Neurobiology of Learning and Memory 100 (2013) 98–107

Contents lists available at SciVerse ScienceDirect

Neurobiology of Learning and Memory

journal homepage: www.elsevier.com/locate/ynlme

Free and nanoencapsulated curcumin prevents cigarette smoke-induced cognitive impairment and redox imbalance

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ARTICLE INFO

Article history:
Received 19 October 2012
Revised 21 November 2012
Accepted 10 December 2012
Available online 20 December 2012

Keywords:
Curcumin
Cigarette
Cognitive impairment
Oxidative stress
Lipid-core nanocapsules

ABSTRACT

Cigarette smoke-exposure promotes neurobiological changes associated with neurocognitive abnormalities. Curcumin, a natural polyphenol, have shown to be able to prevent cigarette smoke-induced cognitive impairment. Here, we investigated possible mechanisms involved in curcumin protection against cigarette smoke-induced cognitive impairment and, due to its poor bioavailability, we investigated the potential of using curcumin-loaded lipid-core nanocapsules (C-LNC) suspension. Rats were treated with curcumin and cigarette smoke, once a day, 5 days each week, for 30 days. Animals were divided into ten groups: I, control (vehicle/corn oil); II, curcumin 12.5 mg/kg; III, curcumin 25 mg/kg; IV, curcumin 50 mg/kg; V, C-LNC 4 mg/kg; VI, tobacco exposed; VII, curcumin 12.5 mg/kg along with tobacco exposure; VIII, curcumin 25 mg/kg along with tobacco exposure; IX, curcumin 50 mg/kg along with tobacco exposure; X, C-LNC 4 mg/kg along with tobacco exposure. Cigarette smoke-exposure impaired object recognition memory ($P < 0.001$), indicated by the low recognition index, increased biomarkers of oxidative/nitrosative stress such as TBARS ($P < 0.05$) and NOx ($P < 0.01$), decreased antioxidant defenses such as NPSH content ($P < 0.01$) and SOD activity ($P < 0.01$) and inhibited the activities of enzymes involved in ion homeostasis such as Na^+,K^+-ATPase and Ca^{2+}-ATPase. Both curcumin formulations (free and nanoencapsulated) prevented the memory impairment, the redox imbalance and the alterations observed in the ATPases activities. Maintenance of ion homeostasis and redox balance is involved in the protective mechanism of curcumin against tobacco-induced cognitive impairment. Our results suggest that curcumin is a potential therapeutic agent for neurocognition and that C-LNC may be an alternative to its poor bioavailability.

1. Introduction

Epidemiological data indicates that nearly 20% of worldwide population, estimated in 1.4 billion of people, uses products derived from tobacco and, as a consequence, around 6 million of people died only in 2011 (Eriksen, Mackay, & Ross, 2012). Cigarette smoke contains over 4000 different chemicals including many carcinogenic compounds (Genbacev-Krtolica, 2005). Besides these compounds, cigarette is also a source of reactive oxygen species (ROS) such as superoxide anion radical ($O_2^{\cdot}$), hydroxyl radical ($HO^{\cdot}$) and hydrogen peroxide (H_2O_2); and reactive nitrogen species (RNS) such as nitric oxide ($NO^{\cdot}$), peroxynitrite ($ONOO^-$) and peroxynitrate (O_2NOO^-) (Pryor & Stone, 1993).

Studies demonstrate that the reactive species generated by the exposure to these compounds and by combustion of cigarettes

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http://dx.doi.org/10.1016/j.nlm.2012.12.007

cause oxidative damage in biological macromolecules (Moriarty et al., 2003) and trigger an inflammatory cascade which can lead to the development and/or facilitate the development of many diseases that involve the central nervous system (CNS) (Almeida et al., 2008; McQuaid, Cunnea, McMahon, & Fitzgerald, 2009). Furthermore, chronic exposure to cigarette smoke alters ion homeostasis (Anbarasi, Vani, Balakrishna, & Devi, 2005), which also can contribute to neurological diseases and memory impairment since it is associated with neuronal injury or death (Xiao, Wei, Xia, Rothman, & Yu, 2002).

Curcumin (diferuloylmethane), a polyphenol, is an active principle of the perennial herb *Curcuma longa* L. (commonly known as turmeric) (Goel, Kunnumakkara, & Aggarwal, 2008). Studies demonstrate its cytoprotective potential against the oxidative damage in neuronal cells (Scapagnini et al., 2006) and neuroprotective in the prevention of cognitive dysfunction (Jaques et al., 2012; Pan, Qiu, Lu, & Dong, 2008; Reeta, Mehla, & Gupta, 2009; Tang et al., 2009), neurotoxicity (Sethi, Jyoti, Hussain, & Sharma, 2009), as well as in the promotion of neuroplasticity and neurogenesis (Begum et al., 2008; Kim et al., 2008).

Biological activity of curcumin, however, is severely limited due to its poor bioavailability (Kelloff et al., 1996). Facing this problem, many study groups have employed nanotechnology to improve oral bioavailability of curcumin and the effects of nanoparticles formulations of curcumin have been promising (Ray, Bisht, Maitra, Maitra, & Lahiri, 2011; Shaikh, Ankola, Beniwal, Singh, & Kumar, 2009; Thangapazham, Puri, Tele, Blumenthal, & Maheshwari, 2008).

In the present study, we investigated the effects of curcumin and C-LNC on ionic and oxidative stress parameters in cerebral cortex of rats exposed to cigarette smoke and also investigated their memory performance.

2. Materials and methods

2.1. Reagents

Ouabain octahydrate (⩾95%, HPLC, Sigma O3125), adenosine 5′-triphosphate disodium salt hydrate (⩾99%, Sigma A2383), 5-5′-dithiobis(2-nitrobenzoic acid) (⩾98%, TLC, Sigma D8130), (−)-epinephrine(+)bitartrate salt (Sigma E4375), malonaldehyde bis-dimethyl acetal (MDA, 99%, Aldrich 108383), 2-thiobarbituric acid (sodium derivative, Aldrich S564508) and (E,E)-1,7-bis(4-Hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione (diferuloylmethane; curcumin; Sigma C1386) were obtained from Sigma–Aldrich 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

Animals were randomly divided into ten groups (5 rats in each group): I, control (vehicle/corn oil); II, curcumin 12.5 mg/kg; III, curcumin 25 mg/kg; IV, curcumin 50 mg/kg; V, curcumin-loaded lipid-core nanocapsules (C-LNC) suspension 4 mg/kg; VI, tobacco exposed; VII, curcumin 12.5 mg/kg along with tobacco exposure; VIII, curcumin 25 mg/kg along with tobacco exposure; IX, curcumin 50 mg/kg along with tobacco exposure; X, C-LNC 4 mg/kg along with tobacco exposure. In their home cages, rats were housed in same-treatment groups. Free curcumin was diluted with corn oil, administered by oral gavage, and did not exceed 1.0 mL/animal. C-LNC was also administered by oral gavage, and did not exceed 2.5 mL/animal. The treatment with curcumin was carried out once a day, 5 days each week, for 30 days (6 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 (Jaques, Rezer, Goncalves, et al., 2011; Jaques, Rezer, Ruchel, et al., 2011; Jaques, Ruchel, et al., 2011; Jaques et al., 2012). Furthermore, this protocol was an attempt to mimic the occupational exposure of people which work 5 days a week. First curcumin or corn oil was administered, and approximately 10 min 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 min. They were exposed to smoke in groups of 5 rats, the whole group. 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 smoke (Thome et al., 2009).

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 as experienced by non-smokers (Thome et al., 2009).

2.5. Preparation of C-LNC

C-LNC were obtained by the interfacial deposition of polymer method (Jager et al., 2009). The organic phase contained poly(ε-caprolactone) as a biodegradable polymer (1.0 g), sorbitan monoestearate (0.383 g), curcumin (0.05 g) and grape seed oil as lipid-core (1.65 mL). These hydrophobic constituents were dissolved in 267 mL of acetone, a water miscible organic solvent, and injected into the 534 mL of aqueous phase containing polysorbate 80 (0.766 g). Then, acetone was eliminated and the aqueous phase concentrated by evaporation under reduced pressure to obtain 100 mL. The formulations were prepared and kept protected from light.

2.6. Characterization of lipid-core nanocapsules

2.6.1. Drug content, encapsulation efficiency and pH

Curcumin was assayed by validated liquid chromatography (LC) method. The mobile phase was composed by acetonitrile: 0.1% trifluoracetic acid (50/50 v/v), (adjusted with pH 3.0 with triethylamine) eluted at the flow rate of 0.6 mL min^{-1}. The column used