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Paraoxonase-2 (PON2) in brain and its potential role in neuroprotection

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Abstract

Paraoxonase 2 (PON2) is a member of a gene family which also includes the more studied PON1, as well as PON3. PON2 is unique among the three PONs, as it is expressed in brain tissue. PON2 is a lactonase and displays anti-oxidant and anti-inflammatory properties. PON2 levels are highest in dopaminergic regions (e.g. striatum), are higher in astrocytes than in neurons, and are higher in brain and peripheral tissues of female mice than male mice. At the sub-cellular level, PON2 localizes primarily in mitochondria, where it scavenges superoxides. Lack of PON2 (as in $PON2^{-/-}$ mice), or lower levels of PON2 (as in male mice compared to females) increases susceptibility to oxidative stress-induced toxicity. Estradiol increases PON2 expression *in vitro* and *in vivo*, and provides neuroprotection against oxidative stress. Such neuroprotection is not present in CNS cells from $PON2^{-/-}$ mice. Similar results are also found with the polyphenol quercetin. PON2, given its cellular localization and antioxidant and anti-inflammatory actions, may represent a relevant enzyme involved in neuroprotection, and may represent a novel target for neuroprotective strategies. Its differential expression in males and females may explain gender differences in the incidence of various diseases, including neurodevelopmental, neurological, and neurodegenerative diseases.

Introduction

The paraoxonases (PONs) are a family of three genes (PON1, PON2, PON3) clustered in tandem on the long arm of human chromosome 7q21–22, and on mouse chromosome 6

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(Primo-Parmo et al. 1996). Phylogenetic analysis suggests that PON2 is the oldest PON family member, from which PON1 and PON3 have evolved (Draganov and LaDu, 2004). The name of these enzymes derives from paraoxon, the active metabolite of the organophosphorus (OP) insecticide parathion, which is hydrolyzed by PON1 *in vitro*, though not efficiently *in vivo* (Li et al. 2000). The name has been extended to the other two PONs, though they do not have OP esterase activity. In contrast, all three PONs are lactonases, displaying overlapping but distinct substrate specificities for lactone hydrolysis (Draganov et al. 2005). For example, all three PONs can hydrolyze a number of acyl-homoserine lactones (acyl-HCL), molecules which mediate bacterial quorum-sensing signals, important in regulating expression of virulence factors and in inducing a host inflammatory response. PON2 has the highest acyl-HCL hydrolytic activity of the three PON isozymes (Draganov et al. 2005; Stoltz et al. 2007; Teiber et al. 2008; Horke et al. 2010). Among the three PONs, the most studied is PON1, because of its long- known role in OP metabolism and in cardiovascular disease, and the presence of several relevant polymorphisms (Costa et al. 2003; Furlong et al. 2008). Table 1 summarizes the main enzymatic activities and localization of the three PONs.

This paper focuses on PON2, a PON isozyme less studied than PON1, which is nevertheless emerging as an important defense system toward oxidative stress and inflammation and, most importantly, was also recently found to be expressed in nervous system tissue. The focus will indeed be on recent findings which have identified and characterized PON2 in brain, identified its potential roles, and discovered gender differences in expression with potential important ramifications and novel modulators of its expression. In peripheral tissues, PON2 is considered important in modulating sensitivity to bacterial infections because of its high acyl-HCL hydrolytic activity, and may represent a pharmaceutical or dietary target for the prevention of infections (Precourt et al. 2011). PON2 also plays a significant role in atherosclerosis, as shown by studies indicating that PON2 over-expression decreases atherosclerotic lesions, while the opposite is true in PON2-null ($PON2^{-/-}$) mice (Ng et al. 2006a; 2006b). In macrophages, PON2 has been suggested to protect against accumulation of triglycerides and oxidative stress, thereby attenuating the development of vascular complications in diabetes (Rosenblat et al. 2009; Meilin et al. 2010). In the gastrointestinal tract, PON2 antagonizes oxidative and inflammatory processes that may affect mucosal integrity (Levy et al. 2007).

Two common polymorphisms have been found in human PON2, an Ala/Gly substitution at position 147, and a Ser/Cys substitution at position 311 (Primo-Parmo et al. 1996; Mochizuki et al. 1998). The PON2 Ser311Cys polymorphism has been shown to affect lactonase activity (Stoltz et al. 2009). Carriers of the Cys311 allele have been found to be at risk for myocardial infarction and other cardiovascular diseases, as well as for Alzheimer's disease in several studies (Chen et al. 2003; Shi et al. 2004; Mackness et al. 2005; Marchegiani et al. 2009; Li et al. 2012).

While PON1 and PON3 are expressed primarily in the liver, and their protein products are associated with high-density lipoproteins (HDL) in the plasma, PON2 is a ubiquitously expressed intracellular enzyme, but is not present in plasma (Mochizuki et al. 1998; Ng et al. 2001; Marsillach et al. 2008; Giordano et al. 2011). PON2 mRNA and/or protein have been

detected in several tissues including liver, lung, kidney, heart, pancreas, small intestine, muscle, testis, endothelial cells, tracheal epithelial cells, and macrophages (Mochizuki et al., 1998; Ng et al., 2001; Rosenblat et al., 2003; Levy et al., 2007; Stoltz et al., 2007; Marsillach et al., 2008; Precourt et al., 2009). In mice, the highest levels were found in lung and small intestine, followed by heart and liver, with lower levels in testis, kidney and brain (Giordano et al. 2011). Of great interest in the latter study is the novel observation that in all tissues PON2 expression was always significantly higher in female mice than in male animals (Giordano et al. 2011).

PON2 as an antioxidant and anti-inflammatory factor

In several tissues, PON2 has been shown to exert an antioxidant effect (Ng et al., 2001). PON2 antagonizes oxidative stress generated by various sources in the intestine of humans and rats (Levy et al., 2007), in human vascular endothelial cells (Horke et al., 2007), in lung epithelial carcinoma cells (Horke et al., 2010), in Caco-2/15 intestinal epithelial cells (Precourt et al., 2009), and in mouse macrophages (Rosenblat et al., 2003). These anti-oxidant effects of PON2 are believed to play a major role in preventing the atherosclerotic process, as shown directly in $PON2^{-/-}$ mice (Horke et al. 2007; Devarajan et al. 2011). Sub-cellular distribution studies have shown that PON2 is localized primarily in the mitochondria (Devarajan et al. 2011; Giordano et al. 2011). Mitochondria are a major source of free radical-related oxidative stress (Higgins et al. 2010), and the preponderant localization of PON2 in mitochondria would support a role for this enzyme in protecting cells from oxidative damage. In HeLa cells, PON2 has been shown to bind to Coenzyme Q_{10} that associates with mitochondrial Complex III, and PON2 deficiency causes mitochondrial dysfunction (Devarajan et al. 2011). In human endothelial cells PON2 has been shown to reduce, indirectly but specifically, the release of superoxide from the inner mitochondrial membrane, without affecting levels of other radicals such as hydrogen peroxide and peroxynitrite (Altenhofer et al. 2010). Also of interest is that the Cys311Ser mutation, which influences lactonase activity (Stoltz et al. 2009), does not appear to affect PON2's antioxidant properties, suggesting independent hydrolytic and antioxidant functions (Altenhofer et al. 2010).

PON2 also appears to exert anti-inflammatory effects. In the gastrointestinal tract, PON2 antagonizes oxidative and inflammatory processes that may affect mucosal integrity (Levy et al. 2007). The absence of PON2 in $PON2^{-/-}$ mice exacerbates the macrophage inflammatory response (Bourquard et al. 2011). Furthermore, PON2 acts as a potent anti-inflammatory agent against the inflammatory response caused by administration of pyocyanin (a quorum sensing signal factor) (Schweikert et al. 2012).

PON2 expression in the central nervous system

PON2 mRNA has been found in mouse and human brain (Primo-Parmo et al., 1996; Mochizuki et al., 1998; Ng et al., 2006a; Mackness et al. 2010), and PON2 protein had been detected in mouse (Ng et al., 2006a; Marsillach et al., 2008) and monkey brain (de Laat et al., unpublished). In a series of recent studies, the expression of PON2 has been characterized in mouse brain (Giordano et al. 2011; 2013a; Costa et al. 2013). The highest

levels of PON2 protein were found in three dopaminergic regions, the substantia nigra, the striatum, and the nucleus accumbens, with lower levels in cerebral cortex, cerebellum, hippocampus and brainstem. In every brain region, PON2 levels were higher (by ~2–3-fold) in female mice than in male mice. The higher levels of PON2 in dopaminergic areas are of interest, as they may be related to the higher level of oxidative stress, due to dopamine metabolism, present in these regions. The regional distribution and gender difference of PON2 was confirmed by measurements of its lactonase activity [measured by dihydrocoumarin (DHC) hydrolysis] and of PON2 mRNA levels (Giordano et al. 2011).

In brain, and to a lesser extent in kidney and testis, but not in all tissues, the PON2 antibody recognized two bands, the lower at MW ~43 kDa, which corresponds to the reported MW of PON2, and an upper band at MW ~55 kDa. This upper band had been found at times by some investigators (Shamir et al. 2005; Ng et al. 2006a; Horke et al. 2007; 2010; Witte et al. 2011), but not by others (Ng et al. 2001; Shiner et al. 2004; Levy et al. 2007; Precourt et al. 2009), and may represent a PON2 alloform, in accordance with the two mRNA splice variants (Mochizuki et al. 1998; Horke et al. 2007; 2010). However, its exact nature has not been defined yet. Though PON2 is known to have four putative N-linked glycosylation sites at asparagine residues (Stoltz et al. 2009), deglycosylation experiments indicated that both putative isoforms are glycosylated (Giordano et al. 2011). Thus, purification of the upper band, and its analysis (e.g. by mass spectrometry) are needed to identify its structural features and other potential post-translational modifications. Nevertheless, neither band was detected in brain from PON2-deficient mice (Giordano et al. 2011).

PON1 was detected at very low levels (20- to 40-fold less than PON2) in all brain areas and did not show any regional or gender difference (Giordano et al. 2011). Such low level in tissue homogenates may be due to residual blood, as no PON1 could be detected in striatal astrocytes or neurons (Costa et al. 2013). PON3 was not detected in any brain region (either homogenate or cells).

PON2 protein (by Western blot), mRNA (by qRT-PCR) and activity (by DHC hydrolysis) levels were also examined in astrocytes and in neurons isolated from several brain regions. PON2 was significantly higher in astrocytes than in neurons in all brain regions, with the highest levels in cells isolated from the striatum. Striatal neurons and astrocytes isolated from female mice expressed higher levels of PON2 that the same cells from male animals. PON2 was also present in cortical microglia, at levels similar to those found in neurons (Giordano et al. 2011). The sub-cellular distribution of the PON2 protein was assessed in cerebellar granule neurons and cerebellar astrocytes, and found to be similar in astrocytes and neurons. Differences were found in the localization of the 43 kDa lower band (the putative PON2) and the upper 55 kDa band (the putative PON2 alternate isoform). In both cell types, the highest levels of 43 kDa PON2 were found in mitochondria, followed by membranes (microsomes), in agreement with previous observations in HeLa cells (Devarajan et al. 2011). PON2 was not detected in the cytosolic, nuclear, or cytoskeletal fractions. In contrast, the upper band PON2 isoform was expressed at highest levels in the nucleus and the cytoskeleton, neither of which contained significant levels of the 43 kDa band (Giordano et al. 2011).

PON2 protein levels were also measured in whole brain from female mice during development. Highest levels were found before birth (gestational day 20) and early after birth (postnatal days 1–7) with a gradual decline with age. A plateau of ~30% of the highest level was reached between 1 and 2 months of age (Giordano et al. 2011). Previously, PON1 hepatic mRNA and plasma paraoxonase activity were shown to increase with age, reaching adult levels at approximately 21 days of age (Li et al. 1997; Cole et al. 2003). This apparent differential developmental profile of PON1 and PON2 is of much interest, and further studies are underway in the authors' laboratories to substantiate and expand the findings. Nevertheless, the high level of PON2 in early brain development may be relevant to protect brain cells from oxidative stress during this critical period (Hayashi, 2009).

To provide a direct indication of whether PON2 exerts a protective effect toward oxidative stress in brain cells, as observed in other tissues and cell types (Ng et al. 2001; Levy et al. 2007; Horke et al. 2007; 2010), the cytotoxicity of two known oxidants, hydrogen peroxide (H_2O_2) and 2,3-dimethoxy-1,4-naphtoquinone (DMNQ), was investigated in cerebellar and striatal astrocytes, and in cerebellar granule neurons (CGNs) and striatal neurons, isolated from wild-type ($PON2^{+/+}$) and $PON2^{-/-}$ mice. Table 2 shows an example of such findings. In all instances, cells from mice lacking PON2 were more susceptible to the toxicity of both compounds, by a factor of 5 to 11-fold. The differential susceptibility was higher in astrocytes than in neurons, and was higher in cells from striatum than in those from the cerebellum, in accordance with the relative levels of PON2 expression. The protection afforded by PON2 to neurons and astrocytes was related to its ability to scavenge reactive oxygen species (ROS) upon exposure to oxidants. For example DMNQ (10 μM) increased ROS to ~400% of basal in CGNs from $PON2^{-/-}$ mice, and only 170% in the same cells from $PON2^{+/+}$ mice (Giordano et al. 2011). Glutathione (GSH) represents the main cellular defense factor against oxidative stress. However, levels of GSH did not differ between cells (either astrocytes or neurons) isolated from striatum and cerebellum of $PON2^{-/-}$ and $PON2^{+/+}$ mice, suggesting that the differential susceptibility to oxidants was primarily due to the presence or absence of PON2 (Giordano et al. 2011).

Gender differences in PON2 expression

As indicated earlier, initial studies found different levels of expression of PON2 protein between male and female mice in all tissues examined; similarly, in brain regions, PON2 levels and lactonase activity were always higher in female than in male mice (Giordano et al. 2011), and the same gender differences in PON2 protein, mRNA and activity were found at the cellular level, in astrocytes and neurons isolated from different brain regions (Giordano et al. 2011; 2013a). As indicated earlier, the antibody recognized two bands (the 43 kDa band, corresponding to PON2, and the 55 kDa band, which may represent a PON2 alloform, but whose identity has not been clarified). The variability in the levels of the upper 55 kDa band is high, possibly due to instability of this protein or to conditions used in the Western blot, which are optimized for the detection of PON2. Of interest, however, is that the abundance of the 55 kDa band appears to differ between genders, though to a lesser extent (e.g. 2-fold vs. 3-fold in striatal astrocytes) than that of the 43 kDa band corresponding to PON2 (Giordano et al. 2013a).

The higher levels of PON2 in brain tissue from female mice may be related to a positive modulatory effect by estrogens. In striatal astrocytes from male mice, 17β-estradiol caused a time-and concentration-dependent increase in the levels of PON2 protein; a 12–24 h exposure with 200 nM estradiol increased PON2 expression to the levels found in female striatal astrocytes (Giordano et al. 2013a). Interestingly, in female astrocytes, estradiol could further increase PON2 expression, by a factor of about 2.5-fold. The estradiol effect was due to transcriptional activation of the PON2 gene, and was mediated by activation of estrogen receptors alpha (Giordano et al. 2013a). In ovariectomized mice, PON2 levels (protein and mRNA) were significantly reduced in striatum, cerebral cortex and liver, approaching the levels found in male mice. Given the previous findings of enhanced susceptibility to oxidative stress due to lack of PON2 (Giordano et al. 2011; Table 2), it was of interest to ascertain whether the 2–3-fold difference in PON2 levels between genders was sufficient to confer differential susceptibility to oxidants. This was indeed the case, as striatal astrocytes and neurons from male mice were more sensitive to H_2O_2 and DMNQ-induced oxidative stress and ensuing cytotoxicity (Giordano et al. 2013a). Though gender-dependent differences in other cell defense mechanisms cannot be excluded, it is noteworthy that levels of GSH did not differ between genders; as expected, they were higher in striatal astrocytes than in neurons (20 vs. 9 nmol/mg protein), but with no gender differences. Another important aspect is related to the lack of gender difference in susceptibility in cells from $PON2^{-/-}$ mice. Striatal astrocytes from $PON2^{-/-}$ mice of either gender were highly susceptible to oxidant-induced toxicity, as expected, but there were no significant female/male differences. Further evidence for a central role of PON2 in mediating gender differences in susceptibility to oxidative stress toxicity was provided by experiments with estradiol. Some of these findings are shown in Table 3. In CNS cells from $PON2^{+/+}$ male mice, exposure to estradiol (200 nM, 24 h) provided protection toward toxicity induced by the two oxidants. This is not surprising, as neuroprotective actions of estrogens are well known (Simpkins et al. 2010; Arevalo et al. 2010; Azcoita et al. 2011; Arnold et al. 2012). However, the protective effect of estradiol was absent in cells from $PON2^{-/-}$ mice, suggesting that a major mechanism of estrogen neuroprotection may be represented by induction of PON2 (Giordano et al. 2013a).

The functional consequences of a higher expression of PON2 in females may have several ramifications. First, though all the findings summarized above have been obtained in mice, initial evidence indicated that similar gender differences were present, at least in astrocytes, also in rats and humans (Giordano et al. 2013a). With regard to neurodegenerative diseases, the role of oxidative stress in the etiopathology of Parkinson's disease (PD) is well established (Surmeier et al. 2011); of note is that the incidence of PD is 90% higher in males (Van den Eeden et al. 2003; Wirdefeld et al. 2011). Even though dopaminergic areas (striatum, substantia nigra, and nucleus accumbens) have the highest level of PON2 in both genders, levels in females are still 2 to 3-fold higher than in males (Giordano et al. 2011; 2013a; Costa et al. 2013). Lower PON2 levels in dopaminergic neurons in males may thus provide fewer defenses against oxidative stress. In this regard, of much interest are the recent findings that activation of dopamine D2 receptors in the kidney positively modulates PON2 expression, leading to a decrease in ROS production (Yang et al. 2012). In the CNS, the highest levels of dopamine D2 receptors are found in the striatum, nucleus accumbens,

substantia nigra and olfactory tubercle (Beaulieu and Gainetdinov, 2011), areas that also have the highest level of PON2 expression (Giordano et al. 2011; unpublished results). If a similar mechanism as observed in kidneys also occurs in the CNS, the loss of dopamine associated with PD would lead to decreased PON2 levels, thus fostering a spiral of events further aggravating neurodegeneration. Of interest in this regard are also *in vitro* results showing an enhanced susceptibility of male mesencephalic astrocytes to the toxicity of the dopaminergic neurotoxin MPP^+ (Sundar-Boyalla et al. 2011). Additional evidence that males may be more sensitive than females to oxidative stress-mediated CNS toxicity is provided by studies with 6-hydroxydopamine (Tamas et al. 2005; Misiak et al. 2010), methamphetamine (Dluzen et al. 2010; Bourque et al. 2011), or manganese (Madison et al. 2011), or by analysis of oxidative changes in brain mitochondria (Guevara et al. 2011). Furthermore, as PON2 is expressed in most tissues, and levels appear to be higher in females in each tissue examined (Giordano et al. 2011), the reported higher sensitivity of males to oxidative stress in heart, the higher susceptibility of males to atherosclerosis and to infections, may all be related to a differential expression of PON2 (Klein et al. 2000; Kardys et al. 2007; Wang et al. 2010).

As discussed earlier, PON2 also appears to have anti-inflammatory properties in peripheral tissues (Levy et al. 2007; Bourquard et al. 2011; Schweikert et al. 2011), and may have similar properties in the CNS. Since most neurodevelopmental and neurodegenerative disorders appear to involve neuroinflammatory processes (Niranjan, 2013; Depino, 2013), gender differences in response to neuroinflammation may also be expected. In support of this hypothesis, we have recently found that neuroinflammation (e.g. increase in levels of pro-inflammatory cytokines such as TNF-alpha and several interleukins) observed in several brain regions of mice upon exposure to diesel exhaust, is significantly more pronounced in male than in female mice (Giordano et al. 2013b).

Modulation of PON2

While there is a substantial amount of work on the modulation of PON1, which has been summarized in several reviews (Costa et al. 2005; 2011; Camps et al. 2009), more limited research has been carried out on PON2. In macrophages, PON2 expression is increased by oxidative stress (Rosenblat et al. 2003), and in vascular cells an endoplasmic reticulum stress element-like sequence was found to be present in the promoter region of PON2 (Horke et al. 2007). Arachidonic acid (Rosenblat et al. 2010), unesterified cholesterol (Shiner et al. 2007a), the licorice phytoestrogen glabidrin (Yehuda et al. 2011), and the hypocholesterolemic drug atorvastatin (Rosenblat et al. 2004) also upregulate PON2 expression in various cell types. Urokinase plasminogen activator upregulates PON2 in macrophages via NADPH oxidase and the transcription factor SREBP-2 (Fuhrman et al. 2008; 2009), while in mouse fibroblasts dexamethasone increases PON2 mRNA levels (Lim and Kim, 2009).

In one study, pomegranate juice was found to increase PON2 in macrophages through activation of the PPAR-γ and AP-1 pathways (Shiner et al. 2007b), while quercetin was reported to increase PON2 mRNA and protein in macrophages *in vitro*, though administration of 150 mg/day to human volunteers for six weeks was without effects

(Boesch-Saadatmandi et al. 2009). Extracts of Yerba mate (*Ilex paraguariensis*) have been reported to increase PON2 mRNA and lactonase activity in macrophages *in vitro* and after *in vivo* administration to healthy women (Stucker Fernandez et al. 2012). A recent study has examined the induction of PON2 by quercetin *in vitro* (Costa et al. 2013). Quercetin was found to increase PON2 protein expression in mouse striatal astrocytes (mixed gender) by ~2-fold, and the effect was antagonized by a JNK/AP-1 inhibitor. Quercetin may thus induce a very low-level of oxidative stress (Halliwell, 2008), which in turn would modulate the JNK/AP-1 pathway (Granado-Serrano et al. 2010), causing an increase in PON2 expression. Alternatively, quercetin may induce PON2 expression by activating estrogen receptors alpha (Galluzzo et al. 2009), though this would need to be investigated further. Independent of the underlying mechanism(s), the ability of quercetin to induce PON2 may play a role in the reported neuroprotective actions of this polyphenol, which have been observed *in vitro* (Mercer et al. 2005; Ossola et al. 2009; Bournival et al. 2009), as well as *in vivo* (Barcelos et al. 2011; Lv et al. 2012; Selvakumar et al. 2012). In striatal astrocytes from $PON2^{+/+}$ mice (mixed gender), exposure for 24 h to quercetin abolished the increase in ROS levels caused by H_2O_2 or DMNQ, and resulted in protection against the cytotoxicity of these oxidants. In contrast very limited neuroprotection was observed in the same cells isolated from $PON2^{-/-}$ mice (Costa et al. 2013). Some of these findings are shown in Table 3.

Conclusion and future perspectives

In recent years, PON2 has emerged as a potentially important intracellular defense mechanism against oxidative stress, particularly given its widespread tissue distribution and mitochondrial localization. Its identification and initial characterization 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.

Gender differences in PON2 expression also represent a finding of much interest. Results so far suggest that brain cells from male mice are intrinsically more susceptible to oxidative stress because of a lower expression of PON2. Gender is a variable that is often ignored in toxicological and neurotoxicological studies, though most scientists would readily acknowledge that major differences may exist between males and females in their response to toxicants, which may be ascribed to differences in exposure, toxicokinetics and metabolism, and to pharmacodynamic factors (Vahter et al. 2007; Wald and Wu, 2010; Weiss et al. 2011). As many adverse health outcomes in the CNS and other organs involve oxidative stress, this finding may explain the gender-dependent differential incidence of several diseases. The lactonase activity of PON2 and its potential anti-inflammatory actions may also pertain to other pathological processes, providing the stimulus for numerous investigations addressing gender effects.

The protective action of PON2 toward oxidative stress and neuroinflammation suggest that attempts aimed at increasing its levels of expression may be useful. So far, limited research has been carried out in this area. However, dietary or pharmacological modulation of PON2 may be of interest and may provide new avenues for neuroprotection or explanations for known neuroprotective effects. A caveat to this strategy is represented by the findings that in

tumor cells PON2 is up-regulated, by still unknown mechanisms (Witte et al. 2011). Though CNS tumors have not been specifically investigated, the finding appeared to be valid for tumors of different tissues. Given its characteristics, it is not surprising that PON2 would thus provide resistance of these cells to apoptosis and that a useful therapeutic strategy would be one causing a decrease of PON2 (Witte et al. 2011).

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References

Altenhofer S, Witte I, Teiber JF, Wilgenbus P, Pautz A, Li H, Daiber A, Witan H, Clement AM, Forstermann U, Horke S. One enzyme, two functions. PON2 prevents mitochondrial superoxide formation and apoptosis independent from its lactonase activity. J Biol Chem. 2010; 285:24398–403. [PubMed: 20530481]

Arevalo MA, Santos-Galindo M, Bellini MJ, Azcoitia I, Garcia-Segura LM. Actions of estrogens on glial cells: implications for neuroprotection. Biochim Biophys Acta. 2010; 1800:1106–12. [PubMed: 19818384]

Arnold S, Victor MB, Beyer C. Estrogen and the regulation of mitochondrial structure and function in the brain. J Ster Biochem Mol Biol. 2012; 131:2–9.

Azcoita I, Arevalo MA, De Nicola AF, Garcia-Segura LM. Neuroprotective actions of estradiol revisited. Trends Endocrinol Metab. 2011; 22:467–73. [PubMed: 21889354]

Barcelos GRM, Grotto D, Serpeloni JM, Angeli JPF, Rocha BA, Souza VVO, Vicentini JT, Emanuelli T, Bastos JK, Antunes LMG, Knasmuller S, Barbosa F Jr. Protective properties of quercetin against DNA damage and oxidative stress induced by methylmercury in rats. Arch Toxicol. 2011; 85:1151–7. [PubMed: 21286687]

Beaulieu JM, Gainetdinov RR. The physiology, signaling, and pharmacology of dopamine receptors. Pharmacol Rev. 2011; 63:182–217. [PubMed: 21303898]

Boesch-Saadatmandi C, Pospissil RT, Graeser AC, Canali R, Boomgaarden I, Doering F, Wolffram S, Egert S, Mueller MJ, Rimbach G. Effect of quercetin on paraoxonase 2 levels in RAW264.7 macrophages and in human monocytes - role of quercetin metabolism. Int J Mol Sci. 2009; 10:4168–77. [PubMed: 19865538]

Bournival J, Quessy, Martinoli MG. Protective effects of resveratrol and quercetin against MPP^+-induced oxidative stress act by modulating markers of apoptotic death in dopaminergic neurons. Cell. Mol. Neurobiol. 2009; 29:1169–80.

Bourquard N, Ng CJ, Reddy ST. Impaired hepatic insulin signaling in PON2-deficient mice: a novel role for the PON2/apoE axis on the macrophage inflammatory response. Biochem J. 2011; 436:91–100. [PubMed: 21361875]

Bourque M, Liu B, Dluzen DE, Di Paolo T. Sex differences in methamphetamine toxicity in mice: effect on brain dopamine signaling pathways. Psychoneuroendocrinology. 2011; 36:955–69. [PubMed: 21236583]

Camps J, Marsillach J, Joven J. Pharmacological and lifestyle factors modulating serum paraoxonase-1 activity. Mini Rev Med Chem. 2009; 9:911–20. [PubMed: 19601886]

Chen Q, Reis SE, Kammerer CM, McNamara DM, Holubkov R, Sharaf BL, Sopko G, Pauly DF, Merz CNB, Kamboh MI. Association between severity of angiographic coronary artery disease and paraoxonase gene polymorphisms in the National Heart, Lung, and Blood Intitute-sponsored Women's Ischemia Syndrome Evaluation (WISE) study. Am J Hum Genet. 2003; 72:13–22. [PubMed: 12454802]

Cole TB, Jampsa RL, Walter BJ, Arndt TL, Richter RJ, Shih DM, Tward A, Lusis AJ, Jack RM, Costa LG, Furlong CE. Expression of human paraoxonase (PON1) during development. Pharmacogenetics. 2003; 13:357–64. [PubMed: 12777966]

Costa LG, Cole TB, Jarvik GP, Furlong CE. Functional genomics of the paraoxonase (PON1) polymorphisms: effects on pesticide sensitivity, cardiovascular disease, and drug metabolism. Annu Rev Med. 2003; 54:371–92. [PubMed: 12525679]

Costa LG, Vitalone A, Cole TB, Furlong CE. Modulation of paraoxonase (PON1) activity. Biochem Pharmacol. 2005; 69:541–50. [PubMed: 15670573]

Costa LG, Giordano G, Furlong CE. Pharmacological and dietary modulators of paraoxonase 1 (PON1) activity and expression: the hunt goes on. Biochem Pharmacol. 2011; 81:337–44. [PubMed: 21093416]

Costa LG, Tait L, de Laat R, Dao K, Giordano G, Pellacani C, Cole TB, Furlong CE. Modulation of paraoxonase 2 (PON2) in mouse brain by the polyphenol quercetin; a mechanism of neuroprotection? Neurochem Res. 2013 in press.

Depino AM. Peripheral and central inflammation in autism spectrum disorders. Mol Cel Neurosci. 2013; 53:69–76.

Devarajan A, Bourquard N, Hama S, Navab M, Grijalva VR, Morvardi S, Clarke C, Vergnes L, Reue K, Teiber JF, Reddy ST. Paraoxonase 2 deficiency alters mitochondrial function and exacerbates the development of atherosclerosis. Antiox Redox Signal. 2011; 14:341–51.

Dluzen DE, McDermott JL, Darvesh AS. Relationships among gender, age, time, and temperature in methamphetamine-induced striatal dopaminergic neurotoxicity. Neuroscience. 2010; 167:985–93. [PubMed: 20211701]

Draganov DI, La Du BN. Pharmacogenetics of paraoxonase: a brief review. Naunyn Schmiedebergs Arch Pharmacol. 2004; 369:78–88. [PubMed: 14579013]

Draganov DI, Teiber JF, Speelman A, Osawa Y, Sunahara R, La Du BN. Human paraoxonases (PON1, PON2 and PON3) are lactonases with overlapping and distinct substrate specificities. J Lipid Res. 2005; 46:1239–47. [PubMed: 15772423]

Fuhrman B, Khateeb J, Shiner M, Nitzan O, Karry R, Volkova N, Aviram M. Urokinase plasminogen activator upregulates paraoxonase 2 expression in macrophages via an NADPH oxidase-dependent mechanism. Arterioscler Thromb Vasc Res. 2008; 28:1361–7.

Fuhrman B, Gantman A, Khateeb J, Volkova N, Horke S, Kiyan J, Dumler I, Aviram M. Urokinase activates macrophage PON2 gene transcription via the PI3K/ROS/MEK/SREBP-2 signalling cascade mediated by the PDGFR-beta. Cardiovasc Res. 2009; 84:145–54. [PubMed: 19497963]

Furlong, CE.; Richter, RJ.; Li, WF.; Brophy, VH.; Carlson, C.; Rieder, M.; Nickerson, D.; Costa, LG.; Ranchalis, J.; Lusis, AJ.; Shih, DM.; Tward, A.; Jarvik, GP. The functional consequences of polymorphisms in the human PON1 gene. In: Mackness, B.; Mackness, M.; Aviram, M.; Paragh, G., editors. The Paraoxonases: Their Role in Disease Development and Xenobiotic Metabolism. Springer; 2008. p. 267-81.

Galluzzo P, Martini C, Bulzomi P, Leone S, Bolli A, Pallottini V, Marino M. Quercetin-induced apoptotic cascade in cancer cells: antioxidant versus estrogen receptor alpha-dependent mechanisms. Mol Nutr Food Res. 2009; 53:699–708. [PubMed: 19194971]

Giordano G, Cole TB, Furlong CE, Costa LG. Paraoxonase 2 (PON2) in the mouse central nervous system: a neuroprotective role? Toxicol Appl Pharmacol. 2011; 256:369–78. [PubMed: 21354197]

Giordano G, Tait L, Furlong CE, Cole TB, Kavanagh TJ, Costa LG. Gender differences in brain susceptibility to oxidative stress are mediated by levels of paraoxonase-2 (PON2) expression. Free Rad Biol Med. 2013a; 58:98–108. [PubMed: 23376469]

Giordano G, Engstrom A, Weldy CS, Kavanagh TJ, Farin F, Costa LG. Higher susceptibility of male mice to diesel exhaust neurotoxicity. Toxicologist. 2013b

Granado-Serrano AB, Martin MA, Bravo L, Goya L, Ramos S. Quercetin modulates Nf-kB and AP-1/JNK pathways to induce cell death in human hepatoma cells. Nutr Cancer. 2010; 62:390–401. [PubMed: 20358477]

Guevara R, Gianotti M, Oliver J, Roca P. Age and sex-related changes in rat brain mitochondria oxidative stress. Experim Gerontol. 2011; 46:923–8.

Halliwell B. Are polyphenols antioxidants or pro-oxidants? What do we learn from cell culture and in vivo studies? Arch Biochem Biophys. 2008; 476:107–12. [PubMed: 18284912]

Hayashi M. Oxidative stress in developmental brain disorders. Neuropathology. 2009; 29:1–8. [PubMed: 19154320]

Higgins GC, Beart PM, Shin YS, Chen MJ, Cheung NS, Nagley P. Oxidative stress: emerging mitochondrial and cellular themes and variations in neuronal injury. J Alzheimers Dis. 2010; 20 (Suppl. 2):S453–73. [PubMed: 20463398]

Horke S, Witte I, Wilgenbus P, Kruger M, Starnd D, Forstermann U. Paraoxonase-2 reduces oxidative stress in vascular cells and decreases endoplasmic reticulum stress-induced caspase activation. Circulation. 2007; 115:2055–64. [PubMed: 17404154]

Horke S, Witte I, Altenhoffer S, Wilgenbus P, Goldeck M, Forstermann U, Xiao J, Kramer GL, Haines DC, Chowdhary PK, Haley RW, Teiber JF. Paraoxonase-2 is down regulated by the *Pseudomonas aeruginosa* quorum-sensing signal N-(3-oxododecanyl)-L-homoserine lactone and attenuates oxidative stress induced by pyocyanin. Biochem J. 2010; 426:73–83. [PubMed: 19925453]

Kardys I, Vliegenthart R, Oudkerk M, Hofman A, Witteman JCM. The female advantage in cardiovascular disease: do vascular beds contribute equally? Am J Epidemiol. 2007; 166:403–12. [PubMed: 17566064]

Klein SL. The effects of hormones on sex differences in infection: from genes to behavior. Neurosci Biobehav Rev. 2000; 24:627–38. [PubMed: 10940438]

Levy E, Trudel K, Bendayan M, Seidman E, Delvin E, Elchebly M, Lavoie JC, Precourt LP, Amre D, Sinnett D. Biological role, protein expression, subcellular localization, and oxidative stress response of paraoxonase 2 in the intestine of human and rats. Am J Physiol Gastrointest Liver Physiol. 2007; 293:G1252–61. [PubMed: 17916643]

Li BH, Zhang LL, Yin YW, Pi Y, Yang QW, Gao CY, Fang CQ, Wang JZ, Li JC. Association between paraoxonase 2 Ser311Cys polymorphism and ischemic stroke risk: a meta-analysis involving 5,008 subjects. Mol Biol Rep. 2012; 39:5623–30. [PubMed: 22183305]

Li WF, Matthews C, Disteche CM, Costa LG, Furlong CE. Paraoxonase *(Pon1)* gene in mice: sequencing, chromosomal localization and developmental expression. Pharmacogenetics. 1997; 7:137–44. [PubMed: 9170151]

Li WF, Costa LG, Richter RJ, Hagen T, Shih DM, Tward A, Lusis AJ, Furlong CE. Catalytic efficiency determines the in vivo efficacy of PON1 for detoxifying organophosphates. Pharmacogenetics. 2000; 10:767–79. [PubMed: 11191881]

Lim JA, Kim SH. Transcriptional activation of an anti-oxidant mouse Pon2 gene by dexamethasone. BMB Rep. 2009; 42:421–6. [PubMed: 19643039]

Lv C, Hong T, Yang Z, Zhang Y, Wang L, Dong M, Zhao J, Mu J, Meng Y. Effect of quercetin in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced mouse model of Parkinson's disease. Evid Based Complem Altern Med. 2012:6.10.1155/2012/928643

Mackness B, McElduff P, Mackness MI. The paraoxonase-2 310 polymorphism is associated with the presence of microvascular complications in diabetes mellitus. J Intern Med. 2005; 258:363–8. [PubMed: 16164576]

Mackness B, Beltran-Debon R, Aragones G, Joven J, Camps J, Mackness M. Human tissue distribution of paraoxonases 1 and 2 mRNA. IUBMB Life. 2010; 62:480–2. [PubMed: 20503442]

Madison JL, Wegrzynowicz M, Aschner M, Bowman AB. Gender and manganese exposure interactions on mouse striatal neurons morphology. NeuroToxicology. 2011; 32:896–906. [PubMed: 21641932]

Marchegiani F, Spazzafumo L, Provinciali M, Cardelli M, Olivieri F, Franceschi C, Lattanzio C, Antonicelli R. Paraoxonase 2 C311S polymorphism and low levels of HDL contribute to a higher mortality risk after acute myocardial infarction in elderly patients. Mol Genet Metab. 2009; 98:314–8. [PubMed: 19540141]

Marsillach J, Mackness B, Mackness M, Riu F, Beltran R, Joven J, Camps J. Immunohistochemical analysis of paraoxonase-1, 2 and 3 expression in normal mouse tissues. Free Rad Biol Med. 2008; 45:146–57. [PubMed: 18440321]

Meilin E, Aviram M, Hayek T. Paraoxonase 2 (PON2) decreases high glucose-induced macrophage triglyceride (TG) accumulation, via inhibition of NADPH-oxidase and DGAT1 activity: studies in PON2-deficient mice. Atherosclerosis. 2010; 208:390–5. [PubMed: 19748094]

Mercer LD, Kelly BL, Horne MK, Beart PM. Dietary polyphenols protect dopamine neurons from oxidative insults and apoptosis; investigations in primary rat mesencephalic cultures. Biochem Pharmacol. 2005; 69:339–45. [PubMed: 15627486]

Misiak M, Beyer C, Arnold S. Gender-specific role of mitochondria in the vulnerability of 6-hydroxydopamine-treated mesencephalic neurons. Biochim Biophys Acta. 2010; 1797:1178–88. [PubMed: 20416276]

Mochizuki H, Scherer SW, Xi T, Nickle DC, Majer M, Huizenga JJ, Tsui LC, Prochazka M. Human *PON2* gene at 7q21. 3: cloning, multiple mRNA forms, and missense polymorphisms in the coding sequence. Gene. 1998; 213:149–57. [PubMed: 9714608]

Ng CJ, Wadleigh DJ, Gangopadhyyay A, Hama S, Grijalva VR, Navab M, Fogelman AM, Redd ST. Paraoxonase-2 is a ubiquitously expressed protein with antioxidant properties and is capable of preventing cell-mediated oxidative modification of low density lipoprotein. J Biol Chem. 2001; 276:44444–9. [PubMed: 11579088]

Ng CJ, Bourquard N, Grijalva V, Hama S, Shih DM, Navab M, Fogelman AM, Lusis AJ, Young S, Reddy ST. Paraoxonase-2 deficiency aggravates atherosclerosis in mice despite lower apolipoprotein-B-containing lipoproteins. Antiatherogenic role for paraoxonase-2. J Biol Chem. 2006a; 281:29491–500. [PubMed: 16891303]

Ng CJ, Hama SY, Bourquard N, Navab M, Reddy ST. Adenovirus mediated expression of human paraoxonase 2 protects against the development of atherosclerosis in apolipoprotein E-deficient mice. Mol Genet Metab. 2006b; 89:368–73. [PubMed: 16935014]

Niranjan R. The role of inflammatory and oxidative stress mechanisms in the pathogenesis of Parkinson's disease: focus on astrocytes. Mol Neurobiol. 2013 in press.

Ossola B, Kaariainen TM, Mannisto PT. The multiple faces of quercetin in neuroprotection. Expert Op Drug Saf. 2009; 8:397–409.

Precourt LP, Seidman E, Delvin E, Amre D, Deslandres C, Dominguez M, Sinnett D, Levy E. Comparative expression analysis reveals differences in the regulation of intestinal paraoxonase family members. Int J Biochem Mol Biol. 2009; 41:1628–37.

Precourt LP, Amre D, Denis MC, Lavoie JC, Delvin E, Seidman E, Levy E. The three-gene paraoxonase family: physiologic roles, actions and regulation. Atherosclerosis. 2011; 214:20–36. [PubMed: 20934178]

Primo-Parmo SL, Sorenson RC, Teiber J, La Du BN. The human serum paraoxonase/arylesterase gene (PON1) is one member of a multigene family. Genomics. 1996; 33:498–507. [PubMed: 8661009]

Rosenblat M, Draganov D, Watson CE, Bisgaier CL, La Du BN, Aviram M. Mouse macrophage paraoxonase-2 activity is increased whereas cellular paraoxonase 3 activity is decreased under oxidative stress. Arterioscler Thromb Vasc Biol. 2003; 23:468–74. [PubMed: 12615656]

Rosenblat M, Hayek T, Hussein K, Aviram M. Decreased macrophage paraoxonase 2 expression in patients with hypercholesterolemia is the result of their increased cellular cholesterol content: effect of atorvastatin therapy. Arterioscler Thromb Vasc Biol. 2004; 24:175–80. [PubMed: 14592851]

Rosenblat M, Coleman R, Reddy ST, Aviram M. Paraoxonase 2 attenuates macrophage triglyceride accumulation via inhibition of diacylglycerol acyltransferase 1. J Lipid Res. 2009; 50:870–9. [PubMed: 19091699]

Rosenblat M, Volkova N, Roqueta-Rovera M, Nakamura MT, Aviram M. Increased macrophage cholesterol biosysnthesis and decreased cellular paraoxonase 2 (PON2) expression in Δ6-desaturase knockout (6-DS-KO) mice: beneficial effects of arachidonic acid. Atherosclerosis. 2010; 210:414–21. [PubMed: 20042190]

Schweikert EM, Amort J, Wilgenbus P, Forsterman U, Teiber JF, Horke S. Paraoxonases-2 and -3 are important defense enzymes against *Pseudomonas aeruginosa* virulence factors due to their anti-oxidative and anti-inflammatory properties. J Lipids. 2012

Selvakumar K, Bavithra S, Suganthi M, Benson CS, Elumalai P, Arunkumar R, Krishnamoorthy G, Venkataraman P, Arunakaran J. Protective role of quercetin on PCBs-induced oxidative stress and apoptosis in hippocampus of adult rats. Neurochem Res. 2012; 37:708–21. [PubMed: 22127757]

Shamir R, Hartman C, Karry R, Pavlotzky E, Eliakim R, Lachter J, Suissa A, Aviram M. Paraoxonases (PONs) 1, 2 and 3 are expressed in human and mouse gastrointestinal tract and in Caco-2 cell line: selective secretion of PON1 and PON2. Free Rad Biol Med. 2005; 39:336–44. [PubMed: 15993332]

Shi J, Zhang S, Tang M, Liu X, Li T, Han H, Wang Y, Guo Y, Zhao J, Li H, Ma C. Possible association between Cys311Ser polymorphism of paraoxonase 2 gene and late-onset Alzheimer's disease in Chinese. Mol Brain Res. 2004; 120:201–4. [PubMed: 14741412]

Shiner M, Fuhrman B, Aviram M. Paraoxonase 2 (PON2) expression is upregulated via a reduced nicotinamide-adenine-dinucleotide-phosphate (NADPH)-dependent mechanism during monocytes differentiation into macrophages. Free Rad Biol Med. 2004; 37:2052–63. [PubMed: 15544923]

Shiner M, Fuhrman B, Aviram M. Macrophage paraoxonase 2 (PON2) expression is upregulated by unesterified cholesterol through activation of the phosphatidylinositol 3-kinase (PI3K) pathway. Biol Chem. 2007a; 388:1353–8. [PubMed: 18020951]

Shiner M, Fuhrman B, Aviram M. Macrophage paraoxonase 2 (PON2) expression is up-regulated by pomegranate juice phenolic anti-oxidants via PPARγ and AP-1 pathway activation. Atherosclerosis. 2007b; 195:313–21. [PubMed: 17292903]

Simpkins JW, Yi KD, Yang SH, Dykens JA. Mitochondrial mechanisms of estrogen neuroprotection. Biochim Biophys Acta. 2010; 1800:1113–20. [PubMed: 19931595]

Stoltz DA, Ozer EA, Ng CJ, Yu JM, Reddy ST, Lusis AJ, Bourquard N, Parsek MR, Zabner J, Shih DM. Paraoxonase-2 deficiency enhances *Pseudomonas aeruginosa* quorum sensing in murine tracheal epithelia. Am. J. Physiol. Lung Cell. Mol. Physiol. 2007; 292:L852–60.

Stoltz DA, Ozer EA, Recker TJ, Estin M, Yang X, Shih DM, Lusis AJ, Zabner J. A common mutation in paraoxonase-2 results in impaired lactonase activity. J Biol Chem. 2009; 284:35564–71. [PubMed: 19840942]

Stucker Fernandez E, de Oliveira Machado M, Minuzzi Becker A, de Andrade F, Maraschin M, Luiz da Silva E. Yerba mate (*Ilex paraguariensis*) enhances the gene modulation and activity of paraoxonase-2: in vitro and in vivo studies. Nutrition. 2012; 28:1157–64. [PubMed: 22964087]

Sundar-Boyalla S, Victor MB, Roemgren A, Beyer C, Arnold S. Sex-and brain region specific role of cytochrome c oxidase in 1-methyl-4-phenylpyridinium-mediated astrocyte vulnerability. J Neurosci Res. 2011; 89:2068–82. [PubMed: 21598289]

Surmeier DJ, Guzman JN, Sanchez-Padilla J, Goldberg JA. The origins of oxidant stress in Parkinson's disease and therapeutic strategies. Antioxid Redox Signal. 2011; 14:1289–301. [PubMed: 20712409]

Tamas A, Lubics A, Szalontay L, Lengvari I, Reglodi D. Age and gender differences in behavioral and morphological outcome after 6-hydroxydopamine-induced lesion of the substantia nigra. Behav Brain Res. 2005; 158:221–9. [PubMed: 15698888]

Teiber JF, Horke S, Haines DC, Chowdhary PK, Xiao J, Kramer GL, Haley RW, Draganov DI. Dominant role of paraoxonases in inactivation of the *Pseudomonas aeruginosa* quorum-sensing signal N-(3-oxododecanoyl)-L-homoserine lactone. Infect. Immun. 2008; 76:2512–9.

Vahter M, Gochfield M, Casati B, Thiruchelvam M, Falk-Filippson A, Kavlock R, Marafante E, Cory-Slechta D. Implications of gender differences for human health risk assessment and toxicology. Environ Res. 2007; 104:70–84. [PubMed: 17098226]

Van den Eeden SK, Tanner CM, Bernstein AL, Fross RD, Leimpeter A, Bloch DA, Nelson LM. Incidence of Parkinson's disease: variation by age, gender, and race/ethnicity. Am J Epidemiol. 2003; 157:1015–22. [PubMed: 12777365]

Wald C, Wu C. Of mice and women: the bias in animal research. Science. 2010; 327:1571–2. [PubMed: 20339045]

Wang F, He Q, Sun Y, Dai X, Yang XP. Female adult mouse cardiomyocytes are protected against oxidative stress. Hypertension. 2010; 55:1172–8. [PubMed: 20212261]

Weiss B. Same sex, no sex, and unaware sex in neurotoxicology. Neurotoxicology. 2011; 32:509–17. [PubMed: 20875453]

Wirdefeld K, Adami HO, Cole P, Trichopoulos D, Mandel J. Epidemiology and etiology of Parkinson's disease: a review of the evidence. Eur J Epidemiol. 2011; 26:S1–S58. [PubMed: 21626386]

Witte I, Altenhöfer S, Wilgenbus P, Amort J, Clement AM, Pautz A, Li H, Förstermann U, Horke S. Beyond reduction of atherosclerosis: PON2 provides apoptosis resistance and stabilizes tumor cells. Cell Death Dis. 2011; 2:e112.10.1038/cddis.2010.91 [PubMed: 21368884]

Yang Y, Zhang Y, Cuevas-Gonzalez S, Villar VA, Escano C, Asico L, Yu P, Grandy DK, Felder RA, Armando I, Jose PA. Paraoxonase 2 decreases renal reactive oxygen species production, lowers blood pressure, and mediates dopamine D2 receptor-induced inhibition of NADPH oxidase. Free Rad Biol Med. 2012; 53:437–46. [PubMed: 22634053]

Yehuda I, Madar Z, Szuchman-Sapir A, Tamir S. Glabridin, a phytoestrogen from licorice root, up-regulates manganese superoxide dismutase, catalase and paraoxonase 2 under glucose stress. Phytother Res. 2011; 25:659–67. [PubMed: 21053390]

Highlights

- Paraoxonase-2 (PON2) is a mitochondrial enzymes present in most tissues including the brain
- PON2 has antioxidant effects in cells and protects cells from oxidative stress-mediated toxicity
- Levels of PON2 are higher in brain from female mice
- Level of expression of PON2 correspond to degree of neuroprotection
- Estradiol and the polyphenol quercetin positively modulate PON2 expression

Table 1

Enzymatic activities and localization of PONs

Characteristic	PON1	PON2	PON3
Lactonase	++	+++	++
Paraoxonase	+++	–	–
Arylesterase	+++	+	+
Statinase	–	–	+++
Antioxidant	+++	+++	+++
Localization	Liver, plasma HDL	**Wide tissue distribution**	Liver, plasma HDL, other tissues
Chromosome:			
Human	7	**7**	7
Mouse	6	**6**	6

Table 2

PON2 protects neurons and astrocytes from oxidant-induced toxicity

Striatal neurons	**PON2$^{+/+}$**	**PON2$^{-/-}$**
ROS (% of basal)	180	600
Toxicity (IC_{50}, μM)	9.6	1.2
Striatal astrocytes		
ROS (% of basal)	130	500
Toxicity (IC_{50}, μM)	42.0	3.7

Striatal neurons or astrocytes from PON2$^{+/+}$ and PON2$^{-/-}$ mice were treated for 60 min (for ROS measurements) or 24 h (for cytotoxicity) with 10 μM H_2O_2 or 5 concentrations of H_2O_2, respectively. ROS levels were measured by the dichlorofluorescein method, while cytotoxicity was assessed by the MTT assay. Adapted from Giordano et al. (2011).

Table 3

Neuroprotection by estradiol and by quercetin against oxidant-induced toxicity is significantly reduced in striatal astrocytes from PON2$^{-/-}$ mice

	PON2$^{+/+}$	PON2$^{+/+}$ (+E/Q)	PON2$^{-/-}$	PON2$^{-/-}$ (+E/Q)
Estradiol				
PON2 levels (% of basal)	100	320*	0	0
Cytotoxicity (μM)	20	53*	3	3
Quercetin				
PON2 levels (% of basal)	100	210*	0	0
Cytotoxicity (μM)	37	131*	6	8

Striatal astrocytes from PON2$^{+/+}$ or PON2$^{-/-}$ mice were treated for 24 h with estradiol (E, 200 nM) or quercetin (Q, 20 μM). PON2 levels were measured by Western blot and are indicated as percent of control. After washout, cells were incubated for 24 h with DMNQ (4–5 concentrations), and cytotoxicity was determined by the MTT assay. Numbers shown represent IC_{50} values. Experiments with estradiol were carried out in astrocytes from male mice, while those with quercetin utilized mixed gender culture. Results show also the effect of gender (i.e. mixed gender cultures are less sensitive to DMNQ toxicity). Adapted from Giordano et al. (2013a) and Costa et al. (2013).