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Altered glucose homeostasis and oxidative impairment in pancreas of rats subjected to dimethoate intoxication

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Abstract

The primary objective of this study was to investigate the effect of repeated sublethal doses of dimethoate (DM), an organophosphorus insecticide on glucose homeostasis, oxidative stress induction in pancreas and pancreatic damage in adult rats. Daily oral administration of DM (20 and 40 mg/kg b.w.) for 30 days induced a significant increase in blood glucose levels which was associated with impaired glucose tolerance. DM treatment resulted in elevated levels of pancreatic tissue specific markers such as activities of amylase and lipase in serum and pancreatic tissue indicating pancreatic dysfunction. Further, the activities of DT-diaphorase and NADPH-diaphorase in pancreas of DM treated rats were also found to be elevated. Interestingly, these biochemical dysfunctions were accompanied by a marked dose-related enhancement of lipid peroxidation and ROS levels in the pancreatic tissue indicating significant induction of oxidative damage. Additional evidence such as depletion in reduced glutathione levels and significant alterations in enzymic antioxidant defenses in pancreas among DM treated rats suggested induction of oxidative stress. Taken together, these findings provide experimental evidence that dimethoate at subchronic oral doses has the propensity to impair glucose homeostasis, induce significant pancreatic damage and also provide an account of the associated oxidative damage to pancreatic tissue in adult rats.

Keywords: Dimethoate; Organophosphorus insecticide; Oxidative impairment; Pancreas; Blood glucose; Pancreatic enzymes; Lipid peroxidation; Antioxidant enzymes

1. Introduction

The widespread use of pesticides in public health and agricultural programs has caused severe environmental pollution and potential health hazards, including acute and chronic cases of human poisoning. Organophosphate insecticides (OPI) constitute one of the most widely used classes of pesticides being employed for both agricultural and landscape pest control. Use of OPI has increased considerably due to their low toxicity and low persistence in the mammalian system compared to organochlorine pesticides. OPI are primarily recognized for their ability to induce toxicity in mammals through inhibition of acetylcholinesterase (AChE) and subsequent activation of cholinergic receptors (Costa, 2006).

Various complications have been reported in OPI intoxication cases (Hsiao et al., 1996). In fact, the OPI are currently responsible for more poisonings than any other single class of pesticides (Sultatos, 1994; Gulr et al., 1996). Hyperglycemia has been widely reported as one of the adverse effects in poisoning by OPI in humans and animals (Abdollahi et al., 2003; Hagar et al., 2002; Seifert, 2001; Shoba and Prakash, 2000; Kalender et

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al., 2004). Although the precise mechanism/s of OP-induced hyperglycemia is not known, it is speculated to be due to inhibition of acetylcholinesterase of central and peripheral synapses that act in the endocrine regulation of glucose metabolism (Matin and Siddiqui, 1982; Kant et al., 1988). Acute pancreatitis is also well known complication of OP poisoning in both humans and animals (Dressel et al., 1979; Frick et al., 1987; Hsiao et al., 1996). Epidemiological findings indicate that the incidence of pancreatitis is high in OPI intoxication based on various pathophysiological reports (Gokalp et al., 2005). However, the precise molecular mechanisms underlying OPI-induced acute pancreatitis are still undefined, although it is believed to involve obstruction of pancreatic ducts and/or enhanced reactive oxygen species (Dressel et al., 1982; Sultatos, 1994; Sevillano et al., 2003). Involvement of oxidative stress following acute exposure to OPI has been reported recently (Banerjee et al., 2001) and it has been demonstrated unequivocally that lipid peroxidation is one of the molecular mechanisms involved in OPI-induced cytotoxicity (Ranjbar et al., 2002; Akhgari et al., 2003; Abdollahi et al., 2004a).

Dimethoate (*O*,*O*-dimethyl *S*-*N*-methyl carbomyl methyl phosphorodithioate) (DM) is one of the most important OPI used extensively on a large number of crops against several pests. The residue of DM and its analog were found in number of foods including cow's milk (Srivastava and Raizada, 1996). While data on acute, subchronic and chronic toxicity of DM in laboratory animals are well documented, its potential to alter glucose homeostasis and impair the endocrine function of pancreas in mammals is less understood. Interestingly, DM is reported to cause various toxic effects on rat pancreas following chronic exposure (Hagar et al., 2002) as well as pancreatitis in humans following dermal exposure (Panieri et al., 1997). In view of the above, in the present study, we have focussed our attention on the potential of DM to cause alterations in glucose homeostasis and also on the toxic effects in pancreas of adult rats subjected to daily oral administration at sublethal doses for 1 month. Further, we have examined the various oxidative impairments in pancreas in terms of lipid peroxidation, generation of reactive oxygen species (ROS), response of antioxidant enzymes and examined its correlation with pancreatic acetylcholinesterase activity.

2. Materials and methods

2.1. Chemicals

Thiobarbituric acid (TBA), xanthine oxidase, ethylenediamine tetraacetic acid (EDTA), hydrogen peroxide (H_2O_2), glutathione reductase (GR), 5,5-dithio-bis-2-nitrobenzoic acid (DTNB), 2′,7′-dichlorofluorescein diacetate (DCFH-DA), 2′,7′-dichlorofluorescein (DCF) and cytochrome *C* were procured from M/s Sigma Chemical Co. (St. Louis, MO, USA). Xanthine, NADPH, nicotinamide adenine dinucleotide-reduced (NADH), trichloroacetic acid (TCA), reduced glutathione (GSH), oxidized glutathione (GSSG), 1-chloro-2,4-dinitrobenzene (CDNB), and acetylthiocholine iodide were procured from M/s Sisco Research Lab (Mumbai, India). All other chemicals used were of analytical grade. Dimethoate, technical grade (97.4% pure) was a gift from M/s Hyderabad Chemical Supplies Ltd., Hyderabad, India.

2.2. Animals and care

Adult male rats (CFT-Wistar strain, 12–14-week old, 280 ± 5 g) were randomly drawn from the stock colony of our institute animal house facility and were housed individually in polypropylene cages under standard housing conditions (controlled atmosphere with 12:12-h light/dark cycles, $50 \pm 5\%$ humidity and an ambient temperature of $25 \pm 2\,^{\circ}C$). The rats were acclimatized for 1 week prior to the start of the experiment. Rats were maintained on commercial pellet diet (Gold Mohur, supplied by M/s Lipton India Pvt Ltd.) *ad libitum* and had free access to water. All procedures with animals were conducted strictly in accordance with approved guidelines by the Institute Animal Ethical Committee, regulated by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India. During the experiments, maximum care was taken to minimize animal suffering and in addition, the number of rats used was kept at minimum.

2.3. Animal treatment and experimental protocol

Adult male rats were grouped by randomized design into three groups ($n = 6$). Rats of the first group (negative control) received saline daily, while rats of the treatment groups were orally administered dimethoate (DM) at dosages of 20 and 40 mg/kg b.w./day (corresponding to 1/20 and 1/10 of LD50 value: 400 mg/kg b.w., determined in a preliminary study) (0900–1100 h) for 30 days to non-fasted rats. Both control and DM treated rats were subjected to oral glucose tolerance test at the end of the 30 days and were subsequently killed. Body weights were recorded weekly and at autopsy. After the treatment period, the rats were sacrificed and blood was collected for separation of serum. Pancreas and other vital organs were excised and their weights were recorded. Pancreas was rinsed in ice-cold saline, homogenized to obtain 10% homogenate in phosphate buffer (0.1 M, pH 7.4) and the homogenate was centrifuged at $9000 \times g$ at $4\,^{\circ}C$ for 20 min. The supernatant was used for various biochemical analyses.

2.4. Oral glucose tolerance test

Twenty-four hours after the last dose of DM, oral glucose tolerance was conducted in control and dimethoate treated rats.