Chapter

Paraoxonase in Nervous System

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Abstract

The paraoxonase (PON) family consists of—PON1, PON2 and PON3 which are anti-oxidative, any dysfunction in their action, has been suggested to play a role in the pathobiology of diseases having a chronic inflammatory component. PON1 is the most studied which has paraoxonase, arylesterase, thiolactonase, and anti-oxidant actions. Studies have shown the association between lowered PON1 activity and increased incidence of ischemic stroke, dementia, Parkinson disease, multiple sclerosis, and amyotrophic lateral sclerosis. It may occur due to increased oxidative stress and/or prolonged exposure to organophosphates, and reduced capacity of the body to counter these stresses due to reduced PON1 function. PON2 has arylesterase, lactonase, and antioxidant properties. Under-expression of PON2 is associated with Parkinson Disease and Amyotrophic Lateral Sclerosis, and over-expression with tumors with glioblastoma. Various mechanisms have been proposed for the role of PON2 in the pathobiology of the said diseases. PON3 is least studied. The PON family, to some extent, interacts with acetylcholine esterase (AChE), as both share the same locus, and PONs degrade the inhibitors of AChE, especially the organophosphates. This could probably have significant role in the development of Parkinson disease and the prognosis of the treatment of Alzheimer disease by AChE inhibitors.

Keywords: paraoxonase, arylesterase, lactonase, nervous system, PON1, PON2, PON3

1. Introduction

The paraoxonase (PON) multi-gene family consists of three enzymes: PON1, PON2 and PON3, the genes for which are located adjacent to each other on human chromosome 7q21.22 [1]. The amino acid sequences of the three forms are considerably similar—within the range of 79–95%. All PON genes have nine exons, eight introns and TATA-less promoters. This enzyme came into focus with the notion that PON1 protects low-density lipoproteins (LDL) and high-density lipoproteins (HDL) from lipid peroxidation by the virtue of its arylesterase, lactonase and paraoxonase activities [2]. Paroxonases are HDL-associated, and their antioxidant property plays a vital role in prevention of various microvascular complications due to oxidative stress and also provides protection from various toxic chemicals. All PON family enzymes require calcium to exhibit the action and are able to delay lipid peroxidation in lipoproteins and cell membranes. Thus, polymorphism or any other imbalance in the activity of PON has been suggested to play a role in the pathogenesis of rheumatic

diseases, cancers and cardiovascular diseases—i.e. the diseases having a chronic inflammatory component [3, 4]. Although these enzymes have been named paroxonases, the paroxonase activity is significant only for PON1; in others the activity has not been detected. PON1 is a hydrolase and can hydrolyse a wide range of substrates including organophosphorus triester pesticides, lactones, thiolactones, cyclic carbamates, nerve gases like sarin, soman, arylesters, aromatic carboxylic acid and unsaturated aliphatic esters, estrogen-esters, and glucuronide drugs [5].

PON enzymes synthesized in the liver and distributed throughout the human body. They are present in different tissues, and are associated with cell membranes and some lipoproteins, although literature reports free enzyme found in the blood. Paroxonases were named after the ability of PON1 (first to be discovered) to hydrolyze paraoxon, a compound of the organophosphate insecticides class, to the metabolite p-nitrophenol. In vivo, paraoxon, the most toxic form, is an oxidized product of biotransformation of parathion, organophosphate insecticide in the context of which it was found first. As explained above, the name can be said to be a misnomer.

Of the three members of the family, PON1 is the most studied one. It is a circulating Ca^{2+} dependent enzyme with a molecular mass of 43 kDa and containing 354 amino acids and is classified as an aryldialkyl phosphatase [4]. Synthesis of PON1 is mainly hepatic. From liver, it is secreted into the bloodstream, where it is tightly bound to HDL particles [6]. Structurally, it is a six-bladed beta-propeller with a central tunnel containing two calcium ions—a structural one which is necessary for the conformational stability of the enzyme, and a catalytic one. Addition of EDTA (which removes calcium by complexing with it) resulted in the inactivation of paraoxon and phenyl acetate hydrolysis of PON1, showing that they are Ca^{2+}-dependent activities. However, there was no effect on the ability of PON1 to protect low density lipoprotein (LDL) from oxidation, thus implying that the antioxidant property of PON is independent of Ca^{2+} [7, 8]. This leads to the possibility of existence of different active sites on PON1 for those dependent on Ca^{2+}-dependent, and for those independent of the same, like protection against oxidation [9]. The amino terminal end of the protein contains hydrophobic amino acid residues that play a role in its binding to HDL and to other proteins such as apoA1 as well as in its self-aggregation. Recently, it has been shown that modulating the hydrophobicity of PON1 can affect organophosphatase activity of the enzyme [10].

A histidine-histidine (His) catalytic dyad is proposed to be involved in the catalytic mechanism of PON1 in which His-115 acts as a general base to deprotonate a single water molecule while His-134 increases His-115 basicity via a proton shuttle mechanism; however, some researchers found that it may participate in the substrate binding and/or orientation [11, 12]. Due to such a wide range of activities as well as being the first to be discovered, PON1 is the most studied one compared to other members. PON1 is thought to play an important role in a variety of disorders including metabolic syndrome, diabetes, atherosclerosis which results in cerebrovascular and cardiovascular events, because it is closely associated with the prevention of oxidative stress and inflammation, and is a determinant of HDL dysfunctionality. There are evidences suggestive of its atheroprotective effects through various mechanisms—maintaining cholesterol homeostasis, regulating cholesterol efflux from macrophages, as an effective xenobiotic metabolizer, and by participating in endothelial homeostasis [5, 13–16].

N-acylhomoserine γ-lactones (AHL) are produced by gram negative bacteria and regulate bacterial virulence and biofilm formation. All three PONs hydrolyse AHL with PON2 having the greatest efficacy, the resulting metabolites are inactive therefore the PON family could be important in preventing bacterial infections [17].

2. Paraoxonase 1

Oxidative stress due to reactive oxygen species results in oxidation of LDL particles and phospholipids, especially phosphatidylcholine, of the cell membrane of macrophages. This leads to a state of cellular damage and inflammation. It is possible that PON1 acts on oxidized phosphatidylcholine to produce lactone which further taken care by PON1 lactonase activity.

PON1 also exhibits homocysteine-thiolactonase activity. Homocysteine (Hcy) is a four-carbon amino acid with free thiol group formed by demethylation of methionine. Plasma Hcy levels are affected by both acquired and genetic factors [18, 19]. High levels of Hcy have been implicated in the development of cardiovascular and cerebrovascular disorders. Elevated Hcy has been shown to cause homocysteinylation, induction of oxidative stress and excitotoxicity, leading to atherosclerotic and thrombotic effects [20]. Hyperhomocysteinemia results in excess production of Homocysteine-thiolactone. This modifies proteins of coagulation, lipoproteins, endothelial receptors and is an important risk factor for adverse vascular events [21, 22]. Thus hyperhomocysteinemia, encompassing higher concentrations of homocysteine-thiolactone, may be an added risk factor for enhanced atherogenesis. PON1 has been postulated to detoxify the Hcy with its homocysteine-thiolactonase activity. At the same time, it can also protect macrophages from oxidation and prevents further inflammatory cascades. Hence the patients with low PON1 arylesterase and lactonase activity are more susceptible for the deleterious effects of lipid peroxidation, homocysteine-thiolactone toxicity and macrophage activation, which would increase the risk of neurovascular disease [23].

As a xenobiotic metabolizer, PON1 provides link between exposure to pesticides and adverse effects. The products formed by action of PON1 are considered to be the markers of pesticide exposure due to which they can be useful in the assessment of severity of pesticide exposure. Animal studies have demonstrated the potential application of PON1 in tackling the effect of pesticide poisoning. But more advanced and stringent clinical trials are required to support the definitive role in pesticide poisoning [5, 24]. PON1 response to pesticides depends upon the genetic polymorphism like Q192R, L55M. Literature reports that 192Q is more protective than 192R towards prevention of LDL oxidation.

2.1 PON1 and ischemic stroke

Globally recognized among the common causes of death, ischemic stroke accounts for major disabilities too. It is a classical multi-factorial disease with major risk factors such as hypertension, smoking, hyperlipidemia, obesity, diabetes, and atrial fibrillation. Many studies have been conducted to search for role of PON1 in ischemic stroke. Literature reports R allele and RR genotype of Q192R PON1 polymorphism carries higher risk of ischemic stroke. Many researchers have found that decrease in activity of PON1 is associated with vascular events [25, 26]. Some report, paraoxonase and arylesterase activities and their ratio can be used either to predict or to access the severity of ischemic stroke [27, 28]. In general there is significant negative correlation of PON1 activities with adverse vascular events. Lower the activities, more advanced are the vascular lesions [29, 30].

2.2 PON1 and dementias

Erstwhile dementia is now termed as major neuro-cognitive disorder (MND). It describes an overall decline in memory and cognitive skills severe enough to reduce

person's ability to perform everyday activities. Previously thought to affect elderly only now it is affecting the younger age group too. It is characterized by significant decline in any of the cognitive domain including, executive function, complex attention, language, learning, memory, and perceptual-motor or social cognition. Major neuro-cognitive disorder is diagnose by the decline in patient's previous cognitive ability without delirium, which should be persistent and progressive over the time. At present disease burden supposed to be 43 million worldwide which is expected to escalate to 131 million by 2050. Alzheimer disease (AD) which is responsible for nearly 70–80% cases of dementia worldwide, is one of the important cause of death over the age of 65 years [31].

One of the characteristic neuro-pathological features of AD is the presence of amyloid plaques which comprise aggregates of β-amyloid derived from the amyloid precursor protein [32]. Increasing evidence suggests that cholesterol plays a role in the pathophysiology of Alzheimer's disease, and elevated serum total-cholesterol level has been shown to be a risk factor for AD [33]. Abnormal phosphorylation of tau proteins is thought to be responsible for pathogenesis of AD. In addition to phosphorylation, neuronal degeneration is caused by a combination of beta amyloid production, oxygen deficiency. Lipid peroxidation too plays role in pathogenesis of AD. Amyloid is responsible for oxidative stress through free radicals. This oxidative stress is responsible for conversion of soluble amyloid proteins to insoluble fibrils and further in to polymerization of tau proteins [34, 35]. Some researchers report that deficient serum paraoxonase activity is a significant risk factor for AD and that paraoxonase activity is governed in part by at least 2 distinct variants, one located in the PON1 region and another in PON2 [36]. Some reports suggest that low PON1 activity is associated with cognitive decline, especially in AD [37]. There are many opposite results regarding polymorphism In MND. Some says there is definite correlation between the Q192R and L55M polymorphism and risk of AD and MND while others differ in their views as they found no correlation [38, 39].

2.3 PON1 and Parkinson's disease

Parkinson's disease (PD) is an idiopathic disease of the nervous system characterized by both motor and non-motor system manifestations. It is a chronic progressive neurodegenerative disorder that occurs mostly in older persons but that can appear in much younger patients. It is the second most common neurodegenerative disease. Sometimes called "paralysis agitans", PD is uncommon in young people, especially those under 40 years of age [40, 41]. The pathological definition of PD is loss or degeneration of the dopaminergic neurons in the substantia nigra and development of Lewy Bodies in dopaminergic neurons. Pathologic changes may precede obvious symptoms by two decades or more. This preferential loss of dopamine producing neurons and simultaneous lack of cholinesterase inhibition results in marked imbalance acetylcholine and dopamine along with impairment of motor control. Lewy Bodies, or abnormal intracellular aggregates, contain various proteins including α-synuclein and ubiquitin that impair optimal neuron functioning [42, 43].

While the exact pathogenesis of the disease has not been completely elucidated, several theories implicating the association of genetic and environmental toxic elements such as exposure to pesticides or an oxidative cell environment conspire to trigger the neuron degeneration. Recent reports suggest that the chronic low-grade inflammation due to various sources like pesticides, drugs, aging process etc. is responsible for the cellular senescence in nervous tissue [44, 45]. From a pathologic

perspective, the brain's substantia nigra pars compacta and pontine locus ceruleus are affected by typical abnormalities of PD including depigmentation, neuronal loss and gliosis. Nearly 60–70% neurons are lost by the time symptoms appears [46, 47].

Pesticides have been implicated in the development of PD by inhibition of ubiquitine proteosome system. Probably upon exposure to pesticides, mitochondrial dysfunction and α-synuclein, which is a neuronal protein, undergo conformational change which leads to symptoms of PD. One hypothesis states that as a result of mitochondrial dysfunction, there is release of cytochrome-c in the cytosol which binds to apaf1 and starts apoptosis. This results into neuronal degeneration and ultimately to PD [48].

As PON1 has antioxidant capacity and is capable of hydrolyzing toxic substances, literature links PON1 polymorphism with PD. PON1 polymorphism as Met54 may be an independent risk factor for PD. This mutation could possibly be responsible for decreased PON1 activity which results in lessened metabolism of environmental neurotoxins and could play a role in neuro-degeneration. Researchers have found that there is an association between L55M polymorphism of PON1 and PD, whereas Q192R polymorphism was unlikely to be a major risk factor for susceptibility to PD [49]. Recently a researcher found MM PON1–55 genotype exhibit greater than 2-fold increase in PD risk when exposed to organophosphates, compared with subjects who had the wild type or heterozygous genotype and no exposure [50].

2.4 PON1 and multiple sclerosis

Multiple sclerosis (MS) is one of the most common neurological disorders, occurring mainly in young adults in age 20–40 years and more commonly in women. MS is a chronic inflammatory disease characterized by demyelinating lesions in the brain, spinal cord, and optic nerve [51, 52]. Some studies have suggested a role of the oxidative stress and lipid peroxidation in the pathogenesis of MS. Due to the pathogenetic role of reactive oxygen species and the oxidation of lipoproteins in MS pathology, antioxidants prevent free-radical mediated tissue destruction [53]. PON1 is shown to play an important antioxidant role in the blood. Variability of PON1 activity depends on polymorphism in the coding region. There are only a few studies that describe the relationship between PON1 polymorphism and the risk for MS [54]. The relationship between MS and oxidative stress due to oxidized lipoproteins in cholesterol transport has been discussed. It has been proposed that increased number of oxidized HDL particles due to decreased PON activity may be unable to protect LDL against oxidation increasing the risk of atherogenesis which may lead to MS [55].

Oxidative stress is a critical factor in pathogenesis of MS as it promotes leukocyte migration, participates in oligodendrocyte damage and axonal injury. Reactive oxygen species and reactive nitrogen species are produced in the CNS of MS patients chiefly by activated macrophages and microglia could account for demyelination and axonal disruption, the hallmarks of the disease [56, 57].

2.5 PON1 and amyotrophic lateral sclerosis

Amyotrophic lateral sclerosis (ALS) is a dangerous neurodegenerative disease, characterized by progressive motor neurons loss, paralysis, and inflammation with an average survival of 3–5 years after diagnosis. 5–10% of cases are familial, and 70% of the familial cases can be explained by identified gene mutations, e.g. the *C9orf72* repeat expansion [58]. ALS is a degenerative disease of adult-onset

and fatal outcome characterized by the simultaneous loss of motor neurons in the cerebral cortex, brainstem and spinal cord. In the familial cases, a large number of mutations have been found, most prominent of which is the mutation in the gene that encodes for the cytosolic enzyme superoxide dismutase [59, 60]. It was found in recent studies that PON1 gene is down-regulated in the central nervous system and peripheral cell like lymphocytes and fibroblasts. Some genetic mutations also are identified in the patients of ALS [61, 62]. It is well established fact that the oxidative stress is responsible for degeneration of the motor neurons in ALS. The CNS as a whole is particularly susceptible to oxidative stress because the neuronal membrane contains a high abundance of polyunsaturated fatty acids, especially arachidonic and docosahexaenoic acids; it consumes oxygen at a high rate; and it contains high concentrations of redox-active transition metals but a relatively low concentration of antioxidants. In ALS, at the cellular level, genetic factors, excitotoxicity, apoptosis, inflammation, mitochondrial dysfunction, protein aggregates, and oxidative stress are among the primary hypotheses put forth to explain motor neuron degeneration. Among these factors, oxidative stress appears intimately linked to a series of cellular events in motor neurons that contribute to neuronal degeneration and death [63, 64]. Individuals that had the homozygous genotypes RR and heterozygous GQ had a lower survival rate when compared to the homozygote genotype QQ. Moreover, the allele R was associated with bulbar onset. Some authors have mentioned the increased chance of ALS following the exposure to pesticides and the susceptibility increases two folds with single nucleotide polymorphisms (SNPs) like G-832A, G-162A and C-108 T [65, 66].

3. Paraoxonase 2

The PON2 isoform is highly expressed in several different types of human cells and tissues, mainly in macrophages and hepatocytes, lower lung airways, brain, cardiac and gastrointestinal systems. It is found in association with the endoplasmic reticulum and mitochondria, specifically associating with complex III of the inner mitochondrial membrane. PON2 deficiency alters mitochondrial function by decreasing mitochondrial complex I and III activity and total ATP levels and alters mitochondrial oxidative stress by increasing mitochondrial superoxide production, increasing lipid peroxidation and decreasing reduced glutathione levels. In vascular cells, PON2 has been found to be a cell-based enzyme and appeared in two glycosylated isoforms of approximately 40–43 kDa. PON2 is not detectable in plasma. In brain tissue, PON2 is an antioxidant intracellular enzyme against oxidative stress. In CNS, PON2 expression has been found in nucleus accumbens, striatum and substantia nigra. PON2 is found in astrocytes and neurons in different amounts. However, the loss of PON2 expression in both cells negatively modifies the cellular ability to recover from oxidative damage and subsequently death [67, 68].

PON2 is the oldest yet least studied variant of the paraoxonase family – its intracellular location has made studies challenging. Its three-dimensional structure has not been elucidated, nor has its intracellular compartmental distribution been determined. It has been found in multiple subcellular compartments. PON2 is highly expressed in vital organs such as the heart, brain, and the lungs, and ubiquitously found throughout the body in multiple different tissue types. It is not found in blood/plasma [69]. At mRNA level, it is enhanced in level in the liver and ubiquitous; at the

protein level its occurrence is ubiquitous. It is found on the endoplasmic reticulum, in the perinuclear region, on the membrane of mitochondria, and on the plasma membrane. It is overexpressed in cancer cells. Expression in the elderly has been shown to be lower [70].

PON2 is degraded by the ubiquitin-proteasome pathway and by ADP-ribosylation. In all cell types, its expression is upregulated by Arachidonic acid, unesterified cholesterol, pomegranate juice, Antioxidants, the licorice phytoestrogen glabidrin, and atorvastatin. Its activity is decreased by glycated compounds. The two common polymorphisms found in PON2 are [71] position 147—an Ala/Gly substitution and position 311—a Ser/Cys substitution.

An apparently benign, heterozygous frameshift mutation in PON2 is present within the general population. This suggests that haploinsufficiency of PON2 is not obviously pathogenic. Thus, only after more profound loss of function than is predicted by eliminating one functional PON2 allele occurs, as in a homozygous defect, will PON2 mutants be pathogenic [72]. Plasma membrane PONs are transmembrane proteins, with the N-terminal region forming a part of the transmembrane anchoring domain on the cytoplasmic side, and C-terminal region as the extracellular catalytic site. Like PON-1, it may counteract lipid peroxidation as well as form the first line of defense for the cell against any microbial invasion [69].

PON2 has multiple enzyme functions as:

- Lactonase—hydrolyzes quorum sensing signaling molecules of bacteria. These signaling molecules, in simple terms, detect cell population density and respond to it by gene regulation. They have lactonase group in structure and are involved in autoinduction. Lactonase activity results in inhibition of these, leading to inhibition of microbial mechanisms of pathogenesis, as well as biofilm inhibition, thus forming a defense against infection, especially on the plasma membrane. Its lactonase activity has been found to be higher than PON-1 and PON-3. The Ser/Cys substitution polymorphism at position 311 affects the lactonase activity.

- Antioxidant—reduces oxidative stress in mitochondria and endoplasmic reticulum, and reduces the amount of oxidized LDLs. Deficiency of PON2, and even single nucleotide polymorphisms of the enzyme, have been shown to increase susceptibility to oxidative stress and the injury caused thereby. It is associated with mitochondrial ETC helping in sequestering ROS; however, the enzymatic nature of its overall antioxidant activity has not been proven. In endothelial cells, PON2 has been shown to reduce the production of specifically the superoxide radical but not of that of the others. Its ROS-eliminating function is probably independent of lactonase activity since the 311 Ser/Cys mutation does not affect antioxidant property and is associated with Coenzyme Q in bacteria. The exact mechanism is not understood.

It may also hydrolyze arylesters and other esters, however, unlike PON1 and PON3, its paraoxonase and statinase activities have not been detected. As said earlier, PON2 has antioxidative function, which leads to reduce oxidized LDL levels by preventing LDL oxidation and reversing the oxidation of mildly reduced LDL. This leads to inhibition of the monocyte chemotaxis associated with oxidized LDL, and increased efflux of cholesterol. Thus, PON2 is antiatherogenic.

3.1 Distribution of PON2 in the nervous system

PON2 is the only PON to be expressed in the brain. Distribution in the spinal cord has not been described adequately. Most information of the probable role of PON2 in brain is extrapolated from studies on mice [69].

PON2 is an intracellular protein [70]. It is postulated to be involved in neuroprotection by the virtue of its anti-inflammatory and antioxidant properties, and exclusive intracellular location. Measurement of its lactonase activity has been used to study the regional distribution and sex differences in mice and the findings have been extrapolated to humans. Higher levels are seen in [73].

- Females as compared to males. Oestradiol has been shown to increase PON2 expression level, since removing the ovaries of mice lead to reduction of PON2 levels. There is evidence that this occurs through activation of estrogen-α receptors. The oestrus cycle may play a role in regulating the dopamine levels; however, this not been verified [74]. Quercetin, a phytoestrogen, has been found to increase the levels of neuronal PON2 by increasing its synthesis. The functional consequences of higher expression of PON2 in females may have several ramifications, as multiple neurodegenerative diseases involve oxidative stress and neuroinflammation in their etiopathology and are divided on sex. For example, the incidence of Parkinson's disease (PD) is 90% higher in males, pointing to a protective mechanism in females that may involve PON2.

- Astrocytes as compared to neurons. PON2 has been found in the cell membranes of both of these, along with in the ER and mitochondria, but its role in the cell membrane is not known [74]. PON2 deficient neurons and astrocytes exhibit significantly higher levels of ROS when exposed to oxidative compounds H2O2 and DMNQ.

- Dopaminergic regions, where oxidative stress is higher due to dopamine metabolism. PON2 is found in the highest amount in the substantia nigra, striatum, and the nucleus accumbens. Lower levels were found in the cerebral cortex, hippocampus, brainstem and cerebellum [71]. Neurons and astrocytes in the striatum have exhibited higher susceptibility to oxidative stress than those in cerebellum in PON2 deficiency.

- Premature infants and young ones as compared to the elderly. It is postulated that the levels of high PON2 in younger ages prevent neuronal oxidative stress during the period of development of brain. The age groups who had lower presence of PON2 showed higher susceptibility to oxidative stress [70]. PON2 may also serve to regulate apoptosis or play other signaling role/—s during maturation of brain.

These differences in quantification correspond to the comparative levels of neuroprotection seen in the said groups [69]. The major mechanism by which PON2 is said to reduce oxidative neuroinflammation is by regulating mitochondrial CoQ. The mechanism is applicable in all cells PON2 is present. During transport of electrons in the electron transport chain of the inner mitochondrial membrane, CoQ accepts electrons and becomes unstable. It regains its stable form by transfer of electrons.

Most electrons are transferred to Complex III and energy is generated. However, some electrons may be transferred by CoQ to oxygen molecules when reduced CoQ comes in contact with them, given rise to reactive oxygen species. PON2 has been postulated to reduce this transfer of electrons to oxygen, thereby reduced oxidative stress and consequent neuroinflammation [70].

The role of PON2 in the cytoplasm is postulated to be different than that in the mitochondria in the nervous system; however, research on this topic is next to none [73]. Further, the role of PON2 in the endoplasmic reticula of the nerves has not been studied [69]. In the brain, the loss of PON2 in both neurons and astrocytes impairs their ability to recover from toxic levels of oxidative stress generated by oxidants hydrogen peroxide (H2O2) or 2,3-dimethoxy-1,4-naphthoquinone (DMNQ) [73].

3.2 Modulation of PON2

Quercetin has been found to increase the levels of astrocyte PON2 in mice approximately two times. It is postulated that since quercetin is a phytoestrogen, it may activate estrogen-α receptors like oestradiol, leading to increased PON2 synthesis. Alternatively, the JNK/AP-1 pathway has also been suggested to be the central underlying mechanism—it was shown that inhibition of this pathway lead to antagonization of the effect of quercetin on PON2 levels [71].

3.3 Impact on motor behavior

Mice deficient in PON2 showed slightly more activity during the dark cycle than mice having PON2. There were no differences in food consumption. During the rotarod experiment, PON2 deficient mice had significant shorter latency to fall, suggesting impaired motor co-ordination [73].

3.4 Probable role in dopaminergic system

Oxidation of dopamine during its metabolism generates free radicals and reactive quinones in the dopaminergic neurons. Due to this additional oxidative burden, the dopaminergic neurons are more susceptible to additional oxidative stress, neuroinflammation and cellular death. PON2 reduces the oxidative stress here by inhibition of superoxide formation by mitochondrial CoQ [74]. PON2 was also found to be modulated by dopamine receptor activity in a receptor-specific manner, with protein and transcript upregulated in neurons upon exposure to a dopamine receptor 2 agonist, but not an agonist for dopamine receptor 1 [73].

PON2 has been shown to interact with PARK7 in vivo. The protective antioxidant effects of PARK7 are partly mediated by expression of PON2 [74]. *In vitro*, PON2 has been shown to reduce the increased susceptibility of striatal neurons to oxidative stress in cases of PARK7 deficiency [73].

In mice, in PON2 deficient striatum, the levels of tyrosine hydroxylase protein were found to be lower and that of tyrosine hydroxylase transcript were found to be higher than in the wild type (with PON2 present). The levels of Vesicular Amine Transporter 2 (VMAT2) transcript were found to be increased but there was no change in protein levels. The levels of Dopamine Transporter, which is involved in the reuptake of dopamine form the synaptic cleft, were found to be unchanged at both the transcript and protein level. Dopamine receptors DRD-1, DRD-2, and DRD-5 were found to be upregulated at the transcript level but not

at the protein level in PON2 deficiency. Thus, PON2 deficiency significantly upregulates the transcript of multiple dopaminergic related genes in the striatum of mice.

Transcript levels of antioxidant enzymes, heme-oxygeneases-1 and -2 and MADPH-oxidase 2, were increased in PON2 deficient mice, although the protein levels were not altered. This implied that in absence of PON2, the oxidative stress in these neurons increased, due to which levels of antioxidant enzymes were increased [74].

3.5 PON2 and Parkinson disease

As said above, PON2 interacts with the DJ-1 (PARK7) gene. Of all cases of familial Parkinson's disease (PD), loss-of-function mutations in DJ-1 (PARK7) gene account for about 1%. The actions of this gene are said to reduce the oxidative stress-mediated damage; however, the mechanisms for the same are unknown. It was shown that *in vivo*, PON2 associates with DJ-1.

MPTP is one of the causative agents of Parkinson Disease, and it, along with its metabolite MPP is used for research regarding Parkinson disease. MPP is a complex I inhibitor which leads to oxidative stress and death of a number of different neurons. Exposure to MPP lead to increase in the PON2 lactonase activity in mice. It was found that in deficiency of DJ-1 both the basal and MPP-induced lactonase activity of PON2 was blocked. Loss of DJ-1 thus impairs PON2 activity. However, it was also noted that DJ-1 does not alter PON2 levels in neurons, implying that it increases PON2 activity by increasing the rate of enzyme action, and not the amount of enzyme synthesized. The exact mechanism is unclear.

Absence of either of PON2 or DJ-1 leads to increased sensitivity of the neuron towards oxidative stress by MPP. Interestingly, PON2 expression effectively rescues DJ-1 deficiency-mediated hypersensitivity to oxidative stress, although DJ-1 expression cannot do so for PON2. This suggests PON2 to be a downstream target of DJ-1. Thus, PON2 expression protects neurons against MPP and can also reverse the hypersensitivity observed with DJ-1 loss [75].

3.6 PON2 and amyotrophic lateral sclerosis

ALS is a neurodegenerative disorder of spinal tract. It is a multifactorial disease characterized by cerebral cell dysfunction and mitochondrial alteration. It is associated with the progressive increase in neuro-inflammation, generalized oxidative stress and metabolic alterations. The C allele of the C311S PON2 has been associated with sporadic ALS. In addition, the expression of messenger RNA of the PON2 gene was decreased in spinal cord and trunk tissue of patients with ALS [76]. ALS-associated variant in PON2 is present as a homozygous defect.

However, since there are multiple substrates for PONs, it is difficult to ascertain the exact role of PONs in the pathogenesis of AML. ALS-related mutations have been found in all the three forms of PON, implying a higher probability that the property of the PONs which plays a role in pathogenesis is likely to be a common property/feature. One of the theories says that loss of antioxidant functions of the PONs leads to the inability of nervous tissue to detoxify the abnormal oxidative stresses the motor neurons and spinal cord are exposed to, leading to neurotoxicity. Another theory says that mutations in PONs may lead to failure to inhibit some unknown exotoxin, which leads to ALS [72].

3.7 PON2 and glioblastoma

In all cancers, PON2 is overexpressed. Due to its antioxidant effect, PON-2 reduces cellular oxidative damage and influences redox signaling, which promotes cellular survival [70]. PON2 in tumor cells probably protects the intracellular membranes against oxidation and, possibly, prevents free radicals from percolating through the nuclear envelope and damaging the genetic material contained in the cells [74]. Elevated PON-2 levels may stabilize tumor cells by enhancing cellular stress resistance, attenuating mitochondrial ROS-mediated apoptosis [70].

The highest expression of PON2 is observed in liver and brain cancers. PON2 was localized in the perinuclear region. In glioblastoma, PON2 gene is amplified. The level of PON2 was a negative prognostic factor in glioblastoma [77]. Valproic acid has been shown to inhibit the growth of glioblastoma and increase the production of reactive oxygen species in glioblastoma cells. This was attributed to inhibition of PON2 by valproic acid at the transcriptional level. This deceased PON2 expression could potentiate the cytotoxic effects of ROS and enhance VPA-induced cell cycle arrest. The use of the model of transfectants overexpressing PON2 provided further support for VPA-induced GBM cell growth suppression being mediated by increased ROS production and that the effect was augmented by decreased PON2 [78].

3.8 Developmental expression of PON2

In mice, PON2 protein appears to be lowest directly after birth, steadily increasing with age up until Post-natal Day (PND) 21. A significant decrease in both protein and mRNA is noted from PND 21 to 30 and continuing to PND 60. In monkeys, PON2 protein levels were lower at mid-gestation and gradually increased up to infant age. This was followed by a decrease in the juvenile stage and a stabilization, in which similar levels were found in young adult and in aged monkey brains. The overall trend observed in both species reveals a possible window of susceptibility to oxidative stress in young adult mice and monkeys which may point to a change in cellular environment driving a decrease in PON2 expression [73].

3.9 PON2 and age

Expression of PON2 decreases with age. Thus, with growing age, the susceptibility to oxidative damage is increased, in turn increasing the risk for neurodegenerative diseases. In mice, concurrent to PON2 deficiency, transcripts of glucose transporter 4, insulin receptor and tau were upregulated, while butyrylcholinesterase was significantly downregulated, potentially having neurodegenerative consequences. However, the findings supported a lack of oxidative stress in the aged brain with potential impacts to endogenous signaling and host immunity through a reduction in cytokine expression [73].

3.10 PON2 and future perspectives

The identification and initial characterization of PON2 in brain tissue suggest that this enzyme may play a relevant role in determining susceptibility to oxidative stress and neuroinflammation, and that its positive modulation may represent a novel strategy for neuroprotection. Attempts to elevate PON2 levels could be neuroprotective. For tumor cells, PON2 overexpression probably provides resistance of these cells to apoptosis and that a useful therapeutic strategy would be one causing a decrease of PON2 [71].

4. Paraoxonase 3

PON3 is an antioxidant hydrolase enzyme with approximately 40-kDa, synthesized in the liver. In plasma PON3 is bound to HDL and apolipoprotein—A1 and possesses strong anti-oxidant properties but its concentration is about two orders of magnitude less abundant than PON1. PON3 is also expressed at low levels in the kidney. PON3 was the last enzyme in the paraoxonase family genetic cluster to be described. Currently, very little is known about its function and physiological characteristics in humans. The enzymes PON3 and PON1 show some similarities in structure and hydrolase activity. Regarding the structure, both enzymes have three highly conserved cysteine (Cys) residues in positions -41; -283 and -351 in the protein chain. As for enzyme activity, PON3 can hydrolyze cyclic carbonate esters and lactones rapidly, mainly drugs such as statin lactones. The arylesterase activity of PON3 is almost undetectable when compared to PON1 [79, 80].

PON3 protein expression in the white-matter brain areas of healthy C57BL/6J mice. One study reports that there is strong evidence of PON1 and PON3 expression surrounding Aβ plaques, and intense positive staining in star-shaped cells that resembled glial cells in areas with an abundance of Aβ plaques [81]. It was suggested in the study that localization of PON1 and PON3 in astrocytes or oligodendrocytes, at the same time there is colocalization of PON3 in microglia which indicates a potential antioxidant role of PON1 and PON3 in decreasing levels of ROS and/or preventing lipid peroxidation in these cells in Alzheimer disease pathology.

Although there is no documented *PON1* or *PON3* gene expression in any of the mouse- or human-brain regions, one of the study indicated that PON1 and PON3 proteins are expressed in myelinated fibers in the brain tissue of healthy C57BL/6 mice. This suggests that PON1 and PON3 are somehow transferred from blood circulation to the brain [82]. Overall the PON3 is much less studied as compared to PON1 & PON2. So much less information is available in the literature than others.

4.1 Paraoxonases and acetylcholine esterase

PON1 and Acetylcholine esterase are both serum ester hydrolases. Genes for both are located on the same chromosome—7q1-22—near to each other [83]. At the genetic level, due to the proximity in their locations, it is thought that the genes for both would be regulated by a locus control region. It has been shown that PON1 and acetylcholinesterase share an inverse relationship. This relationship is not shown by pseudocholinesterase. It is thought that the same locus control region may control their interactions. Acetylcholine esterase is especially susceptible to oxidative stress, and paraoxonase is an antioxidant. This also contributes to their interactions.

Organophosphates are inhibitors of acetylcholine esterase (AChE). They are metabolized in liver to form oxones, which irreversibly inhibit AChE. The usual form of AChE is oligomeric and it is this form which is inhibited by organophosphate compounds (OPCs). On inhibition, up-regulation at genetic level causes increase in synthesis of AChE; however, this AChE is unable to oligomerize. This form of AChE, also called $AChE_R$, is thought to act in inflammatory processes, contributing to the disease pathobiology. PON1 is a key detoxifier of organophosphates and organophosphate exposure has been linked to the development of neurological disorders where acetylcholine plays a significant role [84].

PON1 is thought to protect AChE through its antioxidant action by neutralizing superoxide radicals. They also spare AChE from organophosphates by lysis of

organophosphate compounds. Exposure to OPCs usually results from chronic domestic pesticide exposure. PON1 polymorphisms result in hypofunction of PON1. In such states, OPCs irreversibly inactivate circulating AChE. PON1 usually is not a vital enzyme, but in case of a person with PON1 polymorphism, exposure to OPCs may trigger detrimental effects of the polymorphism present. In absence of functional PON1, the organophosphate exposure results in irreversible inhibition of the circulating oligomeric AChE and up-regulation of $AChE_R$. Thus, presence of PON1 is thought to prevent this by its antioxidant and paraoxonase activities, and genetic variations in PON1 affect the individual's susceptibility to OPC exposure [85].

The nigrostriatal pathway of dopamine secretion degenerates with increasing age. This leads to minor DA depletion. In people with intact and functional AChE and PON1, this minor depletion does not cause any significant detrimental alteration in health, even in presence of OPCs exposure. However, in presence of polymorphisms in either of AChE or PON1, this can predispose to Parkinson disease. In Parkinson disease, the acetylcholine and dopamine levels are imbalanced with respect to each other due to damage to the substantia nigra. Chronic exposure to OPCs aggravates this imbalance by the means explained above. PON1 counters this imbalance through its antioxidant and paraoxonase activities [84].

Alzheimer disease, on the other hand, is linked to cholinergic deficiency. Hence, AChE inhibitors (AChEIs) are used to treat it. However, the response of patients to these AChEIs is not uniform—many patients have been found not to respond to the same. It was discovered that the AChEI activity was inversely related to PON2 esterase activity. Further investigations [85] revealed that this was most likely because the esterase activity of PON2 lead to the lysis of the AChEIs. While PON1 mutations did not have this esterase effect on these drugs, the PON2 polymorphic forms, 311C and 148G, showed increased esterase activity. Thus, PON2 analysis in patients with Alzheimer disease may indicate whether the AChEI therapy would be successful or not [86].

We can conclude that the paraoxonase is the molecule which has implications in the neurodegenerative disease and other aspects of nervous system. Mostly the role of paraoxonase is protective one by the virtue of prevention of oxidative stress which arises due to the imbalance in the redox system. But this is only the tip off the iceberg as more research is required in this aspect.

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