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Neuronal, astroglial and locomotor injuries in subchronic copper intoxicated rats are repaired by curcumin: A possible link with Parkinson's disease

Abdellatif Abbaoui, Halima Gamrani*

Cadi Ayyad University, Faculty of Sciences Semlalia, Neurosciences, Pharmacology and Environment Unit, Marrakesh, Morocco

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ABSTRACT

We aim herein to assess the neurotoxic effects of subchronic Cu-exposition (0125%) for 6 weeks on dopaminergic and astroglial systems then locomotor activity in rats as well as the probable therapeutic efficiency of curcumin-I (30 mg/kg B.W.). We found that intoxicated rats showed a significant impairment of Tyrosine Hydroxylase (TH) within substantia nigra pars compacta (SNc), ventral tegmental area (VTA) and the striatal outputs together with loss expression of GFAP in these structures. This was linked with an evident decrease in locomotor performance. Co-treatment with curcumin-I inverted these damages and exhibited a significant neuroprotective potential, thus, both TH expression and locomotor performance was reinstated in intoxicated rats. These results prove a profound dopaminergic and astroglial damages following subchronic Cu exposition and new beneficial curative potential of curcumin against subchronic Cu-induced astroglial and dopaminergic neurotoxicity. Consequently, we suggest that Cu neurotoxicity may be strengthened in vivo firstly by attacking and weaking the astroglial system, and curcumin could be prized as a powerful and preventive target for the neurodegenerative diseases related metal element, especially Parkinson's disease.

1. Introduction

Copper (Cu) is a necessary trace element for numerous physiological functions in mammals. Cu is essential to the action of many enzymatic systems specifically cytochrome c oxidase, superoxide dismutase (SOD) (a key enzyme in defense against excessive oxidation phenomena) and lysyl oxidase. In this respect, it intervenes in the metabolism of several nutrients: carbohydrates (sugars), lipids (fats) and iron. It contributes to oxygen transportation, neurotransmitters synthesis, the formation of red blood cells, immune defenses, bone mineralization, neurotransmitter regulation, and the production of melanin. (Uauy et al., 1998; Letelier et al., 2005; Desai and Kaler, 2008; Banci, 2013).

However, Cu is known to be the most toxic metal element to living beings. In fact, excessive Cu incite the generation of reactive oxygen species (ROS), inducing lipids and proteins damages (Wang et al., 2013), DNA injuries and impairment of mitochondrial roles (Desai and Kaler, 2008). Cumulative evidences have reported the intervention of excessive Cu in the physiopathology of neurodegenerative disorders in humans such as Parkinson's disease (PD), Alzheimer's disease, wilson's disease and prion disease (Strausak et al., 2001; Barnham and Bush, 2008; Dusek et al., 2015; Valensin et al., 2016). PD is a chronic neurodegenerative pathology well known by the occurrence of lewy bodies, augmented synucleinopathy, degeneration of dopaminergic neurons in the SNc and dopamine diminution in the striatum (Cheng et al., 2016; Okita et al., 2017). Such damages leads to motor dysfunctions, including rigidity, walking difficulty, tremor dyskinesia, instability and tremors. Moreover, Cu may interact with α-synuclein (αSyn) and induces its aggregation in PD, then, stimulates oxidative damage of αSyn related to death of dopamine neurons (Binolfi et al., 2010; Zhang et al., 2015; Dell'Acqua et al., 2015). Furthermore, Barnham and Bush (2008) has reported that high concentration of Cu has been detected in substantia nigra and in cerebrospinal fluid of PD patients.

The bioactive molecules, isolated from herbal plants, are continuously used to treat various tremors and pathologies. Curcumin, or diferuloyl-methane, also known as saffron in India, is a natural polyphenolic compound plentiful in the rhizome of Curcuma longa L. (Mythri and Srinivas Bharath, 2012). Traditional Pharmacopeia, habitually, used curcumin in many countries such as India and china, and is known to assure various therapeutic potentials such as anti-inflammatory, anti-cancerous, antimutagenic, antibacterial, as well as a powerful antioxidant (Miquel et al., 2002; Maheshwari et al., 2006; Mythri and Srinivas Bharath, 2012; Esatbeyoglu et al., 2012). Curcumin has a special polyphenolic structure, may cross the blood-brain barrier, able as a treatment for neurodegenerative diseases (Garcia-Alloza et al.,

* Corresponding author at: Neurosciences, Pharmacology and Environment Unit, Faculty of Sciences Semlalia, Cadi Ayyad University, Avenue My Abdellah, B.P. 2390, Marrakesh, Morocco.
E-mail address: gamrani@uca.ac.ma (H. Gamrani).

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2007). The protective effect of curcumin was described in vitro and in animal models, it can defend against free radicals damages and brain injury. Other epidemiological studies have associated the curcumin intake and feeble occurrence of neurodegenerative disorders specially PD and AD (Cole et al., 2007; Mythri and Srinivas Bharath, 2012). Furthermore, curcumin proves a beneficial effect against harms lead (Shukla et al., 2003; Benammi et al., 2014, 2016, 2017) aluminum (Sethi et al., 2009; Laabbar et al., 2014) and MPTP (Rajeswari, 2006). However, according to the best of our knowledge, no studies have explored the implication of subchronic Cu exposition in Parkinsonism as well the efficacy of curcumin in preventing chronic Cu damages. Hence, the objective of this investigation was, in one hand, to assess the toxicological effects of subchronic Cu exposition on dopaminergic and astroglial systems, as well as locomotion behavior, in the other hand, the possible restorative potential of curcumin against subchronic Cu-induced neurobehavioral dysfunctions linked to PD.

2. Material and methods

2.1. Animals

In this study, we used male Wistar rats, aged three months, delivered by the central animal-care facilities of Cadi Ayyad University, Marrakech (UCAM), Morocco. The use of the animals was requested and approved by the central animal-care. Animals were kept at constant temperature (25∘C) on a 12-h dark–light cycle with *ad libitum* access to food in a special room for animal lodging. Animals were treated in agreement with the guidelines of the UCAM. All procedures were in arrangement to the European decree, related to the ethical evaluation and authorization of projects using animals for experimental procedures, 1 February 2013, NOR: AGRG1238767 A. Consequently, we made all efforts to minimize the number and animals suffering.

Our animals were divided into 4 groups. Group-I: control rats (C) (n = 10). Group-II: rats (Cu) (n =10) received Cu at a dose of 0125% in drinking water for 6 weeks. Group-III: rats (Cur + Cu) (n =10) received Cu at a dose of 0125% in drinking water for 6 weeks and treatment with curcumin-I (suspended in olive oil) at a dose of 30 mg/kg BW by oral gavage daily for 6 weeks. Group-IV: rats (Cur) (n = 10) had only curcumin-I (suspended in olive oil) at a dose of 30 mg/kg B.W. by gavage daily for 6 weeks.We based, for experimental protocol and doses, on our preceding data and the literature (Daniel et al., 2004; Abbaoui et al., 2016; Benammi et al., 2016).

2.2. Chemicals

We gotten Cu-(II) acetate trihydrate from (Riedel-de Haen, Seelze, Germany; code No. 25038, Lot No. 83370). Curcumin, 95% (total curcuminoid content), from Turmeric rhizome, molecular formula $C_{21}H_{20}O_6$ and formula weight 368.39 was purchased from Alfa Aesar (Karlsruhe, Germany, code No. B21573).

2.3. Open-field test

We used The “open field” experiment to assess locomotor performance. The apparatus comprises 25 identical boxes of 20 cm per side (100 cm × 100 cm × 40 cm). We placed each animal in the center of the field and the rearings (Vertical locomotion) and the number of squares (horizontal locomotion) traversed by the rat was checked in for 5 min to habituate animals. Before the test, Rats had habituation sessions for 10 min on three successive days.

2.4. Immunohistochemistry study

After experiments, rats were sacrificed for the immunohistochemical study. Each animal was anesthetized with urethane via the intraperitoneal route (40 mg/kg i.p.) and perfused transcardiacally with cool physiological saline (NaCl 0.9%) (Sigma-aldrich, St.Louis,MO, USA, Cas-No. 7647-14-5, lot-No. BCBH2237 V) and paraformaldehyde (4%) (Panreac Quimica SA, Barcelona, Spain, catalog No.141451.1211, lot No.0000078736) in phosphate buffered saline (PBS, 0.1 M, pH 7.4) (Riedel-de Haen, Seelze, Germany). Then, Brains were quickly detached and post-fixed for 12 h at 4∘C, after, dehydrated by means of ordered ethanol series (70–100%), passed through serial polyethylene glycol (Merck-Shuchardts, Hohenbrunn, Germany, Cas-No.25322-68-3) (PEG: 20–100%) solutions and rooted in pure PEG. We made the coronal sections of 20 µm, using microtome according to stereotaxic coordinates, and then were collected in phosphate buffered saline (PBS). Slices were selected throughout the midbrain, through (SNc), (VTA) (Bregma – 5.3 mm) and the dorsal striatum (Bregma 0.20 mm). Slices were preincubated for 2 h in PBS with 0.3% triton and 0.1% bovine serum albumin (BSA) (Sigma-Aldrich, St Louis, USA, CAS-No. 9048-46-8, Lot NO. 078K0729), then incubated overnight at 4 °C in a solution of monoclonal TH antibody (Santa Cruz, CA, USA; catalog No.SC-25269) and monoclonal Glial Fibrillary Acidic Protein (GFAP) antibody, diluted 1/1000 containing PBS (0.1 M, pH 7.4), Triton (0.3%), and BSA (1%). Next, sections were washed for 5 min with PBS (0.1 M, pH 7.4) three times and incubated with the secondary antibody (rabbit anti-immunoglobulins, 1/500) (Vector Labs, Burlingame, CA, USA, Catalog NO.BA-1100, lot No.WO611) for 2 h at room temperature. Afterward the slices were incubated for 1h30 min in PBS buffer containing Triton (0.3%) and the Avidin-biotine peroxidase complex (Kit ABC 1/500) (Vector Laboratories Burlingame, Californie, USA, Catalog No.PK-6101). TH and GFAP were revealed ensuing the peroxidase enzymatic reaction, with the 3,3-diaminobenzidine(0.03%) (Sigma-Aldrich; Oakville, Canada, CAS No. 868272-85-9) and hydrogen peroxide (0.006%) in Tris buffer (0.05 M; pH 7.5). When, slices were dehydrated and mounted in Eukit for microscopy observation. To evaluate the specificity of the antibodies, negative controls were performed. We expose the sections to the similar immunohistochemical method as defined overhead and by way of the pre-immune serum or omitting the primary antibodies, we note the absence of immunostaining. These tests revealed that the primary antibodies against GFAP and TH exhibit specific marking (Benammi et al., 2014; Abbaoui et al., 2016; El Hiba et el., 2016; Abbaoui et al., 2017a, 2017b, 2017c).

2.5. Immunolabeling quantification

GFAP- immunoreactivity (GFAP-ir) and TH-immunoreactivity (TH-ir) quantification were executed allowing to the protocol described by Vilaplana and Lavialle (1999). We used a Zeiss-Axioskop 40 microscope (Carl Zeiss; Oberkochen, Germany) (objective magnification ×100) accompanying with a Nikon digital camera for digitization and images storing. Images were digitized into 512 × 512 pixels with eight bits of gray resolution and were stored in TIFF format. Subsequently, Adobe Photoshop v.6.0 (Adobe Systems, San Jose, CA, USA) was used for quantification and images analysis. Each image was transformed to the binary mode and the percentage of black pixels was got by the image histogram option of the software package. The obtained percent corresponds to the GFAP and TH immunopositive areas in the whole nucleus or the processes. We used a sample of 5 randomized sections/animal/group and the average of animals/group was used for statistical analysis. The sampled area for the immunohistochemical analysis is of 225 mm^2 from all studied structures.

2.6. Statistical analysis

Our results were subjected to a one-way analysis of variance (ANOVA). Then Tukey test was used to evaluate Post hoc differences between group means. Data are stated as mean ± S.E.M. and p values < 0.05 were deliberated significant. Statistical studies were achieved by means of the computer software SPSS 10.0 for Windows (SPSS, IBM, Chicago, IL, USA).