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Effect of repeated dermal application of α-cypermethrin on lipid peroxidation and antioxidant system in rats

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Abstract

α-Cypermethrin, a pyrethroid insecticide has a wide use for crop protection and ectoparasitic control in domestic animals. Excessive dermal exposure due to environmental contamination or inadvertent use may result in hazardous effects in man and animals. In present study the various biomarkers for the oxidative stress have been studied in Wister rats. SOD and catalase activities in erythrocytes were significantly decreased after 30 day of dermal exposure although these were initially over expressed. There was significant decrease ($p < 0.05$) in blood GSH level and GPx activities in all the three groups. Enzymatic activity of GST was not significantly affected on any day of the exposure. Significant increase in lipid peroxidation of erythrocyte membrane from 10 days onwards exposure was also observed. The evaluated enzymatic and non-enzymatic components of the cell suggest that dermal exposure of ?-cypermethrin produces oxidative stress in rats.

Keywords: α-cypermethrin, antioxidant system, dermal application, lipid peroxidation

Introduction

Pyrethroids are highly active insecticides with low mammalian toxicity as compared to other commonly used insecticides. α-cypermethrin, [(R)-cyano-3-phenoxybenzyl (1R)-cis-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecar-boxylate] is an active pyrethroid, due to high bio-efficacy and lipid solubility, enhanced stability and considerably low mammalian toxicity. It is widely used insecticides for crop protection in agricultural practices and ectoparasitic control in veterinary practices (Elliot and Janes, 1978). Inadvertent use for ectoparasitic control or environmental contamination is the common source of dermal exposure in man and animals (Daniel and Moser, 1993).

Mammalian cells are endowed with extensive antioxidant defense mechanisms which counteract the damaging effects of toxic reactive oxygen species (ROS) produced during the oxidative stress (Halliwell and Gutteridge, 1989). Mitochondria are the major physiological source of ROS, which arise as a consequence of oxygen reduction (Chance *et al.*, 1979). The excess ROS cause cellular and sub-cellular damage by peroxidation of membrane lipids, by denaturing cellular proteins and by breaking DNA strands and thereof disrupting cellular functions (Halliwell and Gutteridge, 1985; Cross, 1987; Maxwell, 1995). These dysfunctions may be responsible for the variety of pathological conditions ranging from aging to Parkinson's disease (Kushnareva *et al.*, 2002; Trojanowski, 2003). Enzymatic components viz. superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione-S-transferase (GST) and others non enzymatic components like reduced glutathione, ascorbic acid, vitamin E etc. are the main endogenous defense in the mammalian body to combat with oxidative stress (Mohammad, 2002). The GPx and GST are involved in maintenance of the cellular proteins and peptides by scavenging free radicals (Meister and Anderson, 1983). GPx catalyze the reaction between H_2O_2 and reduced glutathione to form oxidized glutathione and water (Sen *et al.*, 2006). SODs are metalloenzymes responsible for the conversion of superoxide free radical anion to hydrogen peroxide (Fridovich, 1995). CAT is capable of scavenging the hydrogen peroxide radical, which is formed in various biochemical and metabolic reactions (Radi *et al.*, 1991). The reduced glutathione (GSH) is the main oxidant in the cell, which directly scavenges free radical and protects bio-molecules from free radical attack.

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The present study was undertaken to elucidate the effect of sub-acute dermal application of α-cypermethrin induced oxidative stress in Wister rats at different predetermined time intervals.

Materials and Methods

Pesticide

α-cypermethrin 10% solution commercially obtained from Meghmani Organic Limited, Ahmedabad was used. The reported LD50 value for acute dermal toxicity of a-cypermethrin in rats is 500 mg kg^{-1} b. wt (Luty *et al.*, 1998).

Animals

Wister rats (200 – 250 gm b. wt.) of either sex were purchased from IIIM (CSIR, Lab) Jammu, and maintained under standard environmental conditions with ad lib feed and water. a-cypermethrin was applied by dermal application at a dose of 50 mg kg^{-1} b. wt (1/10 of LD_{50}) daily for 30 days on inter-scapular area (Punareewattana *et al.*, 2001). During the entire experiment, the body weight of rats was recorded once a week and dosage for application were accordingly adjusted. The animals were divided into 4 groups of 6 rats in each group. Blood was collected from orbital sinus in clean sterile heparinized tubes in different groups on 0 day and 10, 20 and 30 days after the culmination of dermal exposure. The experimental protocol was approved by Institutional Animal Ethics Committee.

Blood enzyme assays

Whole blood is used for the estimation of blood glutathione and hemoglobin. Erythrocyte lysate 1% was used for the catalase, SOD, GPx, GST whereas 33% lysate was used for the determination of lipid peroxidation activity of the erythrocyte membrane. The extent of lipid peroxidation was estimated as the concentration of thiobarbituric acid reactive products (malondialdehyde) by the method of Ohkawa *et al.* (1979). The activity of SOD and catalase was measured according to the method described by Marklund and Marklund, (1974) and Aebi *et al.* (1987), respectively.

The GPx and GST activity in erythrocyte lysate was assayed by the methods of Hafeman *et al.* (1974) and Habig *et al.* (1974) respectively. The blood glutathione (GSH) was determined by the method of Beutler *et al.* (1975). Hemoglobin (Hb) in the blood was determined by cyanmethemoglobin method (Gowenlock, 1996).

Statistical analysis

The data were expressed as mean ± SE. Statistical analyses were done by one-way ANOVA followed by Dunnet's test with $P \leq 0.05$ as a limit of significance.

Results and Discussion

Excess generation/accumulation of free radicals and ROS or impaired endogenous oxidant mechanisms are the important factors that have been implicated in various diseases like atherosclerosis, cancer, diabetes mellitus and myocardial infarction (Braughler and Hall 1989; Butterfield *et al.*, 2001). In present study different endogenous antioxidant parameters that help in assessing the antioxidant status of the rats are presented (Table 1; Figure 1a, 1b). In the present study significant ($p < 0.05$) increase in the catalase activity was after 20th day after whereas reduction in the activity of enzyme was observed ($p < 0.05$) after 30 days of dermal application. The activity of SOD increased up to 10 days, which appears to be an adaptive mode in the form of increased antioxidant defense. Various reports also suggest that the expression of Mn-SOD induced by agents that cause oxidative stress (Tsan *et al.*, 2001). However the levels of SOD in red blood cells of rats decreased after 20 and 30 days indicate decreased ability of the tissues to handle excessive free radicals generated (Andreyev *et al.*, 2005). Significant decrease in GSH level in the present study after a-cypermethrin application may indicate the inhibition of GSH synthesis and/or increased utilization of GSH for detoxification of toxicant induced free radicals. Similarly decrease in the GSH/GSSG ratio indicates that the conversion of GSSG to GSH has been severely affected (Singh *et al.*, 2001). Contrary to our findings acute oxidative stress has been reported to enhance glutathione levels and GPx activities, which also can be may be attributed to adaptive response of the tissues to the oxidant challenge resulting from exposure of cypermethrin in rats (Edes *et al.*, 1986; Chow and Tappel, 1972). GSTs are a major family

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Table 1. Effects of sub-acute dermal exposure of α-cypermethrin on different enzymes, blood glutathione and lipid peroxidation in Wister rats

Parameters	Units	No of days after dermal application			
		0	10	20	30
Catalase	μmole of H_2O_2 decomposed min^{-1} $mgHb^{-1}$	61.62 ±4.03[a]	197.86 ±16.61[b]	223.59 ±12.25[b]	131.44 ±9.41[c]
Superoxide Dismutase	units $mgHb^{-1}$	4.61 ±0.30[a]	8.55 ±0.37[c]	7.31 ±0.40[b]	7.39 ±0.24[b]
Glutathione Peroxidase	units $mgHb^{-1}$	7.59 ±0.25[c]	5.68 ±0.21[b]	1.85 ±0.30[a]	1.68 ±0.16[a]
Glutathione-S-transferase	μmole of conjugate of GSH-CDNB min^{-1} $mgHb^{-1}$	0.005 ±0.001[a]	0.006 ±0.002[a]	0.005 ±0.001[a]	0.006 ±0.003[a]
Blood glutathione	n moles ml^{-1}	110.85 ±8.53[c]	89.21 ±5.41[b]	75.72 ±3.98[b]	34.46 ±4.34[a]
Lipid peroxidation	n moles MDA produced gm Hb-1 hr^{-1}	1.41 ±0.084[a]	2. 83 ±0.168[b]	4.01 ±0.221[c]	4.04 ±0.296[c]

Values are expressed as mean ± S.E. of 6 animals
a, b, c Means with different superscript differ significantly at 5% (P = 0.05) level of significance

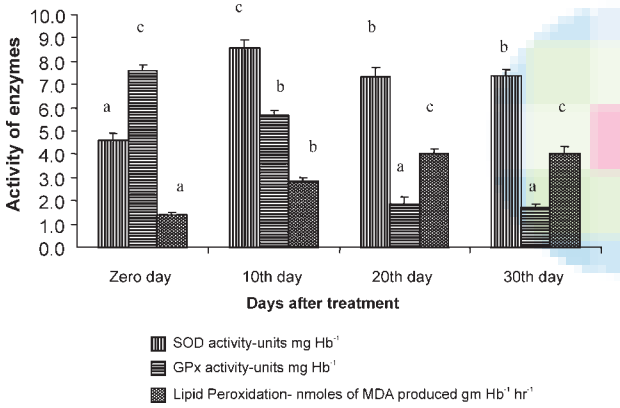


Fig 1a. Effect of a-cypermethrin on the activity of SOD, GPx, and lipid peroxidation after repeated dermal exposure at different time intervals

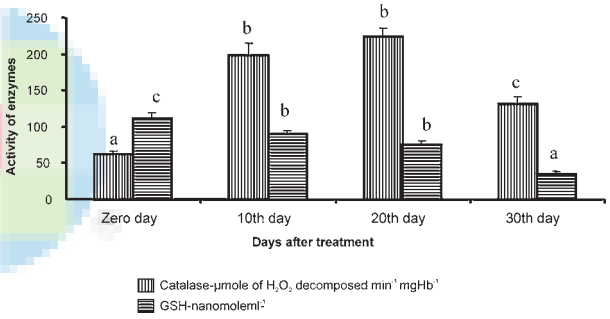


Fig 1b. Effect of a-cypermethrin on the activity of Catalase and blood glutathione (GSH) after repeated dermal exposure at different time intervals.

of detoxification enzymes in cells and catalyze the conjugation of the reduced glutathione to electrophilic centers of lipophilic compounds which further help in excretion of the toxicant from the cells and protect the tissues from oxidative stress (Hayes and Pulford, 1995). However, in present study activity of GST was not affected significantly by the exposure of toxicant.

The significant increase in SOD and catalase activity and decreased levels of GSH and GPx activity suggest that the dermal exposure increases the concentration of ROS and other free radicals. Excess free radicals attack the thiol group of cysteine residue and polyunsaturated fatty acid (PUFA) chain of phospholipids of biological membrane (Chow and Tappel, 1972). Free radical-induced lipid peroxidation serves to propagate and amplify oxidant-mediated damage resulting in deterioration of biological membranes (Sen *et al.*, 2006). Time dependent increase ($p<0.05$) in lipid peroxidation from day 10 onwards in present study suggests peroxidation of membrane lipids by the excess free radicals. Cypermethrin has been reported to cause lipid peroxidation in rats and such peroxidation is prevented by the supplementation of allopurinol and Vitamin E (Belma *et al.*, 2001). The enzymatic and non-enzymatic components of the

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cell suggest oxidative stress in Wister rats as a result of dermal exposure of α-cypermethrin.

References

Aebi, H (1983): Catalase, In: Methods Enzymol (Eds. Bergmeyer. H.U.) Vol.3, pp, 276-286); Academic Press, New York.

Andreyev, AY, Kushnareva, YE and Starkov, AA (2005): Mitochondrial metabolism of reactive oxygen species. *Biochem. (Mosc)*, **70:** 200-214.

Belma, G, Aylin, G and Filiz, H (2001): Cypermethrin-induced oxidative stress in rat brain and liver is prevented by Vitamin E or allopurinol. *Toxicol. Letters,* **118(3):** 139-146.

Beutler, E, (1975): Red cell metabolism. In: A manual of biochemical methods, New York: Grune Strottan, 67-69.

Braughler, JM and Hall, ED (1989): Central nervous system trauma and stroke. I. Biochemical considerations for free radical formation and lipid peroxidation. *Free Rad. Biol. Med.*, **6:** 289-301.

Butterfield, DA, Darke, J, Pocernich, C and Castegna, A (2001): Evidence of oxidative damage in Alzheimer's disease brain: Central role of Amyloid beta-peptide. *Trends Molecular Med.*, **7:** 548-554.

Chance, B, Sies, H and Boveris, A (1979): Hydroperoxide metabolism in mammalian organs. *Physiolog. Rev.*, **59:** 527-605.

Chow, CK and Tappel, AL (1972): An enzymatic protective mechanism against lipid peroxidation damage to lungs of ozone-exposed rats. Lipids, pp. 7518-7524.

Cross, CE (1987): The spectrum of diseases, in: Cross CE, moderator Oxygen radicals and human disease. *Ann. Intern. Med.*, 107:526-45.

Daniel, KL and Moser, VC (1993): Utility of a neurobehavioral screening battery for differentiating the effects of two pyrethroids, permethrin and cypermethrin. *Neurotoxicol. Teratol.*, **15:** 71-83.

Edes, I, Piros, G, Toth, GK, Csanady, M and Kovacs, K (1986): Effects of long term alcohol ingestion on glutathione metabolizing enzymes and taurine contents in the myocardium of rats. *Cardiovascular Res.*, **20:** 918-21.

Elliot, M and Janes, NF (1978): Synthetic pyrethroid a new class of insecticides. *Chem. Soc. Rev.*, **7:** 473-505.

Fridovich, I (1995): Superoxide radical and superoxide dismutases. *Annu. Rev. Biochem.*, **64:** 97-112.

Gowenlock. AH (1996): Varley's practical clinical biochemistry, 6th edition, New Delhi: CBS publishers 664-665.

Habig, WH, Pabst, MJ and Jakoby, WB (1974): Glutathione S-transferase. The first enzymatic step in mercapturic acid formation. *J. Biol. Chem.*, **249:** 7130-7139.

Hafeman DG, Sunde RA and Hoekstra WG (1974) Effect of dietary selenium on erythrocyte and liver glutathione peroxidase in the rat. *J. Nutr.*, **104:** 580-587.

Halliwell, B and Gutteridge, JMC (1985): Oxygen radicals and the nervous system. *Trends Neuroscience*, **8:** 22-26.

Halliwell, B and Gutteridge, JMC (1989): Free radicals in biology and medicine, 2nd edition, Clarendon press, Oxford. pp 188-276.

Hayes, JD and Pulford, DJ (1995): The glutathione S-transferase super gene family. Regulation of GST and the contribution of the isoenzymes to cancer chemoprotection and drug resistance. *Crit. Rev. Biochem. Mol. Biol.*, 30:445-600.

Kushnareva, Y, Murphy, AN and Andreyev, A (2002): Complex mediated reactive oxygen species generation: modulation by cytochrome c and NAD(P)+ oxidation-reduction state. *Biochem. J.*, **368:** 545-553.

Luty, S, Latuszynsika, J, Halliop, J, Tochman, A, Przylepa, OE and Korczak, E (1998) Toxicity of dermally applied alpha-cypermethrin in rats. *Ann. Agric. Environ. Med.* **5:** 109-115.

Marklund, S and Marklund, M (1974): Involvement of superoxide anion radical in autoxidation of pyrogallol and a convenient assay of superoxide dismutase. *European J. Biochem.*, **47:** 469-474.

Maxwell, SR (1995). Prospects for the use of antioxidant therapies. *449 Drugs*, **49:** 345-361.

Meister, A and Anderson, ME (1983): Glutathione. *Ann. Rev. Biochem.*, **545(52):** 711-760.

Mohammad, A (2002): Oxidative stress and experimental carcinogenesis. *Indian J. Exp. Biol.*, **40:** 656-667.

Ohkawa, H, Ohishi, N and Yagi, K (1979): Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal. Biochem.*, **95:** 351-358.

Punareewattana, K, Smith, BJ, Blaylock, BL, Longstreth, J, Snodgrass, HL, Gogal, RM, Prater, RM and Holladay, SD (2001): Topical permethrin exposure inhibits antibody production and macrophage function in C57Bl/6N mice. *Food and Chem. Toxicol.*, **39:** 133-139.

Radi, R, Turrens, JF, Chang, LY, Bush, KM, Crapo, JD and Freeman, BA (1991): Detection of catalase in rat heart mitochondria. *J. Biol. Chem.*, **266:** 22028–22034.

Sen, T, Sen, N, Tripathy, G, Chatterjee, U and Chakraborty, S (2006): Lipid peroxidation associated cardiolipin loss and membrane depolarization in rat brain mitochondria. *Neurochem. Int.*, **49:** 20-27.

Singh, SN, Vats, P, Kumria, MM, Ranganthan, S, Shyam, R, Arora, MP, Jain, CL and Sridharan, K (2001): Effect of high altitude (7620m) exposure on glutathione and related metabolism in rats. *European J. Physiol.*, **84:** 133-137.

Trojanowski, JQ (2003): Rotenone neurotoxicity: a new window on environmental causes of Parkinson's disease and related brain amyloidoses. *Exp. Neurol.*, **179:** 6-8.

Tsan, MF, Clark, RN, Goyert, SM and White, JE (2001): Induction of TNF-? and MnSOD by endotoxin: role of membrane CD14 and Toll-like receptor-4. *American J.Cell Physiol.*, **280:** C1422-1430.

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