Original Paper

Alteration in Protein Metabolic Profiles in Gastrocnemius Muscle Tissue of Rats during Cypermethrin Toxicosis

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ABSTRACT

Cypermethrin is the most widely used Type II pyrethroid pesticide in India because of its high efficacy against target species, and its reportedly low mammalian toxicity. It is a fast-acting neurotoxin and is known to cause free radical-mediated tissue damage.

This paper is an attempt at estimating its toxicity in rats at a molecular level.

Following exposure to oral, sublethal doses (41 mg/kg bw) of cypermethrin as single dose, double dose, and multiple dose with 48 h interval, the various profiles of protein metabolism were studied in different groups of rat muscle tissue. Total proteins showed decrement, whereas free amino acids, and the activity of protease, aspartate aminotransferase, alanine aminotransferase and glutamate dehydrogenase as well as ammonia significantly increased in cypermethrin-exposed rats. Urea content increase at all doses of exposure was not statistically significant. These effects on the protein metabolism of rats exposed to cypermethrin, which cause impairment of protein synthetic machinery, indicate its toxic effects on cellular functioning.

Key Words

Cypermethrin, Protein metabolism, Gastrocnemius, Rat

Introduction

Pesticides have made valuable contributions to human health by increasing food and fibre production, and by reducing the occurrence of vector-borne diseases.[1] An increase in global food demand has resulted in a significant increase in the use of pesticides in agriculture. This has caused great concern among health and environmental scientists, since some of these chemicals induce mutations (somatic as well as germ-line) in experimental systems.[2] In humans, exposure to pesticides has been associated with cancer.[3]

Synthetic pyrethroid pesticides account for over 30% of the global pesticide use.[4] Two distinct classes of pyrethroids have been identified based on different behavioural, neuropsychological, and biochemical profiles. Type I pyrethroids mainly cause hyper-excitation and fine tremors, while Type II pyrethroids possess a cyano-group and produce a more complex syndrome, including clonic seizures.[5] These compounds have gained popularity over organochlorine and organophosphate pesticides due to their high efficacy against target species,[6] their relatively low mammalian toxicity,[7] and rapid biodegradability.[8] Cypermethrin is a composite synthetic pyrethroid, a broad spectrum, biodegradable insecticide, and a fast-acting neurotoxin with good contact and stomach action. It is used to control many pests, including moths, and pests of cotton, fruit, and vegetable crops. Consistent with its lipophilic nature, cypermethrin has been found to accumulate in body fat, skin, liver, kidneys, adrenal glands, ovaries, and brain.[9]

The present study critically examines the magnitude and relationships of the metabolites and enzymes involved in

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the metabolism of proteins in muscle tissue of rats treated with sublethal doses of cypermethrin, since the farmers, pesticide applicators, industrial workers and other pesticide users will be exposed to the pesticides repeatedly.

Materials and Methods

Test Chemical

Technical grade cypermethrin (92% purity; cis:trans ratio 40:60) was obtained from Tagros Chemicals India Limited, Chennai.

Experimental Animals

About 40 adult, healthy, wistar strain albino rats (70±5 days, 175±10 g) were obtained from the Indian Institute of Science (Bangalore, India) breeding colony, and raised on a commercial pellet diet (Sai Durga Feeds and Foods, Bangalore, India), and water ad libitum. The animals were housed at constant temperature (28±2 ºC) and relative humidity (60±10%), with a 12 h light: 12 h dark cycle.

Experimental Design

The study design comprised four groups consisting of ten rats each. Toxicity evaluation was conducted by static bioassay method,[10] and the LD50 for 48 h value of cypermethrin to rats was found to be 205 mg/kg bw. 1/5 LD50 value (41 mg/kg bw) was selected as sublethal dose and administered as single, double, and multiple dose with one day interval in between. The first group of animals was treated as vehicle controls, and administered corn oil. To the second group of animals, single dose of cypermethrin (i.e., on 1st day) was administered orally (41 mg/kg bw). Double doses (82 mg/kg bw) were given with 48 h interval to the third group of animals on 1st and 3rd day. To the fourth group of animals, multiple doses (164 mg/kg bw) were given with 48 h interval i.e., on 1st, 3rd, 5th and 7th day. After 48 h, both control and experimental animals were sacrificed and gastrocnemius muscle tissues isolated and stored at -80 ºC for biochemical analysis.

Estimation of Organic Constituents

One percent homogenate of the muscle tissue was prepared in 0.25M ice-cold sucrose solution, using a motor-driven Teflon-coated pestle control homogenizer for the estimation of total proteins (TP) with Folin phenol reagent,[11] using bovine albumin serum as standard. This homogenate was precipitated with 10% trichloroacetic acid, and the protein-free supernatant was processed for free amino acids (FAA) estimation by the addition of ninhydrin reagent.[12] Tyrosine was used as standard.

Analysis of Nitrogenous end Products

Five percent homogenate of the tissues was prepared in distilled water for ammonia, and in 15% perchloric acid for urea. Levels of ammonia were deduced using ammonium chloride as standard, and urea by diacetyl monoxime method.[13]

Assay of Enzymes

Five percent homogenate of the tissues was prepared in 0.25M ice-cold sucrose solution for aspartate aminotransferase (AST), alanine aminotransferase (ALT) and glutamate dehydrogenase (GDH); in ice-cold distilled for protease, and these were centrifuged at 2500 rpm for 10 min in a refrigerated centrifuge at 4ºC to remove cell debris, and clear cell-free extracts were used as enzyme source. Protease activity was measured with the reaction mixture containing 100 µm of phosphate buffer (pH 7.0) and 12 mg of denatured protein.[12] AST (E.C.2.6.1.1) and ALT (E.C.2.6.1.2) activities were assayed following the method of Reitman & Frankel.[14] The incubation mixture for AST contained 100 µm of phosphate buffer (pH 7.4), 2 µm of ketoglutarate, and 50 µm of L-aspartic acid (pH 7.4). For ALT, incubation steps followed were the same as described for AST, except that the substrate used was D-alanine (50 µm). The standard graph was prepared with sodium pyruvate. GDH activity was measured by the method of Lee & Lardy.[15] Incubation mixture contained 100 µm of phosphate buffer (pH 7.4), 40 µm of sodium glutamate, 0.1 µm of NAD, 4 µm of 2,4-iodophenyl-3-(nitrophenyl)-5-phenyltetrazolium chloride (INT), and the enzyme source. This was incubated for 30 min at 37ºC, and stopped with 5.0 ml of glacial acetic acid. The colour was extracted by shaking with 5.0 ml of toluene. After keeping the tubes overnight at 4ºC, the colour extract was measured. All spectrophotometric measurements were determined using Hitachi U-2800 model spectrophotometer.

Evaluation of Results

An average of six individual estimations were taken after pooling them, and the mean values of control and experimental albino rats were subjected to statistical analysis using Duncan test for multiple comparison. The values were considered significant at 5% level.

Results

The results of protein metabolic profiles of the control and experimental rats under cypermethrin are mentioned in **Table 1**. The experimental rats exposed to cypermethrin showed statistically significant ($p<0.05$)

decrease of total protein content; whereas FAA, the activities of protease, ALT, AST and GDH and ammonia significantly ($p < 0.05$) increased. Urea content increase was not statistically significant. Alteration in protein metabolic profiles was in the form of a dose- and time-dependent manner in treated rats.

Discussion

Since proteins are involved in the architecture and physiology of the cell, they appear to occupy a key role in cell metabolism.[16] Catabolism of proteins and amino acids make a major contribution to the total energy production in rats. The depletion of total protein content observed in this investigation can be correlated with this fact. These results are in agreement with the earlier report of David et al (2004), [17] who demonstrated a similar situation in Cyprinus carpio exposed to cypermethrin. Bradbury et al (1987)[18] pointed out that the decreased protein content might also be attributed to the destruction or necrosis of cellular function, and consequent impairment in protein synthetic machinery. Protein depletion in tissues may constitute a physiological mechanism, and may play a role of compensatory mechanism under cypermethrin stress, to provide intermediates to the Krebs cycle. It

Table 1 Biochemical and Enzymological Changes in Gastrocnemius Muscle Tissue of Control and Cypermethrin-treated Rats

Gastrocnemius muscle	Control	Single dose	Double dose	Multiple dose
Total proteins (mg/g wet weight of the tissue)	127.9300^{d} ±1.2665	116.8417^{c} ±1.2647 (-8.67)	109.3192^{b} ±0.9687 (-14.55)	101.7072^{a} ±1.5333 (-20.50)
Free amino acids (μ moles of tyrosine/g wet weight of the tissue)	65.8195^{a} ±0.5282	69.6527^{b} ±0.4065 (5.82)	74.1390^{c} ±0.6072 (12.64)	79.4723^{d} ±0.5289 (20.74)
Protease (μ moles of tyrosine /mg protein/h)	0.5297^{a} ±0.0699	0.5535^{a} ±0.0387 (4.49)	0.6008^{a} ±0.0389 (13.42)	0.6718^{b} ±0.0697 (26.83)
AST (μ moles of pyruvate /mg protein/h)	0.4917^{a} ±0.0058	0.5287^{b} ±0.0070 (7.52)	0.5663^{c} ±0.0050 (15.17)	0.6097^{d} ±0.0209 (24.00)
ALT (μ moles of pyruvate /mg protein/h)	0.7758^{a} ±0.0102	0.8170^{b} ±0.0109 (5.31)	0.8770^{c} ±0.0152 (13.04)	0.9505^{d} ±0.0114 (22.52)
GDH (μ moles of formazone /mg protein/h)	0.2463^{a} ±0.0098 (11.73)	0.2752^{b} ±0.0111 (24.73)	0.3072^{c} ±0.0122 (41.17)	0.3477^{d} ±0.0100
Ammonia (μ moles of ammonia /g wet weight of the tissue)	7.8710a ±0.1094	8.4483b ±0.1036 (7.33)	8.8932c ±0.0959 (12.99)	9.3965d ±0.1172 (19.38)
Urea (μ moles of urea/g wet weight of the tissue)	0.1232^{a} ±0.0390	0.1335^{a} ±0.0464 (8.36)	0.1437^{a} ±0.0318 (16.64)	0.1540^{a} ±0.0337 (25.00)

Values are means ± SD (n=6). Values with different superscripts are significantly different ($p<0.05$), and those without, are not significantly different, as determined by Duncan's Multiple Range test. Values in parentheses indicate percent change over control.

has also been reported that this trend of proteins may be to enhance osmolality, to compensate for osmoregulatory problems encountered due to the leakage of ions and other essential molecules during pyrethroid toxicity.[19] The increase in protease activity observed at different doses of cypermethrin was clearly reflected in the breakdown of proteins. Under proteolysis, enhanced breakdown dominates over synthesis, while in the case of anabolic processes, increased synthesis dominates the protein breakdown.[16] Moreover, histopathological damage and hydromineral imbalance during pesticide stress has been reported to account for the elevated protease activity.[20]

Enhanced protease activity and decreased protein level resulted in a marked elevation of FAA content in the muscle tissues at different doses of cypermethrin-exposed rats. Presumably, the degradation of proteins led to FAA accumulation. This higher level of FAA can also be attributed to the decreased utilization of amino acids, and is also suggestive of its involvement in the maintenance of osmotic and acid base balance.[20]

The elevation of AST and ALT activities observed in this study offers an excellent corroboration of the above trend. This is a clear indication of shunting of amino acids into TCA cycle through oxidative deamination and active transamination. Such a phenomenon is necessary to cope up with the energy crisis during pyrethroid stress. It has also been suggested that stress conditions in general induce elevation in the transamination pathway.[21] Involvement of alternate pathways such as aminotransferase reactions are also possible due to inhibition of oxidative enzymes like succinate dehydrogenase, malate dehydrogenase, isocitrate dehydrogenase and cytochrome-c-oxidase, a situation also demonstrated by Ghosh (1989)[22] in *Labeo rohita* under cypermethrin toxicity.

The elevation observed in the GDH activity indicates its contribution to enhanced ammonia levels and glutamate oxidation during cypermethrin toxicity. Increased free amino acid levels and their subsequent transamination results in greater production of glutamate, thus increasing the intracellular availability of substrate glutamate, for consequent oxidative deamination reaction through GDH. Besides, the elevation in transaminases and GDH helps in supplying keto acids to the TCA cycle, in order to compensate for the energy crisis in muscle tissues during cypermethrin toxicity.

In the present study, ammonia content increased in muscle tissues of rats exposed to sublethal doses of cypermethrin. Elevated activities of proteases, transaminase reactions, and increased deamination reaction (GDH) support the augmented ammonia levels during cypermethrin toxicosis. The enhanced ammonia levels in muscle tissues of cypermethrin-treated rats may lead to ammonotoxaemia, and shows deleterious effects on the animal metabolism. Though ammonia is essential for the synthesis of important compounds such as purines, pyrimidines, and non-essential amino acids, and also functions as a key factor in acid-base regulation, it is toxic in non-physiological concentrations, and excess ammonia therefore has to be disposed off.[16] Ammonia, a toxic nitrogenous end product, is released exogenously into the digestive tract, and endogenously into the tissues through catabolism of amino acids, pyrimidines, and purines.[23] Ammonia cannot be stored for long periods of time in the body as it leads to endogenous ammonoto-xicity. The reduction in ammonia content suggests that the ammonia might have been converted into non-toxic compounds, glutamine and urea in cypermethrin-exposed Cyprinus carpio.[17]

The presence of urea in gastrocnemius muscle tissue might be due to the vascular mobilization and translocation from liver. The elevation in urea levels is in consonance with increased proteolytic activity, enhanced transamination, and elevated ammonia levels during cypermethrin toxicosis. Thus, an increase in urea enunciates the role being played by muscle tissues of exposed rats in the elevation of ammonia toxicity, besides their pivotal role in replenishing the protein nitrogen to synthesize useful precursors for the maintenance of homeostasis and dynamic equilibrium. Increased levels of urea under cypermethrin stress reveal that the rats might have adapted to the biosynthesis of urea as a major pathway of detoxification of ammonia. Probably this pathway may be beneficial to animals in detoxification and physiological compensation, or adjustment to various exogenous and endogenous toxicants.

David et al. (2004)[17] have reported that decreased Na^+, K^+ and Ca^{2+} ions in the tissues of *Cyprinus carpio* under cypermethrin exposure might also account for the elevation in urea and glutamine. Sambasiva Rao (1983)[24] demonstrated that both urea and glutamine compensate for the loss of osmolarity of the internal milieu under stress conditions. The status of protein metabolic profiles changes in muscle tissues in the present study corrobo-

rates the findings of Begum et al (2007).[25] At repeated dose levels of cypermethrin administered orally, a damaging effect on the cell metabolism occurs, thereby leading to impaired protein synthetic machinery.

In conclusion, it can be stated that long term exposure to sublethal doses of pyrethroid pesticides can result in cell metabolism toxicosis.

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REFERENCES

1. Blindauer KM, Jackson RJ, McGeehin M, Pertowski C, Rubin C. Environmental pesticide illness and injury. The need for a national surveillance system. Environ Health 1999; 9-13.
2. Meng Q, Singh N, Heflich RH, Bauer MJ, Walker VE. Comparison of the mutations at Hprt exon 3 of T-lymphocytes from B6C3F1 mice and F344 rats exposed by inhalation to 1,3-butadiene or the racemic mixture of 1,2:3,4-diepoxybutane. Mutation Research 2000; 464:169-184.
3. Dich J, Zahm SH, Hanberg A, Adami H. Pesticides and cancer. Cancer Causes Control 1997; 8: 420-443.
4. Eisler R. Fenvalerate hazards to fish, wildlife and invertebrates: A synoptic review. In: Report 24, Contaminant Hazards Reviews, US Department of the Interior, Fish and Wildlife Service, Washington, DC. 1992; p3.
5. Verschoyle RD, Aldridge WN. Structure-activity relationships of some pyrethroids in rats. Arch Toxicol 1980; 45: 325-329.
6. Eillot M, Janes NF, Potter C. The future of pyrethroids in insect control. Ann Rev Entomol 1978; 23: 744-769.
7. Parker CM, Patterson DR, Van Gelder GA, Gordon EB, Valerio MG, Hall WC. Chronic toxicity and carcinogenicity evaluation of fenvalerate in rats. J Toxicol Environ Health 1984; 13: 83-97.
8. Leahey JP. Metabolism and environmental degradation. In: Leahey JP (ed). The Pyrethroid Insecticides. 1985. Taylor and Francis, London. 133-224.
9. Hall BE, Vickers JA, Hopkins JA. A study to determine the bioaccumulation of 14C-cypermethrin radioactivity in the rat following repeated oral administration. Report No. 2487-72/20. WHO, Geneva. 1980.
10. Finney DJ. Probit Analysis. 3rd edn, 1971. Cambridge University Press, London.
11. Lowry OH, Rosenbrough NJ, Farr AL, Randall RS. Protein measurement with folin-phenol reagent. J Biol Chem 1951; 193: 265-273.
12. Moore S, Stein WH. A modified ninhydrin reagent for the photometric determination of amino acids and related compounds. J Biol Chem 1954; 221: 907-913.
13. Natelson S. In: Charless G (ed). Techniques of Clinical Chemistry. 1971. Spring Field III Illinois, USA.
14. Reitman S, Frankel S. A colorimetric method for the determination of serum glutamic oxaloacetate and glutamic pyruvic transaminases. Amer J Clin Pathol 1957; 28: 56-63.
15. Lee YL, Lardy AA. Influence of thyroid hormones on L-glycerophosphate dehydrogenases in various organs of the rat. J Biol Chem. 1965; 240: 1427-1430.
16. Murray RK, Granner DK, Mayes PA, Rodwell VW. In: Harper's Illustrated Biochemistry. 26th edn, 2007. McGraw-Hill, USA
17. David M, Mushigeri SB, Shivakumar R, Philip GH. Response of *Cyprinus carpio* (Linn) to sublethal concentration of cypermethrin: Alterations in protein metabolic profiles. Chemosphere 2004; 56: 347-352.
18. Bradbury SP, Mc Kim JM, Coasts JR. Physiological responses of rainbow trout, *Salmo gairdneri* to acute fenvalerate intoxication. Pesticides Biochem Physio 1987; 27: 275-288.
19. Rafat Yasmees M. Physiological responses of freshwater fish, *Anabas scandens* to the toxicity of endosulfan. PhD Thesis, 1986. Osmania University, Hyderabad, India.
20. Moorthy KS, Moorthy B, Kasi Reddy B, Swami SK, Chetty CS. Changes in respiration and ionic content in tissues of freshwater mussel exposed to methylparathion toxicity. Toxicol Letters 1984; 21: 287-291.
21. Awasthi M, Shaw P, Dubale MS, Gadhia P. Metabolic changes induced by organophosphates in the piscine organs. Environ Res 1984; 35: 320-325.
22. Ghosh TK. Influence of cypermethrin on the oxidative metabolism of fish *Labeo rohita*. Proc Indian Acad Bull 1989; 55: 115-120.
23. Lowenstein JM, Goodman MN. The purine nucleotide cycle in skeletal muscle. Fed Proc 1978; 37: 2308-2312.
24. Sambasiva Rao KRS. Impact of technical and commercial grade phenthoate on some selected parameters of oxidative metabolism in fish, *Channa punctatus*. J Environ Biol 1983; 8: 173-177.
25. Begum G. Cypermethrin-induced biochemical perturbations in freshwater fish *Clarias batrachus* at sublethal exposure and after release into freshwater. Drug Chem Toxicol 2007; 30: 55-65.