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Short Communication

Inhibition of monoamine oxidase-B by the polyphenolic compound, curcumin and its metabolite tetrahydrocurcumin, in a model of Parkinson’s disease induced by MPTP neurodegeneration in mice

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Abstract. We investigated the effects of the polyphenolic compound curcumin and its metabolite tetrahydrocurcumin (ThC), in the model of Parkinson’s disease induced in mice by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). In this model depletion of dopamine (DA) and DOPAC (3,4-dihydroxyphenylacetic acid) occurs with increased monoamine oxidase (MAO-B) activity. We used HPLC with electrochemical detection to measure DA and DOPAC respectively while MAO-B was assayed by spectrofluorimetry using the conversion of the fluorogenic substrate, kyruramine. Systemic administration of curcumin (80 mg/kg i. p.) and tetrahydrocurcumin (60 mg/kg i. p.) significantly reversed the MPTP-induced depletion of DA and DOPAC. The MAO-B activity was also significantly inhibited by these compounds. The results showed that curcumin and tetrahydrocurcumin reversed the MPTP induced depletion of DA and DOPAC which may in part be due to inhibition of MAO-B activity. In conclusion, both curcumin and its metabolite ThC exert neuroprotection against MPTP induced neurotoxicity.

Key words: MPTP – dopamine – DOPAC – curcumin – tetrahydrocurcumin – MAO-B

Introduction

Curcumin (1,7-bis(α-hydroxyl-3 methoxphenyl)-1,6-heptadiene-3,5-dione) is the principal yellow pigment extracted from turmeric, a commonly used spice, derived from the rhizome of the herb Curcuma longa Linn. (Ireson et al., 2002). This naturally occurring polyphenolic phytochemical is currently being examined in preclinical trials for cancer chemotherapy (Chan et al., 1998). Among its pharmacological actions curcumin has antiinflammatory effects (Chan et al., 1998) and anti-amyloidgenic neuroprotection (Oni et al., 2005). Tetrahydro-curcumin (ThC) is one of the major metabolites of curcumin which has potent bioactivity. ThC has been identified in the intestinal mucosal and hepatic cytosol from humans and rats (Holder et al., 2002; Okada et al., 2001; Ireson et al., 2001) and has similar in some of its properties to that of curcumin. The hydroxyl groups of ThC enable it to serve as sites for conjugation with glucuronic acid.

Recently, attention has focused on the activities of ThC as one of the major metabolites of curcumin, because this compound appears to exert greater antioxidant activity than curcumin in both in vitro and in vivo systems (Okada et al., 2001; Pari, Murugan, 2004). Structurally, ThC and curcumin (Figs. 1a, 1b) have identical β-diketone, structures and phenolic groups but differ in that ThC lacks the double bonds of curcumin (Sugiyama et al., 1996; Okada et al., 2001). Sugiyama et al. (1996) demonstrated that ThC exhibited similar physiological and pharmacological properties as the active form of curcumin in vivo. Natio et al. (2002) showed the involvement of ThC in biochemical and molecular actions of curcumin by ameliorating oxidative stress in cholesterol-fed rabbits. Curcumin and ThC have both been shown to have direct free radical scavenging properties and also function as antioxidants (Khopde et al., 2000; Okada et al., 2001). We, therefore, decided to investigate whether curcumin and ThC can prevent the dopamine (DA) induced depletion and increased monoamine oxidase-B (MAO-B) activity caused by the MPTP treatment in mice. One of the proposed cellular mechanisms underlying neurodegeneration caused by the potent Parkinson-like agent (MPTP) is the development
of oxidative stress (Mohanakumar et al., 2002) which may lead to depletion of DA in the striatal area (Samantaray and Mohanakumar, 2003). In the brain MPTP is converted to its biologically active metabolite 1-methyl-4-phenylpyridinium (MPP+) by the action of MAO-B which is toxic to neurons (Chiba et al., 1984). Animals with low levels of MAO-B in the brain are resistant to this neurotoxin (Mitra et al., 1994).

Materials and methods

Adult Swiss male albino mice (25–30 g) from the (RMMC & H) Raja Muthiah Medical College & Hospital Annamalai University, Annamalai Nagar, Tamil Nadu, South India, were used in the present study. They were housed under standard conditions of temperature (26 ± 1 °C) and illumination (12 h light/dark cycles) with access to water and food ad libitum provided as per guidelines of proper care and use of animals in laboratory research (Indian National Science Academy, New Delhi 2000). The study was approved by the Animal Ethics Committee of our Institute, RMMC & H.

Drug treatments

MPTP·HCl was purchased from Sigma Aldrich (Bangalore, India). ThC was a gift from the Biochemistry Department of Annamalai University. A total of 48 animals were divided into six groups. The first group was treated with saline i.p. (0.9%) for seven days. The second group received 10 mg/kg of MPTP i.p. at 1 h intervals with total dose of 40 mg/kg as previously described (Człorkowska, 1996). The third group was administered with curcumin 80 mg/kg (dissolved in DMSO) i.p. every 24 h for seven days. This dose was determined after initially testing various doses of curcumin at 20, 40, 80 and 160 mg/kg and it was found that doses higher than 80 mg/kg could not be employed because the plasma levels of liver enzymes were elevated at the higher dose, so reflecting liver toxicity. The fourth group received a combination of both treatments in which curcumin was given 1 h before administration of MPTP and every 24 h after final MPTP for 7 consecutive days. The fifth group was given ThC 60 mg/kg i.p. for seven days. The sixth group received a combination of both treatments in which ThC was given 1 h before administration of MPTP and every 24 h after final MPTP for 7 consecutive days. The animals were killed by cervical dislocation on the 3rd and 7th day after the last injection. The brains were rapidly removed and the striatum (ST) were carefully dissected on ice-chilled plate. These tissue were taken for assays of the amines, DA and DOPAC, as well as MAO activity.

Measurements of dopamine (DA) and DOPAC

For analysis of DA and its metabolites mice were decapitated and their brains were dissected and frozen on dry ice. Frozen sections of 1 m of striatum were micropunched, the samples were weighed and the tissue sonicated in 0.2 M ice cold perchloric acid containing 0.5% EDTA. The mixtures were centrifuged at 10,000 g for 10 min, and 20 µl supernatant was injected directly into the HPLC system to determine DA and DOPAC. The HPLC system was equipped with an electrochemical detector with an octadecyl silane (ODS 18) column (4.6 mm 1 D 25 cm). The mobile phase contained HPLC grade methanol with the flow rate was 1 ml/minute; the wavelength of detection being 254 nm. The results were presented as µg/g tissue.

Ex vivo assays of monoamine oxidase-B activity

Striatal monoamine oxidase B activity were assayed by fluorometric procedure as described (Morinan and Garratt, 1985). The effects of curcumin and ThC on MAO-B activity were tested using kynuramine as the substrate. The fluorogenic product that is formed, 4-hydroxyquinoline (4-HQ), was measured at excitation/emission wavelengths of 315/380 nm respectively. MAO-B activity was assayed in presence of clorgyline which inhibits specifically MAO-A activity present in the homogenate. For the assay, 100 µl of the enzyme preparation was incubated with 10 mM potassium phosphate buffer, pH 7.2 and the reaction was started by the addition of 3.07 mM kynuramine in presence and absence of 500 nM of clorgyline (equivalent to an IC50 dose to inhibit MAO-A activity). The reaction was terminated at 30 min by addition of 300 µl of ice cold, 4N perchloric acid, and the mixture was then centrifuged at (7,500 g for 5 min). The supernatant was added to twice the volume of 1N NaOH. The product formed 4-HQ, was determined from a standard curve prepared from authentic sample. The enzyme activity is expressed as nmol-4HQ formed/mg protein/h.

Results and discussion

The striatal levels of DA and DOPAC in mice treated with MPTP are shown in Table 1. It was found that the DA concentrations were markedly decreased (P < 0.05) in the striatum 3 days after MPTP treatment compared with saline-treated group. Also, the DOPAC concentration were significantly reduced (P < 0.05) at this period. Seven days after MPTP treatment, the DA and DOPAC concentrations were significantly reduced (73%, 59% respectively) in the striatum. Curcumin and ThC (60 mg/kg) reversed the reduction in striatal DA and DOPAC levels of mice after MPTP treatment; these effects being statistically significant.