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In vivo changes in antioxidant systems and protective role of melatonin and a combination of vitamin C and vitamin E on oxidative damage in erythrocytes induced by chlorpyrifos-ethyl in rats

Received: 6 November 2000 / Accepted: 16 January 2001 / Published online: 16 March 2001
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Abstract Reactive oxygen species (ROS) may be involved in the toxicity of chlorpyrifos-ethyl (CE) [O, O-diethyl-O-(3,5,6-trichloro-2-pyridyl)phosphorothioate]. We have, therefore, examined the in vivo effects of CE on the rat erythrocyte antioxidant system and evaluated the ameliorating effects of melatonin and a combination of vitamin E and vitamin C on the oxidative damage induced by CE. The experimental groups were: (1) control group, (2) CE-treated group (CE), (3) vitamin E plus vitamin C treatment group (Vit), (4) melatonin-treated group (Mel), (5) vitamin E plus vitamin C plus CE treatment group (Vit + CE), and (6) melatonin plus CE treatment group (Mel + CE). Vitamin E and vitamin C were administered intramuscularly once a day for 6 consecutive days at 150 and 200 mg/kg, respectively, in the Vit and Vit + CE groups. Melatonin was administered intramuscularly at 10 mg/kg per day for 6 consecutive days in the Mel and Mel + CE groups. At the end of the fifth day, the rats of CE, Vit + CE and Mel + CE groups were treated orally with the first of two equal doses of 41 mg/kg CE, the second oral dose being given 21 h later. Blood samples were taken 24 h after the first CE administration. Levels of thiobarbituric acid reactive substance (TBARS), antioxidant defence potential (AOP), and the activities of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and catalase (CAT) were determined in erythrocytes. In comparison with the control group, oral administration of CE significantly \( (P < 0.05) \) stimulated TBARS activity while significantly \( (P < 0.05) \) inhibiting AOP and the activities of SOD and CAT. However, GSH-Px activity remained unchanged by CE treatment. Treatment with melatonin and vitamins E plus C significantly \( (P < 0.05) \) reduced the CE-induced increase of TBARS, and overcame the inhibitory effect of CE on SOD and CAT, but not on AOP. Melatonin treatment significantly \( (P < 0.05) \) increased only GSH-Px activity, irrespective of the effect of CE. These results suggest that CE treatment increases in vivo lipid peroxidation and decreases antioxidant defence by increasing oxidative stress in erythrocytes of rats, and melatonin and a combination of vitamin E and vitamin C can reduce this lipoperoxidative effect.

Keywords Chlorpyrifos-ethyl · Erythrocyte · Lipid peroxidation · Melatonin · Vitamin

Introduction

The widespread use of organophosphorus insecticides (OPIs) has long been shown to exert deleterious effects on living organisms. For instance, the exposure of laboratory animals to OPIs, in particular to chlorpyrifos-ethyl (CE) [O, O-diethyl-O-(3,5,6-trichloro-2-pyridyl)phosphorothioate], elicits a number of effects including hepatic dysfunction (Gomes et al. 1999), delayed fever (Gordon et al. 1997), chibototoxicity (Swann et al. 1996), immunological abnormalities (Thrasher et al. 1993; Blakley et al. 1999), embritotocytosis (Muscarella et al. 1984; Muto et al. 1992), genotoxicity (Bagchi et al. 1995; Dam et al. 1998; Roy et al. 1998; Song et al. 1998) and neurochemical and neurobehavioural changes in developing and adult animals (Whitney et al. 1995; Chanda and Pope 1996; Mattsson et al. 1996; Dam et al. 1998; Song et al. 1998; Dam et al. 1999; Hunter et al. 1999). Many reports also indicate that the most commonly used OPIs induce a teratogenic effect in animals (Gupta 1992; Roy et al. 1998).
Several insecticides have been found to bind extensively to human plasma protein fractions (Datta et al. 1992) and to disturb the biochemical and physiological functions within the erythrocytes, thereby affecting membrane integrity (Agrawal et al. 1991). CE is one of the OPIs commonly used against a wide variety of agricultural pests in Turkey.

Recent findings indicate that toxic manifestations induced by OPIs may be associated with an enhanced production of reactive oxygen species (ROS) (Bagchi et al. 1995). Among ROS, superoxide anions, hydroxyl radicals and hydrogen peroxide enhance the oxidative process and induce lipid peroxidative damage in cell membranes. Hydroxyl radicals were previously proposed as initiators of lipid peroxidation (LPO) through an iron-catalysed Fenton reaction in membranes (Halliwell and Gutteridge 1986). Erythrocytes may be susceptible to oxidative damage due to the presence of haem-iron, polyunsaturated fatty acids (PUFA) and oxygen, which may initiate the reactions that induce oxidative changes in red blood cells. It is possible that CE, during its transportation through the blood stream to the liver, may produce cellular damage in erythrocytes.

The cell has several ways to alleviate the effects of oxidative stress, either by repairing the damage (damaged nucleotides and lipid peroxidation by-products) or by directly diminishing the occurrence of oxidative damage by means of enzymatic and non-enzymatic antioxidants. Enzymatic and non-enzymatic antioxidants have also been shown to scavenge free radicals and ROS.

The enzymatic antioxidants in erythrocytes may counteract oxidative stress. For instance, superoxide dismutase (SOD) catalyses the conversion of super oxide radical (\(O_2^-\)) to hydrogen peroxide (\(H_2O_2\)) while catalase (CAT) or glutathione peroxidase (GSH-Px) converts \(H_2O_2\) to \(H_2O\). These antioxidant enzymes can, therefore, alleviate the toxic effects of ROS.

Non-enzymatic antioxidants such as vitamin E, vitamin C and melatonin can also act to overcome the oxidative stress, being a part of total antioxidant system. Vitamin E is an important biological free radical scavenger in the cell membrane (Horwitt 1976). This has been shown to provide a protection against superoxides as well as \(H_2O_2\) (Clemens and Waller 1987). It has been also shown that vitamin C, a water-soluble vitamin and known antioxidant, can react with vitamin E radicals to regenerate vitamin E.

Melatonin (N-acetyl-5-methoxytryptamine), the chief secretory product of the pineal gland, presumably functions as an exclusive synchroniser of seasonal reproduction, an adjuster of the biological clock, a sleep-inducing agent, and an immune system stimulator. More recently, melatonin was found to act as a free radical scavenger and an antioxidant; exogenous melatonin administration was, therefore, shown to protect DNA, membrane lipids and cytosolic proteins from oxidative damage induced by oxygen-derived free radicals (Reiter 1996).

It has been proposed that xenobiotics may produce ROS leading to LPO (Trush and Kenser 1991). Agrawal et al. (1991) suggested that LPO could be a major molecular cause of tissue injury. The level of thiobarbituric acid reactive substances (TBARS) is a sign of membrane lipid peroxidation resulting from the interaction of ROS and cellular membranes (Mihara and Uchiyama 1978).

An earlier study from our laboratory concentrated on the in vitro effect of CE on LPO and antioxidant enzymes (Gultekin et al. 2000b). The results indicated that increasing CE concentration caused a significant reduction in the activities of SOD and CAT and a significant increase in the level of malondialdehyde and activity of GSH-Px. Addition of exogenous antioxidants (butylated hydroxytoluene and vitamin E) led to no change in SOD activity and MDA level, but increased GSH-Px activity and reduced CAT activity under the influence of CE. It appears that in vitro CE induces LPO in erythrocytes by producing significant changes in the endogenous antioxidant defence mechanism, and the addition of exogenous antioxidants may overcome the LPO induced by CE.

We now propose that in vivo administration of CE may induce oxidative stress, which may be involved in CE toxicity, and the use of melatonin and a combination of vitamin E and vitamin C may reduce CE-induced oxidative stress and CE toxicity.

In order to test the above hypothesis, an experiment was conducted to determine the in vivo effect of CE on antioxidant defence potential (AOP), LPO and on antioxidant enzymes, such as SOD, GSH-Px and CAT in erythrocytes of rats. TBARS was determined as an indicator of the level of LPO. Additionally, some antioxidants such as vitamin E, vitamin C and melatonin were administered to rats to evaluate their protective effects on CE-induced oxidative stress.

### Materials and methods

#### Animals and treatment

Thirty male Wistar albino rats weighing 190–240 g were used as animal subjects in the experiment. The rats were individually caged and fed ad libitum without water restriction. The rats were divided into six groups, each containing five rats. The experimental groups were as follows: (1) control group, (2) CE-treated group (CE), (3) vitamin E plus vitamin C treatment group (Vit), (4) melatonin-treated group (Mel), (5) vitamin E plus vitamin C plus CE treatment group (Vit + CE), and (6) melatonin plus CE treatment group (Mel + CE). Vitamin E (\(\alpha\)-tocopherol acetate, Sigma) and vitamin C (sodium-\(\alpha\)-ascorbate; Redoxon, Roche) were administered intramuscularly once a day for 6 consecutive days at 150 and 200 mg/kg, respectively, to the Vit and Vit + CE groups. Although there are many routes used for the administration of exogenous antioxidants, intraperitoneal and subcutaneous being mostly preferred, the intramuscular route has also been successfully used (Kenns et al. 1983; Lisoni et al. 1986; Augustin et al. 1992; Lu et al. 1995; Black and Hidiroglou 1996). Melatonin (N-acetyl-5-methoxytryptamine; Sigma) was administered intramuscularly at 10 mg/kg per day for 6 consecutive days in the Mel and Mel + CE groups. On each occasion, melatonin, vitamin E and vitamin C were injected with an insulin injector in a volume of 50 μl. At the end of the fifth day, the rats of the CE, Vit + CE and Mel + CE groups were treated...