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Aminochrome induces microglia and astrocyte activation

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ABSTRACT

Aminochrome has been suggested as a more physiological preclinical model capable of inducing five of the six mechanisms of Parkinson's Disease (PD). Until now, there is no evidence that aminochrome induces glial activation related to neuroinflammation, an important mechanism involved in the loss of dopaminergic neurons. In this study, the potential role of aminochrome on glial activation was studied in primary mesencephalic neuron-glia cultures and microglial primary culture from Wistar rats. We demonstrated that aminochrome induced a reduction in the number of viable cells on cultures exposed to concentration between 10 and 100 μM. Moreover, aminochrome induces neuronal death determined by Fluoro-jade B. Furthermore, we demonstrated that aminochrome induced reduction in the number of TH-immunoreactive neurons and reactive gliosis, featured by morphological changes in $GFAP^+$ and $Iba1^+$ cells, increase in the number of $OX\text{-}42^+$ cells and increase in the number of NF-κB p50 immunoreactive cells. These results demonstrate aminochrome neuroinflammatory ability and support the hypothesis that it may be a better PD preclinical model to find new pharmacological treatment that stop the development of this disease.

1. Introduction

Neuroinflammation is one of the mechanisms involved in the loss of dopaminergic neurons in PD (Herrero et al., 2015). In substantia nigra of PD patients have been revealed reactive microglia expressing complement receptor 3 (CD11b/OX-42) (McGeer et al., 1988) and increase in the number of amoeboid immunoreactive cells for ionized calcium-binding adaptor molecule 1 (Iba1) (Doorn et al., 2014). A lot of evidences of neuroinflammation have been obtained with exogenous neurotoxins. The 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine has been shown to activate microglia and astrocytes (Tonouchi et al., 2016). 6-Hydroxydopamine stimulates mRNA and protein levels of TNF-α, interleukin-6 and -1β (Yan et al., 2015). Rotenone increases TNF-α, interleukin-1β and -6 in an animal model (Sharma et al., 2016) and another study with microglia cells showed that rotenone stimulated secretion of interleukin 1β -6, 12 and TNF-α (Ye et al., 2016).

It was demonstrated that microglial activation by neuromelanin is NF-κB dependent and involves p38 mitogen-activated protein kinase (Wilms et al., 2003). However, there is no information about neuroinflammation by using the endogenous neurotoxin aminochrome. The dopamine oxidation to neuromelanin is a pathway that involves the formation of several *o*-quinones. The most stable *o*-quinone formed during dopamine oxidation is aminochrome that can be neurotoxic by forming adducts with proteins or when it is reduced by one-electron to leukoaminochrome *o*-semiquinone radical (Segura-Aguilar et al., 2016). Therefore the question being addressed here is whether aminochrome induce microglia and astrocytes activation.

2. Materials and methods

2.1. Synthesis and purification of aminochrome

Aminochrome was produced by incubation of Dopamine (7.5 mmol) with 10 ng of tyrosinase in potassium phosphate buffer (25 mM, pH 6) for 15–20 min at room temperature. To purify, the incubation solution was loaded on a CM-Sephadex C50-1000 (18 × 0.7 cm) column (Sigma-Aldrich, C25120). 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).

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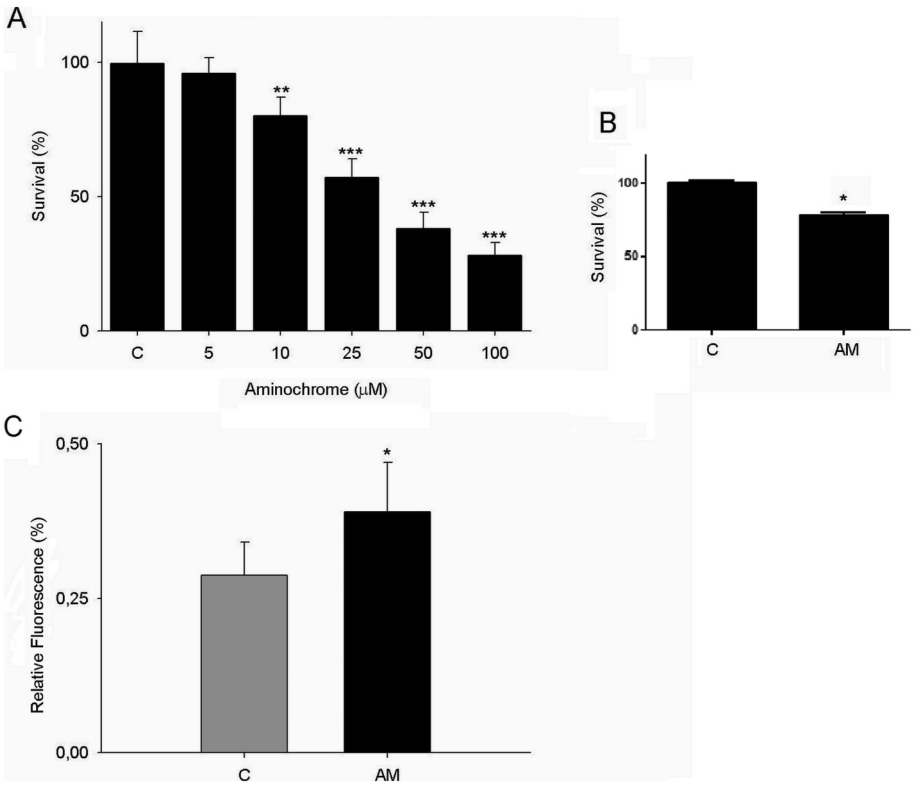

Fig. 1. The effect of aminochrome on cell viability in PMNG cultures. (A) Aminochrome induces decrease in cell survival in a concentration-dependent manner (10 to 100 μM, for 48 h) determined by Trypan blue staining. (B) Aminochrome induces decrease in cell survival since 24 h (10 μM) determined by Trypan blue staining. (C) Aminochrome induces neuronal cell death determined by Fluoro-jade B staining. The values are the mean ± SD (n = 8). In A, the statistical significance was standardized with one-way analysis of variance and postdate by post hoc Student-Newman-Keuls test, in B and C the statistical significance was assessed by using Student's *t*-test of variance between control and one sample (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

2.2. Primary cultures

Primary cultures from Wistar rats were performed according to Brazilian guidelines for production, maintenance and use of animals for teaching activities and scientific research and the local Ethical Committee for Animal Experimentation, protocol number (0272012, ICS - UFBA).

2.3. Mesencephalic neuron-glia (PMNG)

Primary mesencephalic neuron-glia cultures were prepared according to Zhang et al. (2007). Ventral mesencephalic tissues from E14 Wistar rats were dissected out and forced through a sterile 75 μm Nitex mesh. Cells were suspended in DMEM/HAM-F12 medium (Cultilab, Brazil) supplemented with glucose 1 g/L, 2 mM L-glutamine, 1 mM sodium pyruvate, 50 U/mL penicillin, 100 μg/mL streptomycin, 10% SFB (Cultilab) and 10% horse serum (HS) (Cultilab). After that, they were seeded at 5×10^5/well in 24-well culture plate precoated with poly-D-lysine (20 μg/mL) and laminin (2.5 μg/mL) and incubated in a humidified atmosphere with 5% CO_2 at 37 °C. Seven-day cultures were used for treatment with the composition about 55% astroglia, 7% microglia, and 37% neurons, of which about 3% was TH-immunoreactive neurons.

2.4. Microglial cultures

Microglial cells were obtained from cortex of Wistar newborn rats (0–2 days old) Microglial isolation was performed according to Mecha et al. (2011). In brief, after decapitation, the forebrains of newborn Wistar rats were dissociated mechanically and resuspended in DMEM supplemented with 10% FBS, 10% HS, 4 mM L-glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin. The cells were cultured on poly-D-lysine (25 μg/mL)-coated flasks. Upon reaching confluence (7–10 days), adherent microglial cells were harvested by shaking at 165 rpm at 37 °C for 3 h. Isolated microglia were seeded into 24-well plates at a density of $3 \times 10^4/cm^2$, and experiments performed after 24 h. Cells were cultured at 37 °C in 5% CO_2.

2.5. Cell viability

Cell viability was accessed by Trypan blue exclusion test in cultures exposed to aminochrome at different concentrations (5–100 μM) or control conditions (without aminochrome), for 48 h. Floating and adherent cells were harvested after trypsinization (trypsin 0.05%, EDTA 0.02%) and centrifuged at 1300 × *g* for 5 min. The cells were suspended in 200 μL PBS and stained with Trypan blue (0.1%). The proportion of viable cells was determined.

Moreover, floating and adherent cells, cultured in 35-mm Ø plates (TPP Switzerland), were harvested after trypsinization (trypsin 0.025%, EDTA 0.50%) and centrifuged for 5 min at 1300 × *g*.

2.6. Neurodegeneration

Neurodegeneration was assessed using FJ-staining performed ac-