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Quercetin Protects Mouse Brain against Lead-Induced Neurotoxicity

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ABSTRACT: Quercetin (QE), the major bioflavonoid in the human diet, has been reported to have many benefits and medicinal properties. However, its protective effects against lead (Pb)-induced neurotoxicity have not been clarified. The aim of the present study was to investigate the effects of QE on neurotoxicity in mice exposed to Pb. Mice were exposed to lead acetate (20 mg/kg body weight/day) intragastrically with or without QE (15 and 30 mg/kg body weight/day) coadministration for 3 months. The data showed that QE significantly prevented Pb-induced neurotoxicity in a dose-dependent manner. Exploration of the underlying mechanisms of QE action revealed that QE administration decreased Pb contents in blood (13.2, 19.1%) and brain (17.1, 20.0%). QE markedly increased NO production (39.1, 61.1%) and PKA activity (51.0, 57.8%) in brains of Pb-treated mice. Additionally, QE remarkably suppressed Pb-induced oxidative stress in mouse brain. Western blot analysis showed that QE increased the phosphorylations of Akt, CaMKII nNOS, eNOS, and CREB in brains of Pb-treated mice. The results suggest that QE can inhibit Pb-induced neurotoxicity and partly restore PKA, Akt, NOS, CaMKII, and CREB activities.

KEYWORDS: *quercetin, lead, neurotoxicity, PKA, CREB*

INTRODUCTION

Lead (Pb) is a ubiquitous pollutant in the environment. Lead has been commonly used since the prehistoric era, and its production and consumption have increased in recent decades. Human exposure to Pb occurs via food, water, air, and soil. It is also prevalent in occupational settings such as smelters and Pb manufacturing industries.[1] Lead is a systemic toxicant affecting virtually every organ system and, notably, the central nervous system.[2] Oxidative damage is considered an important factor in Pb neurotoxicity. Experimental evidence suggests that Pb induces oxidative stress and exerts some of its toxic effects through the disruption of the pro-oxidant/antioxidant balance, leading to brain injury via oxidative damage.[3] Many papers have been published on the molecular mechanisms of Pb neurotoxicity. One hypothesis is that lead impairs processes involving nitric oxide synthase, with serious repercussions on learning and memory.[3,4] Nitric oxide (NO) plays an important role in the function of brain processes that involve synaptogenesis, cerebral blood flow, neuroendocrine secretion, and neurotransmission.[5] In the central nervous system, NO can be produced by the stoichiometric conversion of L-arginine to L-citrulline via various isoforms of nitric oxide synthases (NOS) (neuronal NOS, nNOS, or NOS-I, endothelial NOS, eNOS, or NOS-III, and inducible NOS, iNOS, or NOS-II).[6] Several studies have reported a decrease in NOS activity and NO production in animals exposed to Pb.[3,7] Pb also affects processes involved in NOS expression. It is possible that the Pb cation negatively acts on Ca^{2+}-dependent transcription elements for nNOS (the cyclic-AMP-response element-binding protein, CREB; cyclic adenosine monophosphate (cAMP) and eNOS (activator protein 1, AP-1) to decrease protein expression.[3] Many studies show that Pb can inhibit CREB phosphorylation by affecting several kinases, such as protein kinase B (Akt), extracellular signal-regulated kinase (ERK), protein kinase A (PKA), and calcium/calmodulin kinase II (CaMKII). These kinases act in signaling pathways that play important roles in learning and memory.[3,4]

The flavonoid quercetin (3,3′,4′,5,7-pentahydroxyflavone, QE), one of the most widely distributed flavonoids in fruits and vegetables, exhibits biological activity against many disorders and diseases, including ischemic heart disease, atherosclerosis, liver fibrosis, renal injury, and biliary obstruction.[8] Many studies have demonstrated that QE had neuroprotective effects in a variety of brain injury models.[9,10] Reports from our laboratory and others have demonstrated that QE possesses a multitude of activities including antioxidative activity,[8,10,11] anti-inflammation,[12−14] and antiapoptosis.[8,11] In addition, QE exerts its neuroprotective effect through the NO signaling pathway.[9,10]

The molecular mechanisms of lead-induced neurotoxicity and the neuroprotective effects of QE are not yet completely understood. Therefore, we have used an experimental model of mice chronically treated with Pb to study Pb-induced neurotoxicity and whether QE can protect rat brain from Pb-induced neurotoxicity. The PKA/Akt/NOS/NO and PKA/Akt/CaMKII/CREB pathways were also investigated. On the basis of our results, a mechanism of action can be suggested.

MATERIALS AND METHODS

Chemicals. Quercetin (3,3′,4′,5,7-pentahydroxy flavone), lead acetate [$(C_2H_3O_2)_2Pb{\cdot}3H_2O$] (PbAc), and sodium acetate [$CH_3COONa{\cdot}3H_2O$] (NaAc) were obtained from Sigma Chemical Co. (St. Louis, MO, USA); anti-nNOS antibody, anti-eNOS antibody, anti-iNOS antibody, antiphospho-Akt antibody, anti-Akt (total) antibody, antiphospho-ERK1/2 antibody, anti-ERK1/2 (total) antibody, antiphospho-CaMKII antibody, and anti-CaMKII antibody were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA); and

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anti-CREB antibody and antiphospho-CREB antibody were obtained from Cell Signaling Technology (Beverly, MA, USA). The total antioxidant capacity (TAC) was determined using brain assay kits from Nanjing Jiancheng Bioengineering Institute (Nanjing, China) and Jingmei Biotech Ltd. (Shenzhen, China); protein concentration was measured using the BCA assay kit from Pierce Biotechnology, Inc. (Rockford, IL, USA). All other reagents unless indicated were obtained from Sigma Chemical Co.

Animals and Treatment. Kunming mice (male, 4 weeks old, weighing approximately 18 g) were purchased from the National Breeder Center of Rodents (Beijing, China). The mice were maintained under constant conditions (23 ± 1 °C and 60% humidity) with free access to rodent food and tap water under a 12 h light/dark schedule (lights on from 08:30 a.m. to 8:30 p.m.).[7,11] After acclimatization to laboratory conditions, the mice were randomly divided into five groups of 10 mice each.

(1) Control group: The mice were given 15 mg NaAc/kg bwt intragastrically once daily. (2) Lead-treated group: The mice received 20 mg PbAc/kg bwt intragastrically once daily. (3) Lead+QE (15 mg) treated group I: The mice received 15 mg QE/kg bwt once daily and were concurrently supplemented with 20 mg PbAc/kg bwt 6 h after QE administration using intragastric intubation. (4) Lead+QE (30 mg) treated group: The mice received 30 mg QE/kg bwt once daily and were concurrently supplemented with 20 mg PbAc/kg bwt 6 h after QE administration using intragastric intubation. (5) QE (30 mg) treated group: The mice received 30 mg QE/kg bwt intragastrically once daily. The choices of PbAc and NaAc doses are based on previous findings.[13] The dose of 20 mg PbAc/kg bwt is about $^1/_{10}$ of the LD_{50}. In these studies, Pb markedly induced neurotoxicity after long exposure.[15] The choice of QE doses is based on previous findings.[14,16,17]

The experiment lasted 3 months. The food intake, water intake, and body weight of mice were measured daily. At the end of treatment, the mice were sacrificed, and 1 mL blood samples were drawn by cardiac puncture with heparinated tubes. Brain tissues were quickly collected, placed in ice-cold 0.9% NaCl solution, perfused with physiological saline solution to remove blood cells, blotted on filter paper, and stored at −70 °C for later use.

This research was conducted in accordance with Chinese laws and NIH publications on the use and care of laboratory animals. Relevant university committees for animal experiments approved these experiments.

Pb Content Assay. Measurement of Pb concentrations in the blood and brain was based on the method of Moniuszko-Jakoniuk et al.[18] Samples of blood collected in heparinized tubes (assigned for Pb determination) were wet-digested with 5% HNO_3. Known amounts of whole brain (approximating 1 g) were dried at 110 °C and mineralized in an electric oven at 450 °C. The resulting ash was dissolved in 1 M HNO_3. Pb concentrations in the prepared samples of blood and brain were measured (after appropriate dilution) by atomic absorption spectrometry (AAS) at the resonance line of 283.3 nm with flameless atomization in a graphite furnace using a Zeeman Z-5000 atomic absorption spectrophotometer (Hitachi, Japan).

Reactive Oxygen Species (ROS) Assay. ROS was measured as described previously on the basis of the oxidation of 2′,7′-dichlorodihydrofluorescein diacetate to 2′,7′-dichlorofluorescein.[7,11]

Lipid Peroxidation Assay. Estimation of lipid peroxidation (TBARS) was performed according to our previous method.[12] A standard calibration curve was prepared by using 1−10 nM 1,1,3,3-tetramethoxypropane. The concentration was expressed in terms of nanomoles of TBARS per milligram of protein.

Total Antioxidant Capacity (TAC) Assay. The total antioxidant capacity in brain was assayed with a commercially available assay kit (Jiancheng Biochemical, Inc., Nanjing, China).[12,19] This method is based on the reduction of iron(III) in acidic medium by intracellular antioxidants. Liberated iron(II) reacts with 1,10-phenanthroline to form a colored complex, which is measured at 520 nm. One unit of TAC is defined as 0.01 optical density (OD_{520}) units per milligram of protein per minute at 37 °C.

NO Assay. The measurement of NO (NO_3^-/NO_2^-) in brain homogenates was performed using the NO_2/NO_3 assay kit (Jingmei Biotech Ltd., Shenzhen, China) according to the manufacturer's instructions. The concentration of total nitrite, expressed as micromoles per milligram of protein, was represented as NO production in the homogenates.[20]

PKA Activity Assay. The PKA activity in the striatum of mice was measured in detergent-soluble extracts of the striatum using the nonradioactive PKA kinase activity kit (Assay Designs, Ann Arbor, MI, USA) according to the manufacturer's instructions. This assay is a solid-phase ELISA that measures the phosphorylation of a synthetic peptide substrate for PKA using a polyclonal antibody against the phosphorylated form of the substrate. The values are expressed as a percentage of the control values, which were considered to represent 100% activity. All of the determinations were performed at least in quintuplicate and repeated twice in separate experiments. One of two experiments with similar results is reported.[21]

Western Blot Analyses. Tissues were homogenized in ice-cold lysis buffer (TBS, 1% NP-40, 0.25% sodium deoxycholate, 1 mM EDTA, 10 mg/mL aprotenin, 10 mg/mL leupeptin, 1 mM PMSF, 10 mM β-glycerophosphate, 1 mM Na_3VO_4, 1 mM NaF). Homogenates were centrifuged at 10000*g* for 10 min at 4 °C. The supernatants were collected and centrifuged again, and the final supernatants were collected. Nuclear and cytoplasmic extracts for Western blotting were obtained by using a nuclear/cytoplasmic isolation kit (Beyotime Institute of Biotechnology, Bijing, China). Protein levels were determined using the BCA assay kit (Pierce Biotechnology, Inc., Rockford, IL, USA). Samples (80 μg each) were separated by denaturing SDS-PAGE and collected on a PVDF membrane (Roche Diagnostics Corp., Indianapolis, IN, USA) by electrophoretic transfer (Bio-Rad Laboratories, Inc., Hercules, CA, USA). The membrane was preblocked with 5% BSA and 0.1% Tween-20 in Tris-buffered saline (TBST) and incubated overnight with the primary antibody (in TBST with 5% BSA). Each membrane was washed three times for 15 min and incubated with secondary horseradish peroxidase-linked antibodies (Santa Cruz Biotechnology, Santa Cruz, CA, USA, and Cell Signaling Technology, Beverly, MA, USA, respectively). Quantitation of detected bands was performed with Scion image analysis software (Scion Corp., Frederick, MD, USA). To correlate protein loading, the blots were analyzed for β-actin expression using an anti-β-actin antibody (Cell Signaling Technology). Each density was normalized using each corresponding β-actin density as an internal control and averaged from three samples. The density of the vehicle control was set at 1.0 to compare other groups.

Statistical Analysis. All statistical analyses were performed using SPSS software, version 11.5. A one-way analysis of variance (ANOVA; $P < 0.05$) was used to determine significant differences between groups, and the individual comparisons were obtained by Tukey's HSD post hoc test. Statistical significance was set at $P \leq 0.05$.

RESULTS

QE Decreased Pb Concentrations in Blood and Brain. As previously reported, Pb-induced neurotoxicity was observed by both diagnostic indicators and behavior analysis. Neither Pb nor QE administration caused mortality during the experimental period.[12,13] No differences were found in food and water consumption, body weight gain, red blood cell number, hematocrit, or biochemical and behavioral parameters after QE administration compared to control groups (data not shown), in accordance with previous research.[12,17] Pb concentrations in blood and brain of mice were shown in Figure 1. The Pb levels in blood and brain of Pb-treated mice were significantly higher than those of control mice ($P < 0.01$). However, treatment with QE decreased the blood and brain Pb concentrations of mice in a dose-dependent manner (Figure 1). There were no significant differences in Pb concentrations of blood and brain between the QE-treated mice and the control mice (Figure 1).

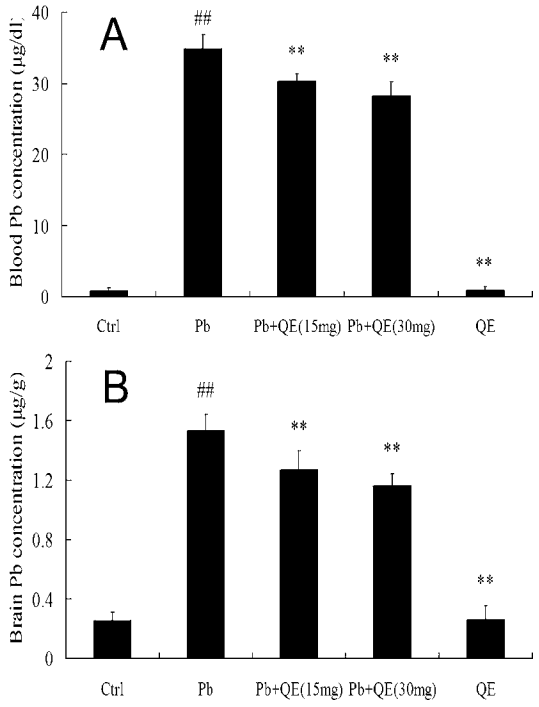

Figure 1. Effect of quercetin on Pb concentrations in mouse blood (A) and (B) brain. All values are expressed as the mean ± SEM (n = 7). (##) $P < 0.01$, compared with the control group; (**) $P < 0.01$, versus the lead-treated group.

QE Inhibited Pb-Induced Oxidative Stress in Brain. QE decreased lead-induced ROS and TBARS levels (Figure 2). On the other hand, Pb treatment markedly increased hepatic ROS and TBARS levels by 73 and 75% as compared with those of the controls, respectively ($P < 0.01$). Interestingly, treatment with QE showed dose-dependent inhibition in this elevation ($P < 0.01$) (Figure 2).

Many studies suggested that the TAC level might be an indicator of oxidative stress. As shown in Figure 2C, the TAC level in lead-treated mice was markedly decreased by 29% as compared with that of the control ($P < 0.01$). However, treatment with QE in lead-treated mice significantly increased the cerebric TAC level in a dose-dependent manner ($P < 0.01$).

No significant difference of levels of ROS, TBARS, and TAC could be seen in the brains from the mice treated with QE only, as compared with vehicle controls.

QE Increased NO Production in the Brain. NO is a signaling molecule with a variety of physiological functions. To determine whether the protective effects of QE are associated with changes in NO production in the brain of Pb-treated mice, we assayed the NO levels. As shown in Figure 3, the amounts of NO in the brains of Pb-treated mice significantly decreased (48%), as compared with those of the controls ($P < 0.01$). However, QE restored the brain NO levels of mice in a dose-dependent manner (Figure 3).

QE-Mediated Protective Action Involves NOS. NO is produced from L-arginine by NOS in the central nervous system.[1] To investigate whether NOS signaling was involved in the action of QE, we determined the effects of QE on nNOS, eNOS, and iNOS expressions in mouse brain. As shown in Figure 4, the expressions of eNOS and nNOS were markedly decreased in the brains of Pb-treated mice, as compared with the vehicle controls. However, QE administration markedly restored the expressions of eNOS and nNOS in Pb-treated

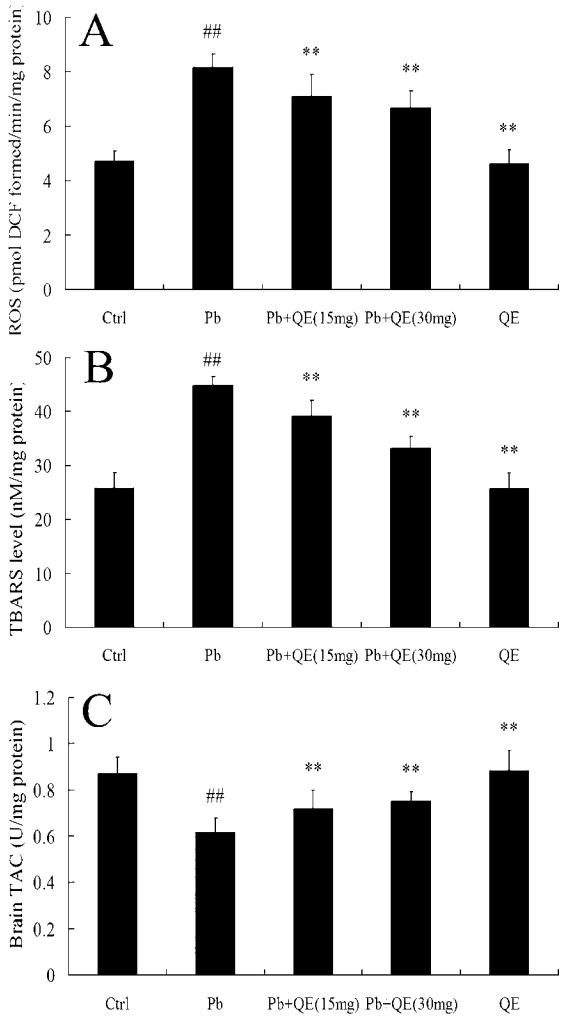

Figure 2. Effect of QE on the levels of (A) ROS, (B) TBARS, and (C) TAC in the brains of Pb-treated mice. Each value is expressed as the mean ± SEM (n = 7). (##) $P < 0.01$, compared with the control group; (**) $P < 0.01$, versus the Pb-treated group.

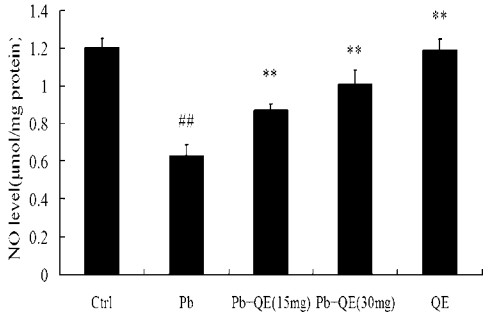

Figure 3. Effect of QE on the levels of NO in the brains of Pb-treated mice. Each value is expressed as the mean ± SEM (n = 7). (##) $P < 0.01$, compared with the control group; (**) $P < 0.01$, versus the Pb-treated group.

mice. No significant changes of iNOS expression were seen among the Pb-treated group, QE+Pb-treated group, QE-treated group, and the control group (Figure 4).

QE Increased PKA Activity in Brain. To investigate the possible mechanisms of protective effects of QE, we assayed brain PKA activity. As shown in Figure 5, PKA activity in the brains of Pb-treated mice decreased by 42%, as compared with

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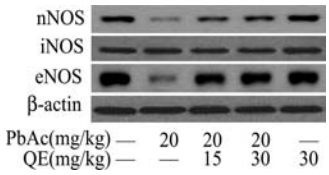

Figure 4. Effect of QE on the nitric oxide synthases (NOS) in the brains of Pb-treated mice.

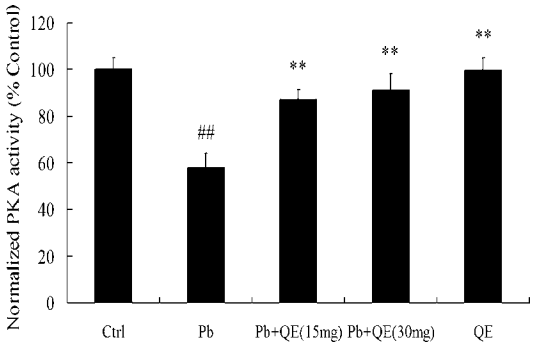

Figure 5. Effect of QE on the PKA activity in the brains of Pb-treated mice. Each value is expressed as the mean ± SEM (n = 7). (##) P < 0.01, compared with the control group; (**) P < 0.01, versus the Pb-treated group.

those of the controls (P < 0.01). However, QE restored the brain NO levels of mice in a dose-dependent manner (Figure 5). ANOVA analysis showed no differences in PKA activity between the QE-treated group and the control group (Figure 5).

Effect of QE on Expression of pAkt, pERK1/2, pCaMKII, and pCREB in Brain Tissues of Pb-Treated Mice. Activations of pAkt, pERK1/2, pCaMKII, and pCREB are known to be involved in regulating synaptic plasticity and dendritic morphology.[3,4,22] Therefore, we determined the effects of QE on expressions of pAkt, pERK1/2, pCaMKII, and pCREB in the brains of Pb-treated mice.

As shown in Figure 6, the expressions of pAkt, pCaMKII, and pCREB were markedly decreased in the brains of Pb-treated

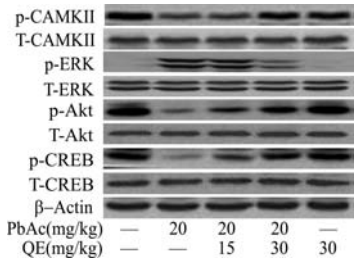

Figure 6. Changes in Akt, ERK, CaMKII, and CREB protein expressions in the brains of mice.

mice, as compared with the vehicle controls. However, down-regulation of pAkt, pCaMKII, and pCREB was markedly suppressed in the QE and Pb cotreated mice. Interestingly, the levels of pERK expression increased in the brain of Pb-treated mice, and administration of QE inhibited this elevation (Figure 6).

DISCUSSION

The central nervous system is the primary site of Pb-induced toxicity. Kunming mice are common laboratory animals for the study of the biological consequences of lead exposure.[23,24] Exposure to Pb is associated with neurobehavioral and psychological alterations, including inhibition of long-term potentiation (LTP), growth retardation, and learning and memory impairment.[25] QE is claimed to provide many health benefits.[17] A previous paper revealed that QE could relieve chronic lead exposure-induced impairment of synaptic plasticity in rat dentate gyrus in vivo, as measured by input/output (I/O) functions, paired-pulse reactions (PPR), and LTP.[26] In this study, our results in behavior and physiology were in accord with previous research[14,17] (data not shown). Moreover, many studies had shown that flavanoids could decrease accumulation of heavy metals in blood and tissue.[12,26,27] Complexes of flavonoids play an important role in limiting metal bioavailability and suppressing metal toxicity. QE, one of the flavonoids, is also a potential metal chelator. The hydroxyl group and the carbonyl group in the C ring of QE may be the main metal coordination domains that interact with Pb.[12,28] By forming complexes, QE can decrease free Pb concentrations in the brain. Therefore, QE appears to be a promising antidote for Pb poisoning in vivo.[7,26,29] The present study also showed that QE markedly decreased lead levels in the blood and brain of mice (Figure 1), which may partly explain the neuroprotective effect of QE.

NO has been shown to play an important role in cell signaling, neurotransmission, cell protection, and regulatory effects in various cells.[3,29] Therefore, alterations in NO production may be a causal factor in the development of neurotoxicity [1] through inhibition of LTP and interference with Ca^{2+}-dependent cellular processes. Several papers have demonstrated that Pb exposure could decrease NO production.[1,3] NO is generated by the NOS enzymes, of which there are three major isoforms: nNOS, eNOS, and iNOS. In this study, we found that lead significantly reduced the expression of nNOS and eNOS in the mouse brain compared to the control group (Figure 4). The effects of Pb may be attributable to this competitive inhibition between Pb^{2+} and Ca^{2+} at Ca^{2+} binding sites on proteins such as calmodulin.[3] However, no expression of iNOS was detected or observed, in accord with a previous study.[3] Perhaps this NO isoform is not regulated by Ca^{2+}[6,30] or the Pb doses used in these experiments are lower than those required to obtain the iNOS immunoreactivity response.[3] Pb did inhibit nNOS and eNOS expression levels, which could reduce NO production in the central nervous system.[3] Notably, in the present study, QE markedly restored the expression levels of nNOS and eNOS and increased NO production in the brains of Pb-treated mice (Figures 3 and 4), which suggests that QE may protect mouse brain by increasing NO production.

Accumulating evidence also showed that Pb caused oxidative stress by inducing the generation of ROS and weakening the antioxidant defense system of cells.[1,29] Our previous study showed that Pb could induce ROS production and decrease antioxidant enzymatic activities in liver and kidney.[7,11,12] In the present study, we found that Pb exposure induced overproduction of ROS and TBARS and decreased the TAC in mice (Figure 2). These changes could lead to brain oxidative damage. Moreover, overproduction of ROS could decrease NO levels in brain, because NO reacts rapidly with $O^{\bullet 2-}$ to produce the peroxynitrite anion ($ONOO^-$), which protonates at

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relevant pH to form peroxynitrous acid (ONOOH). Both ONOO− and ONOOH can cause nitrosative stress, which may lead to nitrosylation reactions that can alter the structure of proteins and so inhibit their normal function.[3] In this research, QE markedly inhibited Pb-induced oxidative stress in brains of Pb-treated mice (Figure 2), which may be due to QE's powerful antioxidant and free radical scavenging activities.[7,8,11,17] Our findings suggest that QE could at least partly attenuate Pb-induced neurotoxicity by inhibiting oxidative stress and increasing NO production (Figures 2 and 3).

Many experiments in rodents have shown that activation of the transcription factor CREB is necessary for learning, memory, and synaptic plasticity. CREB ultimately acts as an important part of the signal propagation from synapses to the nucleus by linking excitatory amino acid receptor (NMDAR) activation and calcium-dependent signaling to the expression of genes necessary for synaptic plasticity.[22] CREB also can regulate the expression of nNOS by interacting with Ca^{2+}-regulated genes, and these interactions are involved in diverse pathological and physiological processes, such as learning and memory.[3,22,31] The activation of CREB by phosphorylation can be regulated by several kinases, such as PKA,[32] Akt,[33] CaMKII,[34] and ERK.[22] The PKA/CREB signal pathway plays an important role in the establishment of short- and long-lasting forms of synaptic plasticity, learning, and memory.[3] Activated PKA can up-regulate Akt. Activated Akt further regulates eNOS and ultimately increases NO production.[7,33] Many studies showed that Pb exposure could decrease PKA activity.[22,35] The serine−threonine kinase Akt and downstream transcription factors such as CREB play an important role in neuronal survival and protection, and their activation can protect against cellular stress and injury.[36] Our previous study had also demonstrated Pb decreased Akt and eNOS activities, leading to the decline of NO production and apoptosis in kidneys of rats.[7] CaMKII is a multimeric kinase composed of four distinct subtypes that are activated when they bind calcium-bound calmodulin, an EF-hand, calcium-sensing protein. CAMKII has been shown to play a pivotal role in learning, memory, and synaptic plasticity. CAMKII is activated by a rise in intracellular calcium, which allows for the binding of CAMKII to a calcium/calmodulin complex.[22] Several studies had shown that Pb exposure coud alter CAMKII activity and expression in rat brain.[22,37] In this study, we also found that Pb decreased CAMKII activity in mouse brain (Figure 6), which may be due to inhibition of the glycine site in the NMDAR by Pb.[38] CREB acts as an important part of the signal propagation from synapses to the nucleus by linking NMDAR activation and calcium-dependent signaling to the expression of genes necessary for synaptic plasticity.[22] Pb can decrease the phosphorylation and binding activity of CREB, perhaps because Pb increased the high-affinity ifenprodil-binding sites in the hippocampus and the cortex and activated functional extrasynaptic NMDAR with a NR1/NR2B composition to result in a dephosphorylation of CREB.[39] Several papers revealed that QE increased Akt activity,[40] PKA activity,[34] and CREB activity.[41] In the present study, we also observed that QE increased PKA activity (Figure 5) and the phosphorylation levels of Akt, CaMKII, and CREB in brains of Pb-treated mice (Figure 6). On the basis of these results, we conclude that PKA, Akt, CaMKII, and CREB have important roles in impairment of learning, memory, and synaptic plasticity in Pb-stimulated mice. QE may exert its neuroprotective effect on mice by increasing the phosphorylation of PKA, Akt, CaMKII, and CREB.

Interestingly, in this study, we found that ERK phosphorylation increased in mouse brain after Pb exposure (Figure 6), which is consistent with previous studies.[40] However, it is in contradiction to the changes in CREB phosphorylation, suggesting that ERK may not be involved in the activation of CREB. One possible explanation for the increase in ERK phosphorylation is that Pb is a potent antagonist of NMDAR. Therefore, inhibition of NMDAR results in increases of ERK phosphorylation, [42] and the increase of ERK phosphorylation after Pb exposure may not be specific to this protein.[22,43]

In conclusion, this study demonstrated for the first time that QE has potent protective effects against Pb-induced impairment of learning, memory, and synaptic plasticity through increasing NO production and CREB phosphorylation, at least in part, by increasing nNOS, eNOS, PKA, Akt, and CaMKII activation. We propose a possible protective effect of QE (Figure 6). Here we demonstrate that QE administration decreased Pb concentrations in blood and brain. QE attenuated Pb-induced oxidative damage by inhibiting ROS generation in the brain of mice. QE seems to be a potent neuroprotective drug. In further studies, we will investigate the effects of Pb exposure on animals at low doses and over longer times. The preventative effects of quercetin on post lead exposure treatment also deserve consideration and further examination. Some recent papers have proposed that QE may have some adverse effects, so the consequences of high doses and long QE administration on animals warrant further study.

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Notes

The authors declare no competing financial interest.

ABBREVIATIONS USED

Akt, protein kinase B; CaMKII, calcium/calmodulin kinase II; CREB, cyclic-AMP-response element-binding protein; eNOS, endothelial nitric oxide synthase; ERK, extracellular signal-regulated kinase; iNOS, inducible nitric oxide synthase; nNOS, neuronal nitric oxide synthase; NO, nitric oxide; Pb, lead; PKA, protein kinase A; QE, quercetin; ROS, reactive oxygen species; TAC, total antioxidant capacity; TBARS, thiobarbituric acid-reactive substances

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