Modulation of paraoxonase 2 (PON2) in mouse brain by the polyphenol quercetin: a mechanism of neuroprotection?

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Abstract

Quercetin is a common flavonoid polyphenol which has been shown to exert neuroprotective actions *in vitro* and *in vivo*. Though quercetin has antioxidant properties, it has been suggested that neuroprotection may be ascribed to its ability of inducing the cell's own defense mechanisms. The present study investigated whether quercetin could increase the levels of paraoxonase 2 (PON2), a mitochondrial enzyme expressed in brain cells, which has been shown to have potent antioxidant properties. PON2 protein, mRNA, and lactonase activity were highest in mouse striatal astrocytes. Quercetin increased PON2 levels, possibly by activating the JNK/AP-1 pathway. The increased PON2 levels induced by quercetin resulted in decreased oxidative stress and ensuing toxicity induced by two oxidants. The neuroprotective effect of quercetin was significantly diminished in cells from PON2 knockout mice. These findings suggest that induction of PON2 by quercetin represents an important mechanism by which this polyphenol may exert its neuroprotective action.

Keywords

Paraoxonase 2; quercetin; polyphenols; neuroprotection

Introduction

A large number of molecules with a polyphenol structure have been shown to exert protective actions in pathological conditions such as cardiovascular disease, metabolic disorders, obesity, diabetes, infections, cancer and neurotoxic / neurodegenerative processes [1–3]. In particular, there has been increasing attention devoted to the possibility that polyphenols may act as neuroprotective agents; polyphenols have an alleged ability to antagonize oxidative stress which is recognized as an important factor in a variety of neurodegenerative diseases, in the adverse effects of a number of neurotoxicants, and as a mechanism for age-related degenerative processes [4–6].

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Quercetin is an important flavonoid polyphenol found in many common fruits and vegetables, such as apples, berries, onions and capers [7]; its estimated dietary intake ranges from 4 to 68 mg/day [8], but can increase to 200–500 mg/day in individuals who consume high quantities of fruits and vegetables rich in flavonols. Furthermore, quercetin is also sold as a dietary supplement, with a recommended dosage of 1 g/day [9].

Evidence exists of a neuroprotective effect of quercetin [10]. *In vitro* studies have shown that quercetin antagonizes cell toxicity induced by various oxidants and other neurotoxic molecules believed to act by inducing oxidative stress [11–15]. In addition, several studies show that quercetin can exert neuroprotection and antagonize oxidative stress when administered *in vivo*. Indeed, oral quercetin has been shown to protect rodents from oxidative stress and neurotoxicity induced by a variety of agents [16–22].

The exact mechanism(s) of quercetin neuroprotective effects have not been elucidated. Though quercetin is a potent scavenger of reactive oxygen and nitrogen species (ROS, RNS) [23], it has been argued that the concentration of quercetin expected to be present in the brain may not be sufficient to exert an appreciable direct antioxidant effect, compared for example to glutathione and vitamin C, which are present at millimolar concentrations [24]. Rather, it has been suggested that quercetin may exert its neuroprotective effects by other mechanisms, particularly by modulating the cell's own anti-oxidant defense systems [25–28]. For example, quercetin has been shown to affect anti-oxidant factors including the Nrf2 pathway, heme oxygenase, and glutamate cysteine ligase (GCL) [14, 29–31]. The present study investigated whether quercetin could modulate paraoxonase 2 (PON2), which has potent antioxidant activity [32–36].

PON2 is the oldest member of the paraoxonase family of three genes (PON1, PON2, PON3) which are clustered in tandem on the long arm of human chromosome 7q21–22, and on mouse chromosome 6 [37, 38]. PON2, which is expressed in most tissues including the brain, has been shown to exert an antioxidant effect in cells [32–36, 39]. Sub-cellular distribution studies have shown that PON2 is localized primarily in the mitochondria, a major source of free radical-related oxidative stress [38, 40, 41]. PON2 has been shown to bind to Coenzyme Q_{10} that associates with Complex III in mitochondria, and PON2 deficiency causes mitochondrial dysfunction [41]. PON2 protein, mRNA and lactonase activity were found in all mouse brain regions, with the highest levels in three dopaminergic regions (striatum, substantia nigra, nucleus accumbens). PON2 levels were higher in astrocytes than in neurons, and in both cell types the ability of two oxidants [hydrogen peroxide (H_2O_2) and 2,3-dimethoxy-1,4-naphthoquinone (DMNQ)] to induce the formation of reactive oxygen species and to decrease cell viability was significantly enhanced in cells from PON2 knockout (PON2$^{-/-}$) mice, despite similar glutathione levels [38].

As higher PON2 levels are associated with increased resistance to oxidative stress-induced toxicity, the possibility of modulating PON2 levels in cells may represent a neuroprotective strategy of much interest. While there is a substantial amount of work on the modulation of PON1 [42], only limited research has been carried out on PON2. In macrophages, PON2 expression was increased by oxidative stress [43], and by urokinase plasminogen activator [44, 45], while arachidonic acid [46], unesterified cholesterol [47], the licorice phytoestrogen glabidrin [48], and the hypocholesterolemic drug atorvastatin [49] have been reported to up-regulate PON2 expression in various cell types. With regard to polyphenols, pomegranate juice was found to increase PON2 in macrophages through activation of the PPAR- and AP-1 pathways [50], while extracts of Yerba mate (*Ilex paraguariensis*) have recently been reported to increase PON2 mRNA and lactonase activity in macrophages *in vitro* and after *in vivo* administration to healthy women [51]. A single study with quercetin reported that this compound increased PON2 mRNA and protein in macrophages *in vitro*

[52]. Results of the present study indicate that quercetin increases levels of PON2 in mouse brain cells, and that the neuroprotective effects of this polyphenol are greatly diminished in the absence of PON2.

Materials and Methods

Materials

DMEM medium, RPMI 1640 medium, fetal bovine serum (FBS), and Hank's balanced salt solution (HBSS) were from Invitrogen (Carlsbad, CA). Anti PON2, PON1, PON3 antibodies were from Abcam (Cambridge, MA, USA). Dimethylsulfoxide (DMSO), hydrogen peroxide (H_2O_2), 2,3-dimethoxy-1,4-naphthoquinone (DMNQ), mouse anti-β-actin antibody, dihydrocoumarin (3,4-dihydro-2H,1-benzopyran-2-one), 3-(4,5-dimethyltiazol-2-yl)-2,5 diphenyltetrazolium bromide (MTT), 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-1-benzopyran-4-one (quercetin; >95%), and 2-chloro-5-nitro-N-phenylbenzamide (GW9662) were from Sigma-Aldrich (St. Louis, MO). Anthra[1,9–cd]pyrazol-6(2H)-one (SP600125) was obtained from Cayman Chemical (Ann Arbor, MI), while rosiglitazone was from Enzo Life Sciences (Farmingdale, NY).

Animals

Wild-type and PON2 knockout mice, kindly provided by Drs. A.J. Lusis, D.M. Shih and S. Reddy (UCLA) were used in this study. Mice were sacrificed at postnatal day (PND) 0.5 for preparation of primary striatal astrocytes and neurons. Mice were housed in a specific pathogen-free facility with *ad libitum* access to food and water and a 12-h light cycle. All procedures for animal use were in accordance with the National Institute of Health Guide for the Use and Care of Laboratory Animals, and were approved by the University of Washington Institutional Animal Care and Use Committee.

Cell cultures

Primary striatal astrocytes and neurons were obtained from PND 0.5 mice, as previously described [36, 39]. For astrocyte preparation, the striatum was dissected, mechanically dissociated and incubated with trypsin, followed by trituration, repeated washing, and filtration. After counting, cells were plated at a density of 10^7 cells per flask in 75 cm^2 tissue culture flasks pre-coated with poly-D-lysine and grown in DMEM containing 10% FBS, 100 U/ml penicillin and 100 ug/ml streptomycin at 37°C in 5% CO_2 / 95% air. After 10 days in culture, cells were plated in 6-well plates for experiments at a density of 6×10^5 astrocytes/well, or in 48-well plates at a density of 5×10^4 astrocytes/well. Cultures of striatal neurons were prepared from 0.5 day-old mice, as described by Giordano et al. [36, 39]. The striatum was collected in HBSS medium containing 0.02% BSA and 10 mM HEPES. The tissue was digested for 25 min in HBSS containing papain (1 mg/ml) and DNAse (40 ug/ml) and centrifuged at $300 \times g$ for 5 min at room temperature. The supernatant (containing papain) was removed and the pellet was gently triturated in Neurobasal A medium with B27 supplement (Invitrogen), using a Pasteur pipette to dissociate larger aggregates. Cells were centrifuged at $200 \times g$ for 10 min and the cell pellet was gently resuspended. Neurons were then counted, seeded on poly-D-lysine coated 6-well plates at a density of 6×10^5 neurons/well, and cultured in neurobasal medium supplemented with B27 (minus antioxidant). Neurons were cultured for eight days before experiments. Mouse RAW264.7 macrophages were grown in RPMI containing 10% FBS, 100 u/ml penicillin and 100ug/ml streptomycin at 37°C in 5% CO_2/95% air.

Western blots

Immunoblots were carried out as described by Giordano et al. [36, 39]. Twenty-five ug of protein were subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotted using antibodies against PON1, PON2, PON3 and β-actin. After electrophoresis, proteins were transferred to polyvinylidenedifluoride membranes that were incubated with antibodies using the following dilutions: 1:250 (for PON1, PON2, and PON3), and 1:750 (for β-actin). After the transfer blots were rinsed in Tris-buffered saline (pH=7.5), blocked for one hour, and incubated with horseradish peroxidase-conjugated anti-rabbit secondary antibody a dilution of 1:750 (for PON2 and PON3), or incubated with horseradish peroxidase-conjugated anti-mouse secondary antibody at the dilutions of 1:1000 for β-actin, or 1:750 for PON1. Band intensity was measured by densitometry using ImageJ (provided by the National Institutes of Health), and the intensity of the bands was normalized to β-actin content.

RT-PCR

Reverse transcription was performed according to the manufacturer's established protocol using total RNA and the SuperScript® III First-Strand Synthesis System. For gene expression measurements, 4 uL of cDNA were included in a PCR reaction (25 uL final volume) that also consisted of the appropriate forward (FP) and reverse (RP) primers at 360 nM each, 80 nMTaqMan probe, and TaqMan Gene Expression Master Mix. The PCR primers and the dual-labeled probes [6–carboxyfluorescein (FAM) and 6-carboxy-tetramethyl-rhodamine (TAMRA)] for all genes were designed using ABI Primer Express v. 1.5 software (Applied Biosystems Inc., Foster City, CA). Amplification and detection of PCR amplicons were performed with the ABI PRISM 7900 system (Applied Biosystems Inc., Foster City, CA) with the following PCR reaction profile: 1 cycle of 95°C for 10 min, 40 cycles of 95°C for 30 sec and 62°C for 1 min. β-actin amplification plots derived from serial dilutions of an established reference sample were used to create a linear regression formula in order to calculate expression levels, and β-*actin* gene expression levels were utilized as an internal control to normalize the data.

PON2 activity assay

PON2 lactonase activity was measured as described earlier [36, 39, 43]. Briefly, cells were washed and resuspended in 400 uL Tris buffer containing 25 mM Tris/HCl, pH 7.6 / 1 mM $CaCl_2$. The homogenates were sonicated twice for 10 seconds on ice. A 50 uL aliquot was saved for the measurement of protein concentration. Enzyme activity was measured using 200 to 300 ug of protein per mL assay mixture. After 10 minutes of incubation, the lactonase activity was measured kinetically using dihydrocoumarin (DHC) as substrate, by monitoring the absorbance at 270 nm in a NanoDrop 2000 (Thermo Scientific).

Cytotoxicity assay

Cell viability was quantified colorimetrically using the metabolic dye MTT, as previously described [36, 39]. Cells in 24-well plates were pre-treated for 24 h with 20 uM quercetin or solvent control and then washed with PBS and treated for 24 h at 37°C with either H_2O_2 or DMNQ, dissolved in distilled water and DMSO, respectively. At the end of exposure, medium was removed and cells were incubated with 0.50 ml/well of Locke's buffer solution containing 5 ug/ml MTT for 30 min. MTT was removed and the reaction product was dissolved in 0.25 ml DMSO/well. The absorbance was read at 570 nm in a SpectraMax 190 microplate reader, and results expressed as percentage of viable cells relative to controls. Values of IC_{50} were determined from concentration-response curves using 4–5 concentrations of each compound.

Assay of reactive oxygen species (ROS) formation

ROS formation was determined by fluorescence using 2',7'-dichlorofluorescin diacetate ($DCFH_2$-DA), as previously described [36]. $DCFH_2$-DA is readily taken up by cells and is subsequently de-esterified to $DCFH_2$, which can be oxidized to dichlorofluorescein (DCF) by hydrogen peroxide, peroxynitrite, and other ROS or reactive nitrogen species. Cells were pre-treated with 20 uM quercetin for 24 hours or left untreated as controls. Cells were first washed with Locke's solution and then pre-incubated for 30 min at 37°C with $DCFH_2$-DA in Locke's solution. $DCFH_2$ was added from a stock solution prepared in DMSO which was also added to the blank. Cells were then washed with Locke's solution to remove extracellular $DCFH_2$-DA before treatments with H_2O_2 or DMNQ. After treatments (at 37°C), cells were washed twice with Locke's buffer and lysed with 0.1 M KH_2PO_4 and 0.5% Triton X-100, pH 7.2 for 5 min. Cell lysates were then scraped from the dishes, and the supernatant was collected. The fluorescence ($_{EX}$ = 488 nm and $_{EM}$ = 525 nm) was read immediately, using a SpectraMax 190 spectrophotometric plate reader. ROS formation was expressed as the amount of fluorescence per mg of protein measured using the bicinchoninic acid protein assay kit.

Statistical analysis

Data are expressed as the mean ± S.D. of at least three independent experiments. Statistical analysis was performed using one-way or two-way ANOVA followed by a Bonferroni test for multiple comparisons.

Results

We had previously shown that PON2 levels in mouse brain were highest in the striatum [36]. We thus chose to carry out the present studies in astrocytes and neurons from this brain region. Fig. 1A shows that levels of PON2 protein are higher in mixed-gender striatal astrocytes than neurons, confirming previous observations [36, 39]. Data presented in Fig. 1A refer to the 42 kDa band, corresponding to the molecular weight of PON2, though a second band (at 55 kDa) was also evident (Fig. 1B). This second band, found by several but not all investigators, may represent a splice variant of PON2; however, its exact nature has not been determined. Levels of PON2 mRNA were also higher in striatal astrocytes than in neurons (Fig. 1C), as was lactonase activity, measured by dihydrocoumarin hydrolysis (Fig. 1D). All PONs have lactonase activity [53]; however, given the virtual absence of PON1 and PON3 in mouse striatal astrocytes and neurons (Fig. 1A), the measured lactonase activity can be ascribed solely to PON2.

Quercetin (1, 10, or 20 uM) increased PON2 expression in striatal astrocytes in a concentration-dependent manner, with a maximum increase of about two-fold (Fig. 2). No cytotoxicity (assessed by the MTT assay) was observed at these concentrations (not shown). Similar increases of PON2 were also found in mouse striatal neurons (180 ± 6% of control at 20 uM quercetin; n=3; p<0.05), and in mouse RAW264.7 macrophages (168 ± 12% of control at 20 uM quercetin; n=3; p<0.05). The increase of PON2 by quercetin in astrocytes was antagonized by SP600125, an inhibitor of the JNK (c-Jun-N-terminal kinase, also known as SAPK, stress-activated protein kinase)/AP-1 pathway, but not by GW9662, a PPARγ inhibitor (Fig. 3). The PPAR agonist rosiglitazone also increased PON2 expression by about two-fold, and in contrast to quercetin, the increase in PON2 expression by rosiglitazone was abrogated by incubating the astrocytes with the PPAR inhibitor GW9662 (Fig. 3).

To assess the functional consequences of PON2 induction by quercetin, striatal astrocytes (mixed gender) were pre-treated with quercetin (20 uM for 24 h, which increased PON2

protein levels by ~2.5-fold) followed by washout, and the ability of two oxidants, H_2O_2 and DMNQ, to induce oxidative stress was determined. Fig. 4 shows that quercetin pre-treatment significantly decreased ROS production induced by both oxidants. The relevance of PON2 induction by quercetin as a mechanism of neuroprotection was further probed by assessing cytotoxicity induced by H_2O_2 and DMNQ in striatal astrocytes (Table 1). As seen previously (Giordano et al. 2011), striatal astrocytes from PON2$^{-/-}$ mice were much more sensitive than astrocytes from wild-type mice to the toxicity of the two oxidants (~6-fold decrease in their IC_{50} values; Table 1). In wild-type astrocytes, pre-treatment with quercetin significantly decreased the toxicity of the two oxidants, as evidenced by a 4-fold increase in their IC_{50} value (Table 1). In contrast, the same treatment with quercetin in striatal astrocytes from PON2$^{-/-}$ mice afforded a much lower degree of protection (<2-fold; Table 1).

Discussion

Quercetin is a common flavonoid polyphenol shown to have neuroprotective actions *in vitro* and *in vivo* [11–22]. Mechanism(s) of such neuroprotection remain elusive, though it has been suggested that quercetin, as well as other polyphenols, may induce the cell's own ability to counteract oxidative stress-induced toxicity [25–28]. We have recently characterized PON2 in brain, and have found that this enzyme confers protection against oxidative stress-induced neurotoxicity [36, 39]. As quercetin had been shown to increase PON2 levels in macrophages [52], we sought to investigate whether a similar effect would be seen in brain cells, and to determine its functional consequences. Our findings indicate that quercetin increases PON2 expression in mouse striatal astrocytes (chosen because they express the highest levels of PON2 among brain regions and cell types; [36]), and in mouse striatal neurons, in addition to macrophages [52]. Such induction of PON2 by quercetin was paralleled by an increased ability of the cells to scavenge ROS and to antagonize oxidant-induced toxicity. Sub-cellular distribution studies have shown that PON2 is localized primarily in the mitochondria [36, 41]. Mitochondria are a major contributor of cellular ROS; ROS produced in the mitochondria can also target the electron transport chain (e.g. complex I), resulting in a cycle generating more ROS, followed by ATP depletion and ultimately cell death [2, 54]. Oxidative stress is recognized as an important factor in a variety of neurodegenerative diseases, in the adverse effects of a number of neurotoxicants, and as a mechanism for age-related degenerative processes [4–6]. 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 Complex III in mitochondria, and PON2 deficiency causes mitochondrial dysfunction [41]. 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 [55]. Of relevance is the fact that mitochondria, together with the cytoplasm and the nucleus, are also preferential accumulation sites for quercetin in cells [56–58].

Shiner et al. [50] reported that polyphenols in pomegranate juice could increase PON2 levels through the PPAR and JNK/AP-1 pathways. In astrocytes, inhibition of PPAR did not affect quercetin's induction of PON2; in contrast, the effect of quercetin was completely antagonized by SP600125, a compound which inhibits JNK by inhibiting the ATP binding site of the kinase [59]. This would suggest an involvement of JNK in the effect of quercetin. JNK is usually activated by a variety of stimuli, including oxidative stress, and phosphorylates various transcription factors such as c-Jun, Elk-1, and AP-1, leading to changes in gene expression [60]. As recently suggested [61, 62], quercetin may thus induce a very low-level of oxidative stress (as shown for example, in hepatoma cells; [63]), which

in turn would activate the JNK/AP-1 pathway [64], causing an increased expression of PON2.

PON2 levels have been recently shown to be modulated by estrogens through activation of estrogen receptor (ER)-alpha [39]. Though quercetin has been shown to exert estrogenic effects, its classification as a phytoestrogen is a matter of debate, as contrasting findings have been reported [65, 66] particularly with respect to the involvement of ER-alpha and ER-beta [57, 67, 68]. Thus, the potential contribution of ERs in quercetin's effects on PON2 remains to be investigated.

Independent of the underlying cellular/molecular mechanisms, the ability of quercetin to increase intracellular levels of PON2 has important functional consequences, as shown by the results of Fig. 4 and of Table 1. An increase of PON2 by 2–2.5 fold protects cells against oxidative stress induced by two oxidants (H_2O_2 and DMNQ), and significantly diminishes their toxicity. We had previously shown that a 2–3-fold differential expression of PON2 levels, as observed between brain cells from male and female mice, similarly caused a 3–4-fold difference in susceptibility to the toxicity of these two oxidants [39]. Other intracellular pathways such as Nrf2, heme oxygenase, and GCL [14, 29–31] have been shown to be affected by quercetin and may also contribute to its neuroprotective action. However, experiments done in cells from PON2$^{-/-}$ mice clearly show that the neuroprotective effect of quercetin is significantly reduced, though not completely abolished, by the absence of PON2 (Table 1). This finding provides strong support for an important role of PON2 induction in neuroprotection afforded by quercetin.

A limitation of the present studies is that they show an *in vitro* effect of quercetin, which will need to be confirmed *in vivo*. Quercetin is present in foods as glycosides that can be efficiently absorbed [69]. Total quercetin derived from the diet is normally present in plasma in the nanomolar range (<100 nM), but can be increased in the micromolar range after supplementation [70, 71]. Furthermore, the half-life of quercetin ranges between 11 and 28 h, suggesting the possibility of significantly increasing plasma concentration upon supplementation [71]. *In vitro* studies with blood-brain barrier models consistently indicate that quercetin enters the brain [72–74]. Upon administration of quercetin *in vivo,* nanomolar levels are found in brain tissue [74–77], though bioavailability of quercetin can be increased [78]. In particular, the formulation of quercetin in lipid nanoparticles significantly increases its penetration in the brain [79–81]. Above all, however, oral quercetin *in vivo* has been shown to protect rodents from oxidative stress and neurotoxicity induced by a variety of agents [16–22].

An additional limitation of the present study is that only the aglycone, i.e. quercetin itself, was tested. Quercetin is known to be metabolized to various conjugated metabolites, such as 3'-O-methyl-quercetin (isorhamnetin), quercetin-3-O-glucuronide, 3'-O-methylquercetin-O-glucuronide, and quercetin-3'-O-sulfate [9, 82]. Interestingly, conjugated quercetin has been shown to enter the cell where it can be converted to its non-conjugated form [57, 83]. Nevertheless, the ability of such metabolites to affect intracellular levels of PON2 would be an important avenue of investigation. It is relevant that various other polyphenol catabolites have been shown to exert strong biological activity, particularly in protecting neuronal cells against DMNQ-induced oxidative stress and toxicity [84].

In summary, the common flavonoid polyphenol quercetin, which had been reported to exert neuroprotection *in vitro* and *in vivo*, has been shown to increase the expression of PON2 in brain cells. The mechanism of such induction appears to involve activation of the JNK/AP-1 pathway, but alternative pathways need to be further investigated. The neuroprotective action of quercetin *in vitro* was significantly reduced in cells lacking PON2, thereby

suggesting that PON2 induction by quercetin represents an important component of its neuroprotective action, possibly shared by other polyphenols.

Acknowledgments

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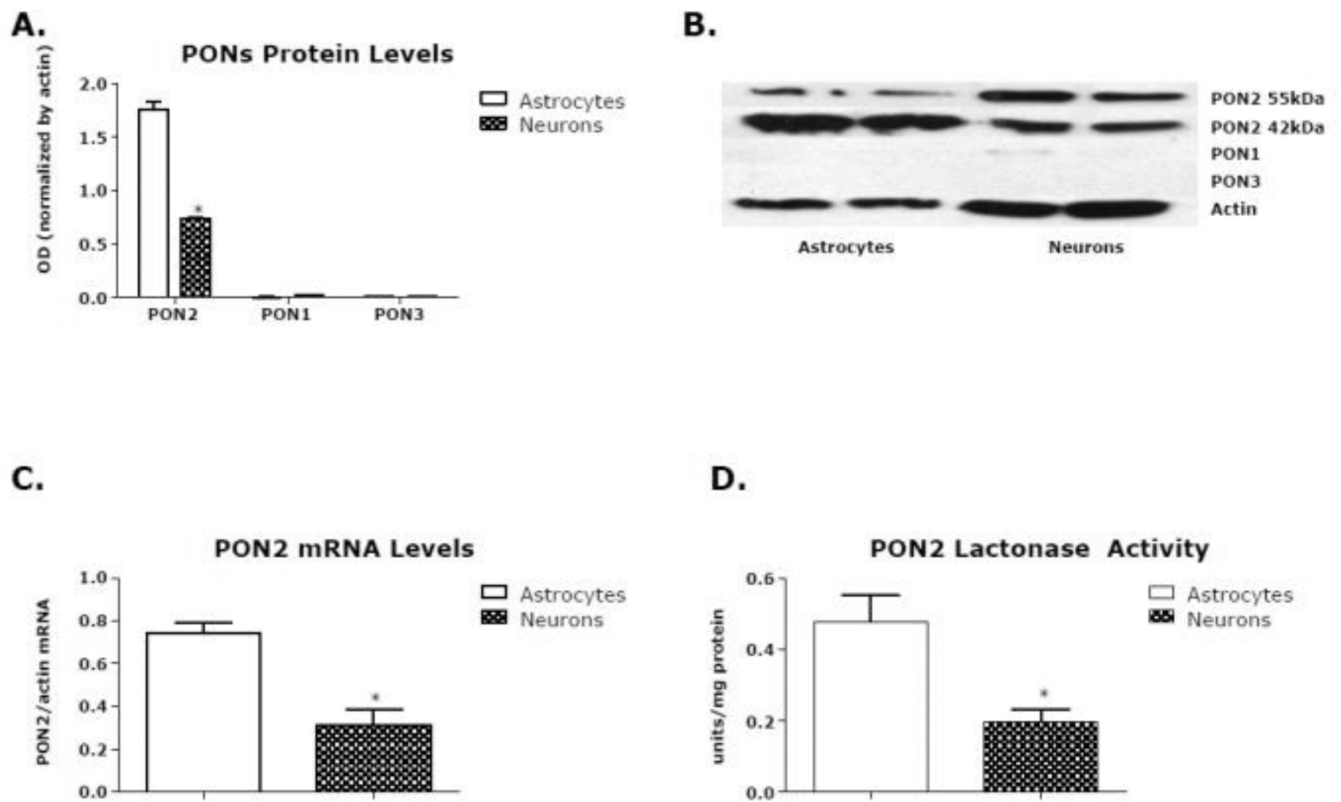
Fig. 1. Levels of PONs in mouse striatal astrocytes and neurons
(A) PON2, PON1, and PON3 protein level in primary mouse striatal astrocytes and neurons.
(B) Example of blot of data of A. (C) PON2 mRNA levels in primary mouse striatal
astrocytes and neurons. (D) PON2 lactonase activity levels in primary mouse striatal
astrocytes and neurons. Results are the mean (± SD) of three independent experiments.
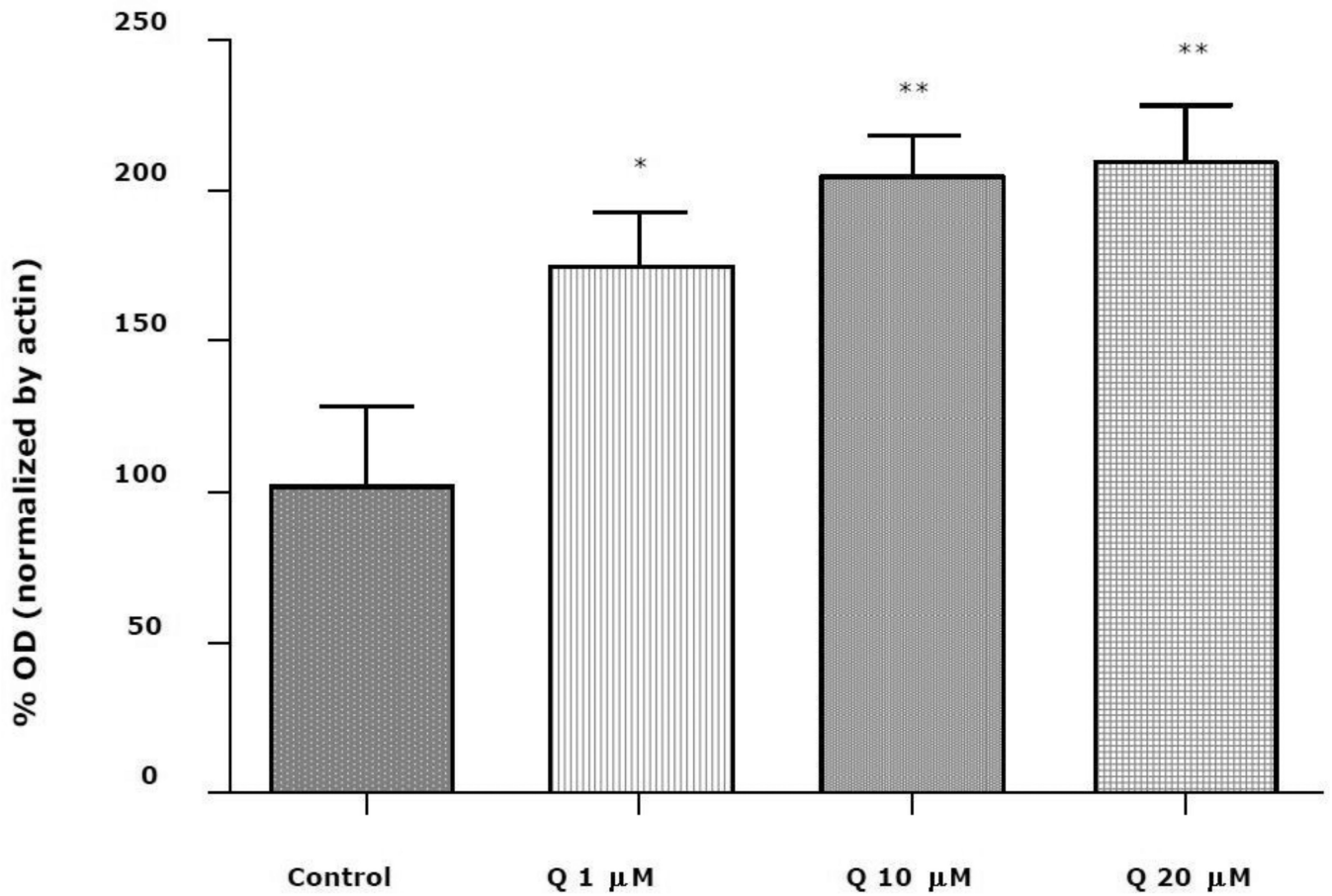*Significantly different from astrocytes, $p < 0.05$.

Fig. 2. Concentration-response effect of quercetin on PON2 protein level in mouse striatal astrocytes
PON2 protein expression in mixed-gender mouse striatal astrocytes following 24 hours exposure to 1, 10, or 20 uM quercetin (Q). Graph shows the quantification of the 42 kDa PON2 alloform. Results are the mean ($\pm$ SD) of at least three independent experiments. Significantly different from control, *p<0.05, **p<0.01
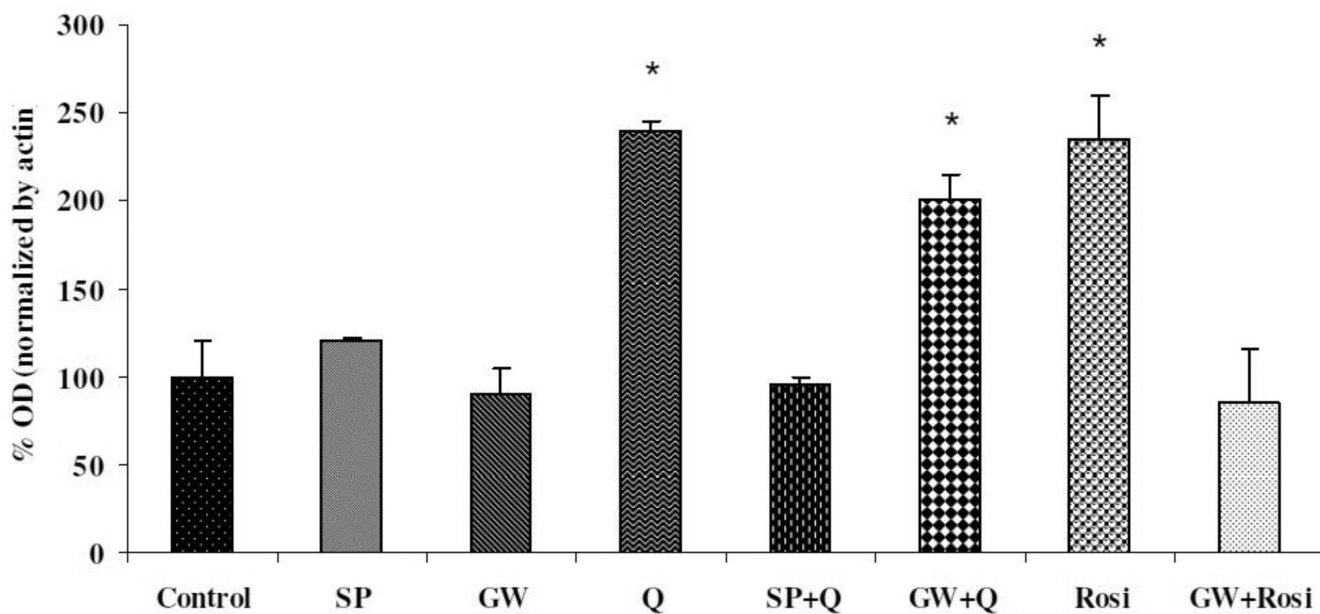
Fig. 3. Effect of JNK/AP-1 and PPAR inhibition on induction of PON2 by quercetin in mouse striatal astrocytes
Mouse striatal astrocytes (mixed gender) were exposed for 24 h to 20 uM quercetin (Q) or 50 uM rosiglitazone (Rosi), with or without 1 hour pre-treatment with 20 uM of the JNK/AP-1 inhibitor SP600125 (SP), or 50 uM of the PPARγ inhibitor GW9662 (GW). Results are the mean (± SD) of three independent experiments. Significantly different from control, *p<0.05.
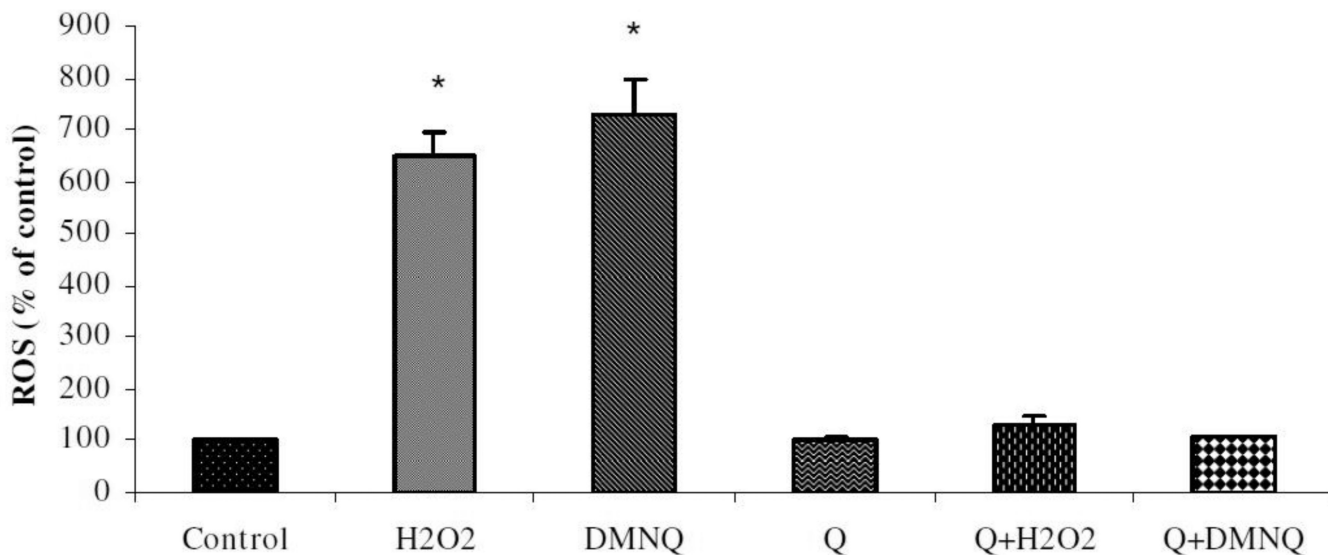
Fig. 4. Oxidative stress in mouse striatal astrocytes treated with quercetin
Mouse striatal astrocytes (mixed gender) were pre-treated for 24 hours with 20 uM quercetin (Q) or solvent control. After wash-out, cells were exposed to 50 uM H_2O_2 or 50 uM DMNQ. ROS formation was measured by fluorescence of dichlorofluorescein (DCF). Results are the mean ($\pm$ SD) of at least three independent experiments. Significantly different from control, $^*p<0.01$.

Table 1

Quercetin protection against oxidative stress-induced toxicity requires the presence of PON2

Cell type/Oxidant	IC_{50} (uM)		Ratio
	Control	**Quercetin**	
Wild-type striatal astrocytes			
H_2O_2	38.9 ± 4.5	157.0 ± 8.1 [*]	4.0
DMNQ	37.5 ± 5.6	131.3 ± 9.2 [*]	3.5
PON2$^{-/-}$ striatal astrocytes			
H_2O_2	6.3 ± 1.3	11.9 ± 1.2	1.9
DMNQ	6.1 ± 1.0	8.3 ± 1.1	1.4

Mixed gender striatal astrocytes were exposed for 24 h to 20 uM quercetin. After washout, cells were treated for 24 h with 4–5 concentrations of oxidants and cytotoxicity as measured by the MTT assay. Values are the mean (± SD) of 3 separate experiments.

[*]
Significantly different from wild-type control, p<0.01.