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Nitric oxide synthase inhibitors protect against brain and liver damage caused by acute malathion intoxication

Omar M.E. Abdel-Salam[1✉], Eman R. Youness[2], Nadia A. Mohammed[2], Noha N. Yassen[3], Yasser A. Khadrawy[4], Safinaz Ebrahim El-Toukhy[2], Amany A. Sleem[5]

[1]*Department of Toxicology and Narcotics, National Research Centre, Cairo, Egypt*

[2]*Department of Medical Biochemistry, National Research Centre, Cairo, Egypt*

[3]*Department of Pathology, National Research Centre, Cairo, Egypt*

[4]*Department of Physiology, National Research Centre, Cairo, Egypt*

[5]*Department of Pharmacology, National Research Centre, Cairo, Egypt*

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ABSTRACT

Objective: To investigate the effect of N^G-nitro-L-arginine methyl ester (L-NAME), a non-selective nitric oxide synthase (NOS) inhibitor, and 7-nitroindazole (7-NI), a selective neuronal NOS inhibitor, on oxidative stress and tissue damage in brain and liver and on DNA damage of peripheral blood lymphocytes in malathion intoxicated rats.

Methods: Malathion (150 mg/kg) was given intraperitoneally (i.p.) along with L-NAME or 7-NI (10 or 20 mg/kg, i.p.) and rats were euthanized 4 h later. The lipid peroxidation product malondialdehyde (MDA), nitric oxide (nitrite), reduced glutathione (GSH) concentrations and paraoxonase-1 (PON-1) activity were measured in both brain and liver. Moreover, the activities of glutathione peroxidase (GPx) acetylcholinesterase (AChE), and butyrylcholinesterase (BChE), total antioxidant capacity (TAC), glucose concentrations were determined in brain. Liver enzyme determination, Comet assay, histopathological examination of brain and liver sections and inducible nitric oxide synthase (iNOS) immunohistochemistry were also performed.

Results: (i) Rats treated with only malathion exhibited increased nitric oxide and lipid peroxidation (malondialdehyde) accompanied with a decrease in GSH content, and PON-1 activity in brain and liver. Glutathione peroxidase activity, TAC, glucose concentrations, AChE and BChE activities were decreased in brain. There were also raised liver aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities and increased DNA damage of peripheral blood lymphocytes (Comet assay). Malathion caused marked histopathological changes and increased the expression of iNOS in brain and liver tissues. (ii) In brain of malathion-intoxicated rats, L-NAME or 7-NI resulted in decreased nitrite and MDA contents while increasing TAC and PON1 activity. Reduced GSH and GPx activity showed an increase by L-NAME. AChE activity increased by 20 mg/kg L-NAME and 10 mg/kg 7-NI. AChE activity decreased by the higher dose of 7-NI while either dose of 7-NI resulted in decreased BChE activity. (iii) In liver of malathion-intoxicated rats, decreased MDA content was observed after L-NAME or 7-NI. Nitrite level was unchanged by L-NAME but increased after 7-NI which also resulted in decreased GSH concentration and PON1 activity. Either inhibitor resulted in decreased liver ALT activity. (iv) DNA damage of peripheral blood lymphocytes was markedly inhibited by L-NAME or 7-NI treatment. (v) iNOS expression in brain and liver decreased by L-NAME or 7-NI. (vi) More marked improvement of the histopathological alterations induced by malathion in brain and liver was observed after 7-NI compared with L-NAME.

✉First and corresponding author: Omar M.E. Abdel-Salam, Department of Toxicology and Narcotics, National Research Centre, Tahrir Street, Dokki, Cairo, Egypt.
E-mail: omasalam@hotmail.com

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Conclusions: In malathion intoxicated rats, the neuronal NOS inhibitor 7-NI and to much less extent L-NAME were able to protect the brain and liver tissue integrity along with improvement in oxidative stress parameters. The decrease in DNA damage of peripheral blood lymphocytes by NOS inhibitors also suggests the involvement of nitric oxide in this process.

1. Introduction

Organophosphate insecticides are widely used compounds in agriculture, gardens, household and in veterinary [1]. These agents pose the risk of causing human neurotoxicity, both acute and chronic [2]. In acute intoxication, exposure to high concentrations of organophosphates, e.g., malathion result in headache, dizziness, ataxia, confusion, agitation, coma, muscle twitching, convulsions, muscle paralysis amounting to respiratory failure and death. These effects are mediated in large part by an inhibitory effect on AChE, the enzyme responsible for the hydrolysis of the neurotransmitter acetylcholine in cholinergic synapses in the central and peripheral nervous system, autonomic ganglia, and motor end-plate [3,4]. Long-term complications of organophosphates have also been described including mood disorders, cognitive and memory impairments, psychiatric manifestations, and delayed polyneuropathy [5,6]. Moreover, exposure to these compounds has been implicated in the development of neurodegenerative disorders like Parkinson's disease [7,8]. Rats treated with malathion showed neuronal degeneration in cortex and hippocampus, reactive gliosis, increased glial fibrillary acidic protein immunostaining in hippocampus [9,10]. Besides their action on AChE, organophosphates are likely to exert their neurotoxic effects through other mechanisms such as impairment of mitochondrial dynamics and mitochondrial bioenergetics [11,12], oxidative and nitrosative stress [9,10,13–15]. In rats, exposure to malathion resulted in increased lipid peroxidation in brain, liver and blood [9,10,14,16,17]. The activities of the antioxidant enzymes glutathione peroxidase (GPx), glutathione reductase and total antioxidant capacity (TAC) decrease in brain as well [9,10,14]. Human erythrocytes showed reduced activities of superoxide dismutase, catalase and GPx [13].

The role of nitric oxide (NO) in the development of neuronal damage in toxic and inflammatory conditions is a subject of paramount importance [18]. The gaseous molecule NO is produced from L-arginine via the action of the nitric oxide synthase enzyme (NOS)in the presence of O_2, nicotinamide adenine dinucleotide phosphate (NADPH) and tetrahydrobiopterin. Under physiological conditions NO is an important signaling molecule involved in neurotransmission, and in maintenance of vascular tone. The endothelial (eNOS) and neuronal (nNOS) isoforms of the enzyme account for constitutively formed low concentrations of NO under physiological conditions. A third isoform that is inducible NOS (iNOS) is not constitutively present but is expressed by glial cells (astrocytes and microglia) and other immune cells by the action of endotoxin lipopolysaccharide or cytokines such as interferon-γ, interleukin-1β or tumor necrosis factor-α(its expression increases) [19,20]. This generates excessive amounts of NO for longer lime with a resultant detrimental impact on tissue function and integrity [21]. This is largely due to the reaction of NO with O_2 to form reactive nitrogen oxide species, e.g., NO_2 and N_2O_3 or with superoxide anion ($O_2^{\bullet -}$) forming the highly reactive peroxynitrite ($ONOO^-$) (with these species being) capable of causing oxidation or nitrosylation of thiols, nitration of tyrosine residues, oxidation of lipids, protein and DNA [21–23]. The neurotoxic effects of acute malathion exposure in rats are associated with increased endogenous NO biosynthesis and iNOS expression in brain. In these studies, neuroprotection was associated with decreased level of NO and lower oxidative stress, thereby, suggesting a role for NO in the malathion neurotoxicity.

NOS antagonists offer the opportunity for evaluating the role of NO in the development of neuronal injury by organophosphates. In this study we investigated the effect of the non-selective NOS antagonist N^G-nitro-L-arginine methyl ester (L-NAME) and the selective neuronal NOS antagonist 7-nitroindazole (7-NI) on oxidative stress and neuronal injury in the rat brain following acute malathion exposure. Since the organophosphate has been shown to cause liver cell necrosis [9,24,25]and DNA damage of peripheral blood lymphocytes [26,27], the study was extended to investigate the role of NO in these effects of malathion.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats weighing 130–140 g from Animal House Colony of the National Research Centre were used in the study. They were allowed free access to standard laboratory food and water. All animal procedures followed the recommendations of the institutional Ethics Committee and the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985).

2.2. Drugs and chemicals

Malathion (Commercial grade: 57%) was obtained from El-Naser Chemical Co. (Cairo, Egypt). L-NAME and 7-NI were purchased from Sigma–Aldrich (St Louis, MO, USA) and dissolved in normal saline. The other chemicals and reagents were of analytical grade and purchased from Sigma–Aldrich.

2.3. Study design

Rats were randomly assigned into six different groups (6 rat/group). Group 1 received i.p. saline (0.2 mL/rat) and served as negative control. Groups 2–6 received i.p. malathion at a dose of 150 mg/kg and were treated i.p. with one of the following: saline (group 2), L-NAME at 10 or 20 mg/kg (groups 3 & 4), 7-NI at 10 or 20 mg/kg (groups 5 & 6). Rats were euthanized by decapitation 4 h post-drug administration; their brains were quickly removed on