European **Rev**iew for **Med**ical and **Pharmacol**ogical **Sci**ences 2006; 10: 157-161

Curcumin protects mouse brain from oxidative stress caused by 1-methyl-4-phenyl-1,2,3,6-tetrahydro pyridine

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Abstract. – We tested the hypothesis that curcumin, a polyphenolic antioxidant, acts as a powerful free radical scavenger in vivo in the brain, and interferes with oxidative stress caused by the parkinsonian neurotoxin, (MPTP) 1 methyl-4phenyl-1,2,3,6-tetrahydropyridine. We measured the (GSH) reduced glutathione levels, (TBARS) glutathione lipid peroxidation, (CAT) catalase and (SOD) superoxide dismutase activity in the (ST) striatum and (MB) mid brain 3rd day and 7th day following MPTP and curcumin administration. MPTP treatment caused a significant depletion in GSH and increased the specific activity of SOD, CAT and lipid peroxidation in both ST and MB on the 3rd and 7th day. MPTP induced GSH depletion and lipid peroxidation in ST and MB was blocked by curcumin treatment. Curcumin exhibited a synergistic effect on SOD and CAT activities in the ST and MB regions. The present study provides direct evidence for the involvement of curcumin in neuroprotection against oxidative stress.

Key Words:

MPTP, Curcumin, Superoxide dismutase, Catalase, Reduced glutathione, Lipid peroxidation.

Introduction

1-methyl-4phenyl-1,2,3,6-tetrahydropyridine (MPTP) is a potent neurotoxin that produces nigral dopaminergic neuronal damage in humans, primates and rodents[1,2,3], MPTP per se is not toxic to neurons, but it has to be oxidized to (MPP+) 1-methyl-2-phenyl pyridinium ion in the astrocytes by the action of monoamine oxidase for it to be active[4,5] MPP+ produces oxidative stress in the lungs, similar to its structural congener, paraquat[6]. The potent parkinsonic neurotoxin MPTP has been show to cause dopaminegic neurodegeneration by generation of free radicals[7,8] leading to oxidative stress as shown by alteration in the states of antioxidant enzymes and molecules[7,9]. Many phenolic, particularly flavonoids, tannins and phenyl propanoids are of particular interest in antioxidant activity. An increasing number of studies shows that nutritional antioxidants such as polyphenols are capable of blocking neuronal death *in vitro* and many therapeutic properties in animal models of neurodegenerative diseases including Alzheimer's and Parkinson's disease[10]. In the present study the neuroprotective of one such polyphenolic antioxidant, curcumin, was investigated, which is an yellow curry spice derived from turmeric, also widely used as dietary curry and herbal medicine in India.

Thus we designed the following study to test the antioxidant properties and neuroprotection of curcumin in brain of Swiss male albino mice.

Materials and Methods

Adult Swiss male albino mice 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 ± 1c) and illumination (12 h lightdark cycles) water and standard rodent food were made available. The experimental protocol met the national guidelines of proper care and use of animals in laboratory research (Indian National Science Academy, New Delhi 2000) and was approved by the Animal Ethics Committee of our Institute, RMMC & H.

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Drug Treatment

A total of 40 animals were divided into five groups. One group was treated with 10 mg/kg of MPTP (intraperitoneally) at 1 hour intervals with total dose of 40 mg/kg as previously described[11]; a second group was administered with dimethyl sulfoxide (DMSO) 4 ml/kg intraperitoneally every 24 hours for 7 days. A third group received curcumin intraperitoneally 80 mg/kg, dissolved in DMSO, intraperitoneally every 24 hours for seven days. A fourth group was administered with saline (0.9%), and a fifth group received a combination of both treatments, in which curcumin was given 1 hour before administration of MPTP and every 24 hours after final MPTP for consecutive 7 days.

Assay of Enzymic Antioxidants

Superoxide dismutase (SOD) activity was determined by the modified method of Kakkar P, et.al.[12]. A single unit of enzyme was expressed as 50% inhibition of NBT (nitroblue tetrazolium) reduction 1 min/mg protein. Catalase (CAT) was assayed colorimetrically as described by Sinha[13] using dichromate acetic acid reagent (5% potassium dichromate and glacial acetic acid were mixed in 1:3 ratio). The intensity was measured at 620 nm and the amount of hydrogen peroxide hydrolysed was calculated for the catalase activity.

Determination of Non-Enzymic Antioxidants

Reduced glutathione (GSH) was determined by the method of Ellman[14]. 1 ml of supernatant (0.5 ml of tissue homogenate precipitated by 2 ml of 5% trichloroacetic acid (TCA) was taken and 0.5 ml of Ellman's reagent (0.0198%) DTNB in 1% sodium citrate and 3 ml of phosphate buffer (pH 8.0) were added. The colour developed was read at 412 nm.

Estimation of Lipid Peroxidation

Lipid peroxidation in brain tissue was estimated colorimetrically by measuring thiobarbituring acid reactive substances (TBARS) and hydroperoxides by the methods of Nichans and Samuelson[15] and Jiang et al.[16], respectively. In brief, 0.1 ml of homogenate was treated with 2 ml of (1:1:1) ratio (TBA) thiobarbituric acid -(TCA) trichloroacetic acid -(HCl) chloridic acid reagent (TBA 0.37%, 0.25 N HCL and 15% TCA) and placed in water bath for 15 min, cooled and centrifuged and then clear supernatant was measured at 535 nm against reference blank. Protein was determined by the method of Lowry et al.[17] using (BSA) Bovin Serum Albumin as standard as 660 nm.

Statistics

Statistical analysis was determined by Student test and value of $p < 0.05$ was used as the criterion for statistical significance. Data are expressed as mean ± SE.

Results

SOD

The values of SOD activity in the striatal and mid brain region are reported in column I and V of Tables I and II. A significant increase in SOD activity, over 50% of the control levels, was observed in MPTP treatments in both the areas on 3rd and 7th day of treatment. Combined treatment of curcumin caused a notable increase ($p < 0.05$) in SOD activity in the striata and mid brain of mice. The augmentation in SOD activity in mid brain was more than double, but not so in striatum. An unassuming change was observed in curcumin alone treatment and DMSO treatments.

CAT

Columns II and VI in Tables I and II exhibit the changes of CAT activity in the striatum as well as in the mid brain regions, which are significantly increased over that of control values ($p < 0.05$). The function of CAT activity was two fold increased during 3rd day and more than three fold increased on 7th day. The combined treatment of MPTP and curcumin further increases the activity of CAT, but a restrained increase was observed when the animal was treated with curcumin and DMSO alone.

Reduced Glutathione

Columns III and VII in Tables I and II shows that the MPTP reduces the level of GSH to 25% on 3rd day and to 50% of further reduction on the 7th day in the striatum region. A significant reduction in MPTP treated group ($p < 0.05$) in respect to controls can be noticed, but the change was only moderate in curcumin alone treated group. A combined treatment of curcumin/MPTP increases the GSH activity ($p < 0.05$) significantly in both striatum and mid brain regions when compared to MPTP treated groups.

Table I. GSH, SOD, CAT and Lipid peroxidation in the striatum of Swiss male albino mice.

Treatment	Striatal area: 3rd Day				7th Day			
	SOD U/1 min/mg of protein	Catalase unit/mg of protein	GSH µmol/mg of protein	Lipid peroxidation nanomol/gm tissue wt	SOD U/1 min/mg of protein	Catalase unit/mg of protein	GSH µmol/mg of protein	Lipid peroxidation nanomol/gm tissue wt
Control	3.76 ± 0.33	0.62 ± 0.22	1.82 ± 0.17	12.35 ± 0.05	3.76 ± 0.33	0.62 ± 0.22	1.82 ± 0.05	12.35 ± 0.05
Curcumin	4.05 ± 0.26[a]	0.75 ± 0.19[a]	1.93 ± 0.56[a]	10.15 ± 0.12[a]	4.10 ± 0.95[a]	0.73 ± 0.06[a]	1.87 ± 0.12[a]	11.27 ± 0.13[a]
MPTP	4.79 ± 0.17[a]	1.23 ± 0.23[a]	1.04 ± 0.93[a]	21.38 ± 0.37[a]	5.15 ± 0.12[a]	2.68 ± 0.05[a]	0.87 ± 0.12[a]	27.39 ± 0.12[a]
MPTP ± CUR	5.15 ± 0.24[b]	2.20 ± 0.46[b]	1.38 ± 0.95[b]	16.18 ± 0.14[b]	5.65 ± 0.3[b]	2.92 ± 0.22[b]	1.45 ± 0.39[b]	15.15 ± 0.17[b]

Swiss male albino mice were treated with MPTP (40 mg/kg) and/or curcumin 80 mg/kg. Saline treated animals served as control. 8 animals treated in each group. [a] Significantly different from the respective control data $P < 0.05$. [b]Significantly different as compared to MPTP treated group, $P < 0.05$. Data are presented as mean ± SEM (n = 8)

Lipid Peroxidation (TBAR)

The values of lipid peroxidation determination in striatum and mid brain were shown in column IV and VIII of Tables I and II. A significant increase ($p < 0.05$) was observed in the MPTP treated groups in striatum and in mid brain regions when compared to control group. The changes in the curcumin alone treated group were moderately decreased in both the regions. However, curcumin and MPTP combined treatments resulted into a significant decrease ($p < 0.05$) in striatum lipid peroxidation and mid brain lipid peroxidation both in the 3rd and 7th day of treatment when compared to MPTP treated group.

Discussion

In this experimental investigation, we found that the intraperitoneal administration of MPTP increases the SOD and CAT activities in the striatal and mid brain regions. The consequent augmented oxidative stress is considered a cardinal feature of MPTP neurotoxicity. Increases in SOD and CAT enzymatic activities were observed in MPTP treated animals[7].

The repeated administration of curcumin causes positive influence on CAT and SOD activities. Both curcumin and MPTP caused a parallel change in the antioxidant enzymatic activities suggesting a repair mechanism in the mice brain.

Table II. GSH, SOD, CAT and Lipid peroxidation in the mid brain of Swiss male albino mice.

Treatment	Striatal area: 3rd Day				7th Day			
	SOD U/1 min/mg of protein	Catalase unit/mg of protein	GSH µmol/mg of protein	Lipid peroxidation nanomol/gm tissue wt	SOD U/1 min/mg of protein	Catalase unit/mg of protein	GSH µmol/mg of protein	Lipid peroxidation nanomol/gm tissue wt
Control	1.74 ± 0.14	1.32 ± 0.56	2.38 ± 0.17	25.63 ± 0.64	1.74 ± 0.14	1.32 ± 0.56	2.38 ± 0.17	25.63 ± 0.64
Curcumin	1.82 ± 0.10[a]	1.54 ± 0.56[a]	2.55 ± 0.5[a]	22.00 ± 1.64[a]	2.01 ± 0.5[a]	1.63 ± 0.43[a]	2.63 ± 0.12[a]	21.14 ± 1.64[a]
MPTP	2.59 ± 0.14[a]	1.84 ± 0.14[a]	1.72 ± 0.10[a]	51.21 ± 0.42[a]	3.64 ± 0.04[a]	2.64 ± 0.14 [a]	1.17 ± 0.61[a]	80.64 ± 0.17[a]
MPTP + CUR	2.70 ± 0.10[b]	1.99 ± 0.14[b]	2.03 ± 0.01[b]	31.89 ± 0.26[b]	4.08 ± 0.24[b]	3.45 ± 0.22[b]	2.14 ± 0.22[b]	35.17 ± 5.73[b]

Swiss male albino mice were treated with MPTP(40 mg/kg) and/or curcumin 80 mg/kg. Saline treated animals served as control. 8 animals treated in each group. [a] Significantly different from the respective control data $P < 0.05$. [b] Significantly different as compared to MPTP treated group, $P < 0.05$.Data are presented as mean ± SEM (n = 8).

The chronic treatment of curcumin improved the levels of two key antioxidant enzymes SOD and CAT[18]. Several studies established the ability of curcumin to mainly eliminate hydroxy radical[19], singlet oxygen[20], nitrogen dioxide[21], and nitric oxide[22]. It has also been demonstrated that curcumin inhibits the generation of the superoxide radicals[23,24].

We observed that intraperitoneal administration of MPTP increases oxidative stress estimated by SOD, and CAT activities. The significantly decreased levels of GSH may impair H_2O_2 clearance. The increase of GSH suggests it's role in neurotoxicity by MPTP, since GSH depleted animals have shown more vulnerability to MPTP insult[7,25].

GSH, a major non protein thiol in living organisms plays a crucial role in co-ordinating the body's antioxidant defence process. The results of the present study indicate that the MPTP administration drastically lowered the levels of GSH in the brain of mice. Our observation confirms the earlier data that the administration of curcumin increases the levels of glutathione reductase in ischemic brains of rats as well as alveola and human leukemia cells[26,27].

Our data proved that the administration of MPTP creates free radicals, which in turn alter the antioxidant systems, and necessarily lead to an increase in lipid peroxidation. However, a combined treatment of curcumin and MPTP led to a significant decrease in lipid peroxidation.

Lipid peroxidation induced by free radicals is believed to be one of the major causes of cell membrane damage leading to lysis of cell[28]. Curcumin inhibits iron catalysed lipid peroxidation in rat brain tissue homogenates by chelation of iron[29]. The effect of curcumin on lipid peroxidation has also been studied in various models by several authors[30]. Curcumin is a good antioxidant and inhibits lipid peroxidation in rat liver microsomes, erythrocyte membrane and brain homogenated[31]. Turmeric can lower lipid peroxidation by maintaining the activities of antioxidant enzymes like superoxide dismutase, catalase and glutathione peroxidase at higher levels. These enzymes play an important role in the regulation of lipid peroxidation[22].

In conclusion, our data suggest that MPTP increases SOD and CAT activities with the formation of oxygen free radicals, irreparable GSH depletion, and elevation of lipid peroxidation. The treatment of curcumin, a polyphenolic compound, has the capacity to rectify the above dangerous changes.

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Acknowledgements

I wish to thank my valuable supporters Dr. L.S. Ranganathan, Dean, Faculty of Science, HOD Department of Zoology, Annamalai University; Dr. M. Sabesan my research guide, for their excellent, tireless and unstinted academic support they showered on me and Dr. S. Sethupathy, HOD, RMMC&H for having permitted me to use his laboratory and some chemicals for my research analysis.