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RESEARCH ARTICLE

Lipid Peroxidation as a Biomarker for Exposure of Pesticides

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Abstract

Oxidative stress by increased production of reactive oxygen species has been implicated in the toxicity of many pesticides. Therefore, the aim of the present study was to investigate the effect of current using pesticides, organophosphate and pyrethroids (Dimthoate, profenofos, chlorpyrifos and cypermethrin), on oxidative stress to establish lipid peroxidation (LPO) as a biomarkers for pesticide exposure in mice. Thirty mice were used in the study and were grouped into two with 10 mice in control group and 20 mice in pesticide treated group. Group A: untreated mice kept as control and served with equal volume of distilled water by gavage method. Group B: mice treated with Dimethoate (EC 30% w/w) 4 mg/kg b.w. was given on day first, profenofos (EC 50% w/w) 3 mg/kg b.w. was given on day second, chlorpyrifos (EC 20% w/w) 10 mg/kg b.w. was given on day third and cypermethrin (EC 25% w/w) 8 mg/kg b.w. was given on day fourth and this cycle was continued for 8 weeks to make chronic exposure of different pesticides. The treated and control group of mice were sacrificed on targeted day. MDA (malondialdehyde), the marker of lipid peroxidation was estimated for exposure of pesticides. The increase in lipid peroxidation marked by elevated levels of MDA in pesticide exposed group indicates oxidative stress. Thus the present study was concluded that lipid peroxidation as a biomarker for pesticides exposure.

Introduction

Present study indicates that the toxic action of pesticides may include the induction of oxidative stress and accumulation of free radicals in the cell. A major form of cellular oxidation damage is lipid peroxidation, which is initiated by hydroxyl free radical through the extraction of hydrogen atom from unsaturated fatty acids of membrane phospholipids (Farber et al., 1990). In recent years it has become apparent that the oxidation of lipids, or lipid peroxidation, is a crucial step in the pathogenesis of several diseases in adult and infant patients. Lipid peroxidation is a process generated naturally in small amounts in the body, mainly by the effect of several reactive oxygen species (hydroxyl radical, hydrogen peroxide etc.). It can also be generated by the action of several phagocytes. These reactive oxygen species readily attack the polyunsaturated fatty acids of the fatty acid membrane, initiating a self-propagating chain reaction. The destruction of membrane lipids and the end-products of such lipid peroxidation reactions are especially dangerous for the viability of cells, even tissues. Enzymatic (catalase, superoxide dismutasse) and nonenzymatic (vitamins A and E) natural antioxidant defence mechanisms exist; however, these mechanisms may be overcome, causing lipid peroxidation to take place. Since lipid peroxidation is a self-propagating chain-reaction, the initial oxidation of only a few lipid molecules can result in significant tissue damage and various studies from several parts of the world revealed the toxic effects of pesticides on human beings especially by elucidating free radical mechanism, which can be confirmed by the direct measurement of lipid peroxidation by products such as malondialdehyde(Feng *et al.*,1997). LPO produces disintegration of biomembrane

and sub cellular organelles. The microsomal membranes rich in PUFA are susceptible to oxidation (Freeman *et al*., 1982). ROS such as superoxide anions (O_2._), hydroxyl radicals (.OH) and H_2O_2 enhance oxidative process and produce lipid peroxidative damage to cell membranes. The .OH radical has been proposed as an initiator of LPO through an iron-catalysed Fenton reaction (Halliwell and Gutteridge, 1986, 1989). The erythrocytes may be susceptible to oxidative damage due to the presence of polyunsaturated fatty acids (PUFA), heme iron and oxygen which may produce oxidative changes in red cells.

Oxidative stress by increased production of reactive oxygen species has been implicated in the toxicity of many pesticides. Therefore, the aim of the present study was to investigate the effect of current using pesticides, organophosphate and pyrethroids (Dimthoate, profenofos, chlorpyrifos and cypermethrin), on oxidative stress to establish lipid peroxidation (LPO) as a biomarkers for pesticide exposure in mice.

Materials and Methods

Animals

In the present investigation, experiments were performed on 12-14 weeks old healthy Swiss albino mice, *Mus musculus*. For the optimal growth and development, the mice were kept in ideal condition under a well regulated light and dark (12h:12h) schedule at 23±1°C in the animal house, Mahavir cancer Institute & Research centre, patna, India (CPCSEA Regd. No. 1129/bc/07/CPCSEA, dated 13/02/2008) and the experiment was duly approved by the IAEC. Animals were given food and water *ad libitum*.

Pesticide - Dimethoate (EC 30% w/w), profenofos (EC 50% w/w), chlorpyrifos (EC 20% w/w) and cypermethrin (EC 25% w/w) pesticides of organophosphate and pyrethroids groups were used and procured from pesticide shop, Jamal Road, Patna.

Methodology

Study Design Thirty mice were used in the study and were grouped into two with 10 mice in control group and 20 mice in pesticide treated group. Group A: untreated mice kept as control and served with equal volume of distilled water by gavage method. Group B: mice treated with Dimethoate (EC 30% w/w) 4 mg/kg b.w. was given on day first, profenofos (EC 50% w/w) 3 mg/kg b.w. was given on day second, chlorpyrifos (EC 20% w/w) 10 mg/kg b.w. was given on day third and cypermethrin (EC 25% w/w) 8 mg/kg b.w. was given on day forth and this cycle was continued for 8 weeks to make chronic exposure of different pesticides. The treated and control group of mice were anaesthetized for blood collection on targeted day. MDA (malondialdehyde), the marker of lipid peroxidation was estimated for exposure of pesticides.

Collection of Blood

The blood from the control and treated mice were obtained from mice by orbital sinus puncture. Mice were anaesthetized for this purpose. Collection of blood from orbital sinus with a Hematocrit tube is one of the most effective methods, which causes least stress to the animal. The blood was collected in EDTA vaccutainer tube for biochemical study. The blood was immediately centrifused at 3000 rpm for 15 minute and the plasma separated. The cells were washed with normal saline and RBC's were subjected to lysis.

Assessment of MDA for Lipid Peroxidation

MDA, as a marker for LPO, was determined by the double heating method of Draper and Hadley (1990). The principle of the method was spectrophotometric measurement of the colour produced during the reaction of thiobarbituric acid (TBA) with MDA. For this purpose, 2.5 ml of 100 g/l triochloroacetic acid (TCA) solution was added to 0.5 ml erythrocytes in a centrifuge tube and placed in a boiling water-bath for 15 min. After cooling in tap water, the mixture was centrifuged at 1000 rpm for 10 min, and 2 ml of the supernatant was added to 1ml 0.67% TBA (w/v) solution in a test-tube and placed in a boiling water-bath for 15 min. The solution was then cooled and the absorbance was measured using a spectrophotometer at 532 nm. The concentration of MDA was calculated by the absorbance coefficient of MDA-TBA complex $1.56x10^5$ $cm^{-1}M^{-1}$ and expressed in nmol/ml blood.

Statistical analysis

Data were analyzed with statistical software (Graph pad Prism 5) and values were expressed as Mean ± SEM and differences between the groups were statistically analyzed by paired t – test.

Results

Data is showing below in the table and values are expressed as Mean ± SEM. The mean ± SD blood level of MDA was 17.24 ± 2.47nmol/TBARS/ml, which was significantly ($p < 0.05$) higher than their control (5.85 ± 0.65). The increase in lipid peroxidation marked by elevated levels of MDA in pesticide exposed group indicates oxidative stress.

Table

Level of MDA (nmol/TBARS/ml blood) in control and pesticides exposed mice	
Control(n=10) Mean ± S.D.	Pesticides exposed (n=20) Mean ±S.D.
5.85 ± 0.65	17.24 ± 2.47

Discussion

The present study was aimed to determine lipid peroxidation as a biomarker for pesticides exposure. Thus organophosphate and pyrethroids group of pesticides have been used to make chronic exposure of pesticides in mice. The present investigation observed elevated level of MDA reflects the increase lipid peroxidation which is in consequence of exposure of pesticides.

It is probable that pyrethroids transported through blood to the liver for metabolism may produce cellular damage to erythrocytes. LPO, a consequence of cellular injury (Spiteller, 1996) was studied in pyrethroid intoxicated rats as an index of oxidative stress. Indiscriminate use of organochlorine, organophosphate, and carbamate pesticides are making harm to human health. These pesticides are known to disturbing the biochemical and physiological function of erythrocytes and lymphocytes (Banarjee *et al*., 1999). It is important to note that many environmental contaminants, such as organochlorine pesticides, accumulate in fatty tissues (Latchoumycandane *et al*., 2002). Tissue degeneration is a free-radical medited process that involves lipid peroxides and lipid peroxidation of polyunsaturated fatty acids (PUFA) of the mammalian tissue (Debnath *et al*., 2000). Therefore, lipid peroxidation has been suggested as one of the molecular mechanisms involved in pesticide-induced toxicity (Banarjee *et al*., 1999). Lipid peroxidation (LPO), which is the major contributor to the loss of cell function, as well as DNA damage, enzyme inactivation, and hormone oxidation are indicators of oxidative cell damage (Ruas *et al.*, 2008). LPO, in particular, has been suggested to be one of the mechanisms of pesticide-induced toxicity (Mansour & Mossa, 2009).

Erythrocytes are a convenient model to understand the membrane oxidative damage induced by various xenobiotic pro-oxidants (Mansour *et al.*, 2009).The treatment with clomazone (*in vitro*) showed increased LPO in human erythrocytes. Polyunsaturated fatty acids of the membrane, an oxygen-rich environment, as well as iron-rich haemoglobin make red cells susceptible to peroxidative damage. ROS initiates LPO reactions that lead to loss of membrane integrity and, consequently, death of the cell. Malondialdehyde (MDA), a highly reactive bifunctional molecule, is an endproduct of membrane LPO. MDA has been shown to cross-link erythrocyte phospholipids and proteins. This process results in impairment of the membrane-related functions, leading ultimately to diminished survival (Cimen, 2008).

The increased MDA levels reflects the increase lipid peroxidation in erythrocytes found in the present investigation may have resulted from an increase of free radicals as a result of stress condition generated by pesticide exposure. It is speculated that oxidative stress in erythrocytes may lead to significant alterations in their structural conformation, which may compromise effective blood flow, oxygen uptake and release (Prasanthi *et al.*, 2005) which is in support of my work. These findings are consistent with results of several other recent investigations (Prasanthi *et al.*, 2005; Duchnowicz *et al.*, 2005).

Conclusion

The aim of the present study was to identify biomarker for exposure of pesticides so that if any person is being exposed to pesticide could be check through biomarker. Since it is observed from present study and support of previous work done that pesticides exposure have more potency to cause oxidative stress consequently produces free

radicals. These free radicals are responsible for degeneration of lipid molecules of plasma membrane and causing lipid peroxidation. Thus lipid peroxidation has been identified as a biomarker for exposure of pesticides.

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