Metabolic Dyshomeostasis in Rats Administered a Single dose of Monocrotophos is not Associated with Oxidative Damage in Liver and Kidney

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ABSTRACT

We have earlier demonstrated the potential of monocrotophos, an organophosphorus insecticide to cause transient hyperglycemia in rats after administration of a single dose. This study was conducted to understand whether hyperglycemia in rats administered a single dose of monocrotophos is associated with oxidative damage in liver and kidney. Oral administration of a single dose of monocrotophos promptly caused classical acute organophosphate toxicity as evidenced by severe inhibition of brain acetylcholinesterase activity. Further, metabolic alterations such as transient hyperglycemia, hypercorticosteronemia, hyperlacticidemia and increase in the activity of hepatic tyrosine aminotransferase were observed in rats treated with monocrotophos. These changes were associated with marginal decrease in glutathione levels in liver and kidney. However, extent of lipid peroxidation and activities of catalase and superoxide dismutase in liver and kidney of monocrotophos-treated rats were comparable to that of vehicle-treated rats. This suggests that single dose of monocrotophos fails to induce oxidative damage in rats in spite of occurrence significant neurotoxicity and metabolic alterations.

Keywords : Acetylcholinesterase Inhibition, Hyperglycemia, Metabolic Dyshomeostasis, Monocrotophos, Oxidative Stress

I. INTRODUCTION

Organophosphorus insecticides (OPI) represent a major class of insecticides used worldwide today for mitigating pest populations in agriculture and other scenario. This group of insecticides includes a large number of compounds of varying toxicities. Mechanistically, OPI act on the target insects by inhibiting the enzyme acetylcholinesterase (AChE) and leading to toxicity underlined by cholinergic overstimulation [1–3]. However, ubiquitous nature and conserved role of AChE in regulation of neurotransmission has rendered human physiology equally vulnerable to the toxicity of OPI. Most often, clinical cases of OPI toxicity are attributable to exposure to OPI at doses sufficient to cause strong AChE inhibition and cholinergic stress [4,5]. However, longer exposure to OPI as a result of occupational hazard or through food chain is a realistic issue. Hence, OPI have come under intense scientific scrutiny for evaluating their effects in milieus other than nervous system.

Both clinical and experimental studies now give evidence for the view that OPI exert disruptive effects on metabolic homeostasis. Hyperglycemic potential of OPI has been established in both clinical [6,7] and experimental [8–11] settings. Hyperglycemic effects of OPI are often associated with physiological changes such as increased glycogenolysis [12,13] and gluconeogenesis [9–11]. An imbalance between oxidants and antioxidants in favour of the oxidants, potentially leading to oxidative damage, is termed 'oxidative stress'. Oxidants are formed as a normal product of aerobic metabolism but can be produced at elevated rates under pathophysiological conditions. Consequences of severe oxidative stress, the oxidative damage can be discerned as free-radical mediated damage to lipids, proteins and nucleic acids. These events essentially are chief contributors to the cellular changes associated with oxidative stress. In addition to causing neurotoxicity and metabolic dyshomeostasis, OPI are now widely recognised for their ability to induce oxidative damage, characterized by free radical mediated changes that can be discerned as lipid peroxidation and DNA damage. Oxidative stress induced by OPI is often associated with alterations in enzymatic and non-enzymatic components of oxidative defence [14–16]. Earlier work from our laboratory demonstrated the propensity of dimethoate to cause oxidative stress in the pancreas of rats after sub-chronic exposure [17,18].

Monocrotophos (dimethyl (e)-1-methyl-2-(methylcarbamoyl) vinyl phosphate) is a fast acting, cholinesterase-inhibiting OPI exerting contact and stomach toxicity. We have earlier demonstrated that a single dose of OPI elicits hyperglycemia in rats as a result of cholinergic stress [10]. The hyperglycemia in rats administered a single dose of monocrotophos is associated with hyperlacticidemia. Further, increased gluconeogenesis appears to be the mechanism of monocrotophos-induced hyperglycemia in rats, as reflected preventive effects of hydrazine sulphate, an inhibitor of gluconeogenesis [9]. With the ability of monocrotophos to induce above said metabolic abnormalities known, the present study was carried out to investigate if metabolic dyshomeostasis induced by a single dose of monocrotophos is associated with oxidative stress in rats.

II. MATERIALS AND METHOD

CHEMICALS

Corticosterone and thiobarbituric acid (TBA) were procured from Sigma Chemical Co. (St. Louis, MO, USA). Technical grade of monocrotophos (dimethyl (E)-1-methyl-2- (methylcarbomoyl) vinyl phosphate) was kindly gifted by Hyderabad Chemical Supplies Ltd., (Hyderabad, India). Acetylthiocholine iodide, 5, 5-dithio-bis-2-nitrobenzoic acid (DTNB), ketoglutarate (alpha), L-tyrosine, pyridoxal- 5-phosphate, trichloroacetic acid (TCA), *O*-phthalaldehyde, quercetin dehydrate and reduced glutathione (GSH) were procured from Sisco Research Lab., (Mumbai, India). All other chemicals used in this study were of analytical grade. Technical grade sample monocrotophos was a gift from Hyderabad Chemical Supplies Ltd., (Hyderabad, India).

ANIMALS AND CARE

This study was performed using male Wistar rats, (CFT strain, 160-200g) obtained from the Central Food Technological Research Institute Animal House. The study was approved by the Institute Animal Ethics Committee and all animal procedures were in strict compliance with the guidelines for the care and use of laboratory animals. They were housed in polypropylene cages at room temperature (25 ± 2°C) with relative humidity of 50- 60% and on a 12h light-darkness cycle.

ANIMAL EXPERIMENTS

Impact of a single dose monocrotophos on blood glucose

Rats were fasted overnight and administered a single oral dose of monocrotophos dissolved in distilled water (1.8mg/kg b.w.). The dosage was based on our previous study [10]. Blood glucose was measured in all rats before and 2, 4, and 6h after administration of monocrotophos using blood collected from a tail-nick. The blood was applied on a commercial glucometer for estimation of glucose levels and results were expressed as mg/dL.

Assessment of impact of a single dose monocrotophos on biochemical parameters

Overnight-fasted rats were randomly divided into two groups. The first group of rats served as control and received oral administration of distilled water while the second group of rats were oral administration of monocrotophos (1.8mg/kg b.w.). Rats were sacrificed under ether anaesthesia 2h after administration of monocrotophos and blood was collected into EDTA-tubes for separation of plasma. Brain, liver and kidneys were collected, rinsed in physiological saline and stored at -80°C for assessment of biochemical parameters.

ASSAYS

Corticosterone and lactate levels were measured in the plasma as described earlier [19,20]. Plasma corticosterone was extracted into chloroform, and the chloroform layer was recovered by centrifugation and washed with 0.1N NaOH. The chloroform extract was the treated with acid-ethanol reagent (2.4 parts conc. H_2SO_4 + 1.0 part 50% Ethanol) and the fluorescence of the acid layer was recorded (Ex: 470nm; Em: 530nm). The amount of corticosterone was calculated from a corticosterone standard graph and the results were expressed as µg corticosterone/100ml plasma. For estimation of lactate, deproteinated plasma (equivalent to 150µl plasma) was added to 1.0ml of 20% $CuSO_4$ and the volume was made up to 10 ml with double distilled water. 1.0 g of $Ca(OH)_2$ was added and tubes were incubated at room temperature for 30min. The tubes were then centrifuged, and 1.0ml of clear solution was taken into glass test tubes containing 50µL of 4% $CuSO_4$. 6.0ml of concentrated H_2SO_4 was added and placed in boiling water bath for 3-4min. Tubes were cooled and 100 µL of 1.5% p-hydroxydiphenyl solution was added. Content of the tubes were mixed well and kept at room temperature for 30min. The tubes were again placed in boiling water bath for 2min, cooled and the absorbance was recorded at 560nm. The amount of lactate in plasma was calculated using a lactate standard graph and results were expressed as mM lactate.

Brain homogenates prepared in phosphate buffer (100mM, pH 7.4) were used for the assay of acetylcholinesterase. Briefly, acetylthiocholine iodide was added to a mixture containing suitable amounts of homogenate (as source of enzyme) and DTNB. Change in absorbance was monitored over 3min in a microplate reader at 405 nm. The amount of enzyme causing a change of 0.001 units of absorbance per minute was considered as one unit of enzyme and the results were expressed as units/mg protein [21]. Liver tyrosine aminotransferase (TAT; EC. 2.6.1.5) was assayed by the method of Diamondstone [22]. The assay mixture in a final volume of 3.2 ml contained 19.2 µmol L-tyrosine, 30µmol α-ketoglutarate, 0.12µmol pyridoxal-5- phosphate and 13µmol diethyldithiocarbamate. The reaction was initiated by addition of liver homogenate (0.025 ml of 5% homogenate) and allowed to run for 10 min at 37°C. The reaction was terminated by addition of 200 µl 10N NaOH and the absorbance was measured at 331nm against null-time blank and the results were expressed as nmol p-hydroxybenzaldehyde (pHBA) formed/min/mg protein.

For quantitation of reduced glutathione levels, protein-free homogenates (0.1ml) of liver or kidney were added to 1.8ml of 100mM phosphate buffer containing 5mM EDTA. To this was added 0.1ml of *o*-phthalaldehyde solution (1mg/ml in MeOH) and

contents were mixed well. Following 15 min of incubation at room temperature, fluorescence was recorded at 420nm using excitation wavelength of 350nm. The amount of GSH was calculated from the GSH standard graph and results were expressed as µg GSH/g tissue [23]. Catalase was assayed by measuring the rate of decomposition of hydrogen peroxide at 240nm [24]. Briefly, tissue was homogenized (5% w/v) in phosphate buffer (0.1M, pH 7.4), centrifuged at 10,000 rpm for 10 min at 4°C. The supernatant (0.075mg protein) was added to a reaction mixture containing 15mM H_2O_2 and the rate of disappearance of H_2O_2 was monitored for 3 min at 240nm. The enzyme activity was expressed as µmol H_2O_2 decomposed/min/mg protein. The superoxide dismutase assay was based on the principle of inhibition of quercetin auto-oxidation by the enzyme [25]. Auto-oxidation of quercetin was monitored at 406nm in a final reaction mixture containing TEMED (0.8mM), EDTA (0.08mM) and quercetin (0.014mM). Inhibition of quercetin auto-oxidation by SOD was monitored under the same conditions after addition of tissue homogenate. One unit of SOD is the amount of enzyme required to bring about 50% inhibition of quercetin auto-oxidation and results were expressed as units/mg protein. The protein content of tissue homogenates was determined by the Lowry method [26].

STATISTICAL ANALYSIS

Mean and SE were calculated for all data and data have been depicted as mean±SE. The degree of significance between different groups was calculated by ANOVA and Tukey test.

III. RESULTS AND DISCUSSION

The class of organophosphate compounds contains a large number of chemicals that find commercial application as insecticides due to their ability to inhibit the enzyme acetylcholinesterase. While effective as insecticides, OPI also exhibit significant toxicity to non-target organisms, including human beings. Neurotoxicity is the major presentation seen in cases that arrive at the clinics after accidental or intentional exposure to OPI. OPI are now recognized to elicit many other forms of adverse reactions, which may or may not be related to their ability to inhibit AChE. The effect on oral administration of a single dose of monocrotophos to fasted rats is depicted in **Fig. 1**. Monocrotophos caused reversible hyperglycemia characterised by peak increase at 2h after exposure (97% above baseline). Thereafter, the glucose levels tended to return to normalcy and were comparable to the baseline at 6h after exposure. Reversible nature of hyperglycemia in experimental animals elicited by a single dose of OPI is a well-documented phenomenon [8,10,11,27,28].
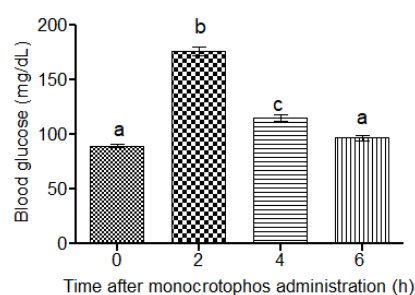

Figure 1: Effect of monocrotophos on blood glucose in rats (mean±SE, $p < 0.05$)

Mechanistic studies performed in our laboratory revealed that hyperglycemic potential of monocrotophos is attributable to AChE inhibition [10] and occurs as a result of adrenergic activation [9]. Single dose of monocrotophos elicited significant hypercorticosteronemia (113.5%) at 2h after administration (**Fig. 2A**) In our previous study, we have demonstrated that acute exposure to monocrotophos causes hypercorticosteronemia as a result of cholinergic stress [10]. Several studies lend support to the view that cholinergic stress associated with poising from AChE inhibitors is strongly associated with increase in the circulating levels of corticosterone [11,29–33]. Acute exposure to monocrotophos was associated with increase in plasma lactate levels (90%, **Fig. 2B**). We have earlier

demonstrated that hyperlacticidemia in monocrotophos treated rats occurs as a result of cholinergic stimulation, with subsequent involvement of adrenergic mechanisms [9,10].
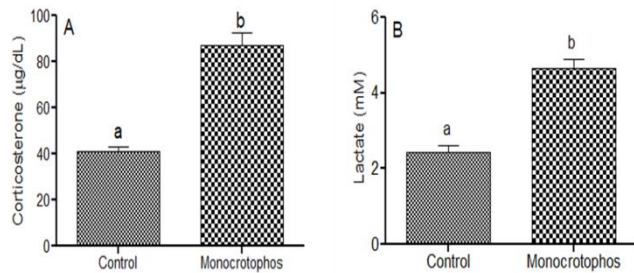

Figure 2: Effect of monocrotophos on plasma corticosterone (A) and plasma lactate (B) in rats (mean±SE, p<0.05)

Corticosterone is a glucocorticoid hormone that exerts its activity by binding glucocorticoid receptor. The receptor-ligand complex migrates to nucleus, where it bring about transcriptional modulation [34]. Tyrosine aminotransferase (TAT) is induced by glucocorticoids [35,36] and has been employed as a classical marker for transactivation potential of glucocorticoids [37,38]. Physiological consequence of hypercorticosteronemia was discernable as increase in the activity of hepatic tyrosine aminotransferase (**Fig. 3A**). We observed severe inhibition of AChE activity in whole brain homogenates of rats exposed to monocrotophos for 2h (83%, **Fig. 3B**), indicating monocrotophos exerts neurotoxicity within 2h of exposure.
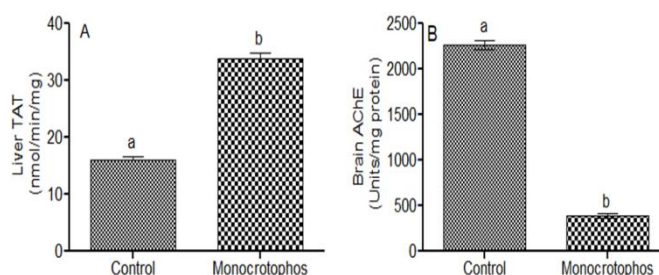

Figure 3: Effect of monocrotophos on liver TAT (A) and brain AChE (B) activities (mean±SE, p<0.05)
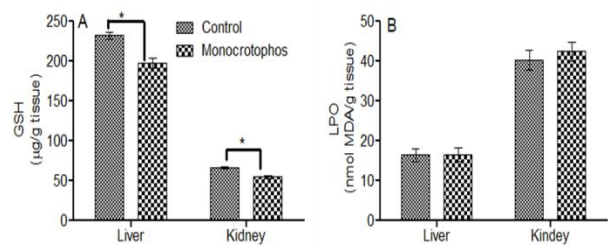

Figure 4: Effect of monocrotophos on reduced glutathione (GSH) levels (A) and extent of lipid peroxidation (LPO) (B) in liver and kidneys (mean±SE, p<0.05)

Effect of monocrotophos on non-enzymatic markers of oxidative stress and damage are depicted in **Fig. 4**. As depicted in **Fig. 4A**, there was marginal but significant reduction in reduced glutathione levels in liver (15%) and kidney (17%). Interestingly, basal level of lipid peroxidation (a marker for oxidative damage of lipids) was nearly 2 folds higher in kidneys as compared to liver. However, monocrotophos failed to enhance extent of lipid peroxidation in comparison to control, indicating that 2h exposure to monocrotophos is not associated with oxidative fatty acid damage in liver and kidneys.
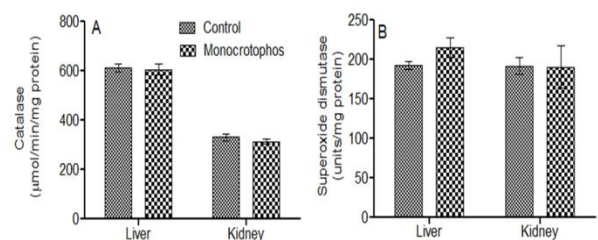

Figure 5: Effect of monocrotophos on catalase (A) and superoxide dismutase (B) activities in liver and kidneys (mean±SE, p<0.05)

Fig. 5 depicts the effect of monocrotophos on activity of anti-oxidant enzymes in liver and kidneys. Basal catalase activity was nearly 2 folds higher in liver as compared to kidneys, while that of superoxide dismutase were comparable in both tissues. Interestingly, we observed that 2h exposure to monocrotophos was not associated with alterations in the activity of catalase and superoxide dismutase in liver and kidneys.

IV. CONCLUSION

Our works clearly demonstrates that early phase of exposure to monocrotophos is associated with metabolic dysregulations and AChE inhibition without overt oxidative damage in kidney and liver.

V. AKCNOWLEDGEMENTS

VI. REFERENCES

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