Published in final edited form as:

Neurochem Res. 2019 April ; 44(4): 751–762. doi:10.1007/s11064-018-02705-0.

Cinnamic acid protects the nigrostriatum in a mouse model of Parkinson's disease via peroxisome proliferator-activated receptor α

Tim Prorok, Malabendu Jana, and Kalipada Pahan

Division of Research and Development, Jesse Brown Veterans Affairs Medical Center, Chicago, USA; Department of Neurological Sciences, Rush University Medical Center, Chicago, USA

Abstract

Parkinson's disease (PD) is the second most common devastating human neurodegenerative disorder and despite intense investigation, no effective therapy is available for PD. Cinnamic acid, a naturally occurring aromatic fatty acid of low toxicity, is a precursor for the synthesis of a huge number of plant substances. This study highlights the neuroprotective effect of cinnamic acid in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mouse model of PD. Oral administration of cinnamic acid protected tyrosine hydroxylase (TH)-positive dopaminergic neurons in the substantia nigra pars compacta (SNpc) and TH fibers in the striatum of MPTP-insulted mice. Accordingly, oral cinnamic acid also normalized striatal neurotransmitters and improved locomotor activities in MPTP-intoxicated mice. While investigating mechanisms, we found that cinnamic acid induced the activation of peroxisome proliferator-activated receptor α (PPAR α), but not PPAR β , in primary mouse astrocytes. Cinnamic acid mediated protection of the nigrostriatal system and locomotor activities in WT and PPAR β ($-/-$), but not PPAR α ($-/-$) mice from MPTP intoxication suggests that cinnamic acid requires the involvement of PPAR α in protecting dopaminergic neurons in this model of PD. This study delineates a new function of cinnamic acid in protecting dopaminergic neurons via PPAR α that could be beneficial for PD.

Keywords

Parkinson's disease; MPTP; Tyrosine hydroxylase; Dopamine; PPAR α

Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disorder after Alzheimer's and the most common movement disorder [1], [2]. The hallmarks of PD involve resting tremor, bradykinesia, muscle rigidity, and postural disturbance [3]. Besides these features, the patient's quality of life decreases and they face an increased risk of falls and their resultant complications as well as possible psychiatric abnormalities [4], [5], [6]. Multiple regions of the brain are attacked by the disease during its course but the region

primarily affected is the Substantia Nigra pars compacta where dopaminergic neurons are lost resulting in substantially reduced dopamine levels in the striatum that correlates with the movement symptoms [7], [8]. Currently treatments for PD treat the symptoms but do not alter the disease course and over time create side effects such as dyskinesia [9]. With rapid increase in aging population and rise in per patient cost burden, it is imperative to find new effective treatments to improve the disease condition and quality of life [10].

Cinnamic acid is an organic compound found in many plants as deaminated product of phenylalanine. This unsaturated carboxylic acid is one of the many pigments giving balsam tree sap its distinctive color. It also finds numerous industrial uses and has a long history of human use as a component of plant-derived scents. While it is found to be beneficial in treating diabetes [11], according to Liu and colleagues [12], cinnamic acid can also induce cytostasis and reverse malignant properties of human tumor cells. Additionally, it is also one of many compounds in the common spice cinnamon which has been used for millennia for its medicinal properties. It has been reported that cinnamon extract contains a compound that may inhibit the buildup of amyloid- β deposits in Alzheimer's disease [13]. According to Hemmati et al [14], cinnamic acid improves memory by reducing the oxidative stress and cholinergic dysfunction in the brain of diabetic mice. In this study, we examined whether cinnamic acid is neuroprotective in the MPTP mouse model of PD and found that oral cinnamic acid exhibits significant protection of the nigrostriatal axis after MPTP intoxication. Moreover, we have also established that cinnamic acid uses peroxisome proliferator-activated receptor α (PPAR α) for its neuroprotective effects. These results provide a mode of action of cinnamic acid against parkinsonian neurotoxin and open an option for treating PD patients with this naturally-available molecule.

Methods:

Animals and MPTP intoxication

Animals were maintained and experiments were in accordance with National Institutes of Health guidelines and approved by the Institutional Animal Care and Use committee of Rush University Medical Center in Chicago, Illinois. C57BL/6 mice (six to eight weeks old) were obtained from Harlan (Indianapolis, IN). Mice were injected with MPTP-HCL (1-methyl-4-phenyl- 1, 2, 3, 6-tetrahydropyridine hydrochloride) obtained from Sigma-Aldrich following the MPTP acute protocol [15]. MPTP-HCL is reconstituted with 0.9% sterile sodium chloride solution obtained from Hospira Inc. Mice received four i.p. injections of MPTP-HCL (18 mg/kg) at 2-h intervals.

Cinnamic acid treatment

Cinnamic acid was obtained from Spectrum Chemicals (Gardena, CA). Starting from 3 h after the last injection of MPTP, mice were treated with cinnamic acid (100 mg/kg body wt/d solubilized in 0.1% methyl cellulose) via gavage. Therefore, one group of MPTP-intoxicated mice received 100 μ l 0.1% methyl cellulose as vehicle.

Western Blotting

Nigral homogenates were electrophoresed, proteins were transferred to nitrocellulose membrane and immunoblot for tyrosine hydroxylase (TH) was carried out using Immunostar anti-mouse TH at a dilution of 1:5,000 as described earlier [16–18].

Immunohistochemistry and quantitative analysis

Mice were perfused with PBS for five minutes and placed into 4% paraformaldehyde for tissue fixation. Sections were cut 40µm thick using a Leica CM3050S Research Cryostat and placed into cryoprotectant and stored at –20°C. Tissue was then stained for TH following the Vectastain DAB protocol, mounted and then counted using 3D stereological method with Optical Fractionator using Stereo Investigator v11 as described earlier [16–18]. Optical Density measures were produced using ImageJ software [19] as described before [20].

HPLC Analysis

Striatal levels of dopamine, 3,4,-dihydroxyphenylacetic acid (DOPAC), and homovanillic acid (HVA) were quantified by HPLC as described earlier [16–18, 21]. Mice were perfused with PBS for 5 minutes and immediately dissected, frozen on dry ice and tissue stored in –80C for processing the next day. Tissues were sonicated in 0.2M perchloric acid containing isopretanol and then spun at 20,000 × g for 30 minutes. After that, 10ul of the supernatant was injected onto a Eicompak SC-3ODS column (complete stand-alone HPLC-electrochemical detection system EiCOMHTEC-500, JM Science Inc., Grand Island, NY) and analyzed following the manufacturer's protocol.

PPRE Luciferase Assay

It was performed as described earlier [22, 23]. Briefly, cells plated at 50–60% confluence in 12-well plates were co-transfected with 500 ng of tk-PPREx3-Luc, a PPRE-dependent luciferase reporter construct, and 50 ng of pRL-TK using LipofectAMINE Plus. Twenty-four hours after transfection, cells were treated with different concentrations of cinnamic acid. After 4 h of incubation, firefly (ff-Luc) and Renilla (r-Luc) luciferase activities were assayed.

Behavioral Analysis

Behavioral analysis was performed as described earlier [16, 17, 21]. Briefly, two types of behavioral testing were conducted. All mice were tested 7 days after MPTP injections. The first is an accelerating Rotarod test for walking. The rotarod test challenges mice to coordinate their movements and stay on an accelerating spinning track. The second is the Open Field test using a Noldus Ethovision Software (Netherlands). The mice were given 5 minute training and then rested to eliminate stress and fatigue before measure taken. For the Open Field, mice were placed in a corner and given 3 minutes free running before being recorded for 5 minutes for parameters of total distance, velocity, acceleration, body fill, and movement. Rearing measures were manually counted by a blinded observer.

Statistical analysis

All statistics were performed on GraphPad Prism 7.02 (GraphPad Software, La Jolla, CA). Statistics performed are one-way ANOVAs with Tukey HSD post hoc test comparing treatment groups within the same genotype. Two-way ANOVAs comparing treatments and genotypes were performed and Bonferroni post hoc testing was done to determine differences. All error bars represent standard deviation. Both Tukey and Bonferroni post hoc tests use alpha set to 0.05.

Results

Cinnamic acid protects nigral TH levels after MPTP intoxication.

The first step was to determine whether cinnamic acid had any effect on the nigrostriatal system in the MPTP mouse model of PD. To do that we injected mice with 18mg/kg of MPTP four times, and then waited three additional hours between the last injection and gavaging the mice with either vehicle or cinnamic acid. The treatments were continued for 7 days at the same time each day and then the mice sacrificed and brains dissected. After evaluation of the SNpc for TH we found that MPTP intoxication drastically reduced TH levels and that cinnamic acid, but not methyl cellulose (vehicle), protected TH levels [$F(3, 16) = 194.2, p < 0.05$] (Fig. 1A–B). Following this we performed TH staining and in conjunction with stereological counting we found that cinnamic acid treatment preserved nigral cells that were lost in both the MPTP and MPTP + vehicle groups [$F(3, 20) = 45.93, P < 0.05$] (Fig. 1C–D). This shows that cinnamic acid is neuroprotective of nigral neurons in the SNpc that are critical to forestalling PD.

Cinnamic acid is protective of striatal innervation and neurotransmitters after MPTP intoxication.

Next, we examined the mouse striata across each group (Fig. 2). Immunohistochemistry was performed on matching sections from all four groups and it was found that the control mice had TH fibers expressed at a homogenous level throughout the striatum. The MPTP group had greatly reduced TH fibers compared to control. However, cinnamic acid treatment protected striatal fibers as compared to control group (Fig. 2A). This result was specific as vehicle treatment had no such protective effect (Fig. 2A). To quantify this we used Optical Densitometry and found significant differences between all groups except for MPTP and MPTP + Veh [$F(3, 16) = 166.2, P < 0.05$] (Fig. 2B). We next investigated whether dopamine and its metabolites are affected by MPTP and possible restorative effects of cinnamic acid. We examined dopamine, DOPAC and HVA via HPLC and found strong depletion of dopamine in the MPTP group and that cinnamic acid group exhibited significant restoration of dopamine as compared to MPTP and MPTP + Veh [$F(3, 26) = 49.63, P < 0.05$] (Fig. 2C). There were also differences in DOPAC and HVA levels however these were not significant (Fig. 2D–E). Together, these results show that cinnamic acid is protective of the neurotransmitters and enzymes of the striatum that are vital to its healthy functioning.

Cinnamic acid restores locomotor deficit induced by MPTP.

Since PD is a movement disorder, to ascertain impairment we performed rotarod testing and open field analysis (Fig. 3). Our results indicated that the unaffected mice stayed on for an average of a little over 200 seconds with the speed accelerating from 4 rpm to 40 (Fig. 3A). The mice receiving only MPTP had an average of 50 seconds before falling off. Mice that received MPTP and cinnamic acid lasted on average 142 seconds, close to three times as long as the MPTP group. Finally the vehicle group lasted approximately 65 seconds which was similar and not significantly different from the MPTP only animals. An ANOVA of the times showed that the cinnamic acid treated mice performed significantly better than either MPTP or MPTP + Veh mice [$F(3, 26) = 20.62$, $p < 0.05$]. Next, we did free roaming behavioral analysis. Multiple parameters such as velocity, total distance and acceleration showed no significant changes which is supported by the literature [24]. The number of stereotypy was observed and counted with the trend of recovery in the MPTP + cinnamic acid found compared to MPTP and MPTP + vehicle [$F(3, 26) = 17.13$, $p < 0.05$] (Fig. 3B). Also counted was the number of rearing events by the mice. There was a significant difference between the cinnamic acid group and MPTP group ($p < 0.05$) but not between cinnamic acid and vehicle groups ($p > 0.05$) (Fig. 3C). Other reports have found differences in rearing number depending on MPTP regimen however our results differed in this regard. During manual scoring, it was noticed that the control and the (MPTP + cinnamic acid) groups displayed increased free standing behavior in that these mice did not stand up and brace themselves on the plexiglass walls of the open field box. When examined the effect was statistically significant [$F(3, 26) = 13.43$, $p < 0.05$] (Fig. 3D). It was found that cinnamic acid restored motor deficits induced by MPTP.

Cinnamic acid increases the activation of Peroxisome Proliferator-Activated Receptor alpha (PPAR α) in primary mouse astrocytes.

Next, we investigated mechanisms by which cinnamic acid exhibits neuroprotection in MPTP mouse model of PD. Although PPAR α being present in the liver controls fatty acid metabolism [25, 26], recently we have seen that PPAR α is present in different parts of the brain and that PPAR α is also involved in neuroprotection [26–30]. Therefore, we examined if cinnamic acid was capable of activating PPAR α (Fig. 4). Accordingly, we monitored PPARE-dependent reporter activity in primary astrocytes isolated from WT, PPAR α (–/–) and PPAR β (–/–) mice. There was an increase in PPARE-driven luciferase activity in the WT and PPAR β (–/–) astrocytes corresponding with the dosing but not in the PPAR α (–/–) cells (Fig. 4). Both WT and PPAR β (–/–) astrocytes exhibited approximately 4½ fold increase in luciferase activity at 100 μ M cinnamic acid [F interaction (8, 30) = 29.98, $p < .001$]. On the other hand, the PPAR α (–/–) astrocytes had very little change in response to different doses of cinnamic acid indicating that cinnamic acid-induced activation of PPARE-luciferase is due to PPAR α .

Cinnamic acid protects striatal TH via PPAR α .

Having established that cinnamic acid induces the activation of PPAR α , our next step was to determine if cinnamic acid requires PPAR α for neuroprotection in MPTP-intoxicated mice. Following the same MPTP intoxication protocol PPAR α (–/–) and PPAR β (–/–) mice were

fed cinnamic acid 3 hours after the final MPTP injection. After 7 days of feeding the mice were tested for behaviors and then sacrificed. We next determined the effects on striatal TH between genotypes after MPTP and cinnamic acid treatment (Fig. 5). It was found that the PPAR α ($-/-$) mice had limited and insignificant protection from the MPTP while the WT mice and PPAR β ($-/-$) mice had protection conferred through the functional PPAR α gene (Fig. 5A). Our findings were confirmed with measuring the Optical Density for TH of matching sections (Fig. 5B). There was significant loss of TH innervation in the striata with MPTP in all three genotypes and expectantly the PPAR α ($-/-$) mice showed no improvement in the MPTP + cinnamic acid group whereas the WT and PPAR β ($-/-$) do have protection. A two-way ANOVA shows that cinnamic acid is protective depending on genotype [F genotype (4, 54) = 11.6, $p < .001$]. These results suggest that cinnamic acid protects striatal fibers in MPTP-intoxicated mice via PPAR α , not PPAR β .

Cinnamic acid restores striatal neurotransmitters in MPTP mouse model of PD via PPAR α .

Next, we examined if cinnamic acid required PPAR α to restore striatal neurotransmitters in MPTP-insulted mice. Dissected striatal tissue from all three genotypes was analyzed by HPLC for dopamine, DOPAC and HVA (Fig. 6). It was found that there was no significant change in dopamine levels in the control animals for each group (Fig. 6A). Furthermore the dopamine loss for all intoxicated groups had similar drops and did not differ from one another. However, after cinnamic acid treatment, the WT and PPAR β ($-/-$) mice had protection of striatal dopamine levels whereas the PPAR α ($-/-$) had no such protection, and had levels similar to the PPAR α ($-/-$) mice receiving only MPTP. There were also changes in DOPAC and HVA (Fig. 6B–C). With measured levels of HVA being very low, less than one nanogram per milligram of tissue, it is difficult to draw any conclusion, however it remains clear that cinnamic acid is protective of striatal dopamine after MPTP insult as long as PPAR α is functional.

Cinnamic acid restores locomotor functions in MPTP-intoxicated mice via PPAR α .

Decreasing functional impairment is ultimate therapeutic goal of neuroprotection in PD. Therefore, we also performed behavioral assessments of all three genotypes. Cinnamic acid treatment improved rotarod performance of MPTP-intoxicated WT and PPAR β ($-/-$) mice. On the other hand, cinnamic acid remained unable to improve rotarod performance of MPTP-insulted PPAR α ($-/-$) animals. While after cinnamic acid treatment, the WT mice lasted approximately 150 seconds and the PPAR β ($-/-$) mice lasted about 175 seconds, PPAR α ($-/-$) mice lasted for around 60 seconds. This is also similar to the PPAR α ($-/-$) mice that only had MPTP and not the cinnamic acid feedings (Fig. 7A). Rotarod two-way ANOVA results were significant for treatment and interaction but not genotype [F treatment (2, 51) = 38.05, $p < .001$], [F genotype (2, 51) = 3.04, $p > .05$] and [F interaction (4, 51) = 3.38, $p < .05$]. The number of unsupported rears where the mouse doesn't rest their forelimbs on the glass was significantly different between cinnamic acid receiving mice genotypes (Fig. 7B). The WT and PPAR β ($-/-$) cinnamic acid groups did not differ from one another, however, these were both significant as compared the PPAR α ($-/-$) cinnamic acid mice that had values similar to the PPAR α ($-/-$) MPTP mice as well as the other MPTP only mice. Counting the number of stereotypy events revealed the same pattern as rotarod and unsupported rearing with large effects seen in the cinnamic acid groups

depending on genotype but none others (Fig. 7C). Stereotypy two-way ANOVA results were significant for treatment, genotype and interaction [F treatment (2, 51) = 25.92, $p < .001$], [F genotype (2, 51) = 5.15, $p < .01$] and [F interaction (4, 51) = 4.93, $p < .01$]. These findings show that cinnamic acid is restorative of the behavioral deficits induced by MPTP but only when PPAR α is present.

Discussion

Parkinson's disease (PD) is the second most common neurodegenerative disorder associated with movement dysfunction with several subtypes. The incidence of PD is 1% in person's age 65 and increasing to 4% at age 85 (Tysnes, 2017). To date, PD has no cure and treatments available only treat the motor symptoms temporarily but do not alter the disease course. Levodopa has been the gold standard of treatment since its introduction as a therapeutic agent. However, within a few years patients frequently begin to have levodopa-induced dyskinesia as well as resurgent motor deficits as the disease continues to progress [31]. Some PD patients go for Deep Brain Stimulation (DBS) that has some advantages since it relies on suppressing inhibition in the brain allowing movements otherwise stunted to occur. Many patients have benefitted from this however the effects are short-lived and the disease continues its insidious destruction of the midbrain and risk of infection or complications with surgery and a recent evaluation found that DBS patients exhibit increased inflammation [32], [33]. With an aging population and other factors such as widespread methamphetamine abuse and pesticide exposure there will be an increase in the number of PD cases [34], [35], [36]. Beyond that the cost of care is also growing on a per patient basis [37]. Between the increasing numbers of patients and the increasing costs associated with care and to alleviate the suffering of the patients it is necessary to find new treatments that are cost effective and widely available. Cinnamic acid is an organic compound that is naturally present in a number of plants. It is the precursor of a wide variety of plant substances, including flavonoids, tannins, lignin, pigments, and many of the flavoring agents. It is also known to affect germination and plant root growth [38]. Here we demonstrate for the first time that cinnamic acid is neuroprotective in the MPTP mouse model of PD. Since cinnamic acid is a non-toxic molecule, our results indicate therapeutic importance of cinnamic acid in PD.

Mechanisms by which cinnamic acid exerts neuroprotective effect are poorly understood. The Peroxisome Proliferator Activating Receptors (PPAR's) are a family of nuclear receptor proteins that have essential roles in carbohydrate, lipid and protein metabolism as well as acting as a transcription regulator following its heterodimerization with the RXR's (Retinoid X Receptors) [26, 39, 40]. Recently we have demonstrated that PPAR α is constitutively present in different parts of the brain to participate in neuroprotection [23, 26, 28–30]. We have seen that cinnamic acid alone is capable of activating PPAR α . Cinnamic acid-mediated induction of PPARE-luciferase activity in astrocytes isolated from WT and PPAR β (–/–), but not PPAR α (–/–), mice indicates that cinnamic acid induces the activation of PPAR α , but not PPAR β . Accordingly, cinnamic acid protected dopaminergic neurons in the nigra, restored striatal fibers and neurotransmitters, and improved locomotor activities via PPAR α , but not PPAR β . This is also the first report that PPAR α is involved in the protection of nigrostriatum from MPTP insult.

However, pathways by which PPAR α exhibits neuroprotection for the nigrostriatum are poorly understood. There are reports of PPAR's being involved with cellular development and differentiation as well as being a regulator of NF- κ B dependent inflammation [41], [42]. Recent studies highlight the importance of neuroinflammation in different neurodegenerative disorders including PD [43], [44]. Therefore, it is important to mention that activation of PPAR α can inhibit NF- κ B activation and prevent the production of proinflammatory cytokines such as tumor necrosis factor α and interleukin 6 [45], [46], [47], [48]. There are also mechanisms by which PPAR α may provide neuroprotection to the vulnerable midbrain dopaminergic cells that are lost in PD. PPAR α is capable of increasing the level of CREB, a multifunctional transcription factor, via direct transcriptional regulation [29]. Activation of PPAR α is also capable of inducing anti-oxidant molecules, which may slow down accumulation of reactive oxygen species detrimental to neurons [46], [49], [50]. Since oxidative stress is associated with different neurodegenerative disorders including PD, PPAR α activation should be helpful for dopaminergic neurons [51], [52]. It has been found that the metabolic pathway of dopamine and other neurotransmitters produce reactive oxygen and nitrogen species which are detrimental to dopaminergic neurons [53]. Another potential mechanism for PPAR α 's protective effects may be its ability to increase neurotrophic factors such as BDNF and NT3 [54], [30]. PPAR α is a focal point of current research since it has numerous beneficial interactions with antioxidant defense, anti-inflammation, neurotrophism, etc. Together, PPAR α may be a linchpin in managing the progression of Parkinson's and be on the path to effective treatments and reversing the disease.

In summary, we have delineated that cinnamic acid, a plant product, activates PPAR α to protect the loss of dopaminergic neurons, restore striatal fibers and neurotransmitters, and improve the behavioral functions in MPTP-intoxicated mice. Therefore cinnamic acid is neuroprotective via PPAR α and further investigation is warranted to determine health benefits of cinnamic acid to PD patients.

Acknowledgements:

This study was supported by a merit award from the Department of Veteran Affairs (1I01BX003033) and grants (NS83054 and AG050431) from National Institutes of Health.

References

1. Mhyre TR, Boyd JT, Hamill RW, Maguire-Zeiss KA (2012) Parkinson's disease. *Subcell Biochem* 65:389–455 [PubMed: 23225012]
2. Wyss-Coray T (2016) Ageing, neurodegeneration and brain rejuvenation. *Nature* 539:180–186 [PubMed: 27830812]
3. Postuma RB, Berg D, Adler CH, Bloem BR, Chan P, Deuschl G, Gasser T, Goetz CG, Halliday G, Joseph L, Lang AE, Liepelt-Scarfone I, Litvan I, Marek K, Oertel W, Olanow CW, Poewe W, Stern M (2016) The new definition and diagnostic criteria of Parkinson's disease. *Lancet Neurol* 15:546–548 [PubMed: 27302120]
4. Opara JA, Brola W, Leonardi M, Blaszczyk B (2012) Quality of life in Parkinson's disease. *J Med Life* 5:375–381 [PubMed: 23346238]
5. Schrag A, Choudhury M, Kaski D, Gallagher DA (2015) Why do patients with Parkinson's disease fall? A cross-sectional analysis of possible causes of falls. *NPJ Parkinsons Dis* 1:15011 [PubMed: 28409181]

6. Diederich NJ, Fenelon G, Stebbins G, Goetz CG (2009) Hallucinations in Parkinson disease. *Nat Rev Neurol* 5:331–342 [PubMed: 19498436]
7. Hindle JV (2010) Ageing, neurodegeneration and Parkinson's disease. *Age Ageing* 39:156–161 [PubMed: 20051606]
8. Seibyl JP, Marek KL, Quinlan D, Sheff K, Zoghbi S, Zea-Ponce Y, Baldwin RM, Fussell B, Smith EO, Charney DS, van Dyck C, et al. (1995) Decreased single-photon emission computed tomographic [123I]beta-CIT striatal uptake correlates with symptom severity in Parkinson's disease. *Ann Neurol* 38:589–598 [PubMed: 7574455]
9. Ellis JM, Fell MJ (2017) Current approaches to the treatment of Parkinson's Disease. *Bioorg Med Chem Lett* 27:4247–4255 [PubMed: 28869077]
10. Kowal SL, Dall TM, Chakrabarti R, Storm MV, Jain A (2013) The current and projected economic burden of Parkinson's disease in the United States. *Mov Disord* 28:311–318 [PubMed: 23436720]
11. Adisakwattana S (2017) Cinnamic Acid and Its Derivatives: Mechanisms for Prevention and Management of Diabetes and Its Complications. *Nutrients* 9
12. Liu L, Hudgins WR, Shack S, Yin MQ, Samid D (1995) Cinnamic acid: a natural product with potential use in cancer intervention. *Int J Cancer* 62:345–350 [PubMed: 7628877]
13. Guo JP, Yu S, McGeer PL (2010) Simple in vitro assays to identify amyloid-beta aggregation blockers for Alzheimer's disease therapy. *J Alzheimers Dis* 19:1359–1370 [PubMed: 20061605]
14. Hemmati AA, Alboghobeish S, Ahangarpour A (2018) Effects of cinnamic acid on memory deficits and brain oxidative stress in streptozotocin-induced diabetic mice. *Korean J Physiol Pharmacol* 22:257–267 [PubMed: 29719448]
15. Jackson-Lewis V, Przedborski S (2007) Protocol for the MPTP mouse model of Parkinson's disease. *Nat Protoc* 2:141–151 [PubMed: 17401348]
16. Chandra G, Roy A, Rangasamy SB, Pahan K (2016) Induction of Adaptive Immunity Leads to Nigrostriatal Disease Progression in MPTP Mouse Model of Parkinson's Disease. *J Immunol* 198:4312–4326
17. Khasnavis S, Pahan K (2014) Cinnamon treatment upregulates neuroprotective proteins Parkin and DJ-1 and protects dopaminergic neurons in a mouse model of Parkinson's disease. *J Neuroimmune Pharmacol* 9:569–581 [PubMed: 24946862]
18. Khasnavis S, Roy A, Ghosh S, Watson R, Pahan K (2014) Protection of dopaminergic neurons in a mouse model of Parkinson's disease by a physically-modified saline containing charge-stabilized nanobubbles. *J Neuroimmune Pharmacol* 9:218–232 [PubMed: 24122363]
19. Varghese F, Bukhari AB, Malhotra R, De A (2014) IHC Profiler: an open source plugin for the quantitative evaluation and automated scoring of immunohistochemistry images of human tissue samples. *PLoS One* 9:e96801 [PubMed: 24802416]
20. Ghosh A, Roy A, Matras J, Brahmachari S, Gendelman HE, Pahan K (2009) Simvastatin inhibits the activation of p21ras and prevents the loss of dopaminergic neurons in a mouse model of Parkinson's disease. *J Neurosci* 29:13543–13556 [PubMed: 19864567]
21. Ghosh A, Roy A, Liu X, Kordower JH, Mufson EJ, Hartley DM, Ghosh S, Mosley RL, Gendelman HE, Pahan K (2007) Selective inhibition of NF-kappaB activation prevents dopaminergic neuronal loss in a mouse model of Parkinson's disease. *Proc Natl Acad Sci U S A* 104:18754–18759 [PubMed: 18000063]
22. Pahan K, Jana M, Liu X, Taylor BS, Wood C, Fischer SM (2002) Gemfibrozil, a lipid-lowering drug, inhibits the induction of nitric-oxide synthase in human astrocytes. *J Biol Chem* 277:45984–45991 [PubMed: 12244038]
23. Roy A, Kundu M, Jana M, Mishra RK, Yung Y, Luan CH, Gonzalez FJ, Pahan K (2016) Identification and characterization of PPARalpha ligands in the hippocampus. *Nat Chem Biol* 12:1075–1083 [PubMed: 27748752]
24. Luchtman DW, Shao D, Song C (2009) Behavior, neurotransmitters and inflammation in three regimens of the MPTP mouse model of Parkinson's disease. *Physiol Behav* 98:130–138 [PubMed: 19410592]
25. Roy A, Pahan K (2009) Gemfibrozil, stretching arms beyond lipid lowering. *Immunopharmacol Immunotoxicol* 31:339–351 [PubMed: 19694602]

26. Roy A, Pahan K (2015) PPARalpha signaling in the hippocampus: crosstalk between fat and memory. *J Neuroimmune Pharmacol* 10:30–34 [PubMed: 25575492]
27. Corbett GT, Gonzalez FJ, Pahan K (2015) Activation of peroxisome proliferator-activated receptor alpha stimulates ADAM10-mediated proteolysis of APP. *Proc Natl Acad Sci U S A* 112:8445–8450 [PubMed: 26080426]
28. Ghosh A, Pahan K (2016) PPARalpha in lysosomal biogenesis: A perspective. *Pharmacol Res* 103:144–148 [PubMed: 26621249]
29. Roy A, Jana M, Corbett GT, Ramaswamy S, Kordower JH, Gonzalez FJ, Pahan K (2013) Regulation of cyclic AMP response element binding and hippocampal plasticity-related genes by peroxisome proliferator-activated receptor alpha. *Cell Rep* 4:724–737 [PubMed: 23972989]
30. Roy A, Jana M, Kundu M, Corbett GT, Rangaswamy SB, Mishra RK, Luan CH, Gonzalez FJ, Pahan K (2015) HMG-CoA Reductase Inhibitors Bind to PPARalpha to Upregulate Neurotrophin Expression in the Brain and Improve Memory in Mice. *Cell Metab* 22:253–265 [PubMed: 26118928]
31. Fahn S (1999) Parkinson disease, the effect of levodopa, and the ELLDOPA trial. Earlier vs Later LDOPA. *Arch Neurol* 56:529–535 [PubMed: 10328247]
32. Hickey P, Stacy M (2016) Deep Brain Stimulation: A Paradigm Shifting Approach to Treat Parkinson's Disease. *Front Neurosci* 10:173 [PubMed: 27199637]
33. Kwiatek-Majkusiak J, Geremek M, Koziorowski D, Tomasiuk R, Szlufik S, Friedman A (2018) Higher serum levels of pro-hepcidin in patients with Parkinson's disease treated with deep brain stimulation. *Neurosci Lett*
34. Xu Y, Yang J, Shang H (2016) Meta-analysis of risk factors for Parkinson's disease dementia. *Transl Neurodegener* 5:11 [PubMed: 27257478]
35. Curtin K, Fleckenstein AE, Robison RJ, Crookston MJ, Smith KR, Hanson GR (2015) Methamphetamine/amphetamine abuse and risk of Parkinson's disease in Utah: a population-based assessment. *Drug Alcohol Depend* 146:30–38 [PubMed: 25479916]
36. Duell EJ, Millikan RC, Savitz DA, Newman B, Smith JC, Schell MJ, Sandler DP (2000) A population-based case-control study of farming and breast cancer in North Carolina. *Epidemiology* 11:523–531 [PubMed: 10955404]
37. Bohingamu Mudiyansele S, Watts JJ, Abimanyi-Ochom J, Lane L, Murphy AT, Morris ME, Iansek R (2017) Cost of Living with Parkinson's Disease over 12 Months in Australia: A Prospective Cohort Study. *Parkinsons Dis* 2017:5932675 [PubMed: 28352490]
38. Salvador VH, Lima RB, dos Santos WD, Soares AR, Bohm PA, Marchiosi R, Ferrarese Mde L, Ferrarese-Filho O (2013) Cinnamic acid increases lignin production and inhibits soybean root growth. *PLoS One* 8:e69105 [PubMed: 23922685]
39. Mangelsdorf DJ, Evans RM (1995) The RXR heterodimers and orphan receptors. *Cell* 83:841–850 [PubMed: 8521508]
40. Dunning KR, Anastasi MR, Zhang VJ, Russell DL, Robker RL (2014) Regulation of fatty acid oxidation in mouse cumulus-oocyte complexes during maturation and modulation by PPAR agonists. *PLoS One* 9:e87327 [PubMed: 24505284]
41. Cimini A, Ceru MP (2008) Emerging roles of peroxisome proliferator-activated receptors (PPARs) in the regulation of neural stem cells proliferation and differentiation. *Stem Cell Rev* 4:293–303 [PubMed: 18561036]
42. Bensinger SJ, Tontonoz P (2008) Integration of metabolism and inflammation by lipid-activated nuclear receptors. *Nature* 454:470–477 [PubMed: 18650918]
43. Straus DS, Glass CK (2007) Anti-inflammatory actions of PPAR ligands: new insights on cellular and molecular mechanisms. *Trends Immunol* 28:551–558 [PubMed: 17981503]
44. Dehmer T, Heneka MT, Sastre M, Dichgans J, Schulz JB (2004) Protection by pioglitazone in the MPTP model of Parkinson's disease correlates with I kappa B alpha induction and block of NF kappa B and iNOS activation. *J Neurochem* 88:494–501 [PubMed: 14690537]
45. Feinstein DL (2003) Therapeutic potential of peroxisome proliferator-activated receptor agonists for neurological disease. *Diabetes Technol Ther* 5:67–73 [PubMed: 12725709]

46. Toyama T, Nakamura H, Harano Y, Yamauchi N, Morita A, Kirishima T, Minami M, Itoh Y, Okanoue T (2004) PPAR α ligands activate antioxidant enzymes and suppress hepatic fibrosis in rats. *Biochem Biophys Res Commun* 324:697–704 [PubMed: 15474484]
47. Yang Y, Gocke AR, Lovett-Racke A, Drew PD, Racke MK (2008) PPAR α Regulation of the Immune Response and Autoimmune Encephalomyelitis. *PPAR Res* 2008:546753 [PubMed: 18645614]
48. Skoczynska A, Dobosz T, Poreba R, Turczyn B, Derkacz A, Zoledziwska M, Jonkisz A, Lebioda A (2005) The dependence of serum interleukin-6 level on PPAR- α polymorphism in men with coronary atherosclerosis. *Eur J Intern Med* 16:501–506 [PubMed: 16275545]
49. Dias V, Junn E, Mouradian MM (2013) The role of oxidative stress in Parkinson's disease. *J Parkinsons Dis* 3:461–491 [PubMed: 24252804]
50. Kim T, Yang Q (2013) Peroxisome-proliferator-activated receptors regulate redox signaling in the cardiovascular system. *World J Cardiol* 5:164–174 [PubMed: 23802046]
51. Hwang O (2013) Role of oxidative stress in Parkinson's disease. *Exp Neurobiol* 22:11–17 [PubMed: 23585717]
52. Blesa J, Trigo-Damas I, Quiroga-Varela A, Jackson-Lewis VR (2015) Oxidative stress and Parkinson's disease. *Front Neuroanat* 9:91 [PubMed: 26217195]
53. Rodriguez M, Morales I, Rodriguez-Sabate C, Sanchez A, Castro R, Brito JM, Sabate M (2014) The degeneration and replacement of dopamine cells in Parkinson's disease: the role of aging. *Front Neuroanat* 8:80 [PubMed: 25147507]
54. Bento-Abreu A, Tabernero A, Medina JM (2007) Peroxisome proliferator-activated receptor- α is required for the neurotrophic effect of oleic acid in neurons. *J Neurochem* 103:871–881 [PubMed: 17683485]

Figure 1. Oral administration of cinnamic acid protects nigral TH levels in MPTP-intoxicated mice.

Mice receiving cinnamic acid (100 mg/kg body wt/day) via gavage from 3h after the last injection of MPTP were sacrificed 7d after the last injection of MPTP followed by Western blot of TH (n=5 per group) in the nigra (A) and its quantification (B) [$F(3, 16) = 194.2$, $p < .001$]. Mice were sacrificed and immunostained for TH (C), analyzed by stereological counting of TH positive neurons in the SNpc showing protection by cinnamic acid [$F(3,20) = 45.93$, $p < .001$] (D). Results are mean $\pm$ SD of five mice per group and uses Tukey's post hoc at 0.05 significance.

Figure 2. Oral administration of cinnamic acid protects striatal innervation and neurotransmitters in MPTP-intoxicated mice.

Mice (n=5 per group) receiving cinnamic acid (100 mg/kg body wt/day) via gavage from 3h after the last injection of MPTP were sacrificed 7d after the last injection of MPTP followed by striatal TH immunostaining (A). Optical density of TH (B) shows protection by cinnamic acid [$F(3,16) = 166.2$, $p < .001$]. A 1 mm³ piece of striatum was dissected and evaluated by HPLC analysis. Cinnamic acid restores dopamine levels [$F(3, 26) = 49.63$, $p < 0.001$] (C). There was no effect on DOPAC (D) or HVA (E) levels. Results are mean $\pm$ SD of five mice per group and uses Tukey's post hoc at 0.05 significance.

Figure 3. Oral administration of cinnamic acid restores locomotor deficit induced by MPTP. Mice (n=5 per group) receiving cinnamic acid (100 mg/kg body wt/day) via gavage from 3h after the last injection of MPTP were sacrificed 7d after the last injection of MPTP followed by locomotor assays. Cinnamic acid improved time spent on rotarod significantly [$F(3, 26) = 20.62$, $p < 0.001$] (A). The number of mouse stereotypy was also restored with cinnamic acid [$F(3, 26) = 17.13$, $P < 0.001$] (B). Total number of rearing events (C) for the cinnamic acid receiving group was significant vs MPTP ($p < 0.05$) but not the vehicle control ($p > 0.05$). Upon further investigation it was found that cinnamic acid receiving mice had a significant number of unsupported rears (D) compared to MPTP and MPTP + vehicle [$F(3, 26) = 13.43$, $P < 0.01$]. Tukey's post hoc testing done between groups using 0.05 significance.

Figure 4. Cinnamic acid induces peroxisome proliferator response element (PPRE)-driven luciferase activity in primary mouse astrocytes.

Astrocytes isolated from wild type, PPARα (-/-) and PPARβ (-/-) mice cells were plated at 50–60% confluence in 12-well plates followed by cotransfection with 0.25 μg of tk-PPREx3-Luc, a PPRE-dependent luciferase reporter construct, and 12.5 ng of pRL-TK using LipofectAMINE Plus. Twenty-four hours after transfection, cells were treated with different concentrations of cinnamic acid for 4 h followed by measuring luciferase activity in a luminometer. WT and PPARβ (-/-), but not PPARα (-/-), astrocytes show increased luciferase activity after 4 treatment from cinnamic acid. Results are mean ± SD of three independent experiments. Two-way ANOVA found significance for genotype, treatment and their interaction [F genotype (2, 30) = 289.9, p < 0.001], [F treatment (4, 30) = 137.7, p < 0.001], [F interaction (8, 30) = 29.98, p < 0.001]. Bonferroni post hoc analysis was employed at 0.05.

Figure 5. Cinnamic acid protects striatal TH fibers via PPARα, not PPARβ.

WT, PPARα (-/-) and PPARβ (-/-) mice (n=5 per group) receiving cinnamic acid (100 mg/kg body wt/day) via gavage from 3h after the last injection of MPTP were sacrificed 7d after the last injection of MPTP followed by striatal TH immunostaining (A). Optical density of TH (B) shows protection for WT and PPARβ (-/-), but not PPARα (-/-), mice by cinnamic acid. Two-way ANOVA shows that cinnamic acid is protective depending on treatment of genotype [$F(4, 54) = 11.6, p < .001$]. Results are mean $\pm$ SD of five mice per group and use Bonferroni's post hoc analysis set to 0.05.

Figure 6. Cinnamic acid restores striatal dopamine via PPAR α , not PPAR β .

WT, PPAR α (-/-) and PPAR β (-/-) mice (n=6 per group) receiving cinnamic acid (100 mg/kg body wt/day) via gavage from 3h after the last injection of MPTP were sacrificed 7d after the last injection of MPTP followed by HPLC analysis of the striatum (A, Dopamine; B, DOPAC; C, HVA). Cinnamic acid did not restore dopamine levels in PPAR α (-/-) mice unlike dopamine protection in WT and PPAR β (-/-) (A). A two-way ANOVA found significance in treatment, genotype and interaction [F treatment(2, 51) = 127.7, $p < 0.001$], [F genotype(2, 51) = 7.93, $p < .01$] and [F interaction(4, 51) = 4.92, $p < 0.01$]. For DOPAC, a two-way ANOVA found significance in treatment, and genotype but not interaction [F treatment(2, 51) = 24.84, $p < .001$], [F genotype (2, 51) = 4.78, $p < 0.05$] and [F interaction (4, 51) = 1.32, $p > 0.05$] (B). For HVA, a two-way ANOVA found significance in genotype, but not treatment and interaction [F genotype (2, 51) = 13.86, $p < 0.001$], [F treatment (2, 51) = 2.86, $p > 0.05$], and [F interaction (4, 51) = 2.21, $p > 0.05$] (C). All tests use Bonferroni's post hoc at 0.05. Results are $\pm$ SEM of six mice per group.

Figure 7. Cinnamic acid improves locomotor functions in MPTP mouse model of PD via PPAR α , not PPAR β .

WT, PPAR α (-/-) and PPAR β (-/-) mice received cinnamic acid (100 mg/kg body wt/day) via gavage from 3h after the last injection of MPTP. After 7 d of treatment, mice were assayed for rotarod (A), rearing (B) and stereotypy (C). Results are mean $\pm$ SD of six mice per group. Results were analyzed by two-way ANOVA and use Bonferroni's post hoc at 0.05.