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Curcumin administration suppress acetylcholinesterase gene expression in cadmium treated rats

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ABSTRACT

Curcumin, the main polyphenolic component of turmeric (*Curcuma longa*) rhizomes have been reported to exert anticholinesterase potential with limited information on how they regulate acetylcholinesterase (AChE) gene expression. Hence, this study sought to evaluate the effect of curcumin on cerebral cortex acetylcholinesterase (AChE) activity and their mRNA gene expression level in cadmium (Cd)-treated rats. Furthermore, *in vitro* effect of different concentrations of curcumin (1–5 μg/mL) on rat cerebral cortex AChE activity was assessed. Animals were divided into six groups (n = 6): group 1 serve as control (without Cd) and receive saline/vehicle, group 2 receive saline plus curcumin at 25 mg/kg, group 3 receive saline plus curcumin 50 mg/kg, group 4 receive Cd plus vehicle, group 5 receive Cd plus curcumin at 25 mg/kg and group 6 receive Cd plus curcumin at 50 mg/kg. Rats received Cd (2.5 mg/kg) and curcumin (25 and 50 mg/kg, respectively) by oral gavage for 7 days. Acetylcholinesterase activity was measured by Ellman's method and AChE expression was carried out by a quantitative reverse transcriptase polymerase chain reaction (RT-qPCR) assay. We observed that acute administration of Cd increased acetylcholinesterase activity and in addition caused a significant ($P < 0.05$) increase in AChE mRNA levels in whole cerebral cortex when compared to control group. However, co-treatment with curcumin inhibited AChE activity and alters AChE mRNA levels when compared to Cd-treated group. In addition, curcumin inhibits rat cerebral cortex AChE activity *in vitro*. In conclusion, curcumin exhibit anti-acetylcholinesterase activity and suppressed AChE mRNA gene expression level in Cd exposed rats, thus providing some biochemical and molecular evidence on the therapeutic effect of this turmeric-derived compound in treating neurological disorders including Alzheimer's disease.

1. Introduction

Environmental exposure to cadmium (Cd) remains a public health concern due to their potential to cause adverse effects in the human population (Johri et al., 2010). It is a highly accumulative toxicant with very long biological half-life of over 20 years (Johri et al., 2010). Absorption and accumulation of cadmium in tissues is determined by a wide range of factors, like nutritional and vitamin status, age and sex (Salvatori et al., 2004). Studies have shown that Cd is able to cross the blood–brain barrier (BBB) and accumulate in the brain (Sinha et al., 2009) leading to several neurodegenerative diseases (Mendez-Armenta et al., 2001, 2003; Goncalves et al., 2010; Abdalla et al., 2014). In addition, Cd can cause changes in key enzymes of the CNS involved in maintaining the levels of important neurotransmitter and neuroprotective agent (Goncalves et al., 2010; Abdalla et al., 2014).

Acetylcholine (ACh) is a neurotransmitter with an important role in many functions of both the peripheral and central nervous systems acting in the learning and memory processes as well as locomotor control and cerebral blood flow (Goncalves et al., 2010; Deiana et al., 2011; Klinkenberg et al., 2011). Acetylcholinesterase (AChE), is a serine protease enzyme that hydrolyzes the neurotransmitter acetylcholine (ACh). AChE is found mainly in neuromuscular junctions and is the key enzyme in central nervous system of cholinergic brain synapses, where its activity serves to terminate synaptic neurotransmission. ACh levels in synaptic cleft

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are regulated by AChE activity. It has been shown that the AChE activity is implicated in cell proliferation and neurite outgrowth (Chacon et al., 2003). Interestingly, AChE responds to various insults including oxidative stress, an important event that has been related to the pathogenesis and progression of a variety of CNS disorders (Chacon et al., 2003). Thus, this enzyme is a target for the emerging therapeutic strategies to treat cognitive disorders like Alzheimer's disease (AD) (Shen et al., 2011).

It is well known that the metabolism and toxicity of Cd may be modified by many factors, including substances essential for life (Berglund et al., 1994; Brzóska and Moniuszko-Jakoniuk, 1998), one of these substances is phenolic compound. Curcumin is the main bioactive polyphenolic compound in turmeric (*Curcuma longa*) rhizome, a member of the ginger family (Zingiberaceae) (Anamika, 2012). Curcumin has a wide spectrum of therapeutic properties and it has been shown to possess antioxidant, anti-inflammatory, anticancer and anti-Alzheimer's properties (Strimpakos and Sharma, 2008). In addition, a number of studies have demonstrated the anti-cholinesterase activities of curcumin (Pan et al., 2008; Reeta et al., 2009; Tang et al., 2009; Akinyemi et al., 2017). Thus, Cd a potent neurotoxic compound in laboratory animals have been shown to alter AChE activity, however, the molecular mechanisms behind its toxicity are not well understood. On the other hand, curcumin supplementation prior to cadmium administration prevents several of the neurotoxic effects observed when cadmium is added alone by acting as an antioxidant and inhibiting AChE activity. Nevertheless, study on their consequences at the molecular level remains unexplored. Hence, the present study sought to investigate the effect of curcumin administration on mRNA expression level of cerebral cortex acetylcholinesterase (ache) gene in Cd exposed rats using the real-time reverse transcriptase polymerase chain reaction (RT-qPCR) technique.

2. Materials and methods

2.1. Chemicals

Cadmium sulphate was obtained from Oxford Laboratory, Mumbai, India and solubilized in normal saline. Curcumin and acetylthiocholine iodide were purchased from Sigma–Aldrich, St. Louis, MO, USA. All other reagents were of analytical grade and the water used was glass distilled.

2.2. Animals and experimental design

Adult male albino rats (weighing 150–180 g) were obtained from the animal breeding unit at College of Medicine, Afe Babalola University, Nigeria and were housed in cages, at room temperature 25–28 °C, relative humidity 60–70%, and 12 h light/dark cycle. Food (pellet rat chow) and water were available *ad libitum*. Animals were cared according to US National Institute of Health (NIH) ethical guidelines. After two weeks of acclimatization, animals were divided randomly into six groups (n = 10) categorized as follows:

- Group 1: Control (receive saline/vehicle)
- Group 2: Cur A (receive saline plus curcumin at 25 mg/kg)
- Group 3: Cur B (receive saline plus curcumin 50 mg/kg)
- Group 4: Cd (receive Cd plus vehicle)
- Group 5: Cd + Cur A (receive Cd plus curcumin at 25 mg/kg) and
- Group 6: Cd + Cur B (receive Cd plus curcumin at 50 mg/kg)

In this study, the rats received Cd in the form of Cd sulphate at a dosage of 2.5 mg/kg, i.p (Akinyemi et al., 2017) where it induce brain damage while the choice of the curcumin doses (25 and 50 mg/kg) was made based on our preliminary experiment where we obtained beneficial results of this compound in brain of rats. Both solutions were administered for a short term period of 10 days. Curcumin was administered 30 min after Cd and the solutions were freshly prepared. Cd was diluted in saline and the curcumin in 1% ethanol and both were administered (1 mL/kg). It is important to note that controls were performed to correct for vehicle (1% ethanol) interference. However, no significant differences between the results obtained to the vehicle (1% ethanol) and to the control (saline) were observed in the parameter analyzed in this study (data not shown).

After the treatment period, animals were fasted overnight and sacrificed 24 h after the last dose under light ether anesthesia. The whole cerebral cortex was isolated and the same rats were used for both measurements: AChE activity and analysis of gene expression by quantitative RT-PCR. Treatment protocol was in accordance with the ethical requirement of the Animal Use and Care Committee of Afe Babalola University, Ado – Ekiti, Nigeria.

2.3. Determination of acetylcholinesterase (AChE) activity

For AChE assay, cerebral cortex was homogenized in 10 vols 100 mM potassium-phosphate buffer (pH 7.5) and centrifuged at 1000*g* for 10 min. The supernatants were used for AChE activity analysis according to the method of Ellman et al. (1961). The reaction mixture (2 mL final volume) contained 100 mM potassium-phosphate buffer, pH 7.5 and 1 mM 5,5′-dithiobisnitrobenzoic acid (DTNB). The method is based on the formation of the yellow anion, 5,5′-dithio-bis-acidnitrobenzoic, measured by absorbance at 412 nm during 2-min incubation at 25 °C. The enzyme (40–50 μg of protein) was preincubated for 2 min. The reaction was initiated by adding 0.8 mM acetylthiocholine iodide (AcSCh). All samples were run in triplicate and the enzyme activity was expressed in μmol AcSCh/h/mg of protein. Protein was measured by the coomassie blue method according to Bradford (1976) using serum albumin as standard.

2.4. Analysis of acetylcholinesterase (AChE) gene expression by real-time reverse transcription polymerase chain reaction (RT-qPCR)

The analysis of AChE mRNA expression was carried out by a two-step quantitative reverse transcriptase polymerase chain reaction (RT-PCR) assay. Cerebral cortex of five animals per group each was isolated followed by a total RNA extraction using TRIzol Reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer instructions. The total RNA extracted was quantified using NanoDrop 2000 spectrophotometer and the ratio of OD260/OD280 of all extracted RNA samples was between 1.9 and 2.0. For reverse transcription (RT), first strand complementary DNA (cDNA) was synthesized from RNA by using a cDNA synthesis kit (Maxima H Minus First Strand cDNA Synthesis Kit) according to the manufacturer's instructions. Quantitative real-time polymerase chain reaction was performed In 20 μL reaction volumes containing 2 μL RT product (cDNAs) as template, 1× PCR buffer, 25 μM dNTPs, 0.2 μM of each primer (Table 1), 1.5 mM $MgCl_2$, 0.1× SYBR Green I (molecular probes), and 1U Taq DNA polymerase (Invitrogen) (Keseler et al., 2005). The thermal cycle was carried

Table 1
The sequences of oligonucleotide primers used for real-time RT-qPCR analysis.

Gene	Primer	Product size (bp)
AChE	F: CGCACCCCAGCCAGGAACTG R: GCCTCCGTGGGCATGCACAT	466
β-actin	F: AGCAAGAGAGGCATCCTGAC R: GTGGTACGACCAGAGGCATA	268