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Curcumin protects against cigarette smoke-induced cognitive impairment and increased acetylcholinesterase activity in rats

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ABSTRACT

Cigarette smoke, a widely spread habit, is associated with a decline in cognitive function and studies have demonstrated that curcumin (Cur), an Indian spice, possesses a strong neuroprotective potential. Considering the relevance of investigating dietary compounds this study aimed to investigate the effect of Cur on memory and acetylcholinesterase (AChE) activity in brain structures and blood of cigarette smoke-exposed rats. Male Wistar rats were treated with curcumin and cigarette smoke, once a day, 5 days each week, for 30 days. The experimental procedures were divided in two sets of experiments. In the first, the animals were divided into 4 groups: Vehicle (corn oil), Cur 12.5 mg/kg, Cur 25 mg/kg and Cur 50 mg/kg. In the second, the animals were divided into 5 groups: Vehicle (corn oil), Smoke, Smoke plus Cur 12.5 mg/kg, Smoke plus Cur 25 mg/kg and Smoke plus Cur 50 mg/kg. Treatment with Cur significantly prevented the decreased latency and cholinergic alterations in cigarette smoke-exposed rats. These AChE alterations could suggest a role in the memory impairment promoted by cigarette smoke-exposure and point toward the potential of Cur to modulate cholinergic neurotransmission and, consequently, improve cognition deficits induced by smoke. This study suggests that the dietary compound Cur may be involved in cholinergic system modulation and as a consequence exert an effect on learning and memory.

1. Introduction

Several studies have investigated the effects of smoking on cognitive function. In the majority of recent studies, a decline in cognitive function is attributed to the effects of cigarette smoke exposure [1,2], and a dose–response relationship with the amount smoked has been observed [3]. Many authors have studied inhibitors of acetylcholinesterase (AChE), a key enzyme involved in cognitive function, and verified an improvement in global cognitive functioning [4] by increasing the neurotransmitter acetylcholine concentration at cholinergic synapses located throughout the brain [5].

Curcumin (diferuloylmethane) is the major constituent in the most important fraction of the coloring agents found in the rhizomes of turmeric (*Curcuma longa* L.). Turmeric is a perennial herb that belongs to the Zingiberaceae family and is distributed throughout the tropical and subtropical regions of the world; it has been widely cultivated in Asiatic countries, mainly India and China [6]. Curcumin is a polyphenol employed in old Hindu medicine [7], and in traditional Indian and Chinese medicine, it is used for the treatment of many disorders [4,8].

In addition to the well-documented anti-inflammatory, antioxidant and chemopreventive (i.e., growth-inhibitory effects on cancer cells) properties [9–12], many studies have demonstrated the neuroprotective potential of curcumin in the prevention of cognitive dysfunction [13–15], neurotoxicity [16], neuroinflammation and Alzheimer's and Parkinson's disease [17], as well as in the promotion of neuroplasticity and neurogenesis [17,18]. The neuroprotective effects promoted by curcumin are

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thought to be partially related to the regulation of important enzymes and molecules involved in inflammation, such as cyclooxygenase-2 (COX-2), lipoxygenase, nuclear factor-kappa B (NF-κB) and cytokines [19]. Furthermore, some studies have demonstrated that curcumin is able to modulate intracellular signaling pathways through the activity of the Ca^{2+}-dependent protein kinase enzymes A, B, and C (PKA, PKB and PKC), as well as the inositol 1,4,5-triphosphate receptor, both of which are important to the neurotransmission [19]. A previous investigation [20] demonstrated that curcumin prevented the induced rise in brain AChE activity.

In the present study, we investigated the effects of curcumin on AChE activity in different cerebral regions and evaluated the memory parameters of rats exposed to cigarette smoke.

2. Material and methods

2.1. Reagents

Acetylthiocholine iodide, 5,5-dithiobis-2-nitrobenzoic acid (DTNB), tris (hydroxymethyl)-aminomethane GR, Coomassie brilliant blue G, and curcumin (curcumin ≥ 80%; curcuminoid content ≥ 94%) were obtained from Sigma Chemical Co (St. Louis, MO, USA). The brand of cigarette used in the experiment was manufactured by Souza Cruz S.A., Brazil. The cigarette contained 10 mg tar, 0.9 mg nicotine, and 10 mg carbon monoxide. All other reagents used in the experiments were of analytical grade and of highest purity.

2.2. Animals

Male Wistar rats (90–110 days) from the Central Animal House of the Federal University of Santa Maria (UFSM) were used in this experiment. They were housed five to a cage (49 × 34 × 16 cm) on a natural day/night cycle (lights on at 19:00 and off at 7:00) at a constant temperature of 21 °C with free access to water and standard chow *ad libitum*. All animal procedures were approved by the animal Ethics Committee from the UFSM (protocol under number: 23081.004963/2009-71).

2.3. Cigarette smoke exposure and treatment with curcumin

Experimental procedures were divided in two sets of experiments. In the first set, animals were randomly divided into four groups (10 rats in each group): Vehicle (corn oil); Cur 12.5 mg/kg body weight; Cur 25 mg/kg body weight; or Cur 50 mg/kg body weight. In the second experimental set, animals were divided into 5 groups (10 rats in each group): Vehicle (corn oil); smoke exposed; smoke and Cur 12.5 mg/kg body weight; smoke and Cur 25 mg/kg body weight; or smoke and Cur 50 mg/kg body weight. Curcumin was diluted with corn oil, administered by oral gavage, and did not exceed 1.0 ml/kg body weight. The treatment with curcumin and cigarette smoke was carried out once a day, 5 days each week, for 30 days (six weeks). We chose these doses of curcumin and time of treatment based on previous studies of our research group in which we observed that cigarette smoke have effects on the immune and central nervous system and curcumin showed a protective effect [21–23]. First, curcumin or corn oil was administered, and approximately 10 minutes later, the smoking groups were exposed to the aged and diluted sidestream smoke of commercial cigarettes inside a whole-body smoke exposure chamber for 15 minutes. Control animals were placed in an equal chamber for the same amount of time, but without exposure to smoke. While the smoke exposure procedure was performed, the control group was always outside, without any contact with the smoke [24].

2.4. Smoke generation

After placing the rats inside the exposure chamber (size 56.4 × 38.5 × 37.1 cm; plastic material), 4 cigarettes were lit, and a stopwatch was turned on. The cigarettes were fixed in a metal holder, allowing them to be fully burned down within a period of 15 min. After lighting the cigarettes, the chamber was immediately closed, with a small opening (371 × 40 mm) in both extremities for ventilation. The smoke generated inside the chamber was suctioned by a noiseless extractor fan to keep an air flow inside the chamber. A metal grille was placed on top of the cigarette holder to avoid direct contact with the cigarettes and, thus, to prevent the rats from injuring themselves. The inhalation exposure of our study was to aged and diluted sidestream smoke, used as a simulation of environmental tobacco smoke (ETS) as experienced by non-smokers [24].

2.5. Behavioral procedure — inhibitory avoidance

Thirty days after the treatment with smoke and curcumin or vehicle, animals were subjected to training and tested in a step-down inhibitory avoidance apparatus [25]. Briefly, the rats were subjected to a single training session in a step-down inhibitory avoidance apparatus, returned to their home cage and tested for retention 24 h later. The apparatus consisted of a 25 × 25 × 35 cm box with a grid floor, and the left portion was covered by a 7 × 25 cm platform 2.5 cm high. The rat was placed gently on the platform facing the rear left corner, and when the rat stepped down with all four paws on the grid, a 3 s 0.4 mA shock was applied to the grid. Test step-down latency was taken as a measure of retention, and a cut-off time of 600 s was established.

2.6. Behavioral procedure - open field

Immediately after the inhibitory avoidance test session, animals were transferred to an open-field measuring 56 × 40 × 30 cm, with the floor divided into 12 squares measuring 12 × 12 cm each. The open field session lasted 5 min, and during this time, the number of crossing and rearing responses was recorded. This test was carried out to identify motor disabilities, which might influence performance during the inhibitory avoidance test.

2.7. Brain tissue preparation

After the behavioral tests, animals were anesthetized and euthanized. Brain structures were quickly removed from skull, rinsed in ice-cold Tris–HCl buffer (10 mM, pH 7.4), placed on filter paper moistened with the same buffer on top of a Petri dish filled with ice and the following brain regions were dissected: hypothalamus, cerebellum, cerebral cortex, hippocampus and striatum, using consistent anatomical landmarks as criteria for dissection. Placing the brain ventral side up hypothalamus was dissected with the help of a scalpel. Cerebellum was dissected by cutting the cerebellar peduncles at the surface of the brainstem. Cerebral cortex comprised all regions dorsolateral to the olfactory tract, excluding the hippocampus, and was dissected from each hemisphere by peeling it away from the striatum. The brain structures were homogenized in a glass potter in a Tris–HCl solution. Aliquots of resulting brain structure homogenates were stored at −20 °C until utilization. Protein was determined previously in a range that varied for each structure: cerebral cortex (0.7 mg/ml), striatum (0.4 mg/ml), hippocampus (0.8 mg/ml), hypothalamus (0.6 mg/ml) and cerebellum (0.6 mg/ml) as determined by the Coomassie blue method [26], using bovine serum albumin as standard solution.