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Morphological and biochemical perturbations in rat erythrocytes following in vitro exposure to Fenvalerate and its metabolite

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Abstract

Erythrocytes are a convenient model to understand the membrane oxidative damage induced by various xenobiotic-prooxidants. In this investigation, we have examined the potency of Fenvalerate (FEN) and its metabolite, *p*-chlorophenyl isovaleric acid (*p*-CPIA) to induce oxidative stress response in rat erythrocytes in vitro in terms of lipid peroxidation and effects on selected antioxidant enzymes. Susceptibility of erythrocytes to FEN exposure was further investigated in terms of morphological alterations by scanning electron microscopy and protein damage by gel electrophoresis of erythrocyte ghosts. Following in vitro exposure, FEN caused a significant induction of oxidative damage in erythrocytes at concentrations beyond 0.1 mM as evidenced by increased thiobarbituric acid reactive substances (TBARS) levels. The response was both concentration and time dependent. At higher concentrations, significant decreases in the activities of vital antioxidant enzymes viz., catalase, superoxide dismutase, glutathione transferase and glutathione reductase were also discernible clearly suggesting the potency of both, parent compound and its metabolite to induce oxidative stress in erythrocytes. Scanning electron micrographs of erythrocytes following FEN exposure at higher concentrations revealed various degrees of distortion in shape and ruptured membranes. Furthermore, gel electrophoresis studies revealed consistent and significant aggregation of only band 3 protein in erythrocyte membranes exposed to either FEN or *p*-CPIA at higher concentrations. These in vitro findings show that FEN and its metabolite have the propensity to cause significant oxidative damage in rat erythrocytes, which is associated with marked damage to membrane proteins. These data suggest that both structural and functional perturbations may ensue in erythrocytes following exposure to FEN at higher concentrations under in vivo situations.

Keywords: Erythrocytes; Fenvalerate; Oxidative stress; Antioxidant enzymes; Scanning electron microscopy; Erythrocyte ghost proteins

1. Introduction

In the recent past, toxic manifestations induced by several xenobiotics are shown to be mediated by oxidative stress mechanisms. Involvement of reactive oxygen species (ROS) has been demonstrated in the toxicity of organochlorine (Bagchi et al., 1992; Hincal et al., 1995) and organophosphorus insecticides (Banerjee et al., 1999). However, studies describing the role of ROS in pyrethroid toxicity are limited (Kale et al.,

Abbreviations: LPO, lipid peroxidation; OS, oxidative stress; ROS, reactive oxygen species; SOD, superoxide dismutase; GPx, glutathione peroxidase; GR, glutathione reductase; MDA, malondialdehyde; TBARS, thiobarbituric acid reactive species; FEN, Fenvalerate; *p*-CPIA, *p*-chlorophenyl isovaleric acid; DMSO, dimethyl sulfoxide; TEP, 1,1,3,3-tetraethoxypropane

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1999; Gupta et al., 1999; Giray et al., 2001). In this regard, we have examined the potential of selected pyrethroid compounds (Type II), for their ability to induce oxidative damage in various mammalian tissues under in vivo and in vitro exposures (Prasanthi, 2001).

Erythrocytes are particularly sensitive to oxidative damage due to the presence of high polyunsaturated fatty acid content in their membranes and high cellular concentrations of oxygen and haemoglobin (Clemens and Waller, 1987; Chiu et al., 1989). Erythrocytes are well equipped with several biological mechanisms to defend against intracellular oxidative stress (Stern, 1985) comprising of many antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR) and glutathione transferase (GST). Despite their well-developed antioxidant defense system, erythrocytes can be oxidatively damaged, due to exposure to toxic chemicals and environmental pollutants. Earlier workers have shown various structural alterations in erythrocytes and associated biochemical perturbations following chronic exposures to insecticides such as HCH (Agrawal and Sultana, 1993) and heptachlor (Suwalsky et al., 1997). More importantly, 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 (Youdim et al., 2000).

Pyrethroids are more hydrophobic than other classes of insecticides (Michelangeli et al., 1990) and therefore their general site of action is biological membranes. Earlier, numerous workers have investigated the metabolism of Fenvalerate in animal models (Lee et al., 1985) and shown CPIA as its major metabolite both in vivo and in vitro (Miyamoto et al., 1986). Further, it has been speculated that the cleavage of FEN and its ester metabolite are reported to release cyanohydrins, which are unstable under physiological conditions and further decompose to cyanides and aldehydes (World Health Organization, 1990), which in turn could act as a source of free radicals. Employing both Fenvalerate (FEN) and its metabolite, *p*-chlorophenyl isovaleric acid (*p*-CPIA), we have observed significant oxidative stress response in various rat tissues in vivo following sublethal oral doses (Prasanthi, 2001). Recently we have reported induction of oxidative damage in erythrocytes of rats fed dietary FEN (Prasanthi et al., 2005). Accordingly, in the present study, the propensity of FEN to induce oxidative stress in rat erythrocytes in vitro has been investigated in terms of lipid peroxidation (LPO) induction response, alterations in selected antioxidant enzymes along with associated morphological/biochemical perturbations.

The results obtained showed that at higher concentrations, FEN and its metabolite induce significant degree of oxidative stress response in erythrocytes as revealed by increased LPO and decreased antioxidant enzymes which were associated with morphological damage as well as specific alterations in erythrocyte membrane proteins.

2. Materials and methods

2.1. Chemicals

Technical grade Fenvalerate (FEN, 94.8% pure) [(*R, S*)-α-cyano3-phenoxy benzyl (*R, S*)-2-(4-chlorophenyl)-3-methyl butyrate] was a gift from M/s Searle India (Mumbai, India). *p*-CPIA was prepared in the laboratory by alkaline hydrolysis of FEN. Thiobarbituric acid (TBA), xanthine oxidase, cytochrome 'c' was procured from M/s Sigma Chemical Co. (St. Louis, MO, USA). Reduced glutathione (GSH), oxidized glutathione (GSSG), 1-chloro-2,4-dinitrobenzene (CDNB), sodium dodecyl sulfate (SDS), coomassie brilliant blue and dimethyl sulfoxide (DMSO) were purchased from M/s SISCO Research Laboratories (Mumbai, India). All other reagents used were of analytical reagent grade. Stock solutions (50 mM) of FEN, and the metabolite of FEN, *p*-CPIA was prepared in DMSO.

2.2. Animals and care

Adult male rats (CFT-Wistar strain, 8–10 weeks old, 180–200 g) were obtained from our 'animal house facility'. They were housed in polypropylene cages (two per cage) at room temperature (25 ± 2 °C) with a relative humidity of 50–60% and on a 12 h light-darkness cycle. The animals had free access to commercial pellet diet (Gold Mohur, supplied by M/s Hindustan Lever Ltd., India) and water *ad libitum*. All animal procedures were in strict conformation with the 'Institute Ethical Committee guidelines' for the care and use of laboratory animals.

2.3. Preparation of erythrocytes and erythrocyte ghosts

Rats were sacrificed under mild diethyl ether anaesthesia. Blood was drawn by cardiac puncture into tubes containing 3.8% trisodium citrate solution. Blood was centrifuged at 3000 rpm for 10 min at 4 °C, and the plasma and buffy coat were removed. The erythrocytes were washed three times with ice-cold phosphate buffered saline (PBS: 145 mM NaCl, 1.9 mM NaH_2PO_4, 8.1 mM Na_2HPO_4) and centrifuged. The packed erythrocytes after the final wash were resuspended in phosphate buffer (0.1 M, pH 7.4) at 1:9 dilution and used for incubations. Erythrocyte ghosts were prepared according to the method of Dodge et al. (1963). The erythrocyte ghosts were frozen at −20 °C until use. The protein content of erythrocyte ghosts was determined prior to use, and aliquots equivalent to 300 μg protein were incubated with FEN/*p*-CPIA for 60 min at 37 °C.