Acetyl-L-Carnitine in Parkinson's Disease

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Acetyl-L-Carnitine in Parkinson's Disease

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1. Introduction

Parkinson's disease (PD) is the most common neurodegenerative movement disorder that is estimated to affect approximately 1% of the population older than 65 years of age (deRijk et al., 2006; Saunders, 2000). PD was first described in 1818 by the British physician J. Parkinson. Before that date, no one had ever described the symptoms of this disease; so many researchers theorize that this pathology is the product of the English Industrial Revolution (Parris, 2000; Perlmutter, 2000). Some authors speculate that new neurotoxic contaminants produced by the industries can have been the cause of this chronic and progressive disease. PD is characterized by the progressive depletion of pigmented dopamine-containing neurons in the region known as the substantia nigra pars compacta and by the presence of intraneuronal aggregates called Lewy bodies (LBs), which are enriched in filamentous α-synuclein and other proteins that are often ubiquinated (Lee & Trojanowsky, 2006). Approximately 80% of dopaminergic neurons in the substantia nigra are already irreversibly destroyed when the symptoms of PD becomes significantly visible. Depletion of dopamine causes dysregulation of the motor circuits that project throughout the basal ganglia (BG), resulting in the cardinal clinical manifestations of PD: bradykinesia (extreme slowness), tremor, rigidity, and postural instability. Consequently, patients experience increasing difficulty in daily living functions along the course of the disease. Additional neuronal fields and neurotransmitter systems are also involved in PD, including the locus coeruleus, the dorsal motor nucleus, the autonomic nervous system and the cerebral cortex. Consequently, noradrenergic, serotoninergic, and cholinergic neurons are also lost. These widespread neuronal changes led to complex and variable progressive non-motor symptoms such as cognitive decline, sleep abnormalities, and depression which dominate the later stages of PD (Braak, 2003). In any case, PD is primarily a sporadic disorder and its etiopathogenesis is still not fully understood, but the recent discovery of genes associated with rare monogenic forms of the disease, together with earlier studies and new experimental animal models, has provided important and novel insight into the molecular pathways involved in disease pathogenesis (Wood-Kaczamar, 2006). Increasing evidence indicates that deficits in mitochondrial function, oxidative and nitrosative stress, accumulation of aberrant or misfolded proteins, and ubiquitin-proteasome system

dysfunction can represent the principal molecular pathways or events that commonly underlie the pathogenesis of sporadic and familial forms of PD (Schapira, 2008). However it is possible that multiple factors contribute to the cascade of events leading to cells death in patients with PD and that different factors might be more important in different individuals (Olanow, 2009) (Fig. 1).

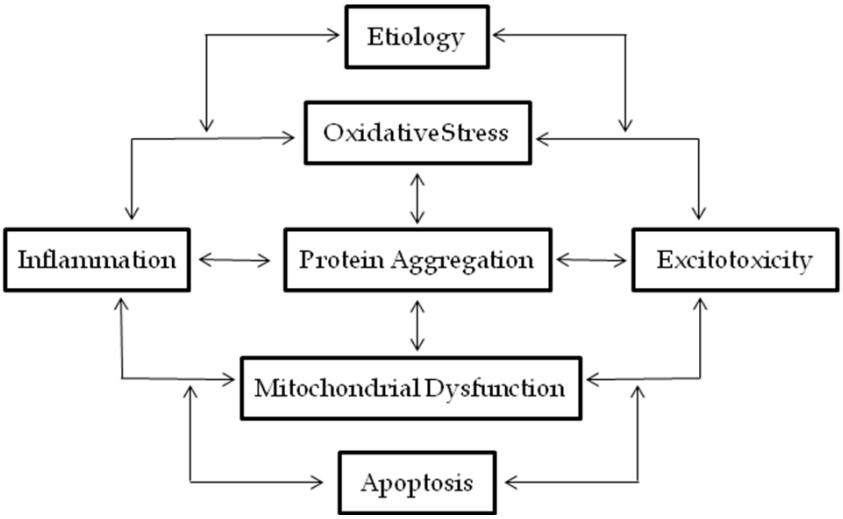


Fig. 1. Schematic illustration of factors that might be involved in the pathogenesis of cells death in PD. (Adapted from Olanow, 2007).

Several pharmacological agents are currently available for the management of PD (Table 1). These drugs can provide symptomatic relief but no agents capable to halt the progression of the neurodegenerative process or reverse the neuronal degeneration have been developed yet. Moreover, the neuroprotective effects suggested for many of the approved drugs have not been convincingly demonstrated in PD patients. Furthermore, although PD also involves degeneration of non-dopaminergic neurons, the treatment of the resulting predominantly non-motor features remains a challenge. The leading therapeutic strategy pursued in PD management is the so called dopamine replacement therapy (DRT), which employs drugs acting on dopamine (DA) circuits to restore the deficient dopaminergic tone existing in this pathology. Pharmacological agents have also been developed which can indirectly boost DA transmission, based on the functional interactions existing between DA and other neurotransmitters in the BG. L-Dopa is the key compound in the treatment of PD, acting as a precursor of DA. It has a long clinical record as the most effective antiparkinsonian drug, and it is still considered the "gold standard" in pharmacological treatment of PD (Mercuri & Bernardi, 2005). However, besides offering only symptomatic relief for patients, motor and non-motor untoward effects are often observed in the course of L-dopa therapy, which can be severe and limit its therapeutic potential (Encarnation & Hauser, 2008; Fox & Lang, 2008). Furthermore, exposure of patients to L-dopa, results in fluctuations of motor responses in approximately 30–50% of patients exposed to therapy for as little as 5 or more years. The most common fluctuation experienced is the so-called "on-

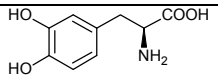
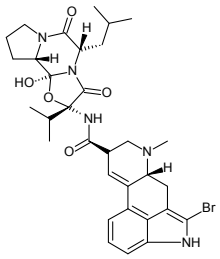
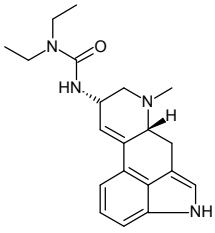
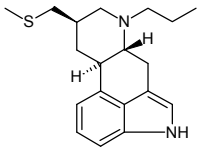
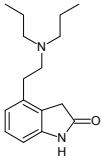
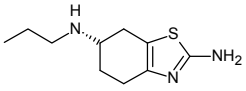
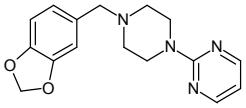
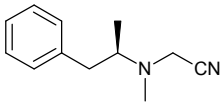
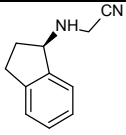
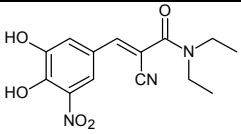
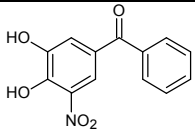
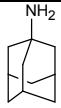
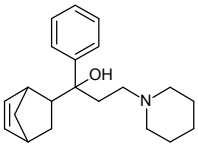
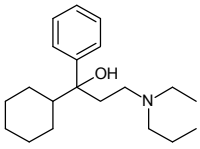
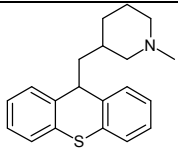
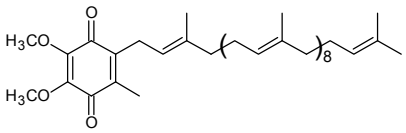
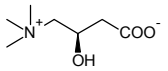
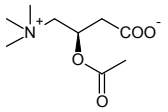
Drugs	Mechanism of action
 L-dopa	Precursor of DA
Ergot derivatives	Agonist at D_2-like receptors
 Bromocriptine	 Lisuride
 Pergolide	
Non-Ergot derivatives	
 Ropinirole	 Pramipexole
 Piribedil	
 Selegiline	 Rasagiline
MAO-B inhibitors	
 Entacapone	 Tolcapone
COMT inhibitors	
 Amantadine	NMDA glutamate receptor antagonist
 Biperiden	 Triexyphenidyl
 Metixene	Muscarinic receptor antagonists

Table 1. Currently available pharmacological therapies for PD treatment.

off" phenomenon that results in an unpredictable transient loss of therapeutic effect. Apart from L-dopa, drugs that are currently prescribed for the management of PD include DA receptor agonists, selective monoaminooxidase type B inhibitors (MAO-B), catecol-O-methyltransferase (COMT) inhibitors, amantadine (an antiviral agent that also bears action as an antiglutamatergic agent), and anticholinergics. DA receptor agonists counteract PD-associated motor impairment chiefly stimulating the D_2-like receptors, though some of them can also bind non–dopaminergic receptors. They may be used alone to delay the need for L-dopa or as multiple-medication therapy (MMT) with L-dopa to increase its effectiveness (Cavalli et al. 2008). Neuroprotective properties have been suggested for some dopaminergic agonists (e.g. bromocriptine and pramipexole), although the clinical evidence collected so far does not convincingly support this hypothesis (Schapira, 2003). Certain other available drugs, like MAO-B (Fernandez & Chen, 2007) and COMT inhibitors (Canesi et al., 2008; Schrag, 2005), are used mainly as MMT with L-dopa, since they alter the *in vivo* metabolism of DA by increasing its plasma half-life. Functional interactions between glutamate and DA receptors exist in BG, and evidence suggests that the loss of DA in PD may lead to glutamatergic hyperactivity, which participates in the manifestation of motor impairment accompanying the disease (Chase & Oh, 2003). On this basis, glutamatergic antagonists have been extensively investigated as potential antiparkinsonian drugs (Johnson et al. 2009). Among these, amantadine is the best characterized antiglutamatergic agent used in PD management. In addition to the blockade of ionotropic *N*-methyl-D-aspartate (NMDA) receptors, amantadine posses other mechanisms of action which contribute to its effects: anticholinergic activity, stimulation of DA release, modulation of the affinity of post-synaptic DAergic receptors for DA (Metman et al., 1998, Peeters et al., 2003). Anticholinergic compounds were the first, and for a long time the only, pharmacological agents available to treat motor deficits accompanying PD (Brocks, 1999). They were intend to correct the imbalance between DA and acetylcholine levels that take place in the BG, where a reduction of cholinergic tone may amplify DA-mediated signal (Cragg, 2006). Although these drugs produce some beneficial effects on PD symptoms, they are associated with adverse cognitive effects (Cancelli et al., 2009). All the anticholinergics used against PD bind to the central muscarinic receptors, having no affinity for the nicotinic ones, although they also block peripheral muscarinic receptors, and this triggers many of their adverse effects, which include nausea, constipation and urinary retention (Lees, 2005). Nevertheless, currently available pharmacological therapies are unable to arrest or to reverse the progression of this relentlessly progressive and severely debilitating condition. PD is currently an incurable disease, and the number of subjects afflicted with this disease is constantly increasing due to the increasing global geriatric population. Therefore, the need for newer and more effective agents is receiving a great deal of attention and, consequently, being subjected to extensive research. The vast amount of information gained regarding the pathogenesis of PD has fuelled numerous developments and vast range of investigated agents have demonstrated immense potential for preventing and eventually providing cure for this condition. Clinical and biochemical evidences suggest that PD involves multifactorial, oxidative neurodegeneration and that L-dopa therapy aggravates the oxidative burden. Strong evidence now exists to support an aberrant role for mitochondrial functions, as well as increased oxidative stress, in the pathogenesis of PD. If mitochondrial defects and oxidative damage play a role in the pathogenesis of PD, then one would suspect that

agents that may improve mitochondrial function or exert antioxidative effects could be neuroprotective. There are several agents that are currently under investigation for their potential neuroprotective effects based on their capacity to modify mitochondrial dysfunction. These include creatine, coenzyme Q10, nicotinamide, lipoic acid and acetyl-L-carnitine, etc. (Table 2). These agents are therefore promising candidates for neuroprotective drugs against PD (Beal, 2003). Acetyl-L-carnitine, (3*R*)-3-(acetyloxy)-4-(trimethylammonio)butanoate (table 2), is an ester of the trimethylated amino acid, L-carnitine, and is synthesized in the brain, liver and kidney by the enzyme acetyl-L-carnitine transferase. Acetyl-L-carnitine facilitates the transport of fatty acids and other moieties across the membranes of mitochondria, thereby participating in the production of energy and mitochondrial function within the brain. Acetyl-L-carnitine has been proposed to have beneficial effects in preventing the loss of brain functions which typically occur during aging and neurodegenerative disorders. The main mechanism of action of acetyl-L-carnitine is the improvement of mitochondrial respiration which allows the neuron to produce ATP necessary to maintain the normal membrane potential. However, acetyl-L-carnitine has been shown to be neuroprotective through a variety of other effects such as the increase in protein kinase C (PKC) activity (McDaniel, et al. 2003). Moreover acetyl-L-carnitine has also been reported to attenuate the occurrence of parkinsonian symptoms associated with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) *in vivo*, and protects *in vitro* against the toxicity of neurotoxic 1-methyl-4-phenylpyridinium (MPP^+), a metabolite of MPTP (Hongyu et. al., 2010). Therefore, acetyl-L-carnitine with its well known antioxidant energizing protective activities and with its trophic effects, might be an effective and safe prevention strategy for PD.

Agent	Structure
Coenzyme Q10	
Carnitine	
Acetyl-L-carnitine	
Lipoic acid	

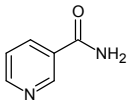
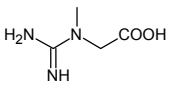
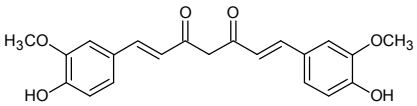
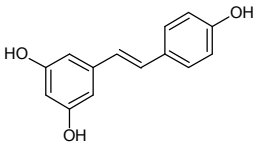
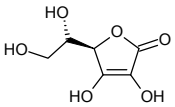
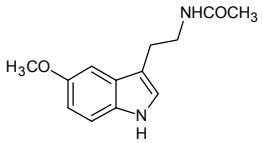
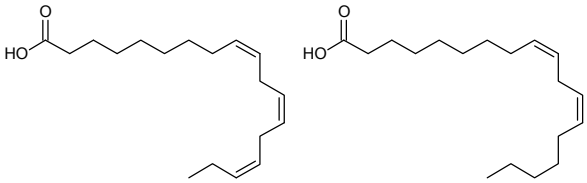
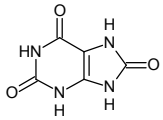
Agent	Structure
Nicotinammide	
Creatine	
Curcumin	
Resveratrole	
Vitamine C	
Melatonin	
Omega-3 and Omega-6 fatty acids	 Linolenic acid Linoleic acid
Uric acid	

Table 2. Neuroprotective agents effective in PD models.

2. Nutritional and biochemical aspects of acetyl-L-carnitine

Carnitine has been discovered in the bovine muscle in 1905, but its structure was defined only in 1927. In 1958 the role of carnitine has been discovered by I. Frizz, who demonstrated that this substance is important in stimulating the oxidation of long chain fatty acids into the mitochondria. L-Carnitine (–)-3-hydroxy-4-(trimethylammonio)butanoate is a highly polar, water-soluble quaternary amine that exists as a zwitterion under physiological conditions. It was initially called vitamin T, because it is necessary for the growth of the *Tenebrio Molitor* warm. Although it is structurally similar to an amino acid it is not involved in the formation of proteins, and it is more similar to acetylcholine. Carnitine is synthesized *in vivo* from the amino acids lysine and methionine, especially in liver, kidney, and muscle, and it is stored mainly in skeletal and cardiac muscles (Marquis & Fritz, 1965). Exogenous carnitine, taken predominantly with the meat of the diet, is about 75% of the body carnitines, while the daily requirement is about 200—300 mg. *In vivo* synthesis of carnitine, supplemented by carnitine from diet, provides sufficient carnitine to maintain metabolic functions. However, in cases of excessive loss of carnitine (low carnitine intake with the diet, altered carnitine metabolism or disease states such as in neurodegenerative diseases and geriatric depression), supplementation with acetyl-L-carnitine may be beneficial. Tissue levels of L-carnitine in animals and humans decrease with age, due to reduced integrity of the mitochondrial membranes. Acetyl-L-carnitine, an ester of the L-carnitine, is synthesized in human brain, liver, and kidney by the enzyme acetyl-L-carnitine transferase. Carnitine, acetyl-L-carnitine and acyl-L-carnitine are responsible for many biological actions. Several authors have suggested that acetyl-L-carnitine has beneficial effects on brain functions during aging and in conditions of neurodegenerative diseases such as Parkinson's and Alzheimer's diseases. It has been demonstrated that acetyl-L-carnitine plays a role in increasing the potency of cholinergic and anti-cholinergic actions, by reacting with the electrophilic or cationic site of the cholinergic receptor (Sinicropi et al., 2010). Carnitine as acyl-L-carnitine is important in the mitochondrial process of β-oxidation of fatty acids (Bremer, 1962; Bremer et al., 1983) and the acetyl moiety can be utilized to maintain acetyl-CoA levels. Acetyl-L-carnitine promotes acetylcholine production and release, and stimulates membrane phospholipid synthesis (Pettegrew et al., 2000). In addition, the acetyl moiety of acetyl-L-carnitine can acetylate –NH_2 and/or –OH functional groups of lysine, serine, threonine, tyrosine and *N*-terminal amino acids in proteins modifying their structure, function and activity. Moreover acetyl-L-carnitine modulates glucose metabolism and stimulates glycogen synthesis, restores ammonia induced depletion of brain energy stores in sparse-fur mice with elevated ammonia and glutamine levels (Rao et al., 1997) and, with carnitine, maintains progressive spermatozoa motility (Jeulin et al., 1988). This molecule acts also on the mitochondrial redox reactions that allow neurons to produce ATP, required to maintain normal membrane potential (McDaniel et al., 2003). L-carnitine and acetyl-L-carnitine are administrated orally, intravenously and/or intramuscularly. These compounds are absorbed in the jejunum by simple diffusion. Transport into tissues and cells is via an active transport mechanism and acetyl-L-carnitine and carnitine plasma concentrations reach equilibrium via carnitine acetyl-transferase activity. Both intravenous and oral administrations result in a corresponding increase of cerebrospinal fluid (CSF) concentrations of acetyl-L-carnitine, indicating that it readily crosses the blood-brain barrier (Kido et al., 2001; Thal et al., 1996).

3. MPTP and neuroprotective effect of acetyl-L-carnitine in pathogenesis of Parkinson's disease

In the mitochondria of all cells redox reactions produce free radicals. High levels of reactive oxygen species (ROS) and reactive nitrogen species (RNS), especially in Parkinson's disease, can result in damage to phospholipids and polyunsaturated fatty acids, which are both abundant in the brain and therefore susceptible to oxidative damage. Therefore, there are many evidences for increased oxidative damage also to DNA and proteins (Dexter et al., 1994). Many evidences have accumulated implicating mitochondrial defects and oxidative stress damage in the pathogenesis of Parkinson's disease. In this contest, fundamental role is attributed to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a neurotoxin that is able to produce an experimental model of Parkinson's disease (PD) in humans and laboratory animals (such as primates and mice). It replicates most of the clinical features of PD as well as the main biochemical and pathologic hallmarks of the disease. The apparent neurotoxic specificity of MPTP is mediated through its conversion into 1-methyl-4-phenylpyridine (MPP^+) (Fig. 2) by the action of the mitochondrial enzyme MAO B (Javitch & Snyder, 1984; Javitch et al., 1985).

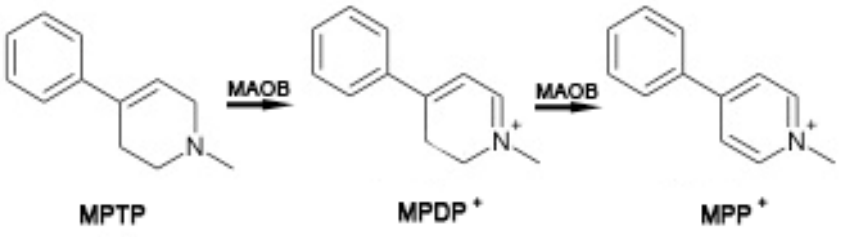


Fig. 2. Conversion of MPTP in MPP^+

The neurotoxicity of MPTP was discovered in 1976 when B. Kidstone, a 23 years old student of chemistry in Maryland, synthesized MPTP and injected it himself. He was contaminated by the MPTP and three days later he showed all the symptoms of Parkinson's disease. Studies on MPTP toxicity showed that it is mediated by inhibition of respiratory chain complex I activity (Bloem et al., 1990). There are at least three ways that MPP^+ can follow once inside dopaminergic neurons (Przedborski & Vila, 2003). It can: a) take the vesicular pathway, bind to dopamine transporters to be translocated into synaptosomal vesicles (Liu et al., 1992); b) interact with various cytosolic enzymes by remaining into the cytosol (Klaidman et al., 1993); c) be concentrated in the mitochondria (Ramsay & Singer, 1986). MPP^+ can passively enter the mitochondria through the transmembrane potential of the mitochondrial membranes and it can accumulate into the mitochondrial matrix. First of all, MPP^+, after being entered the mitochondrial matrix, inhibits the Krebs cycle enzyme α-ketoglutarate dehydrogenase (Mizuno et al., 1987), but the main cause of mitochondrial dysfunction involves the respiratory chain complex I (Ramsay et al., 1991). The MPP^+ toxicity is associated with oxidative damage. In fact MPP^+ induces superoxide production and increases lipid peroxidation. Important studies of Dexter and colleagues, showing increases in both malondialdehyde and in cholesterol hydroperoxides, led to a direct evidence of oxidative damage in Parkinson's disease (Dexter et al., 1994). Even the concentration of 8-hydroxy-2′-deoxyguanosine has been found three to four times higher in

the caudate and substantia nigra of Parkinson's disease subjects (Sancher-Ramos et al., 1994). Shergill and co-authors have found a significant increase of nitrosyl complexes in Parkinson's disease substantia nigra (Shergill, 1996). However, recent studies suggest that MPP^+ toxicity, at least in the initial stages, is primarily due to a decrease in mitochondrial ATP synthesis rather than the formation of free radicals (Fonck & Baudry, 2003). Subsequently, the protective effect of acetyl-L-carnitine in Parkinson's disease, induced by MPTP, has been studied in a group of primates by the research group of Bodis-Wolner (Bodis-Wolner et al., 1991). For their studies Bodis-Wolner and colleagues used three groups of primates, the first of which was treated just with MPTP. To the second group was administered acetyl-L-carnitine before the MPTP, while the third group had a control role. Their results have shown that primates treated only with MPTP developed the classic symptoms of parkinsonism. In the second group, only in a primate a weak form of Parkinson's disease has evolved to signify the protective effect of acetyl-L-carnitine on the disease development. It is conceivable that the effect of MPP^+ results in altering the mitochondrial respiration processes linked to NAD (Nicklas et al., 1985, 1987; Heikkila et al, 1985; Ramsey et al., 1986; Mizuno et al., 1988).

4. Toxic and antioxidant compounds in Parkinson's disease

Oxidants, as hydrogen peroxide and superoxide radicals, are produced as by-products of oxidative phosphorylation into mitochondria, making these organelles the main site of ROS generation within the cells. In fact, mitochondria are a major source of ROS, with up to 2-3 % of all oxygen consumed by mitochondria being converted to hydrogen peroxide (Boveris et al., 1972). This is the normal condition and basal levels of ROS can be limited by the presence of efficient antioxidant defence systems, including the enzymatic antioxidants (superoxide dismutase, catalase, peroxidases, and heme oxygenase) and the non-enzymatic redox-regulating antioxidants (glutathione and vitamin C). However, in pathological neurodegenerative conditions, like in Parkinson's disease, where mitochondrial respiratory defects occur, the amount of ROS produced by the electron transport chain dramatically increases, abolishing the antioxidant protection systems (Parker et al., 1989). In studies on brain tissue in patients with PD, the activity of complex I is reduced in the substantia nigra, without any decrease in other brain regions. As shown by Haas et al. (Hass et al., 1995), the activity of complex I is reduced also in PD platelets of un-medicated patients. A decrease of coenzyme Q10 levels in platelets mitochondria, which is correlated with reduced complex I of respiratory chain, is reported in Shults et al. (Shults et al., 1997). In parkinsonian subjects platelets mitochondria were found to have lower levels of coenzyme Q10 than mitochondria from age/sex-matched controls. As shown in certain clinical studies, coenzyme Q10 appears to slow the progressive deterioration of function in PD (Shults et al., 2002). Coenzyme Q10 is necessary for the normal activity of the respiratory chain and transfers electrons from complexes I and II to III. It can be worthwhile to use coenzyme Q10 to restore the functions of the respiratory chain and scavenge ROS. Nevertheless, coenzyme Q10 protects primary dopaminergic neurons *in vitro* against cell death induced by MPTP (Gille et al., 2004), and seems that at least partially restores the function of complex I in the tissues of patients with PD (Shults et al., 1998; Storch et al., 2007). Moreover, it has antioxidant properties; it has been also shown to prevent peroxidation of membrane lipids and protect mitochondrial DNA from free oxygen radicals. Important results on the defects of complex I activity in the pathogenesis of PD are derived from studies with the toxin rotenone. Rotenone (Table 3) is a

natural compound extracted from the roots of certain plants and has been used as an insecticide for vegetables. Rotenone rapidly crosses the blood-brain barrier due to its lipophilic structure, and rapidly crosses the biological membranes of mitochondria into the cells, in which the toxin reduces the activity of oxidative phosphorylation, by binding to PSST subunit of respiratory chain complex I (Schuler & Casida, 2001). It is known that rotenone is a highly specific inhibitor of complex I of the electrons transport chain. The possibility that rotenone and other pesticides are involved in the pathogenesis of PD stems from epidemiological studies (Gorell et al., 1998; Seidler et al., 1996). In fact, an atypical Parkinson's syndrome has developed in populations of the French West Indies, taking fruit and herbal tea containing insecticides (Caparros-Lefebvre & Elbaz, 1999). Pesticides and herbicides become highly suspect as potential PD triggers. A connection was long suspected between PD and rural living, including the drinking of contaminated well water or exposure to pesticides and herbicides (Hancock et al., 2008; Stephenson, 2000). Recently it was observed that high blood levels of homocysteine (Table 3) are present in patients with PD receiving L-dopa.

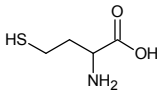
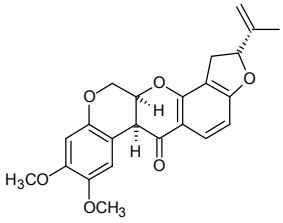
Agent	Structure
Homocysteine	
Rotenone	

Table 3. Neurotoxic agents in Parkinsons's disease

On the other hand it is known that the increase in homocysteine is a risk factor for atherosclerosis, stroke, vascular disease, and dementia. There are many proposed mechanisms for toxicity of homocysteine in promoting neurodegenerative diseases such as of Parkinson's and Alzheimer's diseases (Postuma & Lang, 2004; Seshadri et al., 2002): free radicals formation, induction of inflammation, and altered vulnerability of complex I mitochondrial respiratory chain. The formation of homocysteine occurs from methionine which is converted to *S*-adenosylmethionine and then demethylated to *S*-adenosylhomocysteine, which forms homocysteine. Homocysteine itself is reused to form methionine by the action of two enzymes rate-limiting methylenetetrahydrofolate reductase (MTHFR) and betaine homocysteine methyltransferase (BHMT). Homocysteine can be metabolized to cysteine due to the cystathionine-betasintethase (CBS). The MTHFR requires cofactors such as vitamin B12 and folate, while the CBS requires vitamin B6. The administration of L-dopa urges COMT activities causing methylation of L-dopa to 3-O-methyl-dopa and, at the same time, the demethylation of *S*-adenosylmethionine to S-adenosylhomocysteine, which rapidly forms homocysteine. Therefore, since the

demethylation of *S*-adenosylmethionine results in an increase of homocysteine, it is easy to understand why Parkinson's patients treated with L-dopa have higher levels of homocysteine. Consequently, any substance that can reduce blood levels of homocysteine should be administered to Parkinson's patients who require L-dopa: COMT inhibitors, vitamin B6, folate and vitamin B12. The toxicity of these compounds can be prevented with the administration of antioxidants. Complex I of respiratory chain is genetically coded for the ring-shaped mitochondrial DNA (mtDNA). A line of evidence, implicating mitochondria and mitochondrial genome (mtDNA) in PD pathogenesis, comes from "cybrid cells". While many proteins and enzymes of all electron transport chain complexes are coded from nuclear genes, 13 of them are coded in the small circular double-stranded mtDNA, located within the mitochondrial matrix. The human mitochondrial genome contains 37 genes (16,560 base pairs), including 13 that encode subunits of proteins of respiratory chain, and in particular 7 subunits of complex I. Mitochondrial genes exhibit a much higher mutation rate compared to nuclear genes and mtDNA is exposed to ROS generated during respiration. It is believed, therefore, that the oxidative damage to mitochondrial DNA and its mutation can play a role for mitochondrial dysfunction in PD. To determine if complex I is genetically abnormal, Swerdlow and colleagues (Swerdlow et al., 1996) devised an experiment with cybrid cells, generated to uncouple potential effects of a damaged mtDNA from effects of the nuclear DNA. These Swerdlow cybrid cells are hybrid cells which combine the nuclear genome from neuroblastoma cells with the mitochondrial genome from platelets of PD patients or healthy control subjects. Using these cybrid cells, Swerdlow and colleagues confirmed that PD mitochondria were less efficient in complex I activity (- 20%) associated with increased free radical production and apoptotic cell death (Gu et al., 1998; Swerdlow et al., 1996). Since only mtDNA is derived from the donor platelets, the Swerdlow experiment can be interpreted as a suggestion for mtDNA transmission of the mitochondrial defect. Besides, many authors suggest that alterations in processes of ubiquitination and degradation of proteins by the 26S proteasome can play a primary role in the PD pathogenesis (McNaught & Ienner, 2001; McNaught et al., 2001). Products of oxidative damage can contribute to substantia nigra degeneration in PD. The oxidized proteins can not be adequately ubiquitinated and recognized by the proteasomes and accumulated within the cells. The accumulation of ubiquitinated proteins and the loss of proteasomal activity can induce mitochondrial dependent apoptotic death of dopaminergic neurons in a manner similar to that occurring in the substantia nigra in PD. Although rare, some genetic cases of Parkinson's disease are linked to mutations in a synaptic protein called α-synuclein that was originally identified from smaller peptides isolated in amyloid-containing fractions of Alzheimer disease brains (Hong, 2005). The α-synuclein protein is another aggregating, fibril-forming protein that is a major component of the Lewy body lesions characteristic of PD as well as certain cases of Alzheimer (AD) and several other neurodegenerative conditions. α-Synuclein aggregates show evidence of nitration-based oxidative damage that might play a critical role in aggregate formation (Giasson et al., 2000). Recent studies have shown that the polyphenol curcumin (the active principle of turmeric *Curcuma longa*) can reduce the aggregation of α-synuclein, and its administration to cultured cells with α-synuclein aggregate formation results in fewer aggregates (Ono & Yamada, 2006; Pandey & Galvin, 2005). Also the pesticide rotenone leads to the presence of Lewy bodies with aggregation of α-synuclein (Sherer et al., 2003). The evidence that rotenone, a inhibitor of mitochondrial respiratory chain complex I, causes

aggregation of Lewy bodies may means that mitochondrial dysfunction has a role in the development of these pathologic fibril-forming proteins in Parkinson's disease (Dawson & Dawson, 2003a). Rajeswary has shown that curcumin protects mouse brain from MPTP-induced neurotoxicity by virtue of its scavenger activity (Rajeswary, 2006). Moreover, curcumin has been shown to protect PC12 cells from MPP^+ by inducing bcl-2, a antiapoptotic protein, preventing the dissipation of membrane potential of mitochondria and reducing then ROS and iNOS levels (Chen et al., 2006). The importance of mitochondria in the neuroprotective effect of curcumin has been also emphasized by Mythri et al. (2007), who demonstrated that curcumin inhibits the formation of peroxynitrite responsible for the damage of respiratory chain complex I. Studies with humans and rodents have demonstrated that after oral administration curcumin is transformed to curcumin glucuronide and curcumin sulphate, not only in the liver (Rahaman et al., 2006) but also in the intestinal tract (Ireson et al., 2002). In these organs, curcumin is also reduced into dihydrocurcumin (DHC), tetrahydrocurcumin (THC), hexahydrocurcumin, octahydrocurcumin and hexahydrocurcuminol (Ireson et al., 2002; Rahaman et al., 2006); curcumin, DHC and THC can be further converted in glucuronide conjugates. It is important to note that curcumin and THC have anti-inflammatory activity; in humans and rodents curcumin inhibits the activity of cytochrome P450 enzymes, glutathione-transferase and UDP-glucuronosyl transferases (Basu et al., 2004; Hayeshi et al., 2007; Thapliyal & Maru, 2001). Moreover, it has been shown that a large number of polyphenolic antioxidants have a protective effect against the degeneration induced by high levels of ROS and RNS in cases of mitochondrial dysfunction. It has been proven that green tea polyphenols have a protective effect against 6-hydroxydopamine toxicity in SH-SY5Y cells (Guo et al., 2005). 6-Hydroxydopamine is a hydroxylated analogue of dopamine, extensively used in rodents. 6-Hydroxydopamine possess a high affinity for many membrane transporters of catecholamines and norepinephrine, allowing the drugs to freely enter both dopaminergic and noradrenergic neurons (Bovè et al., 2005). The efficacy of the green tea component epigallocatechin 3-gallate has been demonstrated in the MPTP mouse model of Parkinson's disease. It has been shown that in these treated rats there is both loss of dopaminergic neurons and attenuation of striatal dopamine levels (Choi et al., 2002). Choi et al. (2002) suggest that this protection is mediated by inhibition of NOS expression. Epidemiological evidence shows that two caps a day of green tea have a protective effect against the Parkinson's disease development (Chan et al., 1998). Another polyphenol used in the fight against Parkinson's disease is the oxyresveratrol, found in large amounts in mulberry wood, which has shown potent scavenger activity against ROS and RNS in glial cells exposed to hydrogen peroxide (Lorenz et al., 2003). In addition, in a study on 6-hydroxydopamine-treated neuroblastoma SH-SY5Y cells has been found that the oxyresveratrol drastically reduces the production of ROS and reduces also the apoptotic activity of caspase-3 caused by damaged mitochondria (Chao et al., 2008). Other important antioxidant is uric acid (Ames et al., 1981). Recent studies and epidemiological researches have shown a correlation between high levels of uric acid in serum and a lower incidence of Parkinson's disease (Annanmaki et al., 2007, Weisskopf et al., 2007; Winquist et al., 2010). It was also seen that the uric acid protects against the damage caused by free radicals on the mtDNA (Anderson & Harris, 2003), helping to maintain the integrity of the mitochondrial genome and prevent possible mutations. In addition, uric acid prevents the death of dopaminergic cells treated with rotenone and homocysteine; treatments that increase the production of ROS and act on

mitochondrial membrane depolarization (Duan et al., 2002). Most likely uric acid neutralizes ROS through the Fenton reaction, thus providing dopaminergic neuroprotection. But we have to balance the benefits of dietary supplementation of uric acid on parkinsonism and the possible risk of developing gout and cardiovascular problems.

5. Acetyl-L-carnitine and other nutrients in age-dependent neurodegenerative diseases

A broad spectrum of both genetic and environmental factors has been suggested as contributing to the initiation and progression of PD. Among these, an important risk factor for the disease is the aging (Parris, 2000). It contributes to PD progression, perhaps because of accumulative oxidative damage and decrease of antioxidant capacity. Many evidences support the validity of the oxidative stress hypothesis, which suggests that lowered functional capacity in aged organisms is the result of an increased generation of reactive species. The increased levels of ROS and RNS can cause damage to intracellular macromolecules, as DNA, proteins and lipids and consequently impairing the function of vulnerable tissues and leading to the accumulation of altered gene products (Calabrese et al., 2006a). In addition, protein, lipid or glucose oxidation disrupts redox homeostasis and leads to accumulation of unfolded or misfolded proteins in the aging brain. For this reason Parkinson's and Alzheimer's diseases, having a common denominator, production of abnormal proteins, mitochondrial dysfunction and oxidative stress, are called "protein conformational diseases" (Calabrese et al., 2008). In particular, an unfolded protein response conformational disease is condition that arise from dysfunctional aggregation of proteins in non-native conformations. This is often associated with multiple metabolic derangements that result in the excessive production of ROS and oxidative stress (Zhang et al., 2006). Genetic studies have also revealed that aging can be controlled by changes in intracellular NAD/NADH ratio regulating sirtuins, a group of proteins linked to aging, metabolism and stress tolerance in several organisms. Consistently, the neuroprotective roles of dietary antioxidants including for example, curcumin, carnosine, resveratrol and acetyl-L-carnitine have been demonstrated through the activation of these redox-sensitive intracellular pathways. In particular, acetyl-L-carnitine has been proposed to have beneficial effects in preventing the loss of brain function which typically occurs during aging and neurodegenerative disorders. In fact, acetyl-L-carnitine treatment has been shown to prevent age-related changes in mitochondrial respiration and decrease oxidative stress biomarkers thorough the up-regulation of HO-1 (heme oxygenase-1), Hsp70 (heat shock protein 70) and superoxide dismutase-2 in senescent rats (Calabrese et al, 2006c). It acts through the activaction of transcription factor Nrf2, which after binding to the ARE (antioxidant responsive element) in the HO-1 gene, up-regulates both HO-1 and thioredoxin reductase (TrxR), thus counteracting pro-oxidant conditions. Heme oxygenase-1 is, in fact, a key enzyme in the prevention of brain damage (Calabrese et al., 2006; Maines, 1997; Mancuso, 2004). The neuroprotective effects of over-expressed HO-1 has been attributed to several factors such as: a) increased level of both cGMP and bcl-2 in neurons; b) inactivation of the pro-apoptotic transcription factor p53; c) increase in antioxidant sources, i.e. the iron sequestering protein, as ferritin (Panahian, 1999). Hsp70 is, instead, a member of the stress protein. Hsc70 (heat shock cognate, the constitutive form), Hsp70 (the inducible form, also referred to as Hsp72) and GRP-75 (a constitutively expressed glucose-regulated protein) are included in this family (Calabrese et al., 2006b; Yenari et al., 1999). Recently it has been

demonstrated that overproduction of Hsp70 leads to protection in several models of nervous system injury (Fig. 3). Oxidative stress, which has been suggested to be involved in the pathogenesis of PD, may originate in glial cells (Jenner, 2003). This is supported by post-mortem studies demonstrating the capacity of oxidative stress and oxidizing toxin to induce nigral cell degeneration (Olanow et al., 1998). There is evidence to support that there are high levels of basal oxidative stress in the substantia nigra pars compacta in the normal brain and that this is increased in PD. The brain is therefore particularly sensitive to oxidative stress. This is due to several factors: a) the neurons are particularly enriched in polyunsaturated fatty acids, prime targets for oxidative attack (Kidd & Levine, 1985); b) the brain consumes a high share of the body's oxygen intake and consequently results in the oxyradical formation; c) the activity of the antioxidant enzymes catalase and peroxidase is low in the brain, instead the superoxide dismutase is active. They acquire superoxide oxyradical and convert it in hydrogen peroxide (H_2O_2).

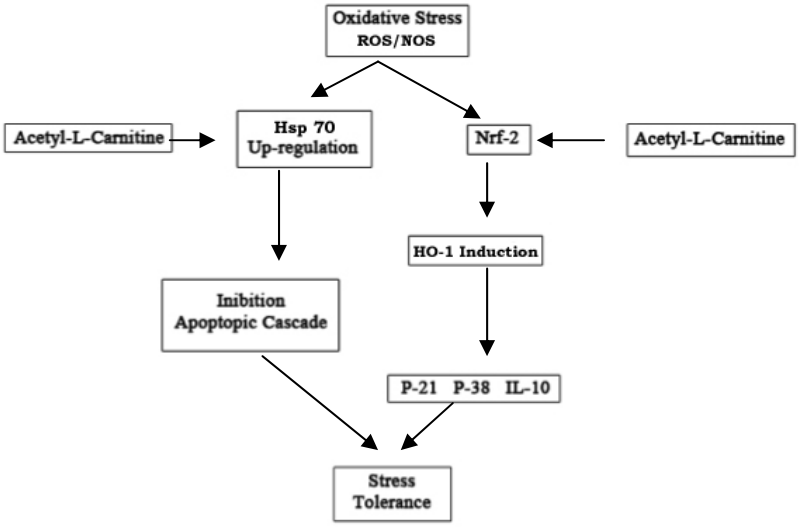


Fig. 3. The role of acetyl-L-carnitine in cell stress tolerance

In the absence of catalase and peroxidase, which normally would detoxify these peroxide products, that are done by glutathione peroxidase enzyme. This enzyme uses glutathione (GSH) as its essential cofactor, and when it is stimulated the brain's GSH reserves are more sensitive to depletion from oxidative attack (Kidd, 1997; Levine & Kidd 1985). As mentioned, the substantia nigra is particularly susceptible to oxidative stress and this is due to varied and many biochemical process that occur in it. It has a high content of dopamine and its metabolism and its "auto-oxidation" could be responsible for the high basal levels of oxidative stress. DA has a strong tendency to "auto-oxidation", generating reactive auto-metabolites, as 6-hydroxydopamine quinone and dopamine aminochrome, the formation of which can be accelerated by free (ionized) iron or by other redox-reactive elements such as copper, zinc or manganese (Pezzella, 1997; Youdim, 1989). Then, the degradation of dopamine by monoamine oxidase to produce H_2O_2 could increase the formation of oxidized glutathione (GS-SG), suggesting the presence of oxidative stress and impairment of the

antioxidant system (Spina & Cohen, 1988). The H_2O_2 generated is also converted (by Fenton reaction), in the presence of the high levels of iron, in toxic hydroxyl radical which can damage DNA and other biomolecules (Youdim et al., 1989). It should be noted that an extremely high content of iron is concentrated in the substantia nigra zona compacta, and various iron-mediated reactions in substantia nigra would potentiate oxidative stress. For example ionized iron, or copper and zinc catalyze transformation of the protein α-synuclein into aggregated form, prominent component of the Lewy aggregates that develop in the SN of Parkinson's patients (Braak & Braak 2000; Paik et al., 2000). Moreover, important role of the iron is for dopamine-melanin, macromolecular material formed from the autoxidation of dopamine and normally scavenger of free radicals. When it is infiltrated with high levels of ionized iron it can drive Fenton reaction converting endogenous hydrogen peroxide to hydroxyl radical. The population of melanin-enriched, dopaminergic neurons found in the SN's zona compacta is the worst affected in PD. In the substantia nigra there are high levels of melanin and it could act as support matrix upon which ionized iron would catalyze oxyradical generation from available hydrogen peroxide or from neuromelanin itself (Youdim, et al., 1990).

6. Conclusion

Although the selective loss of DA neurons and the accumulation of α-synuclein are crucial in the development of PD, many evidences indicate that oxidative stress, and mitochondrial and proteasome dysfunctions have central role in this pathogenesis. Environmental factors, such as exposure to toxins, are also important in late-onset of the disease, whereas in early-onset PD, genetic factors assume predominant importance. Recently, the identification of several genes causing early-onset PD (such as α-synuclein; UCHLl, a ubiquitin carboxy-terminal hydrolase L1; parkin; DJ1, a parkin-associated protein involved with oxidative stress; PINK1, a putative serine threonine kinase) has yielded crucial insights into the possible pathogenic mechanisms (Dawson & Dawson, 2003b). The use of several neurotoxins to produce the clinical symptoms of PD both *in vitro* and *in vivo* has allowed to understand the molecular mechanism of disease. The functions of mitochondria make these subcellular organelles susceptible to oxidative damage, resulting in cell death by apoptosis and mtDNA mutations. In this context, the mitochondria represent, therefore, a highly promising target for the development of disease biomarkers by use of genetic and biochemical approaches.

The mitochondrial antioxidant/nutrient acetyl-L-carnitine, with its antioxidant energizing protective activities and with its trophic effects, at optimal doses, can be an effective and safe prevention strategy for PD, offering the possibility of new and innovative therapeutic strategies for this neurodegenerative disease. Acetyl-L-carnitine is a highly bioavailable molecule, it is thought to penetrate the brain barrier better than carnitine, and it is readily converted to carnitine as needed. Experimental evidences suggest that acetyl-L-carnitine boosts mitochondrial ATP production and helps to protect mitochondria against oxidative attack. This molecule is therefore of great interest for its wide clinical application in various neurological disorders, it has beneficial effects in preventing the loss of brain function which typically occurs during aging, and its neuroprotective benefits have been observed in the hippocampus, prefrontal cortex, substantia nigra and muscarinic receptor portions of the brain. These include antioxidant activity, improved mitochondrial energetics, stabilization of intracellular membranes and cholinergic neurotransmission. In particular, the most

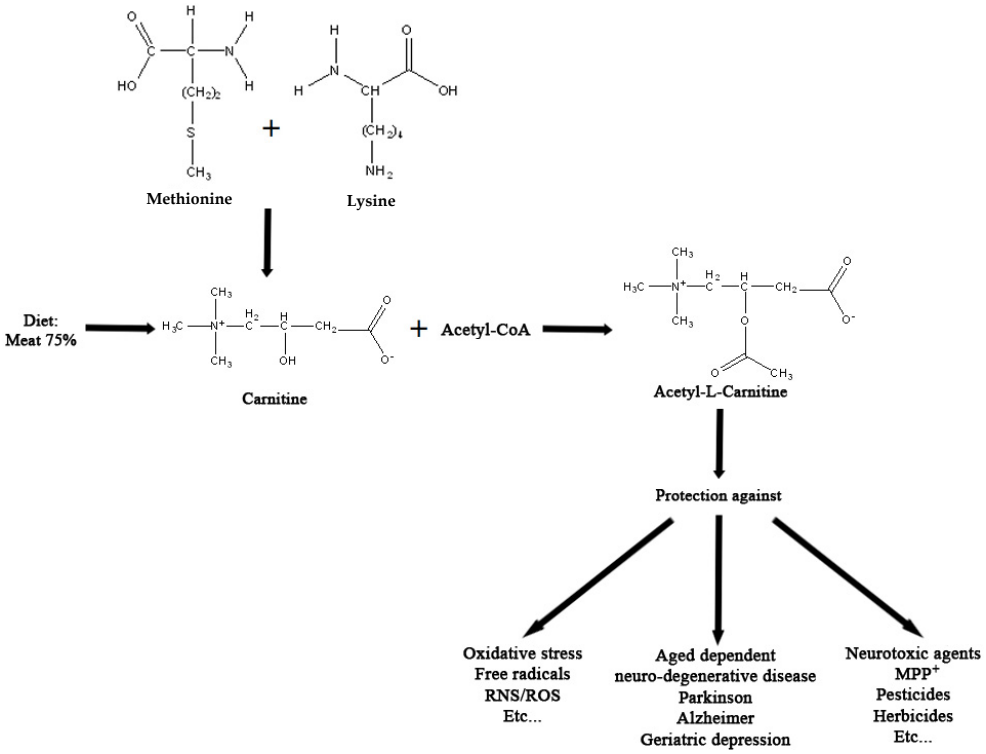


Fig. 4. Biosynthesis and physiological role of acetyl-L-carnitine

common function of acetyl-L-carnitine is the transport of fatty acids across the inner mitochondrial membrane, thereby being involved in the production of energy within the brain and in the maintenance of neuron and repairing of damages. Moreover, it has a variety of other neuronal effects. It increases protein kinase C (PKC) activity and reverse the age-related decline in the number of *N*-methyl-D-aspartate (NMDA) receptors on neuronal membranes. In addition, it is thought that it influences the cholinergic system acting as a cholinergic receptor agonist; it can also promote the synthesis and the release of acetylcholine and stimulates proteins and membrane phospholipids synthesis (Calabrese et al., 2005). Acetyl-L-carnitine can also increase the levels of neurotrophins such as nerve growth factor (NGF) and can reduce the energetic deficits in brain and phospholipids metabolism, probably because it aids mitochondrial functions (Mark et al., 2003). In fact, it increases mitochondrial biogenesis and decreases ROS production through the up-regulation of the PGC-1, as a possible underlying mechanism. In animal models, it partially protects the substantia nigra against 1-methyl-4-phenyl-pyridinium (MPP^+, active metabolite of MPTP) attack, by strengthening the dopaminergic transmission (Bodis-Wollner et al. 1991; Hongyu et. al., 2010; Sinicropi et al. 2010). In fact, brain histology reveals that acetyl-L-carnitine protects neurons in the substantia nigra, which otherwise have been devastated by MPTP attack. It has been well observed that long-term acetyl-L-carnitine administration in rats increases longevity and improves spatial learning, avoidance learning in aged rats, and long-term memory performance (Barnes et al., 1990; Ghiraldi et al., 1989;

Markowska et al., 1990). In summary, the protection provided by acetyl-L-carnitine offers the possibility of new therapeutic strategies for neurodegenerative diseases (including PD) which can share the same final neurotoxic pathway in mitochondria (Fig. 4).

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