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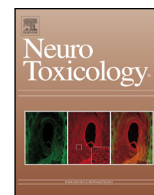
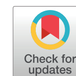


Full Length Article

Aminochrome decreases NGF, GDNF and induces neuroinflammation in organotypic midbrain slice cultures



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ABSTRACT

Recent evidence shows that aminochrome induces glial activation related to neuroinflammation. This dopamine derived molecule induces formation and stabilization of alpha-synuclein oligomers, mitochondria dysfunction, oxidative stress, dysfunction of proteasomal and lysosomal systems, endoplasmic reticulum stress and disruption of the microtubule network, but until now there has been no evidence of effects on production of cytokines and neurotrophic factors, that are mechanisms involved in neuronal loss in Parkinson's disease (PD). This study examines the potential role of aminochrome on the regulation of NGF, GDNF, TNF-α and IL-1β production and microglial activation in organotypic midbrain slice cultures from P8 - P9 Wistar rats. We demonstrated aminochrome (25 μM, for 24 h) induced reduction of GFAP expression, reduction of NGF and GDNF mRNA levels, morphological changes in Iba1^{+} cells, and increase of both TNF-α, IL-1β mRNA and protein levels. Moreover, aminochrome (25 μM, for 48 h) induced morphological changes in the edge of slices and reduction of TH expression. These results demonstrate neuroinflammation, as well as negative regulation of neurotrophic factors (GDNF and NGF), may be involved in aminochrome-induced neurodegeneration, and they contribute to a better understanding of PD pathogenesis.

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1. Introduction

Degeneration of the midbrain is key to understanding motor symptoms in Parkinson's disease (PD) (Braak et al., 2004). However, the mechanisms responsible for the loss of dopaminergic neurons in substantia nigra pars compacta (SNpc) remain unknown. Some cellular and molecular alterations have been described in patient and animal models, such as mitochondrial dysfunction (Exner et al., 2012; Yong-Kee et al., 2012a), protein degradation dysfunction (Ebrahimi-Fakhari et al., 2012), α-synuclein aggregation to neurotoxic oligomers (Kalia et al., 2013; Martinez-Vicente and Vila, 2013; Rohn, 2012), oxidative stress (Gołembiowska and Dziubina, 2012), endoplasmic reticulum stress (Mercado et al., 2013), loss of neurotophic factors (Mogi et al., 1999; Hirsch et al., 2013) and neuroinflammation (Taylor et al., 2013).

The role of neurotophic factors in the maintenance normal brain function in the healthy or neuroprotection in neurodegenerative disease is well documented (Weissmiller and Wu, 2012; Tomé et al., 2017). Among these neurotrophic factors, Glial cell line-derived neurotrophic factor (GDNF), originally isolated from rat glioma cell lines, has demonstrated pronounced neuroprotective effects specifically over dopaminergic neurons in embryos cultures, such as increasing the number of neurons and morphological diferenciation of TH positive neurons and increasing dopamine capture (Lin et al., 1993). Also, nerve growth factor (NGF) is recognized for its association with cholinergic neuron survival and growth (Collier and Sortwell, 1999). However, its relation with dopaminergic neurons in PD has been the target of some studies. Lorigados-Pedre and Bergado-Rosado (2004) showed evidence that primary damage on dopaminergic neurons

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affects NGF synthesis. Furthermore, Mogi et al. (1998) showed that striatum lesion induced by MPTP is associated with a decrease in NGF levels. Other studies showed that NGF protects dopaminergic neurons, in midbrain neuronal culture, against rotenone induced toxicity (Jiang et al., 2006).

Post-mortem evidence from Parkinson's diseased brains suggests the presence of neuroinflammation. Activated microglia cells, astrocytosis, T lymphocytes infiltration, increase in tumor necrosis factor α (TNF-α), interleukin 1β (IL-1β), interferon γ, the presence of inducible nitric oxide synthase and cyclooxygenase 2 were all detected in the substantia nigra. In the striatum TNF–α, β 2 microglobulin, IL-1β, IL-6 and IL-2 were detected (Hirsch and Hunot, 2009). In studies of serum from patients with idiopathic parkinsonism, an increase of TNF-α was detected (Dobbs et al., 1999). In studies of dopaminergic and striatal regions the concentrations of IL-1β, IL-6, EGF, and TGF-α were higher in parkinsonian patients (Mogi et al., 1994). Other evidence is the neuroinflammation induced by exogenous neurotoxins that produce acute dopaminergic degeneration in animal models, such as the 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (Vijitruth et al., 2006); 6-hydroxydopamine (Cicchetti et al., 2002) and rotenone (Sarkar et al., 2017).

The endogenous neurotoxin aminochrome has been proposed to be a more physiological preclinical model for PD, since this is a product of dopamine oxidation and the precursor of neuromelanin (Arriagada et al., 2004; Paris et al., 2011; Muñoz et al., 2012a,b; Segura-Aguilar and Kostrzewa, 2015). It is known that aminochrome is direct involved in the formation and stabilization of neurotoxic alpha-synuclein oligomer (Muñoz et al., 2015; Norris et al., 2005), mitochondria dysfunction, oxidative stress (Paris et al., 2011; Aguirre et al., 2012), dysfunction of both proteasomal and lysosomal systems for protein degradation (Zafar et al., 2006; Zhou and Lim, 2009; Huenchuguala et al., 2014), endoplasmic reticulum stress (Xiong et al., 2014) and formation of adducts with tubulin (Briceño et al., 2016). In our previous studies, we demonstrated that aminochrome induces microglial and astroglial activation (Santos et al., 2017). However, there is no information about neurotoxic cytokines associated with neuroinflammation or modulation of neurotrophic factor by aminochrome. Therefore, the question being addressed here is whether aminochrome modulates the main neurotrophic factor and neurotoxic cytokynes involved in PD patophisiology.

2. Materials and methods

2.1. Synthesis and purification of aminochrome

Aminochrome was produced by incubation of dopamine (5 mM) with 10 ng of tyrosinase in MES buffer (25 mM, pH 6.0) for 10 min at room temperature. To purify, the incubation solution was loaded on a CM-Sephadex C50-120 (18 × 0.7 cm) column (Sigma-Aldrich, C50120) (Paris et al., 2010). The red–orange solution corresponding to aminochrome was collected and detected by spectrophotometry method measuring the absorbance at 480 nm. Aminochrome concentration was determined by the molar extinction coefficient of 3058 $M^{-1} cm^{-1}$ (Segura-Aguilar and Lind, 1989). We used the pH 6.0 to prevent aminochrome polymerization to neuromelanin.

2.2. Organotypic midbrain slice cultures

Midbrain organotypic cultures from postnatal (P8 – P9) wistar rats were prepared according to Stoppini et al. modified methodology (Stoppini et al., 1991) and performed according to Brazilian guidelines for production, maintenance and use of animals for teaching activities and scientific research (Brasil, 2015) and the local Ethical Committee for Animal Experimentation, protocol number (0272012, ICS - UFBA).

The brains were quickly removed and transferred to a polystyrene plate containing sterile culture medium. The mid-portion of midbrain with a thickness of 450 μm was obtained using the Tissue Chopper McILwain apparatus (prepared in sterile conditions and transferred three slices to each micropore membrane (Millicell - CM, Millipore, Beldford, MA, USA) contained in a six-well polystyrene plate and cultured in DMEM HAM's F12 medium (Cultilab,SP, Brazil), supplemented with 100 IU/mL penicillin G, 100 μg/mL streptomycin, 2 mM L-glutamine, 3.6 g/L HEPES, 33 mM glucose and 10% fetal bovine serum (FBS) (Cultilab, SP, Brazil). Slices were incubated in a humidified atmosphere with 5% CO_2 at 37 °C for 3 days, when treatments with aminochrome were performed for 24 h using fetal bovine serum free culture medium or treatments without aminochrome under control conditions.

2.3. Morphological analysis of slices

The morphological integrity of slices was evaluated by optical microscopy using an inverted microscope (LABOMET TCM400) after 24 and 48 h exposure to aminochrome at final concentration ranging from 0.01 to 25 μM or under negative control conditions (culture medium).

2.4. Measurement of lactate dehydrogenase (LDH)

Membrane integrity was evaluated by measuring the lactate dehydrogenase (LDH) activity in the organotypic culture medium under control conditions or exposed to 25 μM aminochrome for 48 h. After exposure, the culture medium was removed and the LDH activity (UI/L) was measured according to manufacturer protocol (Doles, Brazil). Data were expressed as LDH activity (UI/mL of culture medium).

2.5. Western immunoblotting

The expressions of Tyrosine Hydroxylase (TH), a dopaminergic neuron marker and Glial Fibrilary Acidic Protein (GFAP), an astroglial marker, were determined by western immunoblotting. After aminochrome exposure, or control conditions, culture plates were rinsed twice with PBS, harvested, and lysed in 2% (w/ v) SDS, 2 mmol/L EGTA, 4 mol/L urea, 0.5% (v/v) Triton X-100, and 62.5 mmol/L Tris-HCl buffer (pH 6.8) supplemented with a 0.1% (v/v) cocktail of protease inhibitors (Sigma P8340). Protein content was determined by a method adapted from Lowry et al. (1951) with a DC protein assay reagent kit (Bio-Rad, Hercules, CA, USA). For analysis, 10 μg protein, prepared as described above, was loaded onto a discontinuous 4% stacking and 10% running SDS polyacrylamide gel. Electrophoresis was performed at 200 V for 45 min. Proteins were then transferred onto a PVDF membrane (Immobilon-P, Millipore), at 100 V for 1 h. Subsequently, membranes were blocked for 1 h at room temperature in 20 mmol/L Tris-buffered saline solution (pH 7.5) containing 0.05% Tween 20 (TBS-T) and 5% powdered skimmed milk. Then, membranes were incubated with rabbit IgG polyclonal anti-TH (1:1000; Abcam ab152) or rabbit IgG polyclonal anti- GFAP (1: 1000; DAKO Z0334) diluted in TBS-T containing 1% powdered skimmed milk, overnight. Rabbit anti-GAPDH antibody (1:1000, Sigma G9545) was used as an internal standard band. Horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG was used as a secondary antibody. Immunoreactive bands were visualized by chemiluminescence immunoassay using the Immune Start HRP substrate kit (Bio-Rad). Images were obtained by scanning densitometry (Image Quants LAS 500 – GE

Healthcare Life Sciences) and analyzed with ImageJ 1.33u software (Wayne Rasband, National Institutes of Health, USA).

2.6. Immunohistochemistry

Microglial cell morphology was assessed by immunohistochemistry for Ionized calcium-binding adaptor molecule 1 (Iba1) in slices exposed to aminochrome (25 μM) or control conditions (without aminochrome), for 24 h. Afterwards, slices were fixed with 4% paraformaldehyde (PFA) for 10 min at room temperature following by 20% methanol solution for 5 min at room temperature. Slices were washed three times with PBS, incubated on 0.5% Triton X-100 in PBS (Sigma) solution, for 12 h at 4 °C, and incubated on PBS containing 20% bovine serum albumin (BSA) (Sigma) solution overnight, at 4 °C. After blocking, samples were incubated with primary antibodies, rabbit anti-Iba1 primary antibodies (1:300, Wako Chemicals USA), diluted in PBS containing 1% BSA overnight. Afterwards, slices were washed three times with PBS. Then, secondary antibodies Alexa Fluor 594-conjugated goat anti-rabbit IgG (1:200; Molecular Probes, A11037) were added to slices and incubated for 2 h. Following this, the slices were washed with PBS three times and incubated with 1.0 μg/mL 4,6-diamidino-2-phenylindole (DAPI, Sigma) for nuclear staining. Staining was visualized on a confocal microscope (Leica, TCS-SP5). Images were captured with a 40× objective. After, the shape and the cell diameter analyses were performed in cell body plus proximal processes using ImageJ software. Iba1 positive cells showing rounded shape with cell diameter less than 15 μm were classified as amoeboid, while Iba1 positive cells showing bipolar shape with cell diameter greater than 15 μm were classified as non-amoeboid.

2.7. RNA extraction and real-time PCR

RNA extraction and qPCR were used to investigate GDNF, NGF, TNF-α and IL-1β mRNA levels. After 24 h exposure to aminochrome (25 μM), organotipic cultures were processed for qPCR. Total RNA was isolated using Trizol reagent (Ambiom™ 15,596,018) and subjected to DNase treatment using a TURBO DNA-free™ Kit (Invitrogen, AM1906), following manufacturer's instructions. The concentrations of the RNA samples were determined spectrophotometrically. Complementary DNA was generated from 2.5 μg total RNA using the SuperScript® VILO™ Master Mix according to manufacturer recommendations. Expression of mRNA by target genes and the endogenous controls genes β-Actin and HPRT1 were assessed using real-time PCR (with TaqMan Gene Expression Assay products, Applied Biosystems), according to the manufacturer's recommendations. Expression levels of each gene of interest were calculated by normalizing the quantified mRNA amount for β-Actin and HPRT1. Relative gene expression was determined and used to test significance between different groups. Real-time PCR was performed in QuantStudio™ 7 Flex Real-Time PCR System (Applied Biosystems, CA, USA) using TaqMan Universal PCR Master Mix II (Applied Biosystems™ 4,440,044), TaqMan probes and primers provided by Applied Biosystems. The assay IDs provided by the manufacturer are as follows: GDNF (Rn00569510_m1), NGF (Rn01533872_m1), IL-1β (Rn00580432_m1), TNF-α (Rn01525859_g1), HPRT1 (Rn01527840_m1), β-Actin (ACTB; Rn00667869_m1).

2.8. Enzyme-linked immunosorbent assay

To determinate TNF-α and IL-1β concentration in the culture cell medium, we used an indirect enzyme-linked immunosorbant assay (ELISA). The antigen was incubated for 24 h at room temperature, washed 3 times and primary antibody (1:10,000 dilution range) was subsequently added and incubated for 24 h, followed by secondary antibody incubation (rabbit 1:10,000 dilution range) for 3 h at room temperature. The immunoreactivity (1:1) was detected using TMB spectrophotometric detection kit from BD Biosciences (San Diego, USA); the reaction was stopped with sulfuric acid 2 M, and read at 450 nm. Purified recombinant TNF-α and IL-1β (Abcam — Cambridge, UK) was used for standard curve calculation.

2.9. Statistical analysis

Normal distribution and homogeneity of data variance were tested by the Kolmogorov–Smirnov test, respectively. Significant differences between control groups and one sample were determined by Paired *t*-Test or Mann Whitney test. Kruskal-Wallis test followed by Dunn's Test were used for multiple comparison. In all cases the statistic program used was GraphPad Instat., Version 5, for Windows© (San Diego, CA, USA, www.graphpad.com). Data at the 95% confidence interval were expressed as the mean ± standard deviation (SD). Data not following a normal distribution were expressed as median. Differences were considered statistically significant at $p < 0.05$. All analyses were performed with six independent experiments.

3. Results

Morphological analysis demonstrated integrity of the edges of slices in the negative control group (Fig. 1A), while the slices exposed to aminochrome (0.01–25 μM) for 48 h showed changes in the edge morphology marked by spherical cells (Fig. 1B and C). The number of spherical cells was increased in slices exposed to 25 μM aminochrome for 48 h (median = 46.3, $p < 0.05$) in comparison with slices in the control group (median = 8.5) (Fig. 1C). No morphological difference was observed in slices exposed to aminochrome (0.01–25 μM) for 24 h in comparison with slices in the control group (data not shown). In addition, an increase was observed in LDH activity in the culture medium of slices exposed to 25 μM aminochrome for 48 h (322.3 ± 67, $p < 0.001$), when compared to the negative control group (158.1 ± 32.6) (Fig. 1D).

Western immunoblotting analysis demonstrated that 25 μM aminochrome did not change TH expression after 24 h exposure (Fig. 1E), but did reduce TH expression after 48 h exposure (84.2 ± 8.1, $p < 0.05$), when compared to the negative control group (100 ± 1.9) (Fig. 1F). Moreover, a decrease in GFAP expression was observed in the cultures exposed to 25 μM aminochrome for 24 h (84.5 ± 7.7, $p < 0.01$), when compared to the negative control group (100 ± 4.5) (Fig. 1G).

We observed by qPCR analysis, that aminochrome is capable of inducing a slight decrease of GDNF mRNA relative expression (0.8 ± 0.08, $p < 0.01$), when compared to the negative control group (1.0) (Fig. 2A). We similarly observed that aminochrome induces a decrease of NGF mRNA relative expression (0.5 ± 0.18, $p < 0.001$), when compared to the negative control group (1.0) (Fig. 2B)

Immunohistochemistry assay for Iba1 in slices under control condition revealed a percentage of 5.98% Iba1$^+$ (Iba1 positive) cells (Fig. 3C) and 65% of these Iba1$^+$ cells presented a non-amoeboid morphology (Fig. 3D) whereas 35% presented an amoeboid-like phenotype (Fig. 3E). However, after 24 h exposure to 25 μM aminochrome, slices presented 5.64% Iba1$^+$ cells (Fig. 3 C), and a decrease to 9.8% in the percentage of Iba1$^+$ cells presenting a non-amoeboid morphology (Fig. 3D), associated with an increase to 90.2% in the percentage of Iba1$^+$ cells presenting an amoeboid-like phenotype (Fig. 3E).

Analysis by qPCR also revealed an increase of TNF-α mRNA levels in slices exposed for 24 h to 25 μM aminochrome (3.3 ± 1.8, $p < 0.05$), when compared to the negative control group (1.0)

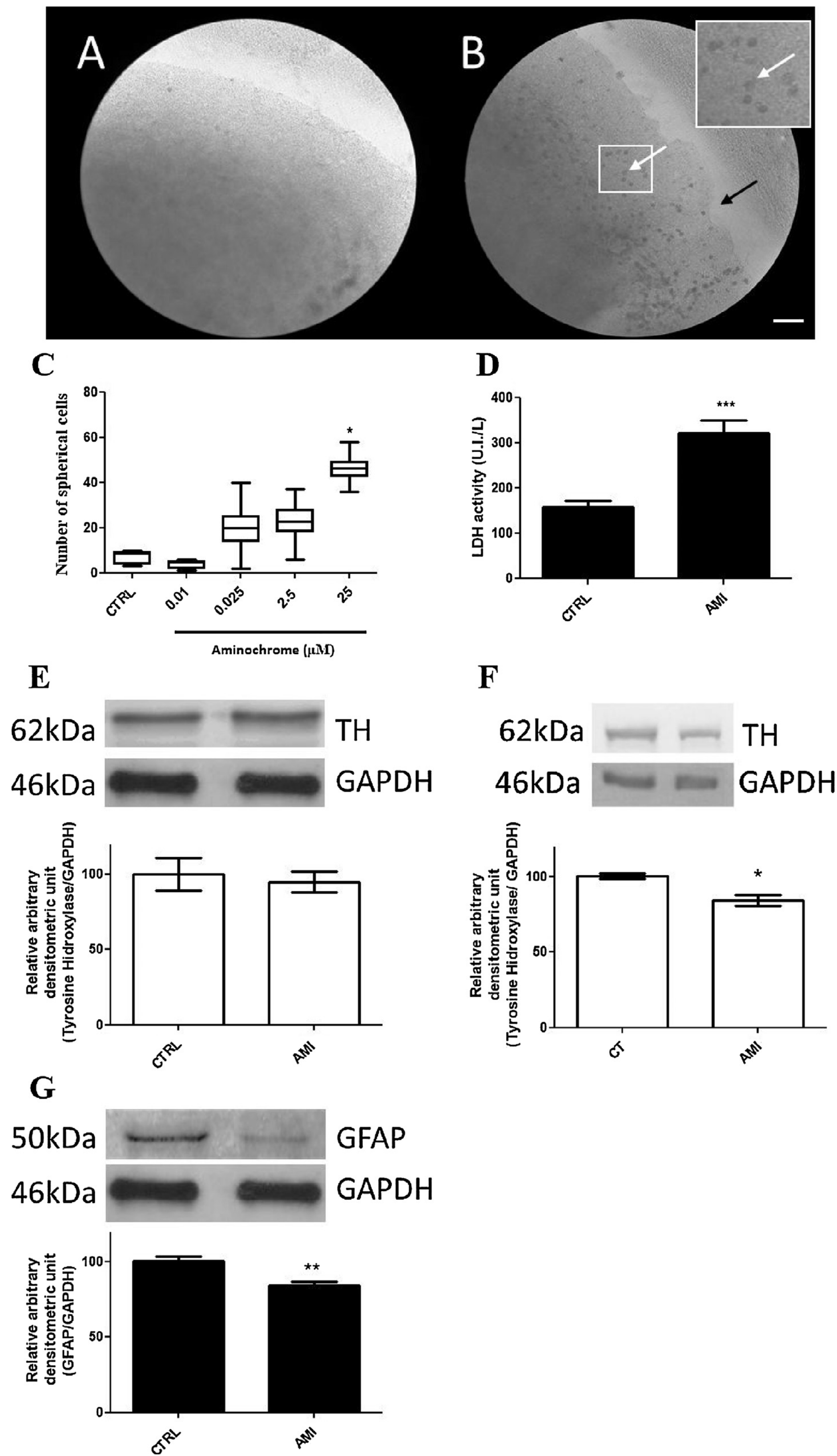

Fig. 1. Aminochrome induces toxicity in organotypic midbrain slice cultures. A: Photomicrograph of slice under control conditions. B: Photomicrograph of slice after 48 h exposure to 25 μM aminochrome. Black arrow shows disformed edge of slice and white arrows show spherical cells in edge of slice. In A–B, Obj. 40×, scale bars = 50 μm. C: Quantification of spherical cells in edges of slices under control conditions or exposed to aminochrome (0.01; 0.025; 2.5; 25 μM) for 48 h. The data were represented by median and range (n = 6). The statistical significance values were analyzed by Kruskal-Wallis test followed by Dunn's test (*$p < 0.05$). D: Measurement of LDH activity in supernatant of culture under control conditions or exposed to 25 μM aminochrome for 48 h. The data were represented by mean ± SD (n = 6). The statistical significance values were analyzed by Student's t-test (***$p < 0.001$). E: Western blotting (WB) showed normal expression of TH in aminochrome exposed slices for 24 h. F: WB showed decreased expression of TH in aminochrome exposed slices for 48 h. G: WB showed decreased expression of GFAP in aminochrome exposed slices for 24 h. In E, F and G, the results were normalized relative to control group considered as 100%. The data were represented by mean ± SD (n = 6). The statistical significance values were analyzed by Student's *t*-test (*$p < 0.05$; **$p < 0.01$).

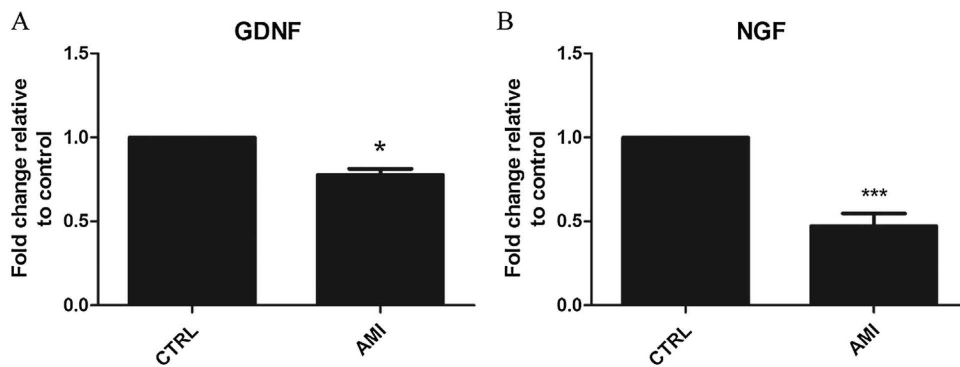

Fig. 2. Aminochrome downregulates neurotrophic factors. A: Analysis by qPCR of GDNF mRNA relative expression in organotypic midbrain slice cultures exposed to 25 μM aminochrome (AMI) for 24 h. B: Analysis of NGF mRNA relative expression in organotypic midbrain slice cultures exposed to 25 μM aminochrome (AMI) for 24 h. The data were represented by mean ± SD (n = 6). The statistical significance values were analyzed by Student's *t*-test of variance between control and one sample (*p < 0.01, **p < 0.001).

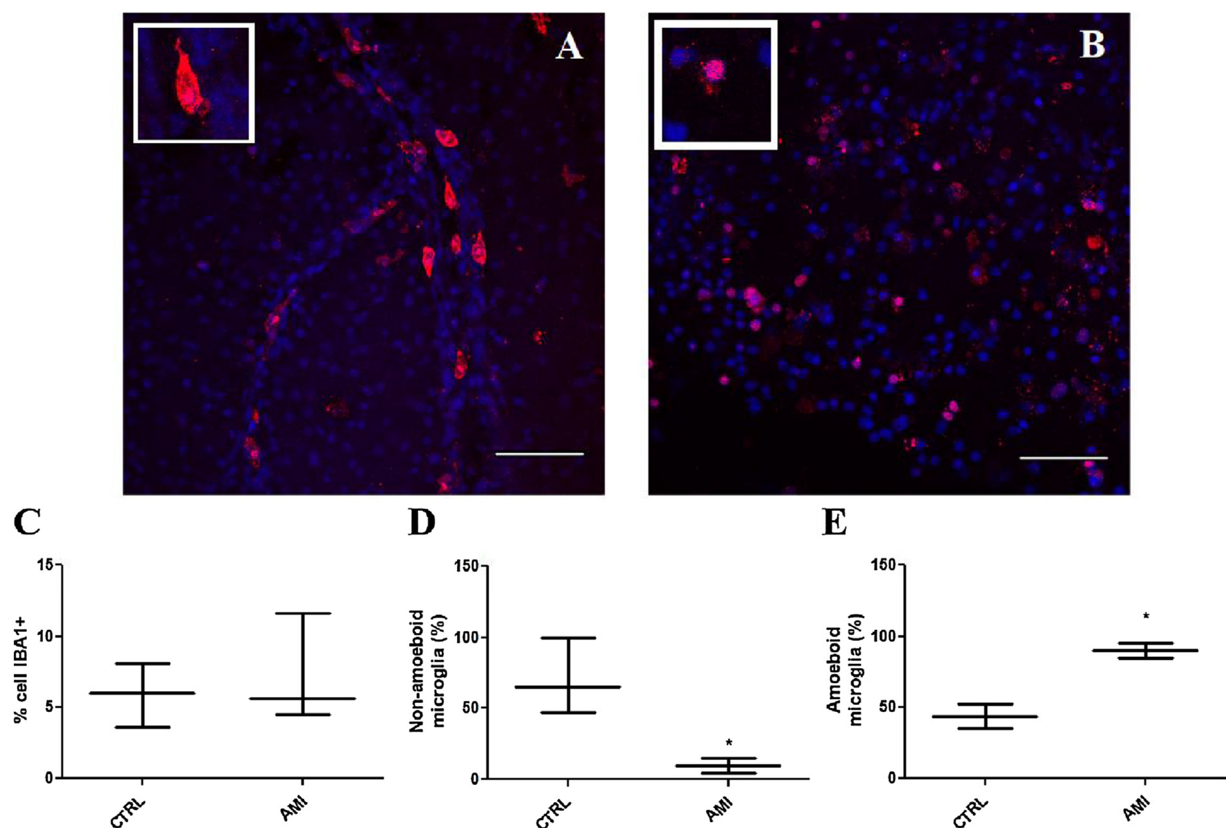

Fig. 3. Aminochrome induces microgliosis. A: Immunohistochemistry in organotypic midbrain slice cultures under control condition showing Iba1$^+$ cells with non-amoeboid morphology in red and DAPI stained nuclei in blue. B: Immunohistochemistry in slices exposed to 25 μM aminochrome (AMI), for 24 h, showing Iba1$^+$ cells with amoeboid-like morphology in red and DAPI stained nuclei in blue. In A and B, Obj. 40×, scale bars = 50 μm. C: Graphic represents median (n = 6) of percentage of Iba1$^+$ cells. D: Graphic represents median (n = 6) of percentage of Iba1$^+$ cells presenting non-amoeboid morphology. E: Graphic represents median and range (n = 6) of percentage of Iba1$^+$ cells presenting amoeboid morphology. Statistical significance values were analyzed by Mann Whitney test (*p < 0.05) (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

(Fig. 4A). Moreover, it revealed that aminochrome (25 μM, for 24 h) induced an increase of IL-1β mRNA levels (33.1 ± 10.9, p < 0.01), when compared to the negative control group (1.0) (Fig. 4B).

ELISA assay revealed TNF-α and IL-1β levels in the supernatant from the slices culture under control conditions of 10.8 ± 3.3 pg/mL and 11.3 ± 4.6 pg/mL, respectively. Increases were noted in the concentrations of TNF-α (22.8 ± 10.6 pg/mL, p < 0.05) and IL-1β (33.4 ± 14.03 pg/mL, p < 0.01) after 24 h exposure to 25 μM aminochrome (Fig. 4C and D).

4. Discussion

Aminochrome is suggested as an inducer of a more physiological animal study model for PD, since it is an endogenous neurotoxin that induces a progressive degeneration of dopaminergic neurons (Muñoz et al., 2015; Norris et al., 2005; Aguirre et al., 2012; Arriagada et al., 2004; Briceño et al., 2016; Cuevas et al., 2015; Huenchuguala et al., 2014; Muñoz et al., 2012a, b; Paris et al., 2011; Segura-aguilar and Kostrzewa, 2015; Xiong et al., 2014; Zafar et al., 2006; Segura-Aguilar, 2017). Studies have been

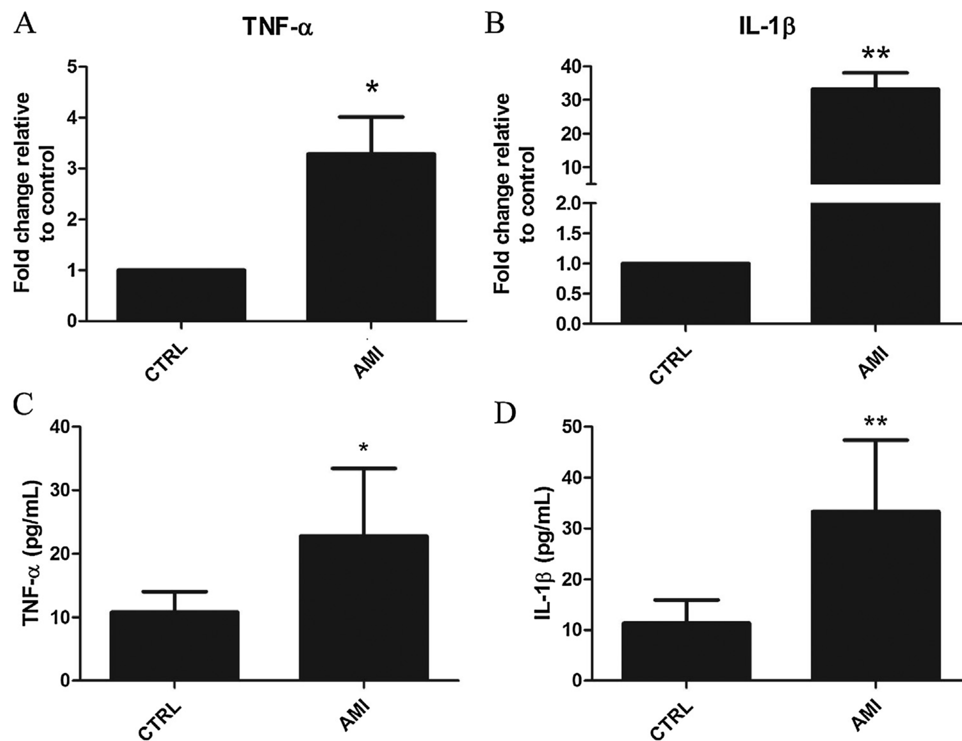

Fig. 4. Aminochrome increases TNF-α and IL-1β production. A and B: Analysis by qPCR for TNF-α and IL-1β mRNA relative expression in organotypic midbrain slice cultures exposed to 25 μM aminochrome (AMI), for 24 h, or under control condition. C and D: Analysis by ELISA for TNF-α and IL-1β levels released on slices culture medium after 24 h exposition to 25 μM AM or under control condition. The data were represented by mean ± SD (n = 6). The statistical significance values were assessed by Student's *t*-test (*p < 0.05, **p < 0.01).

demonstrated that 50 μM aminochrome for 48 h induces neuronal death in cells derived from the substantia nigra of adult rat (RCSN-3 cells) (Paris et al., 2011) and 10–50 μM aminochrome for 24 h induces neuronal death in neuroblastoma cells from human (SHSY-5Y cells) (Cuevas et al., 2015). In this study we demonstrated neurotoxic effects induced by 25 μM aminochrome after 48 h exposure, marked by spherical cell morphological changes of the edges of slices, confirmed by increased LDH activity in the culture medium. Studies have demonstrated that degeneration of rat CNS culture slices is marked by changes in morphology that primarily occur at the tissue edges rather than in central regions (Connelly et al., 2000). Moreover, Paris et al. (2011) reported an apparent volume reduction and change of elongated cell shape to spherical shape in RCSN-3 cells exposed to 50 μM aminochrome for 48 h. These effects were associated with a reduction in the TH expression that is an important indicator of dopaminergic neuronal dysfunction and midbrain degeneration, which contribute towards dopamine deficiency and phenotypic expression in PD (Kastner et al., 1993; Tabrez et al., 2012).

Recent studies demonstrated that astrocytes release human glutathione transferase (GSTM2) into the intersynaptic space where dopaminergic neurons internalize GSTM2 into the cytosol, to protect human dopaminergic neurons against aminochrome neurotoxicity (Cuevas et al., 2015). In our previous study, we demonstrated that aminochrome induces glial cells activation (Santos et al., 2017), but it is not clear what the role of these cells in the aminochrome neurotoxicity mechanism. However, it is known that glial cells might contribute to protective mechanisms by detoxifying oxygen free radicals and secreting neurotrophic factors such as Glial-derived neurotrophic factor (GDNF) and mesencephalic astrocyte-derived neurotrophic factor (MANF) (Lin et al., 1993; Voutilainen et al., 2009). On the other hand, it is known that they might contribute to neuronal degeneration via an inflammatory response associated with the release of neutotoxic factors or phagocytosis (Peterson and Flood, 2012; Hirsch and Hunot, 2009; Barcia et al., 2012). In this study we demonstrated that aminochrome induces downregulation of an important neuroprotective factor produced by astrocytes, GDNF, and reduces GFAP expression. On the other hand, we demonstrated that aminochrome upregulates the main neurotoxic cytokines TNF-α and IL-1β, associated with changes in microglia morphology characteristic of an activated phenotype.

The glial fibrillary acidic protein (GFAP) is a member of the cytoskeletal protein family in astrocytes, which is important to modulate cell motility and shape by providing structural stability of astrocytic processes (Eng et al., 2000). Activation of astrocytes is associated with an increase in GFAP expression, and has beneficial or harmful influence in CNS disorders such as neurodegenerative diseases (Pekny et al., 2014). On the other hand, studies have demonstrated reduction of GFAP expression is associated with autophagy (Tang et al., 2008; Silva et al., 2013); major depressive disorder (Si et al., 2004; Fatemi et al., 2004) and effects of monoamine oxidase B inhibitors, used as an anti-parkinsonian drug to boost the levels of dopamine (Li et al., 1993). We suggest that the reduction of GFAP expression demonstrated in this study is a consequence of aminochrome toxicity to astrocytes. Studies from Huenchuguala et al. (2014, 2016) have demonstrated DT-diaphorase and Glutathione transfarase mu 2 play an important role in preventing aminochrome-induced toxicity in astrocytes. We suggest that more studies must be developed to better characterize the aminochrome-induced toxicity in astrocytes in organotypic culture and its relationship with the pathogenesis of PD.

Knowledge of changes in some neurotrophins levels in PD patients is controversial within the scientific community. Studies on patients with PD using highly sensitive sandwich enzyme-linked immunosorbent assay have demonstrated a decrease in NGF, but not of GDNF levels (Mogi et al., 1999, 2001). However,

studies developed by Chauhan et al. (2001) using immunofluorescence over individual neuromelanin-containing neurons and in areas of neropil demonstrated a depletion of GDNF, but not NGF. In our study, the downregulation of NGF mRNA, observed in rat midbrain slices exposed to aminochrome, was greater than that observed for GDNF mRNA. Futhermore, several studies revealed the neuroprotective role of NGF on dopaminergic neurons in differents animal models, such as MPTP (Garcia et al., 1992); or 6-OHDA (Chaturvedi et al., 2006). Additionaly, evidence shows that GDNF has a neuroprotective role and it is capable of restoring dopaminergic neurons in SNpc damaged by MPTP or 6-OHDA in animal models (Cheng et al., 1998; Aoi et al., 2000; Ding et al., 2004). However, in the first clinical studies GDNF adminstered in patients with PD by intraventricular injection did not induced beneficial clinical effects, and in fact induced side effects, which were attributed to insufficient GDNF concentrations reached in the target tissue (Kordower et al., 1999; Nutt et al., 2003), resolved by use of programmable pumps and catheters implanted into the striatum (Gill et al., 2003).

Also, it is know that GDNF is a potent inhibitor of microglial activation (Rocha et al., 2012). In this study we have evidence that aminochrome induces activation of microglia by changes in morphology of Iba1$^+$ cells. Ionized calcium binding adaptor molecule 1 (Iba1) is a general marker for microglial cells in the central nervous system (Ito et al., 1998), that can be used to evaluate changes in morphology associated with microglial function. Recent studies have demonstrated a major presence of amoeboid Iba1$^+$ cells in the substantia nigra of PD patients (Doorn et al., 2014) and in our previous studies we showed that aminochrome induces an increase in the percentage of amoeboid Iba1$^+$ cells of primary mesencephalic neuron-glia cultures from E14 Wistar rats or directly in microglial cells of isolated primary cultures from P0-2 Wistar rats (Santos et al., 2017). More studies must be performed to clarify if the low level of GDNF induced by aminochrome can contribute to microglial activation in midbrain slices.

There are many different kinds of evidence that support the hypothesis that microgliosis plays, via multiple mechanisms, an important function in the progression of dopaminergic neuron degeneration, for example via production of ROS and NO that results on oxidative stress (Peterson and Flood, 2012), or via secretion of pro-inflammatory cytokines that results in amplification of neuroinflammation and apoptosis (Hirsch and Hunot, 2009). TNF-α and IL-1β are pro-inflammatory cytokines that can induce the expression of the inducible form of nitric oxide synthase (iNOS) or cyclo-oxygenase 2 (COX2) - enzymes to produce toxic reactive species (Boje and Arora, 1992; Chao et al., 1992; Vijitruth et al., 2006; Hirsch and Hunot, 2009). Moreover, TNF-α has been associated with iducible dysfunction of autophagy resulting in α-synuclein accumulation in neurons (Wang et al., 2015). In addition, IL-1β is one of the main molecules involved in non-canonical inflammasome pathway, typically activated by Toll-like receptors (Broz and Monack, 2013). In this present study, both TNF-α and IL-1β were upregulated in midbrain slices exposed to aminochrome. These results support the importance of neuroinflammation in the pathological mechanisms of Parkinson's disease and demonstrate that the product of dopamine oxidation, and the precursor of neuromelanin, is directly involved in several mechanisms of the disease. On the other hand, the use of organotypic slice cultures from postnatal (P8 – P9) wistar rats does not make clear the involvement of aminochrome in inflammaging (Calabrese et al., 2018). Indeed, aging is the major risk factor for Parkinson's disease (Calabrese et al., 2018) and some studies suggest that in aging, aberrant microglia activation leads to neurodegeneration (von Bernhardi et al., 2015). Additional research using brain organotypic cultures from aged animal is needed to make clear aminochrome's contribution to aging-related chronic inflammation (Schommer et al., 2017). Furthermore, it adds weight to the hypothesis that aminochrome may be a better PD preclinical model to find new pharmacological treatments that arrest the development of this disease.

5. Conclusion

Based on the set of results obtained in this work, we conclude that organotypic midbrain slice cultures, from 8 to 9-day-old rats exposed to aminochrome, constitute a good in vitro model to study neuroinflammation and regulation of neurotrophic factors associated with PD. Moreover, neuroinflammation, marked primarily by microglial response and production of cytokines TNF-α and IL-1β, as well as negative regulation of neurotrophic factors (GDNF and NGF), may be involved in aminochrome-induced neurodegeneration. Taken together, these results may contribute to a better understanding of Parkinson's disease pathogenesis.

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References

Aguirre, P., Urrutia, P., Tapia, V., Villa, M., Paris, I., Segura-Aguilar, J., Núñez, M.T., 2012. The dopamine metabolite aminochrome inhibits mitochondrial complex I and modifies the expression of iron transporters DMT1 and FPN1. Biometals 25, 795–803.

Aoi, M., Date, I., Tomita, S., Ohmoto, T., 2000. Single or continuous injection of glial cell line-derived neurotrophic factor in the striatum induces recovery of the nigrostriatal dopaminergic system. Neurol. Res. 22 (8), 832–836.

Arriagada, A., Paris, I., Sanchez delas Matas, M.J., Martinez-Alvarado, P., Cardenas, S., Castañeda, P., Graumann, R., Perez-Pastene, C., Olea-Azar, C., Couve, E., Herrero, M.T., Caviedes, P., Segura-Aguilar, J., 2004. On the neurotoxicity of leukoaminochrome o-semiquinone radical derived of dopamine oxidation: mitochondria damage, necrosis and hydroxyl radical formation. Neurobiol. Dis. 16, 468–477.

Barcia, C., Ros, C.M., Annese, V., Carrillo-de Sauvage, M.A., Ros-Bernal, F., Gómez, A., Yuste, J.E., Campuzano, C.M., de Pablos, V., Fernandez-Villalba, E., Herrero, M.T., 2012. ROCK/Cdc42-mediated microglial motility and gliapse formation lead to phagocytosis of degenerating dopaminergic neurons in vivo. Sci. Rep. 2, 809.

Boje, K.M., Arora, P.K., 1992. Microglial-produced nitric oxide and reactive nitrogen oxides mediate neuronal cell death. Brain Res. 587 (2), 250–256.

Braak, H., Ghebremedhin, E., Rüb, U., Bratzke, H., Del Tredici, K., 2004. Stages in the development of Parkinson's disease-related pathology. Cell Tissue Res. 318, 121–134.

Brasil, 2015. Guia brasileiro de produção, manutenção ou utilização de animais em atividades de ensino ou pesquisa científica 12: 53.

Briceño, A., Muñoz, P., Brito, P., Huenchuguala, S., Segura-Aguilar, J., Paris, I.B., 2016. Aminochrome toxicity is mediated by inhibition of microtubules polymerization through the formation of adducts with tubulina. Neurotoxic. Res. 29 (3), 381–393.

Broz, P., Monack, D.M., 2013. Noncanonical inflammasomes: caspase-11 activation and effector mechanisms. PLoS Pathog. 9 (2), e1003144.

Calabrese, V., Santoro, A., Monti, D., Crupi, R., Di Paola, R., Latteri, S., Cuzzocrea, S., Zappia, M., Giordano, J., Calabrese, E.J., Franceschi, C., 2018. Aging and Parkinson's disease: inflammaging, neuroinflammation and biological remodeling as key factors in pathogenesis. Free Radic. Biol. Med. 115, 80–91.

Chao, C.C., Hu, S., Molitor, T.W., Shaskan, E.G., Peterson, P.K., 1992. Activated microglia mediate neuronal cell injury via a nitric oxide mechanism. J. Immunol. 149 (8), 2736–2741.

Chaturvedi, R.K., Shukla, S., Seth, K., Agrawal, A.K., 2006. Nerve growth factor increases survival of dopaminergic graft, rescuenigral dopaminergic neurons and restores functional deficits in rat model of Parkinson's disease. Neurosci. Lett. 398 (1–2), 44–49.

Chauhan, N.B., Siegel, G.J., Lee, J.M., 2001. Depletion of glial cell line-derived neurotrophic factor in substantia nigra neurons of Parkinson's disease brain. J. Chem. Neuroanat. 21 (4), 277–288.

Cheng, F.C., Ni, D.R., Wu, M.C., Kuo, J.S., Chia, L.G., 1998. Glial cell line-derived neurotrophic factor protects against 1-methyl-4-phenyl-1,2,3,6-

tetrahydropyridine (MPTP)-induced neurotoxicity in C57BL/6 mice. Neurosci. Lett. 252 (2), 87–90.
Cicchetti, F., Brownell, A.L., Williams, K., Chen, Y.I., Livni, E., Isacson, O., 2002. Neuroinflammation of the nigrostriatal pathway during progressive 6-OHDA dopamine degeneration in rats monitored by immunohistochemistry and PET imaging. Eur. J. Neurosci. 15 (6), 991–998.
Collier, T.J., Sortwell, C.E., 1999. Therapeutic potential of nerve growth factors in Parkinson's disease. Drugs Aging 14, 261–287.
Connelly, C., et al., 2000. Metabolic activity of cultured rat brainstem, hippocampal and spinal cord slices. J. Neurosci. Methods 99, 1–7.
Cuevas, C., Huenchuguala, S., Muñoz, P., Villa, M., Paris, I., Mannervik, B., Segura-aguilar, J., 2015. Glutathione transferase M2-2 secretad from glioblastoma cell protects SH-SY5Y cell from aminochrome neurotoxicity. Neurotox. Res. 27 (3), 217–228.
Ding, Y.M., Jaumotte, J.D., Signore, A.P., Zigmond, M.J., 2004. Effects of 6-hydroxydopamine on primary cultures of substantia nigra: specific damage to dopamine neurons and the impact of glial cell line-derived neurotrophic factor. J. Neurochem. 89 (3), 776–787.
Dobbs, R.J., Charlett, A., Purkiss, A.G., Dobbs, S.M., Weller, C., Peterson, D.W., 1999. Association of circulating TNF-alpha and IL-6 with ageing and parkinsonism. Acta Neurol. Scand. 100 (1), 34–41.
Doorn, K.J., Moors, T., Drukarch, B., Berg, W.D.J.V., Lucassen, P.J., Dam, A.V., 2014. Microglial phenotypes and toll-like receptor 2 in the substantia nigra and hippocampus of incidental Lewy body disease cases and Parkinson's disease patients. Acta Neuropathol. Commun. 2, 90.
Ebrahimi-Fakhari, D., Wahlster, L., McLean, P.J., 2012. Protein degradation pathways in Parkinson's disease: curse or blessing. Acta Neuropathol. 124, 153–172.
Eng, L.F., Ghirnikar, R.S., Lee, Y.L., 2000. Glial fibrillary acidic protein: GFAP-thirty-one years (1969–2000). Neurochem. Res. 25, 1439–1451.
Exner, N., Lutz, A.K., Haass, C., Winklhofer, K.F., 2012. Mitochondrial dysfunction in Parkinson's disease: molecular mechanisms and pathophysiological consequences. EMBO J. 31, 3038–3062.
Fatemi, S.H., Laurence, J.A., Araghi-Niknam, M., Stary, J.M., Schulz, S.C., Lee, S., Gottesman, I., 2004. Glial fibrillary acidic protein is reduced in cerebellum of subjects with major depression, but not schizophrenia I. Schizophr. Res. 69 (2–3), 317–323.
Garcia, E., Rios, C., Sotelo, J., 1992. Ventricular injection of nerve growth factor increases dopamine contentin the striata of MPTP-treated mice. Neurochem. Res. 17 (10), 979–982.
Gill, S.S., Patel, N.K., Hotton, G.R., O'Sullivan, K., McCarter, R., Bunnage, M., Brooks, D. J., Svendsen, C.N., Heywood, P., 2003. Direct brain infusion of glial cell line-derived neurotrophic factor in Parkinson disease. Nat. Med. 9 (5), 589–595.
Gołembiowska, K., Dziubina, A., 2012. Effect of adenosine A(2A) receptor antagonists and L-DOPA on hydroxyl radical, glutamate and dopamine in the striatum of 6-OHDA-treated rats. Neurotox. Res. 21, 222–230.
Hirsch, E.C., Hunot, S., 2009. Neuroinflammation in Parkinson's disease: a target for neuroprotection? Lancet Neurol. 4, 382–397.
Hirsch, E.C., Jenner, P., Przedborski, S., 2013. Pathogenesis of Parkinson's disease. Mov. Disord. 28 (1), 24–30.
Huenchuguala, S., Muñoz, P., Graumann, R., Paris, I., Segura-Aguilar, J., 2016. DT-diaphorase protects astrocytes from aminochrome-induced toxicity. Neuro. Toxicol. 55, 10–12.
Huenchuguala, S., Muñoz, P., Zavala, P., Cuevas, C., Ahumada, U., Graumann, R., et al., 2014. Glutathione transferase mu 2 protects glioblastoma cells against aminochrome toxicity by preventing autophagy and lysosome dysfunction. Rev. Autophagy 10 (4), 618–630.
Ito, D., Imai, Y., Ohsawa, K., Nakajima, K., Fukuuchi, Y., Kohsaka, S., 1998. Microglia-specific localisation of a novel calcium binding protein, Iba1. Brain Res. Mol. Brain Res. 57 (1), 1–9.
Jiang, Q., Yaz, Z., Feng, J., 2006. Neurotrophic factors stabilize microtubules and protect against rotenone toxicity on dopaminergic neurons. J. Biol. Chem. 281 (39), 391–400.
Kalia, L.V., Kalia, S.K., McLean, P.J., Lozano, A.M., Lang, A.E., 2013. α-Synuclein oligomers and clinical implications for Parkinson disease. Ann. Neurol. 73, 155–169.
Kastner, A., Hirsch, E.C., Agid, Y., Javoy-Agid, F., 1993. Tyrosine hydroxylase protein and messenger RNA in the dopaminergic nigral neurons of patients with Parkinson's disease. Brain Res. 606, 341–345.
Kordower, J.H., Palfi, S., Chen, E.Y., Ma, S.Y., Sendera, T., Cochran, E.J., Cochran, E.J., Mufson, E.J., Penn, R., Goetz, C.G., Comella, C.D., 1999. Clinicopathological findings following intraventricular glial-derivedneurotrophic factor treatment in a patient with Parkinson's disease. Ann. Neurol. 46 (3), 419–424.
Lin, L.F., Doherty, D.H., Lile, J.D., Bektesh, S., Collins, F., 1993. GDNF: a glial cell line-derived neurotrophic factor for midbrain dopaminergic neurons. Science 21, 1130–1132.
Lorigados-Pedre, L., Bergado-Rosado, J., 2004. Nerve growth factor in neurodegeneration and neurorestorative therapy. Rev. Neurol. 38 (10), 957–971.
Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem. 193, 265–275.
Martinez-Vicente, M., Vila, M., 2013. Alpha-synuclein and protein degradation pathways in Parkinson's disease: a pathological feed-back loop. Exp. Neurol. 247, 308–313.
Mercado, G., Valdés, P., Hetz, C., 2013. An ERcentric view of Parkinson's disease. Trends Mol. Med. 19, 165–175.
Mogi, M., Harada, M., Kondo, T., Riederer, P., Inagaki, H., Minami, M., Nagatsu, T., 1994. Interleukin-1 beta, interleukin-6, epidermal growth factor and transforming growth factor-alpha are elevated in the brain from parkinsonian patients. Neurosci. Lett. 180 (2), 147–150.
Mogi, M., Togari, A., Kondo, T., Mizuno, Y., Kogure, O., Kuno, S., Ichinose, H., Nagatsu, T., 2001. Glial cell line-derived neurotrophic factor in the substantia nigra from control and parkinsonian brains. Neurosci. Lett. 300 (3), 179–181.
Mogi, M., Togari, A., Kondo, T., Mizuno, Y., Komure, O., Kuno, S., Ichinose, H., Nagatsu, T., 1999. Brain-derived growth factor and nerve growth factor concentrations are decreased in the substantia nigra in Parkinson's disease. Neurosci. Lett. 270 (1), 45–48.
Mogi, M., Togari, A., Ogawa, M., Ikeguchi, K., Shizuma, N., Fan, D., et al., 1998. Effects of repeated systemic administration of 1-methyl-4-phenyl- 1,2,3,6-tetrahydropyridine (MPTP) to mice on interleukin-1 beta and nerve growth factor in the striatum. Neurosci. Lett. 250 (1), 25–28.
Muñoz, P., Cardenas, S., Huenchuguala, S., Briceño, A., Couve, E., Paris, I., Segura-Aguilar, J., 2015. DT-diaphorase prevents aminochrome-induced alpha-synuclein oligomer formation and neurotoxicity. Toxicol. Sci. 145 (1), 37–47.
Muñoz, P., Huenchuguala, S., Paris, I., Cuevas, C., Villa, M., Caviedes, P., Segura-Aguilar, J., Tizabi, Y., 2012a. Protective effects of nicotine against aminochrome-induced toxicity in substantia nigra derived cells: implications for Parkinson's disease. Neurotox. Res. 22, 177–180.
Muñoz, P., Huenchuguala, S., Paris, I., Segura-Aguilar, J., 2012b. Dopamine oxidation and autophagy. Parkinsons Dis. 920953.
Norris, E.H., Giasson, B.I., Hodara, R., Xu, S., Trojanowski, J.Q., Ischiropoulos, H., Lee, V.M., 2005. Reversible inhibition of alpha-synuclein fibrillization by dopaminochrome-mediated conformational alterations. J. Biol. Chem. 380 (22), 21212–21219.
Nutt, J.G., Burchiel, K.J., Comella, C.L., Jankovic, J., Lang, A.E., Laws Jr., E.R., Lozano, A. M., Penn, R.D., Simpson Jr., R.K., Stacy, M., Wooten, G.F., 2003. Randomized, double-blind trial of glial cell line-derived neurotrophic factor (GDNF) in PD. Neurology 60 (1), 69–73.
Paris, I., Muñoz, P., Huenchuguala, S., Couve, E., Sanders, L.H., Greenamyre, J.T., Caviedes, P., Segura-Aguilar, J., 2011. Autophagy protects against aminochrome-induced cell death in substantia nigra-derived cell line. Toxicol. Sci. 121, 376–388.
Paris, I., Perez-Pastene, C., Cardenas, S., Iturriaga-Vasquez, P., Muñoz, P., Couve, E., Caviedes, P., Segura-Aguilar, J., 2010. Aminochrome induces disruption of actin, alpha- and beta-tubulin cytoskeleton networks in substantia-nigra-derived cell line. Neurotox. Res. 18 (1), 82–92.
Pekny, M., Wilhelmsson, U., Pekna, M., 2014. The dual role of astrocyte activation and reactive gliosis. Neurosci. Lett. 565, 30–38.
Peterson, L.J., Flood, P.M., 2012. Oxidative stress and microglial cells in Parkinson's disease. Mediat. Inflamm. 2012, 401264.
Rocha, S.M., Cristovão, A.C., Campos, F.L., Fonseca, C.P., Baltazar, G., 2012. Astrocyte-derived GDNF is a potent inhibitor of microglial activation. Neurobiol. Dis. 47 (3), 407–415.
Rohn, T.T., 2012. Targeting alpha-synuclein for the treatment of Parkinson's disease. CNS Neurol. Disord. Drug Targets 11, 174–179.
Santos, C.C., Araújo, F.M., Ferreira, R.S., Silva, V.B., Silva, J.H.C., Granjeiro, M.S., Soares, É.N., Pereira, E.P.L., Souza, C.S., Costa, S.L., Segura-Aguilar, J., Silva, V.D.A., 2017. Aminochrome induces microglia and astrocyte activation. Toxicol. In Vitro 42, 54–60.
Sarkar, S., Malovic, E., Harishchandra, D.S., Ghaisas, S., Panicker, N., Charli, A., Palanisamy, B.N., Rokad, D., Jin, H., Anantharam, V., Kanthasamy, A., Kanthasamy, A.G., 2017. Mitochondrial impairment in microglia amplifies NLRP3 inflammasome proinflammatory signaling in cell culture and animal models of Parkinson's disease. NPJ Parkinsons Dis. 3 (30), 1–15.
Schommer, J., Schrag, M., Nackenoff, A., Marwarha, G., Ghribi, O., 2017. Method for organotypic tissue culture in the aged animal. MethodsX 4, 166–171.
Segura-Aguilar, J., 2017. Aminochrome as preclinical model for Parkinson's disease. Oncotarget 8 (28), 45036–45037.
Segura-Aguilar, J., Kostrzewa, R.M., 2015. Neurotoxin mechanisms and processes relevant to Parkinson's disease: an update. Neurotox. Res. 27 (3), 328–354.
Segura-Aguilar, J., Lind, C., 1989. On the mechanism of the Mn3(+)-induced neurotoxicity of dopamine: prevention of quinoone-derived oxygen toxicity by DT diaphorase and superoxide dismutase. Chem. Biol. Interact. 72 (3), 309–324.
Si, X., Miguel-Hidalgo, Jose Javier, O.' Dwyer, Gillian, Stockmeier, Craig A., Rajkowska, Grazyna, 2004. Age-dependent reductions in the level of glial fibrillary acidic protein in the prefrontal cortex in major depression. Neuropsychopharmacology 29 (11), 2088–2096.
Silva, V.D.A., Pitanga, B.P., Nascimento, R.P., Souza, C.S., Coelho, P.L., Menezes-Filho, N., Silva, A.M., Costa, M.F., El-Bachá, R.S., Velozo, E.S., Costa, S.L., 2013. Juliprosopine and juliprosine from prosopis juliflora leaves induce mitochondrial damage and cytoplasmic vacuolation on cocultured glial cells and neurons. Chem. Res. Toxicol. 16, 1810–1820.
Stoppini, L., Buchs, P.A., Muller, D.J., 1991. A simple method for organotypic cultures of nervous tissue. Neurosci. Methods 37 (2), 173–182.
Tabrez, S., Jabir, N.R., Shakil, S., Greig, N.H., Alam, Q., Abuzenadah, A.M., Damanhouri, G.A., Kamal, M.A., 2012. A Synopsis on the Role of Tyrosine Hydroxylase in Parkinson's Disease. CNS Neurol. Disord. Drug Targets 4, 395–409.
Tang, G., Yue, Z., Talloczy, Z., Hagemann, T., Cho, W., Messing, A., Sulzer, D.L., Goldman, J.E., 2008. Autophagy induced by Alexander disease-mutant GFAP accumulation is regulated by p38/MAPK and mTOR signaling pathways. Hum. Mol. Genet. 17 (11), 1540–1555.

Taylor, J.M., Main, B.S., Crack, P.J., 2013. Neuroinflammation and oxidative stress: co-conspirators in the pathology of Parkinson's disease. Neurochem. Int. 62, 803–819.
Tomé, D., Fonseca, C.P., Campos, F.L., Baltazar, G., 2017. Role of neurotrophic factors in Parkinson's disease. Curr. Pharm. Des. 23, 809–838.
Vijitruth, R., Liu, M., Choi, D.Y., Nguyen, X.V., Hunter, R.L., Bing, G., 2006. Cyclooxygenase-2 mediates microglial activation and secondary dopaminergic cell death in the mouse MPTP model of Parkinson's disease. J. Neuroinflamm. 27 (3), 6.
von Bernhardi, R., Bernhardi, L.E., Eugenín, J., 2015. Microglial cell dysregulation in brain aging and neurodegeneration. Front. Aging Neurosci. 7 (124), 1–21.
Voutilainen, M.H., Bäck, S., Pörsti, E., Toppinen, L., Lindgren, L., Lindholm, P., Peränen, J., Saarma, M., Tuominen, R.K., 2009. Mesencephalic astrocyte-derived neurotrophic factor is neurorestorative in rat model of Parkinson's disease. J. Neurosci. 29 (30), 9651–9659.
Wang, M.X., Cheng, X.Y., Jin, M., Cao, Y.L., Yang, Y.P., Wang, J.D., Li, Q., Wang, F., Hu, L. F., Liu, C.F., 2015. TNF compromises lysosome acidification and reduces α-synuclein degradation via autophagy in dopaminergic cells. Exp. Neurol. 271, 112–121.
Weissmiller, A.M., Wu, C., 2012. Current advances in using neurotrophic factors to treat neurodegenerative disorders. Transl. Neurodegener. 1, 14.
Li, Xin-Min, Qi, Jin, Juorio, Augusto V., Boulton, Alan A., 1993. Reduction in glial fibrillary acidic protein mRNA abundance induced by (−)-deprenyl and other monoamine oxidase B inhibitors in C6 glioma cells. J. Neurochem. 61, 1573–1576.
Xiong, R., Siegel, D., Ross, D., 2014. Quinone-induced protein handling changes: implications for major protein handling systems in quinone-mediated toxicity. Toxicol. Appl. Pharmacol. 280 (2), 285–295.
Yong-Kee, C.J., Sidorova, E., Hanif, A., Perera, G., Nash, J.E., 2012a. Mitochondrial dysfunction precedes other sub-cellular abnormalities in an in vitro model linked with cell death in Parkinson's disease. Neurotox. Res. 21, 185–194.
Zafar, K.S., Siegel, D., Ross, D., 2006. A potential role for cyclized quinones derived from dopamine, DOPA, and 3,4- dihydroxyphenylacetic acid in proteasomal inhibition. Mol. Pharmacol. 70, 1079–1086.
Zhou, Z.D., Lim, T.M., 2009. Dopamine (DA) induced irreversible proteasome inhibition via DA derived quinones. Free Radic. Res. 43, 417–430.