Neuropharmacology 61 (2011) 1193–1201

Contents lists available at SciVerse ScienceDirect

Neuropharmacology

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

Protective efficacy of mitochondrial targeted antioxidant MitoQ against dichlorvos induced oxidative stress and cell death in rat brain

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ARTICLE INFO

Article history:
Received 8 March 2011
Received in revised form
29 June 2011
Accepted 10 July 2011

Keywords:
Organophosphate
Dichlorvos
Oxidative stress
Neurodegeneration
MitoQ

ABSTRACT

Dichlorvos is a synthetic insecticide that belongs to the family of chemically related organophosphate (OP) pesticides. It can be released into the environment as a major degradation product of other OPs, such as trichlorfon, naled, and metrifonate. Dichlorvos exerts its toxic effects in humans and animals by inhibiting neural acetylcholinesterase. Chronic low-level exposure to dichlorvos has been shown to result in inhibition of the mitochondrial complex I and cytochrome oxidase in rat brain, resulting in generation of reactive oxygen species (ROS). Enhanced ROS production leads to disruption of cellular antioxidant defense systems and release of cytochrome c (cyt c) from mitochondria to cytosol resulting in apoptotic cell death. MitoQ is an antioxidant, selectively targeted to mitochondria and protects it from oxidative damage and has been shown to decrease mitochondrial damage in various animal models of oxidative stress. We hypothesized that if oxidative damage to mitochondria does play a significant role in dichlorvos induced neurodegeneration, then MitoQ should ameliorate neuronal apoptosis. Administration of MitoQ (100 μmol/kg body wt/day) reduced dichlorvos (6 mg/kg body wt/day) induced oxidative stress (decreased ROS production, increased MnSOD activity and glutathione levels) with decreased lipid peroxidation, protein and DNA oxidation. In addition, MitoQ also suppressed DNA fragmentation, cyt c release and caspase-3 activity in dichlorvos treated rats compared to the control group. Further electron microscopic studies revealed that MitoQ attenuates dichlorvos induced mitochondrial swelling, loss of cristae and chromatin condensation. These results indicate that MitoQ may be beneficial against OP (dichlorvos) induced neurodegeneration.

1. Introduction

Dichlorvos, an OP pesticide, has been used as a crop protectant as well as general public health insecticide since 1961. Organophosphates (OPs) are used worldwide as pesticides in agriculture, land scape pest control, as plasticizers and flame retardants in the industry. The WHO report on pesticides from agriculture estimated 2,20,000 deaths due to pesticide poisoning in 1990; 99% of which were from the developing countries (Steenland, 1996). Recent years have seen renewed interest in organophosphate poisons because of the gradual increase in accidental and suicidal poisoning that result from easy access to pesticides in agriculture and gardening (Yen et al., 2004). Dichlorvos can be released into the environment as a major degradation product of other OP insecticides, such as trichlorfon, naled, and metrifonate (Hofer, 1981; Pettigrew et al., 1998). Organophosphates, including dichlorvos have been reported to exert their primary pharmacological and toxicological effects through the inhibition of acetylcholinesterase (AChE), required for the transmission of impulse across the cholinergic synapse. The typical clinical symptoms of acute organophosphate poisoning are due to excessive cholinergic effects and include bronchospasms, hypersecretion from cholinergic innervated glands along with cardiac disturbances caused by vagotonus and anoxia (Balali-Mood and Shariat, 1998).

A report of Woodruff et al. (1994) has indicated dichlorvos to be a high risk pesticide particularly in connection with occupational health effects following prolonged exposure. Several groups within the general population may receive potentially higher inhalation

Abbreviations: ROS, reactive oxygen species; cyt c, cytochrome c; MnSOD, Manganese superoxide dismutase; AD, Alzheimer's disease; PD, Parkinson disease.

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doi:10.1016/j.neuropharm.2011.07.008

exposures to dichlorvos, like those living near factories where dichlorvos is produced or processed. Epidemiological studies suggest that pesticide exposure can increase the risk for the development of Parkinson disease (PD) (Gorell et al., 1998; Menegon et al., 1998). It has been reported that exposure to pesticides including paraquat increases the incidence of PD in parts of Canada. Also, several studies have suggested that rural living, use of well water and farming increase the Parkinsonian symptoms such as rigidity as well as bradykinesia (Fiedler et al., 1997). Data from our previous studies on chronic dichlorvos exposure have also shown that it results in the development of severe motor incoordination and deterioration in memory functions (Verma et al., 2009).

Mitochondrial oxidative damage contributes to a range of pathologies including neurodegenerative diseases, ischemia–reperfusion injury, metabolic syndrome, and diabetes (Rodriguez-Cuenca et al., 2010). We have recently reported that chronic dichlorvos exposure causes decreased mitochondrial electron transfer activities of cytochrome oxidase (complex IV) along with altered mitochondrial complex I activity. All this could have been because of enhanced oxidative stress, decreased GSH levels and MnSOD activity in the mitochondria isolated from dichlorvos treated rat brain. Dichlorvos induced enhanced ROS production has been shown to release cyt c from mitochondria to cytosol resulting in DNA damage and finally apoptotic cell death (Kaur et al., 2007). Therefore, protecting mitochondria from oxidative damage should be an effective therapeutic strategy against dichlorvos induced oxidative stress and ensuing apoptotic cell death.

The mitochondria-targeted antioxidant MitoQ comprises of lipophilic triphenyl phosphonium (TPP) cation attached to the ubiquinone antioxidant moiety of the endogenous antioxidant coenzymeQ10. The lipophilic TPP cation enables MitoQ to be taken up rapidly through the plasma and mitochondrial membranes without the requirement for a carrier, and furthermore, the large membrane potential (negative inside) across the mitochondrial inner membrane causes MitoQ to accumulate several hundredfold within mitochondria (Kelso et al., 2001; Murphy and Smith, 2007). Within mitochondria the MitoQ adsorbs to the matrix surface of the inner membrane and is recycled to the active ubiquinol antioxidant by the respiratory chain (Ross et al., 2008). Together these studies conclude that MitoQ inside the cells is several hundredfold more potent at preventing mitochondrial oxidative damage than an untargeted antioxidant. MitoQ has been shown to be protective against amyloid beta (Aβ) induced oxidative stress in Alzheimer's disease neurons (Manczak et al., 2010) MitoQ has also been shown to prevent apoptotic cell death elicited by hydrogen peroxide in Jurkat cells (Kelso et al., 2002). Furthermore, administration of MitoQ results in its accumulation in mitochondria of key organs in rats and mice, including heart, liver, kidney, skeletal muscle, and brain (Adlam et al., 2005; Smith et al., 2003). MitoQ has been tested in a number of animal models of disease: feeding MitoQ to rats decreased heart dysfunction, cell death and mitochondrial damage subsequent to ischemia–reperfusion in isolated heart (Neuzil et al., 2007; Ross et al., 2008). It protected endothelial cell function and damage to mitochondrial enzymes in a rat model of oxidative stress (Esplugues et al., 2006). These data suggest the mitochondria specific protective effects of MitoQ *in vivo* which make it an attractive candidate for testing its potential in ameliorating the dichlorvos induced cell death in rat brain.

Therefore, in this study we determined whether MitoQ was able to reduce oxidative stress and prevent ensuing cell death due to apoptosis in dichlorvos treated rat brains.

2. Materials and methods

2.1. Synthesis of MitoQ

MitoQ was synthesized according to the method of Kelso et al. (2001).

2.2. Animals and their care

Male albino rats (Wistar strain) in the weight range of 100–150 g were procured from the institute animal house. The animals were housed in polypropylene cages, and kept in well ventilated rooms on a 12-h light–dark cycle (lights on 7:00 a.m.). Animals were provided standard rat pellet diet (Hindustan Lever Ltd; Mumbai, India) and water ad libitum. Rats used in this study were maintained and treated in accordance with the guidelines established by the Ethical and Practical Principles of the Use of Laboratory Animals and all procedures used in the present study complied with the Guide for the Care and Use of Laboratory Animals.

2.3. Experimental design

The animals were divided into following groups ($n = 5$).

2.3.1. Control group

In this group, animals received an equal volume of corn oil (vehicle) as administered to the animals in chronic dichlorvos dose group.

2.3.2. Dichlorvos treated group

In this group, animals received dichlorvos (6 mg/kg body wt/day) dissolved in corn oil subcutaneous for 12 weeks.

2.3.3. MitoQ treated group

In this group, animals received MitoQ (100 μmol/kg body wt/day) dissolved in purified water, intragastrically for 12 weeks. The MitoQ dose was given according to previous study (Adlam et al., 2005).

2.3.4. Dichlorvos and MitoQ treated group

In this group animals were given MitoQ (100 μmol/kg body wt/day) and dichlorvos (6 mg/kg body wt/day, s.c.) daily. The MitoQ solution was prepared fresh every 3 days, protected from the light, and stored at 4 °C. The MitoQ was administered in the mornings and the formulation of dichlorvos was administered in the afternoons for 12 weeks.

2.4. Preparation of Mitochondria

After 12 weeks, the animals were anaesthetized with sodium pentothal and sacrificed. Brains were removed, rinsed in ice-cold physiological saline (0.9% NaCl), homogenized and mitochondrial fractions were isolated as described previously (Kaur et al., 2007). The integrity of mitochondria was checked by assessing respiratory control ratio and marker enzymes.

2.4.1. Measurement of ROS levels

ROS levels were measured by the method of Socci et al. (1999). Briefly, mitochondria were isolated from brain and added to respiration buffer containing 5 mM pyruvate, 2.5 mM malate and 10 μM of dichlorodihydrofluorescein diacetae (H_2DCF-DA). After 20 min incubation in dark, mitochondria were pelleted by centrifugation and extra mitochondrial dye was washed off. Fluorescence was quantified using a Cary Eclipse fluorimeter (Varian, Palo Alto, USA) (excitation 488 nm, emission 525 nm) and related to total protein content.

2.4.2. Mitochondrial superoxide dismutase activity (MnSOD)

Mitochondrial Mn-superoxide dismutase (MnSOD) activity was determined by the method of MacMillan-Crow et al. (1996). MnSOD activities in total solubilized mitochondrial extracts were measured by the cytochrome c reduction method in the presence of 1 mM potassium cyanide to inhibit both Cu–Zn SOD and extra cellular SOD as suggested by McCord and Fridovich (1969). The amount of enzyme required to produce 50% inhibition was taken as one unit and results were expressed as U/mg protein.

The reaction mixture consisted of 1.9 ml of 50 mM sodium carbonate buffer (pH 10), 0.75 ml of 96 μM nitrobluetetrazolium (NBT in sodium carbonate buffer), and 0.15 ml of 0.6% triton X-100 in sodium carbonate buffer in presence of 1 mM potassium cyanide. Initial absorbance (X) at 560 nm was taken for 3 min and then, 0.1 ml of mitochondrial sample from brain was added to this reaction mixture. Again the absorbance at 560 nm (Y) was noted for 3 min. Rate of inhibition of the reaction by the enzyme was calculated as $(X-Y \times 100/Y)$.

2.4.3. Mitochondrial reduced glutathione content

Reduced mitochondrial glutathione was estimated by the method of Tietze (1969), as described previously (Kaur et al., 2007). Briefly to an aliquot of mitochondrial fraction, an equal volume of 1% (w/v) sulfosalicylic acid was added, mixed and centrifuged at 10,000 × g for 10 min. The supernatant was collected and 0.4 M Tris buffer (pH 8.9) and 0.1 ml of 0.01 M DTNB was added. The absorbance was then measured within 2–3 min at 412 nm. The results were expressed as nmol GSH/mg protein.

2.4.4. Mitochondrial lipid peroxidation

It was assayed by the method of Wills (1966) as described previously (Kaur et al., 2007). Mitochondrial sample (0.5 ml) was diluted to 1.0 ml using Tris–HCl buffer