the most severe cases, the overall brain weight can decrease by up to 40%.

**Mechanisms of neurodegeneration in HD**

Although not necessarily a direct result of the mutant protein, various mechanisms such as excitotoxicity, Domoic acid (DA) toxicity, metabolic impairment, mitochondrial dysfunction, oxidative stress, apoptosis and autophagy have been implicated in HD pathology. Many of these mechanisms may slowly develop over time, becoming increasingly pronounced by the late stages of the disease.
Metabolic dysfunction, mitochondrial impairment and oxidative stress: Studies in HD patients and HD post-mortem tissue have shown the following.

1) A significant decrease in glucose uptake (consumption) in the cortex (frontal and temporal lobes) and striatum (caudate and putamen) of both pre-symptomatic and symptomatic HD patients.

2) A significant reduction in aconitase activity in the striatum (caudate and putamen) and cerebral cortex. Aconitase is an iron- and sulphur containing enzyme of the tricarboxylic acid cycle and is thought to be particularly sensitive to inhibition by peroxynitrate (ONOO and O2). Thus, a reduction in its activity can be interpreted as an indirect indicator of ROS generation, mitochondrial dysfunction and excitotoxicity.

3) A significant decrease in the activities of mitochondrial complexes II-III and IV in the striatum.

4) An increase in lactate concentrations in the striatum and cerebral cortex.

5) An increase in lactate/pyruvate ratio in the cerebrospinal fluid.

6) A reduced phosphocreatine/inorganic phosphate ratio in skeletal muscle and a significant delay in the recovery of phosphocreatine levels after exercise (a direct measure of ATP synthesis) in HD patients and mutation carriers.

7) Decreased mitochondrial ATP generation.

8) Morphological and morphometric changes, as well as decreased membrane potential in mitochondria from lymphoblasts of both heterozygous and homozygous HD patients.

9) Depletion of mitochondrial DNA in leukocytes from HD patients.

Interestingly, mutant huntingtin was also shown to disrupt mitochondrial-dependent Ca\(^{2+}\) handling and also can affect mitochondrial function by inhibiting the expression of PGC-1\(\alpha\). Reduced expression of PGC-1\(\alpha\) target genes in HD is related to impaired ATP production and a reduction in intact mitochondria, linking transcriptional deregulation and mitochondrial dysfunction in HD. Importantly, mitochondrial dysfunction is also the major contributor to oxidative stress which, along with the excitotoxic activation of neuronal Nitric oxide synthases (nNOS) and the metabolism of Dopamine (DA) can lead to a toxic increase in the levels of ROS. Susceptible neurons, as in the case of HD, may not be able to handle well an increase in ROS production. High ROS levels may promote intracellular cascades of
oxidative stress by oxidizing proteins and DNA and triggering lipid peroxidation. Therefore, it is reasonable to speculate that oxidative stress might play a crucial role in the neurodegenerative process of HD. In support of this hypothesis, several studies have shown an altered pattern of expression and activity of the enzyme nitric oxide synthase as well as of the antioxidants superoxide dismutase and ascorbate. Furthermore, ROS production is increased in the striatum. Moreover, suggested that NO generation is the underlying cause of the observed inhibition of aconitase in human HD brains. Aconitase inhibition is then followed by the inhibition of complex II–III and the initiation of a self-amplifying cycle of ROS generation that results in severe ATP depletion and cell death.

3.1.10 Aetiology

Huntington's disease is an autosomal dominantly inherited disease caused by a prolonged CAG repeated chain on the short arm of chromosome 4p16.3 in the Huntingtin gene. This gene codes as the huntingtin protein and, on exon 1, contains the CAG line. The wild-type contains a CAG repeat, coding for a polyglutamine stretch in the protein at that site in the range 5 to 25. Huntington's disease is associated with 36 repeats or more. Definite clinical manifestation will occur if the number of repeats exceeds 40. The range 36-39 leads to an incomplete penetrance of the disease or to a very late onset. The range between 29 and 35, the so-called intermediate alleles, is unstable, which means that these alleles are prepared for the change during reproduction. Copying the gene may leads to mistake and very often they lead to elongation and shortening. This phenomenon is mainly seen in the males. An inverse correlation has been described between the length of the repeat and the age. The longer the CAG is repeated, the earlier is the onset. When the disease starts before the age of 20 years, so-called juvenile Huntington's disease (JHD), the repeat often exceeds 55. The length of the repeat determines about 70% of the variance in age at onset and gives no indication at all about the initial symptom, the course, or the duration of illness. The only correlation shown is the faster weight loss associated with a longer CAG repeat.

The normal wild-type Huntingtin protein plays a role in synaptic function and is possibly protective against the toxic mutant. There is evidence that the mutant form leads to a gain of function as well as there is a loss of function. The role of the mutation has been studied in many models: cells, fibroblasts, C. Elegans, drosophila,
mice, rat, sheep and monkey. Mice models (more than 10 available) are most commonly used.

The selective neuronal dysfunction and subsequent loss of neurons in the striatum, cerebral cortex, and other parts of the brain seen in the cases of HD.

Several mechanisms of neuronal cell death have been proposed for HD, including

- Excitotoxicity
- Oxidative stress
- Impaired energy metabolism and
- Apoptosis.

- **Excitotoxicity**

  Excitotoxicity refers to the neurotoxic effect of excitatory amino acids in the presence of excessive activation of postsynaptic receptors. N-methyl D-aspartate receptors (NMDA-R) are depleted in the striata of patients with HD, suggesting a role of NMDA receptor-mediated excitotoxicity, but no correlation exists between the distribution of neuronal loss and the density of such receptors.

- **Oxidative stress**

  Oxidative stress is caused by the presence of free radicals (i.e., highly reactive oxygen derivatives) in the large amounts. This may occur as a consequence of mitochondrial malfunction or excitotoxicity and can trigger apoptosis. Striatal damage induced by quinolinic acid can be ameliorated by the administration of spin-trap agents, which reduce oxidative stress, providing indirect evidence for the involvement of free radicals in excitotoxic cell death.

- **Impaired energy metabolism**

  Impaired energy metabolism reduces the threshold for glutamate toxicity and can lead to activation of excitotoxic mechanisms as well as increased in the production of reactive oxygen species.

  In rats, intrastratial injections of 3-nitropropionic acid (3-NP), an inhibitor of succinate dehydrogenase or complex II of the respiratory chain, cause dose-dependent in ATP depletion which was increased in lactate concentration, and neuronal loss in the striatum. Systemic injections of 3-NP into rats produce a selective loss of medium spiny neurons in the striatum.
Apoptosis

Apoptosis is the cell death that is activated normally in the nervous system during embryogenesis to remove supernumerary neurons as part of natural development.

Morphological features of apoptosis have been well characterized. Oxidative stress, excitotoxicity, and partial energy failure can lead to apoptosis. A subset of neurons and glia in the neostriata of patients with HD appears to undergo apoptosis; one theory is that expanded polyglutamine repeats cause neuronal degeneration through abnormal interactions with other proteins containing short polyglutamine tracts.

3.1.11 Juvenile Disease

As mentioned above, when the CAG repeating region is extremely long Huntington’s disease may occur in juveniles. If the polyQ region is greater than 50 or 60 glutamines, a juvenile form of the disease is experienced, with many different symptoms than the general disease symptoms. When Huntington’s disease symptoms develop in a person before age 20, it is considered Juvenile Huntington’s disease. If the person has not reached to age 10, it is considered as childhood-onset. The youngest person who develops the symptoms of Huntington’s disease was two years old. When Huntington wrote his paper about Huntington’s disease, he has not reported any cases on juvenile onset. Whereas in 1863, J.W. Lyon had already published a paper on which is now called as Juvenile Huntington’s disease and soon after Huntington’s reported in 1872. A. Harbinson published the first report of childhood-onset of Huntington’s disease, so it has been known about for many years. When a juvenile develops in Huntington’s disease, the father is the parent with the disease. During spermatogenesis, the CAG repeated length becomes less stable, so the length increases to the level which raises the juvenile disease. Juvenile patients also suffer from seizures and bradykinesia, a retardation of body movements; they usually do not develop chorea. The brain experiences deterioration in the cerebellum, hypothalamus, thalamus, frontal cortex, and hippocampus.

3.1.12 Progression of disease and symptoms

The nuclear symptoms and signs of HD consist of motor, cognitive and psychiatric disturbances. Other less well-known, but prevalent and often debilitating
features of HD include unintended weight loss, sleep- and circadian rhythm disturbances and autonomic nervous system dysfunction. The mean age at onset is between 30 and 50 years, with a range of 2 to 85 years. The mean duration of the disease is 17-20 years. The progression of the disease leads to more dependency in daily life and finally death. The most common cause of death is pneumonia, followed by suicide.

-The motor symptoms and signs-

The characteristic motor changes are involuntary, unwanted movements. Initially, the movements often occur in the distal extremities such as fingers and toes, but also in small facial muscles. For bystanders these muscle twitches are often invisible or can be explained as nervousness. In daily life, walking becomes unstable and the person can look as if he/she is slightly drunk. Gradually the unwanted movements spread to all other muscles from distal to more proximal and axial. Choreatic movements are present all the time the patient is awake. No single pattern exists, but facial choreatic movements can lead to a continuous movement of facial muscles where for instance an eyebrow is lifted, an eye closed, the head is bent or turned while the tongue is protruded with the lips pouting. The most prominent are the extension movements of the long back muscles. Talking and swallowing gradually become more problematic leading to choking at any time in some patients.

The influence of motor disturbance on activities of daily life progresses over time. The presence of hyperkinesia and hypokinesia results in difficulties in walking and standing, and frequently leads to an ataxic gait and frequent falls. Furthermore, daily activities such as getting out of bed, taking a shower, and dressing, toileting, cleaning the house, cooking and eating become more and more difficult. Depending on the kind of work the patient does, motor signs will sooner or later interfere with performance, even if psychiatric and cognitive changes are still in the background.

-Behaviour and psychiatric symptoms and signs-

Psychiatric symptoms are very frequently present in the early stage of the disease, often prior to the onset of motor symptoms. The percentage of patients with psychiatric signs varies between 33% and 76% depending on the methodology of the study. Because of their impact on daily life, these symptoms and signs usually have a highly negative impact on functioning and on the family. The most frequently occurring sign is depression. The diagnosis is difficult because weight loss, apathy
and inactivity also occur in HD. Usually there is low self-esteem, feelings of guilt and anxiety. Apathy is related to disease stage, whereas anxiety and depression are not. Suicide occurs more frequently in early symptomatic individuals and also in premanifest gene carriers. Around the time of the gene test and the stage when independence diminishes are the most risky periods for suicide. Anxiety also occurs frequently (34-61%), sometimes in relation to uncertainty about the start and or the course of the disease. Obsessions and compulsions can disturb the patient's life and also lead to irritability and aggression. Irritability is often the very first sign, in retrospect, but in fact occurs during all stages of the disease. The way irritability is expressed varies enormously from serious disputes to physical aggression. A loss of interest and increasing passive behaviour are seen as part of the apathy syndrome. It can be difficult to discriminate apathy from depression. Psychosis may appear, mainly in the later stages of the disease. In most cases this goes together with cognitive decline. The complete clinical picture is comparable to schizophrenia with paranoid and acoustic hallucinations. In the early stages, hyper-sexuality can cause considerable problems in a relationship. In the later stages hypo-sexuality is the rule.

3.1.13 Symptoms of HD

Huntington's disease usually causes movement, cognitive and psychiatric disorders with a wide spectrum of signs and symptoms. Which symptoms appear first varies greatly among affected people. During the course of the disease, some disorders appear to be more dominant or have a greater effect on functional ability.

- **Movement disorders**

The movement disorders associated with Huntington's disease can include both involuntary movement problems and impairments in voluntary movements, such as:

- Involuntary jerking or writhing movements (chorea)
- Muscle problems, such as rigidity or muscle contracture (dystonia)
- Slow or abnormal eye movements
- Impaired gait, posture and balance
- Difficulty with the physical production of speech or swallowing

Impairments in voluntary movements - rather than the involuntary movements - may have a greater impact on a person's ability to work, perform daily activities, communicate and remain independent.
Cognitive disorders

Cognitive impairments often associated with Huntington's disease include:

- Difficulty organizing, prioritizing or focusing on tasks
- Lack of flexibility or the tendency to get stuck on a thought, behaviour or action (perseveration)
- Lack of impulse control that can result in outbursts, acting without thinking and sexual promiscuity
- Lack of awareness of one's own behaviors and abilities
- Slowness in processing thoughts or "finding" words
- Difficulty in learning new information

Psychiatric disorders

The most common psychiatric disorder associated with Huntington's disease is depression. This isn't simply a reaction to receiving a diagnosis of Huntington's disease. Instead, depression appears to occur because of injury to the brain and subsequent changes in brain function.

Signs and symptoms may include:

- Feelings of irritability, sadness or apathy
- Social withdrawal
- Insomnia
- Fatigue and loss of energy
- Frequent thoughts of death, dying or suicide

Other common psychiatric disorders include:

- **Obsessive-compulsive disorder** — a condition marked by recurrent, intrusive thoughts and repetitive behaviors
- **Mania**, which can cause elevated mood, over activity, impulsive behaviour and inflated self-esteem
- **Bipolar disorder** — a condition with alternating episodes of depression and mania
In addition to the above symptoms, weight loss is common in people with Huntington's disease, especially as the disease progresses.

- **Symptoms of juvenile Huntington's disease**
  The start and progression of Huntington's disease in younger people may be slightly different from that in adults. Problems that often present themselves early in the course of the disease include:

  - **Behavioural changes**
    - Loss of previously learned academic or physical skills
    - Rapid, significant drop in overall school performance
    - Behavioural problems

  - **Physical changes**
    - Contracted and rigid muscles that affect gait (especially in young children)
    - Changes in fine motor skills that might be noticeable in skills such as handwriting
    - Tremors or slight involuntary movements
    - Seizures

- **3.1.14 Causes of HD**
  HD results from genetically programmed degeneration of nerve cells in striatum, cerebral cortex and other areas of the brain responsible for memory storage. Degeneration of neurons causes uncontrolled movements, loss of intelligence, and emotional disturbance. Specifically affected neurons are the cells of basal ganglia, which are deep within the brain that have many important functions, including coordination movement. Within the basal ganglia, HD especially targets neurons of the striatum, particularly those in the caudate nuclei and the pallidum cortex; also gets affected which controls thought, perception, and memory.
Huntington's disease is caused by an inherited defect in a single gene. Huntington's disease is an autosomal dominant disorder, which means that a person needs only one copy of the defective gene to develop the disorder. With the exception of genes on the sex chromosomes, a person inherits two copies of every gene i.e. one copy from each parent. A parent with a defective gene could pass along the defective copy of the gene or the healthy copy. Each child in the family, therefore, has a 50% of chances in inheriting the gene which causes the genetic disorder.

Common causes of death include:

- Pneumonia or other infections
- Injuries related to falls
- Complications related to the inability to swallow

3.1.15 Clinical features

The disease was named as Huntington’s chorea after George Huntington, who gave first detailed description in 1872. The name has changed to HD because chorea is not the only predictive symptom of the disease, there may be many non-motor symptoms which may be more disabling and distressing than the motor symptoms. Imaging and post-mortem studies have shown that the disease is characterized by cerebral atrophy. Atrophic changes are initially seen most prominently in the striatum (part of the basal ganglia) and later become widespread to all muscles.

1. Psychiatric Symptoms

Depression is one of the most common psychiatric symptoms of the disease and it occurs as part of the disease. Other psychiatric symptoms include obsessive-compulsive symptoms and psychosis. It is important to recognize psychiatric symptoms in HD so that symptomatic treatment can be given to the patient. This may be difficult to recognize in the disease later because diagnoses may be unidentified by other features of the disease. For example, depression may be difficult to detect in patients who has altered facial expressions and tone of voice (Paulsen JS, Conybeare R., 2005).
2. **Motor Symptoms**

The motor symptoms of HD can be divided into two categories: 1. Added involuntary movements. Example - chorea and 2. Impaired voluntary movements, which cause limb in coordination and impaired hand function. Motor symptoms get worsened when postural reflexes are lost. The motor symptoms changes over time, chorea decline as the disease progresses while dystonia, rigidity, and bradykinesia becomes more marked in the later stage.

3. **Cognitive Symptoms**

Cognitive impairment includes slowing of thought processes like problem solving and deterioration of executive functions. Typically, patients suffer from difficulty with multitasking, concentration, and short term memory (Duff K., 2010). People with HD are often impulsive and develop psychomotor perseveration. Visuospatial perceptions are also impaired (Nambron R et al., 2016) cognitive dysfunction in HD, not only impairs long-term memory but also impairs executive functions such as organizing, planning, checking, or adapting alternatives, and delays the acquisition of new motor skills. These features are worsened over time; speech deteriorates faster than comprehension (Russell L. et al., 2003)

3.1.16 **Diagnosis**

The diagnosis is based on the clinical symptoms and signs in a person with a parent with proven HD. First, it is obligatory to take a precise history from the person with symptoms followed by a detailed family history. When all information has been obtained the diagnosis is not very difficult, although non-specific clinical pictures can be misleading. Also when the parent is not known or has died due to another cause at a young age, the clinical picture can be difficult to recognise. It is often necessary to request old information in the form of medical records and autopsy reports. The current gold standard is DNA determination, showing a CAG-repeat of at least 36 on the huntingtin gene on chromosome 4. Before 1993, a family history with clinical and morphological verification in at least one of the parents or grandparents was obligatory.
3.1.17 Management of HD

➤ Pharmacological Treatment

There are no disease-modifying treatments available for HD in routine clinical use, and current treatment is therefore given on the basis of symptoms. Many trials were conducted mainly by concentrating on the mechanisms and outcomes associated with movement disorder. Patients reported that their quality of life is decreased by psychiatric manifestations of their condition, including depression, irritability and apathy. Rates of depression may be as high as 40%, and suicide may occur in as many as 10%. Obsessive compulsive symptoms are also common in HD patients (Tommaso M, Serpino C., 2011). Drugs of different categories like anti-psychotics, sedatives and anti-depressants etc. are given after monitoring the symptoms.

★ DA depleting agents

A major neurological symptom associated with HD is chorea. The use of tetrabenazine (TBZ), a specific inhibitor of vesicular monoamine transporter, is approved for chorea in HD patients (Frank S., 2010). TBZ exerts its antichoreic effect by reducing the level of dopamine in brain by preventing its release from vesicle and inhibiting the uptake of monoamines. Uptake of monoamines in vesicle is regulated by vesicular monoamine transporter (VMATs) proteins present on vesicular membrane. TBZ binds to these VMATs and inhibits the storage of dopamine in vesicles and thus prevents the further release of dopamine into synapse. The highest binding density for TBZ is in the caudate nucleus, putamen, and nucleus accumbens, areas known to bear the brunt of pathology in HD. In addition, Secondly, TBZ reduces dopamine by blocking dopamine receptors, as it has been shown in in vitro studies (Huntington Study Group., 2006). TBZ may also act by binding to the receptor of receiving nerve cells and thus blocks the binding of dopamine to these receptors and thus blocks the signaling of neurons (Wang H et al., 2010). Long-term feeding with TBZ (combined with levodopa) alleviated the motor deficits and reduced the striatal neuronal loss in the mouse model of HD, thus suggesting a neuroprotective effect (Delenclos M, Maclean PJ., 2014).

★ Neuroleptics

Both typical and atypical neuroleptics can be used for the treatment of chorea and psychosis in HD. Both are DA D2 receptor antagonist; however the newer atypical antipsychotics are preferred because they cause less extra pyramidal side-
effect. Atypical antipsychotics are often used in the therapy, but evidences showed that clozapine have fewer efficacies with significantly more side effects. While olanzapine gives better results from small open-label studies.

★ **Anti-glutaminergic agents**
Remacemide, a non-competitive NMDA receptor antagonist showed an overall improvement in chorea level.

★ **Acetylcholinesterase inhibitors**
Acetyl cholinesterase inhibitors are used to treat cognitive decline, a common symptoms of HD.

★ **Antidepressant**
Mirtazapine may be preferred for depression as they have anticholinergic activity compared with some other anti-depressants.

★ **Olanzapine and sertraline**
These are reported to cause Improvement in depression and obsessional thinking. Risperidone and Amisulpirid may be used in the treatment of psychosis in HD, while Quetiapine is reported to help in behavioural disturbance (Frank S., 2010).

★ **Transglutaminase inhibitors**
Transglutaminases belongs to a family of closely related proteins that catalyze the cross-linking of a glutamine residue of a protein/peptide substrate to a lysine residue of a protein/peptide co-substrate with the formation of a GGEL $\text{[N}$$\text{-} (\gamma$-L-glutamyl)-L-lysine$]$ cross-link. These bonds may be important in the formation of aggregates and the toxicity of mutant huntingtin. Example: Cystamine is a transglutaminase inhibitor which improves survival, motor phenotype and neuropathology in mouse models, and preliminary dose finding and tolerability trials of Cystamine in humans have been completed.

★ **Chaperones**
Within cells protein are continually degraded in to amino acids and replaced by newly synthesized proteins. These newly synthesized proteins may undergo aberrant folding and aggregation. Protein misfolding can lead to the formation of toxic substances. Cells employ different processes and machinery to prevent the build-up of abnormal proteins. Chaperones and their regulators (co-chaperones) are a group of molecule that contributes to the prevention of aggregation (Delenclos M, Maclean PJ., 2014). Chaperons help proteins to attain more stable conformations and
prevent their aggregation, and their production is increased when subjected to heat-shocks (Imarisio S, Carmichael J., 2008). Chaperones assist other proteins to achieve a functionally active 3D structure and thus prevent the formation of a misfolded protein may be due to loss of function and deleterious gain of function as seen in HD. In which protein misfolding results in the formation of harmful amyloid.

☆ Genetic modification

Currently, a leading strategy among HD researchers are trying to suppress the expression of the mutant gene by introducing fragments of DNA meant to bind with and eliminating the ability of the gene to make the damaging protein. The goal of this approach is to prevent the mHTT from being expressed in the brain and potentially slow the progression of the disease, if not stop (Gusella JF, Lee JM., 2014).

➢ Non-pharmacological treatment of HD

The aim of treatment is to manage symptoms and improve quality of life. No current treatments can slow disease progression, although promising disease modifying treatments are being tested in animal models. There are many effective options for symptomatic management, however, both drug based and non-drug based treatments are available (Mestre T, Sampaio C., 2009). Many non-drug based measures are effective in the management of Huntington’s disease, and these are often more helpful than drugs (Nance MA., 2007).

☆ Physical therapy

In the early stage of HD, people have difficulty in solving problem and making decision but they are able to care for themselves. In the middle stage peoples have difficulty in walking and may fall down. Due to difficulty in thinking and memory, they easily get frustrated and angry. But in the late stage they need help for all of their daily routine activities and requires some special arrangements for their eating, sleeping, sitting due to severe chorea and loss of all controlled movement (Peavy GM., 2010).
Medications for movement disorders (Goizet C, Durr A., 2002)

Drugs to treat movement disorders include the following:

★ Tetrabenazine is specifically approved by the Food and Drug Administration (FDA) to suppress the involuntary jerking and writhing movements (chorea) associated with Huntington's disease. A serious side effect is the risk of worsening or triggering depression or other psychiatric conditions. Other side effects include drowsiness, nausea and restlessness.

★ Antipsychotic drugs, such as haloperidol and chlorpromazine have a side effect of suppressing movements. Therefore, they may be beneficial in treating chorea. However, these drugs may worsen involuntary contractions (dystonia) and muscle rigidity. Other drugs, such as risperidone and quetiapine, may have fewer side effects but still should be used with caution, as they may also worsen symptoms.

★ Other medications that may help to suppress chorea which include amantadine, levetiracetam and clonazepam. At high doses, amantadine can worsen the cognitive effects of Huntington's disease. It may also cause leg swelling and skin discoloration. Side effects of levetiracetam include nausea, stomach upset and mood swings. Clonazepam may also worsen the cognitive side effects of Huntington's disease and causes drowsiness. It also has a high risk of dependence and abuse.

Medications for psychiatric disorders

Medications to treat psychiatric disorders will vary depending on the disorders and symptoms. Possible treatments include the following:

★ Antidepressants include such drugs as citalopram, escitalopram, fluoxetine and sertraline. These drugs may also have some effect on treating obsessive-compulsive disorder. Side effects may include nausea, diarrhoea, drowsiness and low blood pressure.

★ Antipsychotic drugs — such as quetiapine, risperidone and olanzapine these drugs may suppress violent outbursts, agitation, and other symptoms of mood disorders or psychosis.
Mood-stabilizing drugs that can help prevent the highs and lows associated with bipolar disorder include anticonvulsants, such as valproate, carbamazepine and lamotrigine.
Reference:

3.2 Review on 3 - Nitropropionic acid (3-NP)

3.2.1 Introduction

Huntington’s disease (HD) is an inheritable autosomal-dominant disorder whose causal mechanisms remain unknown. Experimental models have begun to uncover these pathways, thus helping to understand the mechanisms implicated and allowing for the characterization of potential targets for new therapeutic strategies. 3-Nitropropionic acid is known to produce in animal behavioural, biochemical and morphologic changes similar to those occurring in HD. For this reason, this phenotypic model is gaining attention as a valuable tool to mimic this disorder and further developing new therapies.

Research in the field of 3-nitropropionic acid (3-NP) toxicity is extensive, so in pragmatic terms, it is impossible to cover all the studies performed with this toxin up to the present. For these reason, we will divide this review into three different parts, each one corresponding to a specific chronological phase of research on 3-NP; and start this review with a brief description of this disorder.

3.2.2 The Past

HD is a neurodegenerative process mainly affecting the basal ganglia in the brain. Symptoms appearing in this disorder have been described for long time (different descriptions can be documented as early as the fourteenth century). Indeed, HD was also known as Saint Vitus’s dance or dancing plague. The disease was first described by Charles Waters as a convulsive disorder, but it was in 1872 (Gonzalez., 2006) when George Huntington formally described it for the first time and referred to as a hereditary chorea.

HD is catalogued as a rare disease, with a stable prevalence in white populations affecting 5-7 individuals per 100,000 (Okun, M.S. et al., 2004). The age of onset ranges between 30 and 40, with death occurring after 15–20 years; onset sometimes occurs early in young people at around 20 and evolves over periods of around five years (Gonzalez-Alegre, P.; Afifi, A.K., 2006), (Leegwater, J.; Jang-Ho, J., 2004), is also known as an autosomal dominant inheritable neuropathological disorder triggered by excessive repetition of the cytosine-adenine-guanine (CAG) triplet, which encodes glutamine present in protein huntingtin (Htt). This triplet is
located in exon 1 at the Huntington gene (HTT), also known as transcript 15 (IT15), located in region 16.3 at the short arm of chromosome 4 (4p16.3).

The number of triplets expressed by the Htt-encoding gene - and therefore the extension of polyglutamines present in the protein - will determine penetrance, age of onset and probability of transmission to descendants and disease severity.

The symptoms appearing and developing during the course and evolution of HD can be classified into three large groups: i) physical symptoms; ii) cognitive symptoms; and iii) psychiatric symptoms. The first evident symptoms are physical inability accompanied by a wide variety of cognitive psychiatric alterations (Johnson, S.A. et al., 2007).

Although several biochemical, molecular, physiological and anatomical changes in HD have been extensively described, these have yet to be fully established and clarified; nevertheless, numerous findings in recent decades have enabled researchers to put forward different hypotheses about different molecular mechanism potentially occurring in this disorder (Brouillet, E. et al., 1999).

Mitochondrial dysfunction is the main source of reactive oxygen species (ROS). ROS-triggered excitotoxicity also induces massive entry of calcium ions (Ca^{2+}) from the extracellular medium, prompting the release of this ion stored in the mitochondrial and endoplasmic reticulum to the cytoplasm, and ultimately resulting in the activation of neuronal nitric oxide synthase or nitric oxide synthase type I (nNOS or NOS-I) with the subsequent release of nitric oxide (NO). In turn, NO is transformed into peroxynitrite (ONOO⁻) after reacting with superoxide anion (O₂⁻) from the ECT. These events, together with dopamine (DA) metabolism, create an imbalance between oxidant and antioxidant systems characterized by excessive production of ROS as O₂⁻, hydrogen peroxide (H₂O₂), ONOO⁻ and a reduction in enzymatic (superoxide dismutase, SOD; glutathione peroxidase, GPx) and non-enzymatic (reduced glutathione, GSH) antioxidant systems, resulting in the appearance of exaggerated oxidative status characterized by macromolecular damage due to oxidative stress (OS). This imbalance promotes typical OS cascades, such as oxidation of proteins and DNA, and lipid peroxidation. This phenomenon is associated with cellular damage and neuronal death and plays a crucial role in the neurodegenerative process of HD by helping explain the strengthening or intensification of the toxic effect of mHtt. In this regard, one important histopathological finding was the discovery of mHtt protein deposits in the form of
inclusion bodies or intraneuronal aggregates in HD. The mechanism triggering aggregation resulting in selective neuronal dysfunction has not yet been determined.

Similarly, the generation of models that mimic, to a greater or lesser extent, the HD phenotype and biochemical-molecular and cellular changes in the disease have allowed researchers to better understand the process and perform a clearer and more detailed study of the biomolecular mechanisms participating in and/or facilitating the development of HD. This has also enabled the development of useful models for studying strategies.

3.2.3 The 3-nitropropionic acid (3-NP) model

Nowadays, researchers are able to study the post-mortem brains of HD patients and analyse biochemical-molecular parameters in biological media, such as blood and cerebrospinal fluid. Nevertheless, animal models have unquestionable value, despite their limitations, due to the considerable amount of anatomopathological, histopathological, physiopathological, biochemical and molecular data they can provide, thereby allowing researchers to obtain better knowledge of these phenomena and further integration in HD. They are also very useful tools for designing and studying new therapeutic targets, procedures and drugs.

Since the 1970's, different animal models have been developed for studying HD. The first was induced by kainic acid (KA) (Schwarcz, R. et al., 1976) based on the vulnerability of striatal neurons to excitotoxicity caused by excessive stimulation of excitatory amino acid receptors with further triggering of neuronal death (Caboche J., 2008). Other models have been developed since then: for instance, the models induced by quinolinic acid (QA), malate (malonic acid; MA) and 3-NP. More recently, transgenic models have also been developed in both rodents (the first transgenic mouse model dates from 1996). However, the most used models are undoubtedly the rodent and non-human primate models.

The central nervous system is particularly sensitive to variations in energy resources due to the high metabolism of neurons, with alterations in oxidative metabolism clearly representing a risk for the viability of this model. Changes in the availability of energy substrates, such as glucose or oxygen, cause an alteration that affects membrane potentials followed by depolarization. In recent decades, many
diseases have been associated with energy metabolism impairment, including HD, which exhibits decreases in glucose and oxygen levels in the basal ganglia and cerebral cortex (Beal et al., 2007).

➢ Treatment with 3-NP and behavioural changes

3-NP is a natural toxin synthesized by fungi (Aspergillus flavus; Astragalus, Arthriniun) and plants (Indigofera endecapylla); it crosses the blood-brain barrier and therefore, it can be administered systemically. The pioneering study in this field was performed by Chinese researchers and Hamilton and Gould. Its administration by means of a subcutaneous osmotic pump or direct subcutaneous injection or intraperitoneal injection are effective methods for systemic infusion of 3-NP, although doses must be adjusted daily according to the weight of the animal since its administration prompts a decrease in the animal’s body weight, up to as much as 20g. Intrastriatal and intraputaminal infusions are also used. Different animal species and strains can be used to develop this model with similar profiles of neurotoxicity to those seen in HD brains. The most common of these are rodent models, where the effect is triggered in different murine (CD1, C57BL/6, BALB/c, Sebster/Swiss, 129SvEMS, etc.) and rat (Fischer, Lewis, Wistar, etc.) strains. Interestingly, the response to this neurotoxin differs according to the species and strain used; doses must therefore be adjusted according to weight, administration period (method and time), species and strain of animal used to obtain the desired biochemical molecular, cellular and phenotypic changes (acute, subacute and chronic) (Brouillet et al., 2005).

The 3-NP model can mimic and reproduce the hyperkinetic and hypokinetic symptoms of HD, depending on the time and dose administered, thus allowing the initial (or early) and late phases of HD to be evaluated. The administration of 3-NP (10 mg/kg intra-peritoneal for more than four doses) induces the onset of similar symptoms to hypokinetic symptoms, while administration in two individual doses display similar symptoms to hyperkinetic symptoms. The effects of acute treatment with 3-NP at a maximum dose of 20 mg/kg are observed after the first two injections, with expression of a phenotype similar to the HD phenotype, although its histopathology is different to that observed in the initial stages of HD, often accompanied by extra-striatal lesions. Chronic administration of 3-NP at low doses
(10 mg/kg/day, 3–6 weeks) (Beal, M.F., 1994), induces a sustained state of metabolic alterations and some other features similar to those displayed by HD patients. For instance, non-human primates under chronic schedules display lip dystonia and chorea form movements, while prolonged administrations of this toxin (for four months) trigger spontaneous dyskinesias and dystonia (Brouillet E et al., 1995).

These results suggest the following limitations in this model:

i) The existence of quite different aberrant movements between non-human primates and rats; and

ii) The differential organization of basal ganglia once again between primates and rodents, the striatum being formed by two well-defined parts in non-human primates and a single structure in rodents, a phenomenon clearly evidenced by certain events such as conduct, where behavioural changes are different in both animals.

Thus, in non-human primates, chronic intoxication with 3-NP triggers a series of movements similar to those observed in HD patients, although these motor alterations have not been reproduced in rats under the same experimental conditions. Moreover, rats treated chronically with 3-NP did not display clear dyskinetic movements resembling chorea (Brouillet, E et al., 1999). Although some motor alterations may constitute resemblances to HD, experimental models must also be able to reconstruct cognitive aspects of this disorder, such as those of memory and attention alterations. In this regard, it has been shown that at the end of a given 3-NP treatment, non-human primates displayed similar cognitive deficit to that observed in HD individuals (Palfi S et al., 1996).

According to the behavioural changes observed in animals administered with 3-NP may be summarised in three major phases:

I) Sleepiness;

II) Unco-ordinated march with stereotypical padding and rolling movements.

III) Lateral and ventral recumbence (Brouillet E).

➢ 3-NP and mitochondria

Despite the fact that 3-NP, a metabolite of 3-nitropropanol, was first described in Chinese children who had eaten contaminated sugar (Ming, L. et al.,
1995). It was first identified a few years earlier after the massive poisoning of cattle in the Western U.S. These animals, after being poisoned with infected legume crops, displayed different motor alterations that evolved towards disco-ordination and paralysis. As mentioned above, 3-NP is a toxin that irreversibly inhibits (suicide inhibitor) the enzyme succinate dehydrogenase, which in turn is present in the internal face of the mitochondrial membrane and is responsible for the oxidation of succinate to fumarate. Inhibition of this enzyme invariably leads to neuronal death in caudate and putamen nuclei, triggering severe dystonia in children. (Figure.1)

**Figure.1 Schematic representation of the effect of 3-NP on ETC**

3-NP irreversibly inhibits succinate dehydrogenase (SDH; complex II) of electron transport chain (ETC) and tricarboxylic acid cycle; IM: inner membrane; IMS: Inter membrane space; OM: outer membrane. Complex I: NADH dehydrogenase; Complex III: Cytochrome bc1 oxytocchrome c reductase; Complex IV: Cytochrome c oxidase; Complex V: ATP synthase.

Since the 3-NP-triggered neurodegeneration model mimics the cascade of processes leading to cell death in HD - mainly mitochondrial alteration - as well as some of its histological and pathological characteristics, it has been proposed as a valuable phenotypic model for studying different aspects related with HD and new drugs.

On the other hand, it is known that alterations in glucose metabolism cause decreased ATP production. Several enzymes involved in the electron transport chain (ETC) and tricarboxylic acid cycle (TCA, Krebs cycle) are indeed, triggered in the
course and evolution of HD. Different studies have also shown that aconitase and complex II, III and IV activities are reduced in the striatal nucleus (caudate + putamen) of HD patients (Browne, et al., 1997). Therefore, damage to mitochondrial complexes occurs simultaneously to the loss of membrane potential, together with a redistribution of cytochrome c. Some of the studies reporting these alterations have also shown that cyclosporine A, an inhibitor of the permeability transition pore, acts as a neuroprotector against 3-NP.

3-NP also induces caspase-9 activation, which in turn requires the simultaneous presence of Apaf-1, cytochrome c and ATP. Overall, these data suggest that neuronal death may occur in the presence of intense ATP depletion. In addition, studies performed in primary neuron cultures show that 3-NP induces the expression of different mitochondrial factors associated with apoptosis, including cytochrome c and Smac/DIABLO protein (Blum D., 2004).

In regard to oxidative damage, it is known that the alteration of oxidative metabolism by 3-NP induces oxidative and nitrative stress due to excessive ROS/RNS production and/or depletion of antioxidant systems. Therefore, oxidative damage has been largely linked with neuronal loss in the 3-NP model. Moreover, calcium ions (Ca^{2+}) play an important role in this process since its homeostasis is altered by the neurotoxin, which triggers cytosolic increases from internal storages that lead to NOS activation via Ca^{2+}/calmodulin, and the subsequent production of NO. Additionally, increased Ca^{2+} concentrations due to the opening of both voltage-gated membrane channels and voltage-gated NMDA receptor-channel complex trigger excitotoxicity and associated events, including the activation of proteases involved in cell death such as calpains (Salcedo M., 2004). Calpains in turn mediate the degradation of different proteins, including Htt. The activity of this protein is unaffected in areas where 3-NP does not cause cell death. (Figure 2)
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Figure 2 Neurotoxicity by 3-NP

3-NP prompts complex-II inhibition and increased sensibility of NMDA-R (excitotoxicity). 3-NP toxicity affects microglia, astrocytes and neurons, and causes secondary excitotoxicity by making neurons more vulnerable to endogenous basal levels of glutamate, while prompting a reduction of ATP availability. This scenario causes relief of voltage-dependent Mg2+ blockade at the NMDA-R pore. In turn, the activation of these receptors leads to massive entry of Ca^{2+} to cytoplasm and further activation of a number of calcium dependent enzymes, including calpains and NOS. Altogether, these events lead to cell death by different pathways: necrosis and/or apoptosis, depending of intensity of insult and cell type. ETC: Electron transport chain; LDH: Lactate dehydrogenase; mΔψ: Membrane potential; NMDA-R: N-methyl D-aspartate (NMDA) receptor; 3-NP: 3-Nitropropionic acid; RNS: Reactive nitrogen species; ROS: Reactive oxygen species.

3-NP and neurotoxicity

Different studies have suggested that glutamatergic innervation due to excitotoxicity plays an important role in 3-NP-induced striatal degeneration. These reports have shown the existence of spontaneous glutamate flow in slices of brain and synaptosomes treated with 3-NP (Brouillet, E.2005). Support to these findings
came from (Storgaard et al., 2000), who showed that treatment with inhibitors to recapture glutamate increased the 3-NP-induced neurotoxicity.

Glutamate levels are regulated by sodium-dependent transporters (Na+) present in the glia and neurons. The activity of these transporters depends on the transmembrane sodium gradient generated by Na+/K+ ATPase. Glutamate internalized in the glial cells is metabolised to glutamine and released into the extracellular space, where it is reconverted into glutamate. Therefore, glutamatergic homeostasis mostly depends on the balance and control of all components involved; its alteration may lead to excessive NMDA-R stimulation, prompting their activation and triggering excitotoxicity induced neuronal death (Figure 2). It has also been reported that during ischemia, NO production starts after initial stimulation of NMDA-R, a phenomenon involving neuronal-NOS (nNOS) activation.

Moreover, the administration of 3-NP to astrocyte cultures is capable of causing substantial increases in intracellular calcium levels; its administration in vivo prompts a reduction in the number astrocytes, as well as a loss of white matter and axons, together with loss of oligodendrocytes (Ryu J.K., 2003). Previous studies have confirmed this observation, showing that 3-NP induces changes in astrocytes prompting a reduction in the release of trophic factors (Brouillet E et al., 1999). Some enlightening information emerged when 3-NP-induced toxicity in astrocytes (Brouillet E et al., 1999) associated with NO. This messenger has been shown to be toxic for CNS in pathological conditions. NO is synthesized by NOS, whose three isoforms are expressed by brain cells:

I. Endothelial NOS (eNOS): also known as type III NOS. This is a calcium-dependent enzyme initially found in the endothelium.

II. Neuronal NOS (nNOS): also known as type I NOS, present in nerve tissue.

III. Inducible NOS (iNOS): also known as type II NOS. This enzyme is calcium independent, plays an important role in immune system modulation and it is regulated by different cytokines. In addition, it produces NO in astrocytes, microglia and macrophages in response to inflammatory reactions.

Microglia, which is activated in the event of intoxication by 3-NP, can also play an important role in the toxic pattern elicited by this molecule. It has been observed that degenerated parts of the striatum are invaded by microglia, the most evident phenomenon from a time and intensity stand point in the case of acute 3-NP treatment (Nagai A., 2003). Microglia activation is accompanied by increased ROS
production, thereby allowing these glial cells to participate in the 3-NP-induced neurotoxicity and neurodegeneration (Nishino H., 2000).

- **3-NP and ROS/RNS**

  Alteration of mitochondrial activity is associated with abnormally high formation of ROS. ETC enzyme inhibition leads to an increase in electrons released from the mitochondria and the subsequent production of ROS, including O$_2$$^-$$^-$$-$ and H$_2$O$_2$. ROS production in turn, alters the balance between oxidants/antioxidants, causing molecular damage that leads to cell death, in a process currently known as OS. Oxidative damage induced by OS affects cell membranes and nucleic acids, as evidenced by the increase in 8-hydroxy-2-deoxiguanosine (8OHdG), carbonylated proteins and lipid peroxides (malondialdehyde, MDA; 4 hydroxynonenals, 4-HDA; thiobarbituric acid reactive substances, TBARS).

  All these events are triggered by acute or chronic treatment with 3-NP through the inhibition of SDH at TCA and ETC. In rats, mice and non-human primates, 3-NP reproduces OS situations similar to those observed in HD (Túnez I., 2004). These events are indirectly revealed by the preventive effect shown by the prior or simultaneous administration of different exogenous and endogenous antioxidants (Collado., 2006). Together with nitrative, nitrosative or nitrergic stress (NS), OS produces substantial ATP depletion and neuronal death. (Figure.3)

  3-NP also induces the release of reactive molecules deriving from NO through stimulation of NOS activity. Thus, subsequent induction of NOS leads to NO production. In turn, NO may react with O$_2$$^-$$^-$$-$ to produce ONOO$$^-$$-. The later molecule is characterized by its high cytotoxicity and its capability to induce both protein nitration and hydroxyl radical (●OH) formation.

- **3-NP, neurochemistry and neuropathology**

  3-NP induces striatal toxicity, causing degeneration of GABAergic medium spiny neurons in the striatum, resembling those processes observed in HD. When administered systemically under chronic conditions, it causes bilateral, symmetric and selective neuronal degeneration of the lateral striatum (Schiffmann., 2004),
being restricted to the dorso-lateral area of the caudate-putamen, and thus mimicking the process that takes place in the dorso-lateral area of the putamen in HD patients.

3-NP-induced cerebral lesions are more or less specific to the striatum, although other areas located in the hippocampus, thalamus and brain cortex are also affected. These lesions display neuronal loss accompanied by moderate gliosis, decreases in cytochrome oxidase activity and a relative sparing of NADPH diaphorase-positive inter neurons and dopaminergic striatal afferents.

In studies with 3-NP, and based on existing data established and characterized three types of striatal lesions, according to histopathological evidence:

i) Type I lesions, described as small lesions distributed randomly in the dorsal striatum with sparing of NADPH-diaphorase neurons;

ii) Type II lesions, characterized by greater loss than in type I, with diaphorase neuronal sparing and shrunken islands of cells; and

iii) Type III lesions, characterized by neuronal loss in the whole dorsal striatum with slight alteration of the ventral area. Lesions in tyrosine hydroxylase fibers have also been reported.

Different studies have shown that DA levels increase after 3-NP administration to animals, which is related with DA release. Indeed, DA represents an additional factor accounting for 3-NP toxicity on GABAergic neurons. Filloux and Townsend reproduced these results and reported that an intrastriatal DA injection triggers a neurotoxic effect, quite similar to the one boosted by simultaneous administration of DA release stimulators such as amphetamines, both in acute and chronic administration models. Additionally, the use of 6-hydroxydopamine (6-OHDA), an agent that causes lesions to the substantia nigra, reduces 3-NP-induced damage (Morton., 1998).

This background prompted Maragos and fellow researchers (Maragos et al., 2004) to propose that endogenous DA contributes to striatal damage caused by excitotoxicity by inhibition of glutamate uptake or by the ROS-independent triggered of mitochondrial complex I. Recently, Villaran et al. found that reserpine and alpha-methyl-p-tyrosine-induced DA depletion prevented, at least partially, the 3-NP induced ROS production in striatal synaptosomes. According to the aforementioned authors, these findings indicate that DA induces mitochondrial ROS production in striatal dopaminergic nerve endings by inhibiting ETC, thus favouring 3-NP toxicity.
Other changes induced by 3-NP in different molecules involved in the neurodegeneration process have been identified. Among them, we can mention: i) consecutive increases in adenosine release upon mitochondrial complex II inhibition, ii) decreases in endocannabinoid levels (anadamide and 2-arachydonoyl-glycerol) after 3-NP treatment, iii) decreases in levels of substance P, enkephalin and choline acetyltransferase (Beal M.F., 2007), and iv) increases in levels of somatostatin, neuropeptide Y and neurotensin (Beal M.F., 2007).

3-NP and death cell

Many mechanisms are involved in striatal MSN degeneration during HD. To determine these mechanisms and processes more accurately, as well as their degree of participation, neuronal toxicity models have been employed, including 3-NP-triggered models (Blum D., 2005). Proposed mechanisms include trophic factors, such as BDNF.

BDNF is produced by cortical neurons and secreted into the striatum. It is, indeed, essential for striatal neuronal survival and maintenance. Its expression is regulated by sequestering of the transcriptional repressor/neuron-restrictive silencing factor (NSR/REST) in the cytoplasm; in this regard, it is known that mHtt enables the translocation of REST to the nucleus, triggering the suppression of BDNF transcription, accompanied by alterations in neuronal transport (Zuccato, C. et al., 2007). This phenomenon is to be expected, bearing in mind that BDNF is the main support of the striatum and produces a variety of neuromodulatory effects in the brain that are more consistent with local actions than with long-distance retrograde signalling. BDNF and other neurotrophins are also involved in chronic potentiation (LTP). Decreases in BDNF levels have been reported in the caudate nucleus and putamen of HD patients compared with healthy subjects of the same age and sex.

Hellweg et al., showed that damage triggered by 3-NP-induced hypoxia is accompanied by increases in NGF and BDNF in the hippocampus of rats, probably, according to the authors, as a neuroprotective response to the repeated action of an inhibited oxidative phosphorylation. Other interesting factors in the HD animal model are the glial-derived neurotrophic factor (GDNF) and neurturin, given the function of the later in growth, development and trophic support of striatal neurons.
It is also important to highlight once again the close relationship between neuronal and cell death in general, and ROS/RNS production in cell and molecular death expression, currently known as OS/nitrative stress (OS/NS). As mentioned previously, intoxication with 3-NP triggers intense OS, accompanied by striatal neuronal loss. ROS/RNS activate mechanisms that intend to prevent, protector recover the tissues from the insult induced by 3-NP. Thus, intoxication with 3-NP with the subsequent ETC complex II inhibition and ROS/RNS production have been linked with activation of the antioxidant response element (ARE), a cis-acting sequence regulating the transcription of several cytoprotective genes. After oxidative insult and subsequent GSH depletion, the nuclear factor erythroid2-related factor (Nrf2) translocates to the nucleus and dimerises with small MAF proteins (MAF) (family of basic-leucine zipper transcription factors) to form a complex linked to ARE proteins, which are activated to coordinate the expression of genes that counteract the pro-oxidant signals triggered by 3-NP (Lee J.M., 2003). (Figure.3) ARE inhibits cell death by apoptosis mediated by Fas (signal transducing adaptor protein that associates with tumour necrosis factor (TNF) receptor complexes), a substrate for proteases similar to caspase-3 and an effector that facilitates cell survival via PK-like ER kinase (Almeida S et al., 2006).

3-NP triggers the transcription activation of vital genes expression, which encoded phase II detoxification enzymes, by mean of antioxidant response element (ARE). COX-2: Cyclooxygenase 2; GST: Glutathione S transferase; HO-1: Hemeoxygenase 1; NADPH: Nicotinamide adenine dinucleotide phosphate; 3-NP: 3-
Nitropropionic acid; NQ01: NADPH quinone oxidoreductase 1; Nrf2: Nuclear Factor-E2-related factor 2; NFκβ: Nuclear factor kappa beta; ROS. Reactive oxygen species; RNS: Reactive nitrogen species; TRXr: Thioredoxin reductase; UGT: Uridine 5′diphosphate glucuronosyl transferase. Ikb: Inhibitor of NFκβ; Keap1: Inhibitor of Nrf2; Small Maf: Transcriptional repressors or transcriptional coactivators.

There is also evidence that 3-NP induces cytochrome c release and activation of both the apoptosis inducing factor (AIF) and cysteine proteases (caspases) 2, 3 and 8 (Almeida S. et al., 2006). In turn, caspase-2 activation induces the release of mitochondrial cytochrome c and alters the interaction of the later with anionic phospholipids, cardiolipin, thereby increasing the release of this hemoprotein to the external domain.

Some studies have shown that 3-NP-induced cytotoxicity is accompanied by elevations of lactate dehydrogenase (LDH) - a characteristic marker of death by necrosis (Tunez I et al., 2006), indicating that this type of cell death is taking place in the toxic insult triggered by molecule, albeit to a lesser extent.

The nuclear factor-κB (NFκB), a protein related with the immune system and inflammatory response, has been associated in neurons with the response to excitotoxicity, metabolic stress and OS, and may be responsible for the induction of both pro- and anti-apoptotic genes, depending on the intensity and nature of the stimulus. Recent findings show that this regulatory factor may be involved in the promotion of nNOS transcription; hence, 3-NP would induce nuclear translocation of NFκB and the simultaneously expression of iNOS and nNOS to create a pro-inflammatory scenario.

3.2.4 Other Huntington’s disease induced models: Emphasis on QA and facilitating models

Comparisons of the phenotypic model of HD produced by 3-NP with other toxic models is relevant. In first instance, it provides contrasting information on how different toxic mechanisms and events might be participating in the human pathology. This is particularly valid when considering that other phenotypic model, such as those produced by endogenous molecules or neurotoxins, are more clearly related with direct excitotoxicity through NMDA-R over-activation. In this regard,
the dissection of the different toxic mechanisms accounting for modeling HD is of major relevance, interesting models combining toxins and mechanisms have been often developed. In addition, it is necessary to mention that some other toxic models might resemble more closely, some alterations seen in HD, so they deserve special attention. This is the particular case of QA.

On the basis of the well described behavioral, morphological, neuro chemical and molecular features of HD, different animal models have been designed for experimental purposes. The animal species in which they have been developed mostly comprehend non-human primates and rodents (mice and rats). Most of them are still currently under investigation in regard to how much they resemble HD features, and for sure, they will continue so, since the results obtained this far constitute valuable approaches for the characterization of mechanistic events underlying the degeneration in the human disorder, as well as for the design of novel pharmacological and molecular therapies.

The most relevant phenotypic models historically explored include the intrastriatal infusions of the glutamate analogues KA, ibotenic acid (IA), NMDA, QA, and glutamate itself, to rodents and nonhuman primates. Despite some classical studies showed that both KA and IA produced degeneration of striatal neurons, as well as reduction of glutamate decarboxylase (GAD) and choline acetyltransferase (CAT) activities – two hallmarks of HD - in animal models, these plant derived agents were simply unable to mimic other specific morphological and neurochemical features of HD. In light of the obvious limitations that these models faced from the beginning, the search for new and more accurate models for HD started in the early 80’s. By far, the most prominent of these models, besides of 3-NP, was the one produced by QA.

It is known as 2, 3-pyridinedicarboxylic acid, QA is a tryptophan metabolite at the kynurenine pathway. This oxidative metabolic pathway, is located in glial cells and produces at least two neuroactive metabolites (QA as excitatory and kynurenic acid as inhibitory) acting on the NMDAR. Given its endogenous nature, QA itself has been directly implicated as a potential pathogenic factor in HD, since it has been recently demonstrated that neostriatal and cortical levels of this toxicant - along with those of the pro-oxidant metabolite 3-hydroxykynurenic acid – are significantly enhanced in brains from the early low-grade HD (Schwarcz R., 2004). QA has been currently shown to exert selective striatal toxicity by means of excitotoxic, pro-
inflammatory and oxidative mechanisms. In fact, both QA and other metabolites from the kynurenine pathway have been involved in the pathogenesis of neurodegenerative, infectious, inflammatory and non-inflammatory diseases. The toxic features leading to the proposal of QA-induced lesions as a model for HD are typically based on its proved capacity to produce a wide variety of events similar to those of the human disorder, including the striatal depletion of the neurotransmitter GABA accompanied by the selective loss of GABAergic neurons, increased levels of cytosolic calcium concentrations, ATP exhaustion, neuronal OS and further massive cell death. Following its intrastrital infusion to rodents, QA has also shown to produce moderate hyperkinetic motor alterations that mimic the early symptoms of HD, while 3-NPA produces more intense changes corresponding to both later symptoms and juvenile onset of HD. Although the effects of QA have been largely related with over activation of NMDA-R, a compelling body of evidence has implicated OS/NS as an integral part of its pattern of toxicity. Despite that part of its oxidative component could be a consequence of excitotoxic events, some reports have deal with the notion that this component might also be an independent factor accounting for cell damage. Indubitably, in the next years, the characterization of signaling pathways through transcription factors, as well as proteomic and genomic analysis, will bring enlightening information on the mechanisms associated with QA toxicity and its role in HD.

More recently, an emerging line of research has provided interesting models to study integrative toxic events occurring in neurodegenerative disorders, including HD. These models comprehend the facilitation of excitotoxic events through the impairment of energy metabolism, and are produced by the combination of toxic molecules in different biological systems and under different experimental conditions, thus turning the neuronal cells more vulnerable to “regular” or moderately high concentrations of excitatory agents, further leading to excitotoxic damage by means of indirect over activation of NMDA-R (“secondary excitotoxicity”), and cell death. In terms of mechanistic events, it has been often assumed that massive extracellular calcium crossing the NMDA-R-associated channel might be responsible for these alterations. However, Jaquard and coworkers (Jacquard C. et al., 2006) recently demonstrated that the energy impairment induced by 3-NP, accompanied by a moderate action of QA, produced together a synergic increase in striatal degeneration in rats that mainly involved the deregulation of
intracellular calcium in absence of NMDA-R hyper sensitization, as well as an increase in calpain activity and cell death. For instance, this suggest that in this specific model, in contrast to other models already mentioned, the mechanisms underlying toxicity might imply different events and signaling pathways, further evoking toxic patterns not only different to other combined models, but also different to the individual models produced by these toxicants (QA and 3-NP). This consideration is under current investigation, and some supporting evidence has been recently collected from a published study demonstrating that intracellular calcium—more than extracellular—is responsible for the oxidative damage to membrane lipids produced by this combined paradigm in synaptic membranes (Perez-de la Cruz V., 2008). In that study, an active role of intracellular calcium was evidenced through the use of a calcium chelating agent, BAPTA-AM. Its efficacy was compared with conditions of either available or deprived extracellular calcium in the incubation media.

Finally, an interesting alternative to explain how these two toxicants together can produce selective mechanisms of damage has emerged since it has been recently proposed that the 3-NPA-induced secondary excitotoxicity is mediated by a component at the NMDA-R that is resistant to antagonists acting at the glycine co-agonist site (Smith R.A., 2008). If this effect is recruiting selective actions of QA in an independent manner of NMDA-R, or even avoiding the effects of QA in a combined model, is a question that remains to be elucidated in further studies. Meanwhile, the information that this combined model can provide for the characterization of toxic events taking place in HD phenotypic models will be of major relevance in the next years.

3.2.5 The Present

This far, we have described evidence from the past (old and recent) that served to build what now can be considered the general concept of 3-NP as a toxic tool to resemble some important features of HD. In this section, we will briefly describe the new approaches that several research groups are currently exploring using this model to direct research toward new horizons. Once again, if some relevant report is unintentionally omitted, this was entirely due to the space limitations of this review.
OS remains as a major expression in this toxic model, as well as a key target to ameliorate nerve tissue damage and the subsequent source of therapeutic designs. Testing the effects of different natural and synthetic molecules with antioxidant properties against 3-NP-induced toxicity is, today more than ever, a valuable approach to characterize this model. In this regard, it has been recently reported that the natural xanthone α-mangostin (isolated from mango fruit) possesses antiperoxidative properties against 3-NP when tested in both rat brain homogenates and synaptosomal P2 fractions. Another report dealing with an antioxidant strategy demonstrated the antiperoxidative and protective effects that an extract of Valeriana officinalis exerted on this model in rat brain homogenates (Sudati J.H. et al., 2009). These approaches are relevant since demonstrate that targeting oxidative damage to lipids may result in structural and functional preservation of nerve tissue. Cyclosporine, an immune depressant, was recently shown to exert neuroprotective and antioxidant effects in an in-vivo model of 3-NP toxicity in the striatum, cortex and hippocampus of rats (Kumar A., 2009). The proposed mechanism by which cyclosporine produced these effects revealed that 3-NP affected the glutathione redox balance in these regions through stimulating NO formation. Another mechanism attributed to 3-NP as part of its toxic pattern is its proved capacity to exert oxidative damage to proteins. In this regard, 3-NP was shown to induce oxidative modification of alpha-synuclein in a transgenic mice model expressing human alpha-synuclein (Ubhi K et al., 2009). Enhanced levels of oxidized and nitrated alpha synuclein were correlated with neurological deficits in these mice. In addition, the active contribution of striatal dopamine to the OS produced in the HD model by 3-NP in rats has been established.

According to this report, dopamine is inducing hydroxyl radical formation, which in turn, contributes to the OS and neurotoxicity evoked by 3-NP, potentiating its effect. Moreover, OS, neurochemical (dopamine metabolism and Heat-shock protein 72 expression) and neurotoxic markers of 3-NP-induced brain damage seem to be all sensitive to thermal modulation, since hyperthermia induced in rats turns the animals more resistant to the toxic insult of this molecule, by mechanisms still to be explored. Other drugs potentially acting as antioxidants and neuro protectants, and recently reported in this model are the cholinesterase inhibitor rivastigmine (Kumar P, Kumar A., 2009), the combination of the pro-bioenergetics coenzyme Q10 plus creatine (Yang L. et al., 2009), the sesame seeds extract sesamol (Kumar P. et al.,
lycopene and epigallocatechin-3-gallate (Kumar A., 2009), the *Withania somnifera* root extract (Kumar A., 2009), the flavonoid kaempferol acting partially as antioxidant, preventing calpain activation and creatine kinase preservation inactivation (Lagoa R. *et al.*, 2009), hesperidin and naringin - acting by potentiation of NOS inhibition, the potent free radical chain breaking antioxidant Trolox (Al Mutairy A. *et al.*, 2009), and tert butyl hydroquinone and the corresponding induction of antioxidant phase 2 enzymes through the Nrf2-ARE pathway (Tasset I. *et al.*, 2009).

Other investigations have enhanced the broad spectrum of 3-NP actions in the Nervous System. For instance, 3-NP has been recently employed as a tool to produce a mouse model of spiral ligament degeneration for studying the pathogenesis of sensory neural hearing loss. Cochleae injected with 3-NP successfully reproduced this pathology in several terms.

3-NP has been used also for exploring the modulation of apoptotic pathways. Histone deacetylase inhibitors (HDACIs) were shown to prevent p53-dependent and p53-independent BAX-mediated neuronal apoptosis by different mechanisms. In particular, when postnatal cortical neurons are challenged with 3-NP and other toxins, they revealed specific pathways since HDACIs prevented caspase-3 cleavage in a mechanism involving Bax, but not p53. Moreover, the toxic inhibition of Complex II at ETC induced by 3NP has been recently shown to be responsible for mitochondrial fragmentation and neuronal cell death via NMDA- and ROS-dependent pathway. Derived for this interesting study, authors concluded that mitochondrial fission is the result of secondary excitotoxicity and OS/NS, but not derived from energy deficit. Furthermore, although discarded in some studies, p53 has been involved in other reports as an important mediator of cell damage, specifically as a factor inducing mitochondria dysfunction-triggered activation and apoptotic cell death in rat striatum lesioned with 3-NP.

Neuro chemical alterations by 3-NP are currently investigated. A recent report shows that *in vivo* dopamine release measured by fast-scan cyclic voltametry is decreased in this toxic model in rats, in contrast with what is observed in transgenic models (Kraft J.C, 2009) Still, the implications of these findings deserve more exploration since it has been mentioned in this review that some reports highlight the contribution of dopamine in 3-NP toxicity. GABAergic system also suffers, in a differential manner, the insults of different toxicants. GABAergic striatal neurons
were recently shown to exhibit caspase independent, mitochondrially mediated programmed cell death, in a study evidencing that 3-NP elicited a mixed profile of caspase and calpain activation (Diwakarla S, Mercer. L.D., 2009).

Protective strategies based on neurotrophic factors are also under current investigation. BDNF was shown to exert protective effects in the 3-NP toxic model in cortical neurons through different potential mechanisms. The same group almost simultaneously reported that sonic hedgehog (a morphogen critical for embryogenesis) mediated BDNF-induced neuroprotection in the rodent 3-NP toxic model is involved in this paradigm. Another explanation for the protective actions of BDNF in this model was offered quite recently, since evidence was collected suggesting that BDNF decreases the levels of the pro-apoptotic protein BIM in mitochondrial and cortical cell lysates through the activation of MEK1/2 pathway (Almeida S, Laço M., 2009).
References

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3.3 Review on Naringenin

- **Drug name**: Naringenin
- **Chemical formula**: C_{15}H_{12}O_{5}
- **IUPAC Name**: (2S)-5,7-dihydroxy-2-(4-hydroxyphenyl)-3,4-dihydro-2H-1-benzopyran-4-one
- **Chemical structure**:

![Chemical structure of Naringenin]

3.3.1 Introduction

Plants have been used by human being since antiquity for diverse purposes such as food and medicine. Many of the currently available drugs have been derived from natural sources. More than 25% of the drugs prescribed worldwide are derived from plants, and 121 such active phytoconstituents are used for different disorders (Patel DK et al., 2011). The term flavonoid is derived from the Latin word "flavus", meaning yellow. It is a plant secondary product that has characteristics in red, blue, and purple pigments in plant tissues. Apart from their physiological roles in the plants, flavonoids are important components of the human diet, even though they are not considered as nutrients. Flavonoidal compounds share the same basic skeleton, the flavan-nucleus, consisting of two aromatic rings with six carbon atoms (ring A and B) interconnected by a hetero cycle including three carbon atoms (ring C) (Peng L et al., 2005, Han L et al., 2009, Procházková D et al., 2011, Kim JS et al., 2012, Schijlen EG et al., 2004). Flavonoids are important natural compounds with diverse biologic activities. Citrus flavonoids constitute an important series of flavonoids.

Flavonoids are plant-derived phytochemicals responsible for the different colours of plant parts like shades of yellow, orange and red in flowers. More than 4,000 flavonoids, such as flavones, flavanols, flavanonols, flavanones, and isoflavones have been reported in the edible plants and are consumed regularly in the
human diet (Galluzzo P et al., 2008) Flavonoids, found in fruits and vegetables, have various health benefits (Han X et al., 2008). The biosynthesis of flavonoids occurs via the combination of shikimic acid and acylpolymalonate metabolic pathways.

A starting compound is phenylpropane, a cinnamic acid derivative derived from shikimic acid, in which three acetate residues are incorporated followed by ring closure. The chalcone structure is an intermediate to the flavone structures, which might be hydroxylated and reduced at different positions (Martin HJ et al., 2003). Recent evidences have indicated that an adult human diet rich in flavonoids leads to a decrease of total cholesterol, low-density lipoproteins, and triglycerides in plasma, as well as a reduced incidence of cardiovascular diseases and osteoporosis (Galluzzo P et al., 2008). Among naturally occurring flavonoids, Naringenin and hesperetin are very common in some edible fruits and vegetables as aglycons and glycosides. Naringenin is most abundant in grapefruit and are used in perfumery, cosmetic and in different pharmaceutical formulations. It has been reported for the hypocholesterolemic, antiestrogenic, hypolipidemic, antihypertensive, and anti-inflammatory activities (Bernini R et al., 2003).

### 3.3.2 Importance of flavonoids

Plants produce a vast array of natural products including phenolic compounds, which are responsible for the major organoleptic characteristics of plant-derived foods and beverages. They play an important role in the color and taste properties of fruits and vegetables (Tapas AR et al., 2008; Horvath CR et al., 2005). Flavonoids, a group of phytochemicals, have diverse beneficial biochemical and pharmacological properties, mainly presented in foods of plant origin, such as fruits, vegetables, tea, wine, seeds, herbs, spices, and whole grains (Hughes LA. et al., 2008). Flavonoids are biological pigments responsible for the colours from red to blue in flowers, fruit and leaves (Erdogdu Y. et al., 2009).

### 3.3.3 Flavonoid biosynthesis

Flavonoids are a major class of plant secondary metabolites that serves a multitude of functions including pigments and antioxidant activity. Flavonoids are synthesized from phenylpropanoid derivatives by condensation with malonyl-CoA.
For example, condensation of p-coumaroyl-CoA (C6-C3) with three malonyl-CoA (C3) molecules results in Naringenin chalcone with a diphenylpropane (C6-C3-C6) unit, which is converted to Naringenin with the flavone (2-phenylchromen-4-one) backbone by conjugate ring closure. These and further modifications yield a variety of structural forms including chalcones, flavanones, dihydroflavonols, and flavans, anthocyanins, flavones and flavonols, and isoflavonoids.

3.3.4 Chemistry of flavonoid Naringenin

Flavonoids are composed of two aromatic rings linked through three carbon atoms that form an oxygenated heterocycle. Variations on the basic structure of flavonoids yield different classes of flavonoidal compounds (Verbeek R. et al., 2004). Flavonoids are a widely distributed group of polyphenolic compounds characterized by a common benzo-pyrone structure. Over 4,000 different flavonoids have been described and categorized into flavonols, flavones, flavanones, isoflavones, catechins, and anthocyanidins. Diverse biochemical properties of flavonoids including naringin, hesperidin, diosmin, and rutin have provoked interest in biology and medicinal chemistry. These compounds have exhibited a broad range of biological and pharmacological activities, such as antioxidant, anti-allergic, antibacterial, anti-inflammatory, antimutagenic and anticancer effects.

Flavonoids represent one of the most prevalent classes of compounds in vegetables, nuts, fruits and beverages, as well as in medical herbs (e.g., Silybum marianum, Alpina officinarum, Hypericum perforatum) (Lee S. et al., 2003). Naringenin belongs to the flavanones and is mainly found in fruits (grapefruit and oranges) and vegetables. Pharmacologically, it has anti-cancer, anti-mutagenic, anti-inflammatory, anti-oxidant, anti-proliferative and anti-atherogenic activities. Naringenin is flavones a type of flavonoid, that is considered to have a bioactive effect on human health. It is the predominant flavones in grape fruit. An inverse association between flavonoid intake and oxidation effect has been suggested by a number of epidemiological studies (Cavia-Saiz M et al., 2010; Dou.w et al., 2009). The chemical name of Naringenin is 2, 3-dihydro-5, 7- dihydroxy-2-(4-hydroxyphenyl) - 4H-1-benzopyran-4-one , and it has a molecular weight of 272.26 (C15 H12 O5). Naringenin is almost insoluble in water and is soluble in organic solvents such as alcohol. Naringenin is derived from the hydrolysis of glycone forms.
of this flavanone, such as naringin or narirutin (Eduardo Madrigal S. et al., 2014). Naringin (Naringenin-7- rhamnoglucoside), the bitter principle of grapefruit (Citrus paradisi), is found in the juice, flower, and rind of the fruit and constitutes up to 10% of the dry weight. Naringin and other Naringenin glycosides can be found in a variety of other sources.

3.3.5 Properties of Naringenin

Low concentrations of Naringenin are also found in tomatoes and tomato based products. Fresh tomatoes, especially tomato skin, also contain Naringenin chalcone, which is converted to Naringenin during processing to tomato ketchup. In vitro studies indicate a wide range of biologic activities for different flavonoids. These studies have mainly been performed with flavonoid aglycones or glycosides. Until very recently, flavonoid metabolites were rarely used, mainly because data about their identity were scarce; moreover, chemical standards for only a few potential metabolites are commercially available (Iris Erlund. et al., 2004).

3.3.6 Sources of Naringenin

Naringenin and its glycoside have been found in a variety of herbs and fruits, including grapefruit, bergamot (Gattuso Giuseppe. et al., 2007), sour orange (Gel-Moreto et al., 2003), tart cherries, tomatoes (Minoggio, M et al., 2003; Vallverdú-Queralt, A et al., 2012), cocoa (Sánchez-Rabaneda et al., 2003), Greek oregano (Exarchou, Vassiliki et al., 2003), water mint (Olsen, Helle T et al., 2008), drynaria as well as in beans. Ratios of Naringenin to naringin vary among sources (Minoggio M. et al., 2003), as do enantiomeric ratios (Krause. M et al., 1992).
3.3.7 Metabolite description

Naringenin is a flavanone that is considered to have a bioactive effect on human health as antioxidant, free radical scavenger, anti-inflammatory, carbohydrate metabolism promoter, and immunity system modulator. This substance has also been shown to repair DNA. Scientists exposed cells to 80 mm of Naringenin per litre, for 24 hours, and found that the amount of hydroxyl damage to the DNA was reduced by 24 percent in that very short period of time. Unfortunately, this bioflavonoid is difficult to absorb on oral ingestion. Only 15% of ingested Naringenin will get absorbed, in the human gastrointestinal tract, in the best case scenario. A full glass of orange juice will supply about enough Naringenin to achieve a concentration of about 0.5 mm per litre. But, one has to wonder, if given more time than 24 hours, would lower concentrations have similar effects.

3.3.8 Reported Pharmacological activities

- **Anti-oxidant effect**

The Naringenin exhibited higher antioxidant capacity and hydroxyl and superoxide radical scavenger efficiency. The glycosylation attenuated the efficiency in inhibiting the enzyme xanthine oxidase and the aglycone could act like a more active chelator of metallic ions than the glycoside. Additionally, Naringenin showed a greater effectiveness in the protection against oxidative damage to lipids in a dose-dependent manner. The flavanone was effective in reducing DNA damage (Kim J.H. et al., 2015).

- **Hepatoprotective effects**

Naringenin has been found to have a hepatoprotective characteristic similar to silymarin. Animal studies have demonstrated turmeric’s hepatoprotective effects from a variety of hepatic insults, including carbon tetrachloride (CCl4), alactosamine, acetaminophen (paracetamol), and Aspergillus aflatoxin. Turmeric’s hepatoprotective effect is mainly a result of its antioxidant properties, as well as its ability to decrease the formation of pro-inflammatory cytokines. The protective capacity of Naringenin on dimethylnitrosamine (DMN) - induced hepatic damage in rats was investigated. Oral administration of Naringenin (20 and 50 mg/kg daily over 4 wk) notably diminished DMN-induced dam-age when the weight of the liver was
evaluated, as well as alanine transaminase (ALAT), aspartate transaminase (ASAT), alkaline phosphatase (ALP), and bilirubin levels. Naringenin also restored natural protein levels in serum and albumin and hepatic malondialdehyde (MDA) levels. The Naringenin had antifibrinogenic and hepatoprotective effects, suggesting that it could be useful in the treatment of hepatic fibrosis.

- **Anti-inflammatory effects**

  The pathogenesis of inflammatory bowel disease (IBD) such as ulcerative colitis (UC) is usually associated with reduced antioxidant capacity. Generation of free radicals like reactive oxygen species (ROS) leads to lipid peroxidation, which inhibits cellular antioxidant capability, resulting in prominent colonic inflammation. There is a great need to search for safe and tolerable compounds for the management of inflammation to reduce patient compliance as well as the adverse effects of conventional treatments. Naringenin is a naturally occurring flavonoid that can be extracted from citrus fruits, tomatoes, cherries, grapefruit, and cocoa. Like most of the flavonoids, Naringenin was experimentally found to have several pharmacological potentials, including anti-inflammatory because of Naringenin has properties to produce sufficient hydroxyl (-OH) substitutions, which give it the capability to scavenge ROS. Thus, it has considered that Naringenin may diminish and/or improve pathological conditions where oxidation or inflammation is deemed to play a vital role.

- **Anti-carcinogenic effects**

  Animal studies involving rats and mice, as well as in vitro studies utilizing human cell lines, have demonstrated Naringenin ability to inhibit carcinogenesis at three stages: tumor promotion, angiogenesis, and tumor growth. Naringenin is also known to cause cytotoxic and apoptotic effects in several cancer cell lines in a dose-dependent manner as well as inhibits tumor growth in sarcoma S-180 implanted mice, suggesting that Naringenin can potentially be used to inhibit tumor growth (14-16). Cytotoxic effects were also induced in human cancer cell lines when high concentrations of Naringenin were administered (50% effective concentration: 150-560 µM). However, the use of flavonoids as cancer chemo preventive or chemotherapeutic agents requires the development of novel flavonoids or Naringenin.
derivatives that can induce cytotoxicity at low concentrations in a cell type-dependent manner.

- **Cardiovascular Effects**
  Naringin showed a range of properties that help protect the cardiovascular system, including antihypertensive, lipid lowering, insulin-sensitising, anti-oxidative and antiinflammatory properties. Naringin prevented the age-related increase in systolic blood pressure in stroke-prone spontaneously hypertensive rats, increased nitric oxide production, improved endothelial function and decreased cerebral thrombotic tendency. Further, naringin prevented oxidative stress in the hearts of rats with isoprenaline-induced myocardial infarction (Rajadurai M. et al., 2009).

- **Obesity**
  It is an important component of metabolic syndrome, is a chronic low-grade inflammatory condition leading to adipocyte differentiation and growth in adipose tissue. In mice fed a high fat diet, naringin decreased visceral adiposity and lowered plasma lipid concentrations, probably by activation of AMP kinase.

- **Gastro-intestinal effect**
  Pre-administration of Naringenin significantly reduced the severity of colitis and resulted in down-regulation of proinflammatory mediators (inducible NO synthase (iNOS), intercellular adhesion molecule-1 (ICAM-1), monocyte chemoattractant protein-1 (MCP-1), cyclo-oxygenase-2 (Cox2), TNF-α and IL-6 mRNA) in the colon mucosa. The decline in the production of pro-inflammatory cytokines, specifically TNF-α and IL-6, correlated with a decrease in mucosal Toll-like receptor 4 (TLR4) mRNA and protein. Phospho-NF-κB p65 protein was significantly decreased, which correlated with a similar decrease in phospho-IκBα protein. Consistent with the in vivo results, Naringenin exposure blocked lipopolysaccharide-stimulated nuclear translocation of NF-κB p65 in mouse macrophage RAW264.7 cells. In addition, in vitro NF-κB reporter assays performed on human colonic HT-29 cells exposed to Naringenin demonstrated a significant inhibition of TNF-α-induced NF-κB luciferase expression (Salim S. et al., 2013).
Naringenin enhances immunity

Natural killer (NK) cells are capable of identifying and killing tumor cells as well as virus infected cells without pre-sensitization. NK cells express activating and inhibitory receptors, and can distinguish between normal and tumor cells. The present study was designed to demonstrate the importance of the expression level of NKG2D ligands on the Burkitt’s lymphoma cell line, in enhancing NK cell cytolytic activity. Various flavonoids were used as stimulants to enhance the expression of NKG2D ligands. NK cell lysis activity against Raji was not changed by pre-treatment of Naringenin with luteolin, kaempferol, taxifolin and hesperetin. However, treatment with Naringenin showed increased sensitivity to NK cell lysis than untreated control cells. The activity of Naringenin was due to enhanced NKG2D ligand expression. These results provide evidence that narigenin’s antitumor activity may be due to targeting of NKG2D ligand expression and suggests a possible immunotherapeutic role for cancer treatment.

3.3.9 Pharmacokinetics

Naringenin is generally present in foods bound to sugars as β-glycosides (i.e., naringin), it was originally thought that absorption from the diet would be negligible. However, a number of studies have detected Naringenin in human urine (Johnson, A.R. et al., 2013; Jong-Hwa P. et al., 2010) and plasma following oral doses of pure naringin (Johnson A.R. et al., 2013) or grapefruit juice. The excretion of Naringenin glucuronides in humans reaches levels more than 100-fold higher than the concentration of Naringenin excreted in the urine. Naringenin present in the bile may either be excreted or reabsorbed, therefore raising the possibility of enterohepatic recycling of Naringenin. Naringenin has been detected in the plasma following oral administration of naringin or grapefruit juice but is generally reported to be below accurate detection limits. However, due to the lipophilic nature of Naringenin, it is possible that it accumulates within tissues, particularly membranes, and eventually reaches greater concentrations than those observed in the plasma. This accumulation would most likely occur in tissues such as the liver and intestine.
3.3.10 Clinical findings for Citrus fruit juices and their active component Naringenin

Most beneficial effects found for citrus flavonoids were mainly based on animal and in vitro cell culture studies, which may be relevant in explaining mechanisms of the bioactive components present in citrus fruits. However, very limited clinical studies have been conducted on citrus flavonoids or various citrus fruit juices in relation to possible cardiovascular and obesity benefits. In 1 study, bergamot extract given orally for 30 d to diet-induced hyperlipemic Wistar rats and in 237 patients suffering from hyperlipemia that was either associated or not associated with hyperglycemia (Aptekmann NP. *et al.*, 2013). Bergamot extract reduced concentrations of total and LDL cholesterol (an effect accompanied by elevation of cholesterol bound to high density lipoprotein) and TGs and significantly decreased blood glucose concentration (Mollace V. *et al.*, 2011). Moreover, bergamot extract inhibited HMG-CoA reductase activity and enhanced reactive vasodilation in hyperlipidemia patients (Mollace V. *et al.*, 2011). Another randomized controlled trial was conducted to evaluate the role of grapefruit in reducing body weight and blood pressure and in promoting improvements in lipid profile in 74 overweight healthy adults (Dow CA *et al.*, 2012). Supplementation of one-half of a fresh Rio Red grapefruit with each meal for 6 wk did not significantly decrease body weight compared with the control condition (Dow CA. *et al.*, 2012). However, supplementation improved blood pressure and lipids, which warrants further studies conducted on grapefruit in the context of obesity and cardiovascular disease prevention. A similar beneficial effect on lipid variables was also observed in a study that showed that long-term orange juice consumption lowered concentrations of total cholesterol, LDL cholesterol, and apoB and the LDL/HDL ratio in comparison with the nonconsumer counterparts (Aptekmann NP. *et al.*, 2013). However, the percentage of abdominal obesity among orange juice consumers did not differ from that of nonconsumers (Aptekmann NP. *et al.*, 2013).

In addition, a previous study showed a positive effect of orange juice on HDL-cholesterol concentrations with the consumption of 750 mL of orange juice. Most of these observed benefits were linked to high amounts of vitamin C and folate present in orange juice. However, the amount of polyphenols, flavonoids, or
Naringenin concentration cannot be determined from these reports. There is also a significant lack of information regarding clinical studies with pure Naringenin or Naringenin. One clinical study found that Naringenin treatment (400 mg · capsule$^{-1}$ · d$^{-1}$ for 8 wk) lowered plasma total cholesterol by 14% and LDL cholesterol by 17%, whereas plasma TG and HDL-cholesterol concentrations remained unaffected (Aptekmann NP. et al., 2013). Moreover, Naringenin significantly increased erythrocyte SOD and catalase activities in the hypercholesterolemic group, whereas GPx activity and plasma TBARS concentrations were not different from baseline measurements (Aptekmann NP. et al., 2013). Recently, the safety of bitter orange (Citrus aurantium) consumption was assessed in a 60-d double-blind, placebo-controlled trial, $p$-Synephrine, which is the primary protoalkaloid in bitter orange, given alone or in combination with Naringenin and hesperidin twice daily to 25 healthy subjects per group showed no significant changes in systolic or diastolic blood pressures, blood chemistries, or blood cell counts in the control or $p$-synephrine–treated groups.
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