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Chronic Toxicity of Menazon and Relation to Oxidative Stress in Red Blood Cells of Rats

[1]A.G. Ebadi and [2]M. Shokrzadeh
[1]Department of Biology, Islamic Azad University of Sari, Sari Branch, Iran
[2]Department of Toxicology, Faculty of Pharmacy, Mazandaran University of Medical Sciences, Sari, Iran

Abstract: The effect of chronic exposure to menazon (Organophosphorus compound) in the production of oxidative stress was assessed in rats. Administration of menazon (50, 100, 500, 1000 ppm) for 2 weeks duration increased catalase (CAT), superoxide dismutase (SOD) activities in red blood cells (RBC). However, acetylcholinesterase (AChE) activity were decreased in these samples. The increase in RBC lipid peroxidation correlated well with the inhibition in RBC AChE activity. Increased activities of CAT and SOD showed significant correlations in RBC samples when different doses of menazon were used. The results of the present study suggest the usefulness of RBC AChE measurement as a good index in the evaluation of menazon-induced oxidative stress affecting blood.

Key words: Menazon, acute toxicity, acetylcholinesterase, oxidative stress, rat

INTRODUCTION

Synthesized in 1937, the organophosphorus insecticides (OPI) include chemicals of organophosphorus structure used as pesticides in both home and agriculture. According to World Health Organization report every year, 1 million serious accidental and 2 million suicidal poisonings with insecticides occur worldwide and of these approximately 220,000 die.

For centuries pesticides have been used in agriculture to enhance food production by eradicating unwanted insects and controlling disease vectors[1]. Among pesticides, Organophosphorus compounds (OPs) are commonly used as insecticides[2]. There is some evidence to suggest the role of oxygen free radicals (OFRs) in the toxicity of certain pyretroids, a commonly used insecticide[3]. Measurement of scavengers of OFRs like superoxide dismutase (SOD) and catalase (CAT) are good markers to study the effects of oxidative stress[4]. The increased activity of SOD reflects the activation of the compensatory mechanisms through the effects of pesticides on progenitor cells and its extent depends on the magnitude of the oxidative stress and hence, on the dose of stressor[2].

Intentional or accidental acute OPIs poisoning is unfortunately very common and many fatal cases are reported. In acute exposure, the main mechanism of toxicity of OPIs is irreversibly binding to the enzyme acetyl cholinesterase and inhibiting its activity that results in accumulation and prolonged effect of acetylcholine and consequently follows with acute muscarine and nicotinic effects. In chronic and subchronic exposures, added to cholinesterase inhibition, induction of oxidative stress has been reported as the main mechanism of toxicity[2,3]. The imbalance between production of oxygen free radicals (OFRs) and antioxidant defenses in the body is called oxidative stress which has important health implications. If there are too many OFRs or too few antioxidants for protection, a condition of oxidative stress develops, which may cause chronic permanent damage. For example, OFRs react with lipids in the cell membrane, a destructive process known as lipid peroxidation. Principally, oxidative stress in human can result from diminished body antioxidants like glutathione peroxidase, superoxide dismutase and catalase or increased production of OFRs which leads to lipid peroxidation and protein oxidation[3]. Oxidative stress is a major mechanism in pathophysiology of several toxins and diseases. Chronic exposure to OPIs for about one year has induced a profound oxidative stress[2,3].

Reactive radicals, such as superoxide anions($^{\bullet}O_2$), hydroxyl ($^{\bullet}OH$) and hydroperoxyl (H_2O_2), can abstract an atom of hydrogen from polyunsaturated fatty acid (PUFA) side chains in biological membranes and produce lipid peroxidative damage[5]. Mammalian cells possess nonenzymatic and enzymatic antioxidant defenses to cope with damaging OFRs. The nonenzymatic defenses include vitamin E, β-carotene and vitamin C and enzymatic ones include SOD, CAT and glutathione peroxidase. Increased free radical formation can cause oxidative stress and resulting cell damage if antioxidant defenses are overwhelmed[6].

Corresponding Author: Dr. Abdol Ghaffar Ebadi, Department of Biology, Islamic Azad University of Sari, Sari Branch, Iran

One of the molecular mechanisms of the toxicity of some pesticides seems to be lipid peroxidation; as a consequence these compounds can disturb the biochemical and physiological functions of the red blood cells[6]. The susceptibility of RBC to oxidative damage is due to the presence of PUFA, haem iron and oxygen, which may produce oxidative changes in RBC[3]. Also, it is well known that acetylcholinesterase (AChE), located in RBC membrane, can be an indicator of exposure to OP compounds[7,8].

The goal of this study was to determination of the effects of menazon in the production of oxidative stress, through the measurement of CAT and SOD activities (as a measure of induction of protective systems) and to investigate potential correlation between AChE inhibition and lipid peroxidation in RBC following chronic exposure to menazon in rats.

MATERIALS AND METHODS

Animals: Male Wistar rats weighing 200-250 g under standard conditions with free access to drinking water and food were selected. The animals were randomly divided into five groups each comprising of five animals and treated for 2 weeks. menazon was dissolved in corn oil and mixed manually with diet to produce 50, 100, 500 and 1000 ppm. The treatment detail is as follows:

Group 1 as control was rats that fed on normal diet, which was mixed completely with corn oil. groups 2 to 5 received 50, 100, 500 and 1000 ppm along with normal diet, respectively for two weeks.

Sample collection: After the treatment period blood samples were collected under anaesthesia by cardiac puncture in vials containing heparin. RBC was separated by centrifugation at 3000 rpm for 15 min. The buffy coat was removed and the RBC was washed three times with physiological saline. Aliquots of RBC were kept at -20°C except the samples for CAT assay[9]. CAT activity in RBC haemolysate was determined spectrophotometrically using hydrogen peroxide (H_2O_2) as substrate at 240 nm and pH 7. Enzyme activity was expressed as K g^{-1} Hb, which K is as follows: K = (2.3/t)(a/b)(log A1/A2), where A1 is A240 at t = 0, A2 is A240 at t = 30 s, a is the dilution factor for Hb concentration and b is the Hb content of blood[9]. The method employed is based on the ability of SOD to inhibit the reduction of nitroblue tetrazolium by superoxide generated by xanthine-xanthine oxidase reaction. One unit is defined as that amount of enzyme causing half the maximum inhibition of NBT reduction. The blood was haemolysed with ice-cold water. Haemoglobin was removed by adding chloroform and ethanol followed by centrifugation. The clear supernatant was used for SOD assay. SOD assay was performed using NBT, xanthine and xanthine oxidase system. The absorbance change at 560 nm was monitored at 25°C for 20 min. Enzyme activity was expressed as units mL^{-1} of blood[10]. The rate of hydrolysis of acetylthiocholine iodide (substrate) by red cell suspension at pH 7.6 was measured at 440 nm by the reaction of thiocholine with DTNB to give the yellow 5-thio-2-nitrobenzoate anion with UV spectro-photometer 160-A. The enzyme activity was expressed as μmol of substrate, which was hydrolyzed per minute per milliliter of packed RBC (μmol min^{-1} mL^{-1})[11].

Statistical analysis: Data were analysed by one-way ANOVA followed by t-test comparison test, $p > 0.05$ were considered insignificant.

RESULTS AND DISCUSSION

RBC CAT activity: CAT activity in RBC was increased in all doses of menazon-exposed animals in comparison to the control group. CAT activity of RBC showed a significant correlation with dose of menazon. total concentration showed significant difference with control group but not seen between test group at $p<0.05$ (Fig. 1).

RBC SOD activity: RBC SOD activity was significantly higher in treated animals in comparison to control group. RBC SOD activity had significant correlation with dose of menazon. Significant difference was seen between concentration of 100, 500 and 500 ppm with control at $p<0.05$ (Fig. 2).

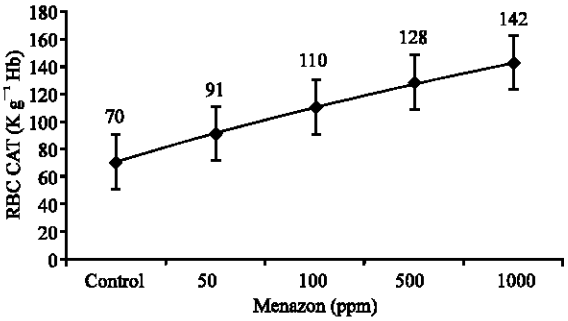

Fig. 1: Effects of menazon on RBC CAT activity. Menazon was administered in different doses of 50, 100, 500 and 1000 ppm for 2 weeks through food. Values are expressed as mean+SD of six animals in each group

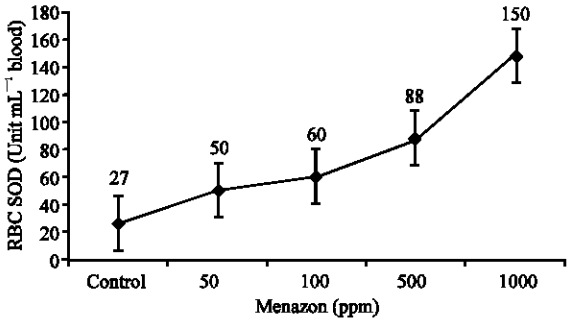

Fig. 2: Effects of menazon on RBC SOD activity. Menazon was administered in different doses of 50, 100, 500 and 1000 ppm for 2 weeks through food. Values are expressed as mean±SD of six animals in each group

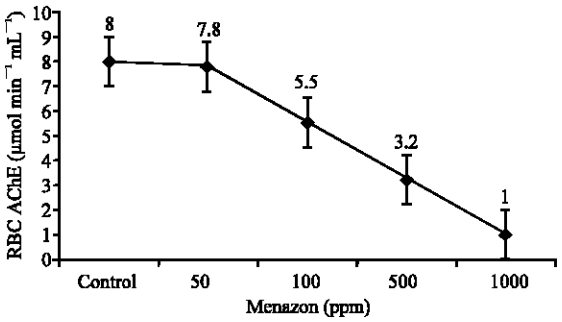

Fig. 3: Effects of menazon on RBC AChE activity. Menazon was administered in different doses of 50, 100, 500 and 1000 ppm for 2 weeks through food. Values are expressed as mean±SD of six animals in each group

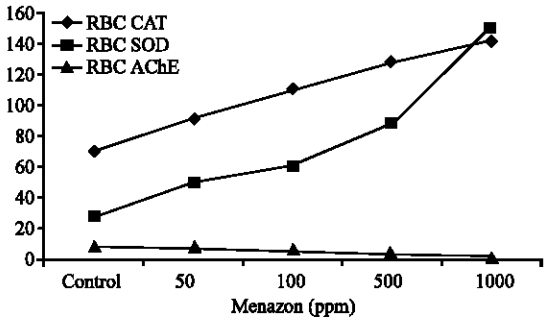

Fig. 4: Comparison of effects of menazon on RBC CAT, SOD and AChE activity. Menazon was administered in different doses of 50, 100, 500 and 1000 ppm for 2 weeks through food. Values are expressed as mean±SD of six animals in each group

AChE activity: RBC AChE activity was significantly lower in treated animals in comparison to the control group except for menazon (50 ppm). RBC AChE activity inhibition had a good correlation with dose of menazon. Concentration of 500 and 1000 showed maximum decrease and significant difference was seen between concentration 500 and 1000 with control group at $p<0.05$ (Fig. 3).

In this study we investigated the effects of menazon chronic exposure on lipid peroxidation in rat RBC by means of estimation of CAT, SOD activities. The present results demonstrated that 2 weeks exposure to menazon with doses of 50, 100, 500 and 1000 ppm increased levels of CAT and SOD activities. We observed significantly higher levels of CAT and SOD activities in RBC of menazon-treated animals than those of control groups (Fig. 4).

The increased activity of SOD reflects an activation of the compensatory mechanism through the effects of pesticides on progenitor cells and its extent depends on the magnitude of the oxidative stress and hence, on the dose of stressor. The elevated activity of CAT is due to the adaptive response to the generated free radicals[12]. indicating the failure of the total antioxidant defence mechanism to protect the tissues from mechanical damage caused by pesticide, which is evidenced by lipid peroxidation[13]. Also SOD activity probably increased to dismutate superoxide anion ($^{\bullet}O_2$) and to decompose H_2O_2[14]. Supporting our results there is evidence that subchronic exposure to malathion (20 ppm) causes an increase in activities of SOD, CAT, glutathione peroxidase (GPx), glutathione reductase (GR) and glutathione S-transferase (GST) in the RBC of rats, suggesting that OP compounds may induce oxidative stress leading to generation of free radicals and alterations in antioxidant or OFR-scavenging enzymes[15]. In humans it has been reported that in acute menazon poisoning, the level of SOD, CAT and GGT are increased. It has been suggested that pesticide-manufacturing workers who are exposed chronically to OPs show decreased thiol group levels in blood as a result of oxidative stress production from these compounds.

Inhibition of AChE that shown in Fig. 3 is believed to be the principal mode of action of OP compounds. menazon inhibited activities of AChE in RBC of rats except for animals that were treated with 50 and 100 ppm of menazon. No signs of toxicity were observed nor any deaths in all doses of menazon used. Rats exhibited normal behaviour even with 90% inhibition in RBC ACHE (1000 ppm) in comparison to the control group. The same result has been reported between inhibition of

AchE activity and lipid peroxidation in pesticide OP-manufacturing workers[15].

The correlation between lipid peroxidation and AChE activity in the present study suggests that inhibition of AChE initiates the accumulation of free radicals leading to lipid per oxidation, which may be the indicator of cell injury[16]. The phospholipid component of biomembranes is believed to be the site of action of OP compounds. AChE forms the link between lecithin and protein in the REG membrane and contributes to the maintenance of membrane integrity and thus AChE inhibition interacts with membrane integrity. Human RBC AChE activity has been reported to be decreased with the decrease in the content of lipid and was restored with the addition of phospholipid. It has been suggested that RBC membrane phospholipid peroxidation interferes with red cell deformability[17]. Thus the variation of lipid composition of biomembranes as well as their structural organization appears to be responsible for the membrane-specific effect of insecticides on AChE activity.

In conclusion, this study suggests that catalase and the superoxide dismutase are important in maintaining the secretory function and the integrity of adrenocortical cells exposed to menazon. Oxidative stress can be prevented by the action of enzymatic antioxidant defenses (CAT, Gpx) and by nonenzymatic antioxidants such as GSH. Under oxidative stress, ROOH are reduced by GSH with concomitant formation of GSSG. GSH also acts in the enzymatic first line antioxidant defense, as a cofactor in GPx mediated reduction of peroxides[16,17].

As a final point it is concluded that menazon can change antioxidant status of biological membranes. The measurement of RBC AChE activity in persons who work with these compounds can be a good toxicity-monitoring factor for OP toxic exposure. The beneficial role of antioxidant vitamins such as vitamin E, α-tocoferol and vitamin C should not be neglected[17].

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