AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

Determination of Enzyme Rate Constant Following Cypermethrin Administration in Male Albino Rat

V. Muthuviveganandavel[1], Hemalatha M[2] Muthuraman Pandurangan[3]

1- Dept of Zoology, Tagoore Arts College, Puducherry, India.

2- Dept of BIN Fusion Technology, Chonbuk National University, Republic of Korea.

3-Dept. of Animal Science, Chonbuk National University, Jeonju, Republic of Korea.

ABSTRACT

Cypermethrin is synthetic pesticide that has been in use for more than a decade. A study of a low-dose exposure of this pesticide in rat administered, followed by determination of enzyme rate constant will highlight early catalytic changes that generally accompanied a toxic response in the animal. Even though, cypermethrin was used at 5 mM, for durations of 6, 12 and 24 h in the different groups of rats, results of the study indicate that simultaneous with changes in enzyme activity and rate constant of selected enzymes in selected tissues and serum. Tissue specific changes in the enzyme rate constant were suggestive of the lack of tissue resistance to pyrethroids.

Key words: Cypermethrin; ALT; AST; ALP; ACP; GGT

*Corresponding Author Email: frenzram80@gmail.com

Received 18 May 2012, Accepted 20 June 2012

Please cite this article in press as: Muthuviveganandavel V *et al.*, Determination of Enzyme Rate Constant Following Cypermethrin Administration in Male Albino Rat. American Journal of PharmTech Research 2012.

INTRODUCTION

A-cypermethrin is known to undergo metabolism through the cytochrome P^{450} microsomal system resulting in oxidative stress. The acute LD_{50} value for α-cypermethrin in DMSO is reported to be 145 mg/kg bwt. It was considered that the increase in protein content could be due to an increase in the rate of translation of protein. Studies involving serum proteins have noted decreased level of total protein in serum of young rabbit due to cypermethrin toxicity [1] there is also claim that the cypermethrin had no significant effect on the total protein content[2.]

The cypermethrin induced increase in specific activity of brain aspartate transaminase (AST) is indicative of an augmented process of oxaloacetate formation from aspartate. Increase in alanine transminase (ALT) and AST in rats treated with 520, 560, 600 mg/kg benomyl for seven days [3.] The low serum AST specific activity can be considered due to increased protein content in the serum as enhanced protein content was noted during the study using cypermethrin. It is well known the alkaline phosphatase (ALP) activity dephosphorylated, phosphorylated organic substrates in the animal tissues. ALP is a hydrolase and a transphosphorylase in function associated with cell membranes (Onikienko, 1963). Increase in the specific activity of this enzyme therefore suggested the existence of a greater depohsphorylation potential within the animal cell.

Cypermethrin was found to be ineffective initially in the various tissues with regard to ALP specific activity. Decrease in ALP activity was taken as an index for parenchymal damage [4]. Gamma-glutamyl transaminase (GGT) catalyzed the transfer of a glutamyl moiety between peptide donors and amino acid/peptide acceptors [5]. GGT was also involved in the transfer of amino acid across the cell membrane, Further, GGT had a role in glutathione metabolism transferring the glutamyl moiety to various acceptor molecule including water, L-amino acids and peptides. Such a process results in the retention of the cysteinyl glycine that was considered to preserve intracellular homeostasis during oxidative stress. GGT is an enzyme activity that is also implicated as a lymphoid cell surface marker and in blastogenesis and differentiation. It is also implicated in the synthesis of glucotrine D'4. Shukla et al [6] were reported that the increase in serum GGT level of rat was determined.

Several inferences could be derived based on these observations. Where GGT specific activity was found increased, all GGT related molecular processes would be enhanced in the rat, as well as in their resulting metabolic consequences. It is possible that even though the rate of transpeptidation would become enhanced, the more significant aspect of increased GGT activity

would be borne by the leukotriene metabolism. Systemic changes due to this however remain uninvestigated and are beyond the scope of this work. However, since the time dependent observations using the pesticides were limited to 24 h. The current study is focused on the effect of cypermethrin on selected enzyme rate constant at different time intervals following administration.

MATERIALS AND METHODS

Materials

Alfa-cypermethrin (95.6% pure) was a gift from Gharda Chemicals Ltd., Mumbai, India. All chemicals used in this study were of analytical grade. Double distilled water was used for the preparation of all reagents. Male Wistar strain-albino rats weighing 150–200 g were used for the investigation. The animals were housed under controlled temperature and hygiene conditions with 12 h of light and dark cycle throughout the experimental period. All the procedure dealing treatment and sacrifice of animal was carried out according to international ethical committee regulations. Commercial rat chow with free access to drinking water *ad libitum* was provided for the animals.

Methods

The experimental approach aimed to investigate the effect of the insecticide α-cypermentrhin on the enzyme rate constant in selected tissues and serum of male albino wistar strain rats. Tissues such as the brain, heart, liver, kidney and testis were selected due to each of their metabolic importances. Control and treated groups of rats contained six animals each. Dose level of compound used was 5 mM. The time intervals selected for investigation were 6, 12 and 24 h following administration of cypermethrin. Blood was collected by cardiac puncture into a tube and serum was prepared.

Preparation of tissue homogenate as crude enzyme source

Specific tissues of the rat were surgically removed, immediately rinsed in ice cold 1.15% KCL solution, pre-cut into small pieces and taken for homogenization employing several strokes in a potter-Elvejham homogenizer using a Teflon pestle, in the appropriate buffer, to obtain 10% (w/v) tissue homogenate. Throughout the homogenization process, the tissue homogenates were maintained on crushed ice in an ice bucket. The tissue homogenates were then centrifuged in a refrigerated high-speed centrifuged at $4^{o}C$ and at 10,000 x g for 20 minutes. The clear supernatant obtained from each tissue homogenate was used an enzyme source for the investigations. ALT, AST, ALP, ACP (acid phosphatase), GGT and Hexokinase enzyme activity

were measured by standard methods [7, 8, 9, 10, 11]. The rate constant of these enzymes were calculated by standard method. Protein concentration was estimated by the method of Lowry [12] with BSA as the standard protein.

Statistical analysis

All values expressed were as mean ± SEM. Statistical analysis was done using SPSS 14 program. The statistical significance of differences between the two means was assessed by one way ANOVA. P values < 0.05 were considered to be significant.

RESULT AND DISCUSSION

Determination of each rate constant as a ratio of molar concentration of product to substrate yielded results in the millimolar range for ALT and AST catalytic activities whereas for ALP, ACP, GGT and HK, the rate constant, were in the micromolar range both for control as well as in the treated tissue samples. An order of magnitude difference was observed between the former and the latter types of catalytic rates. The reported values were mean of three independent determinations **(Table 1-6).**

ALT enzyme rate constant increased significantly at 6 h in liver ($p<0.05$), increased significantly at 12 h in brain, liver and kidney ($p<0.05$, $p<0.01$) and increased significantly at 24 h in brain, liver, kidney and testis ($p<0.05$, $p<0.01$) following 5 mM of cypermethrin administration (Table 1). AST enzyme rate constant increased significantly at 6 hr in brain, heart and testis ($p<0.01$, $p<0.001$), increased significantly at 12 h in brain and kidney whereas reduced in testis ($p<0.05$, $p<0.001$). At 24 h, AST enzyme rate constant increased in heart, testis and serum ($p<0.05$) following 5 mM of cypermethrin administration (Table 2).

GGT enzyme rate constant significantly reduced in brain, heart, kidney and serum ($p<0.05$, $p<0.001$) whereas increased in liver at 6 h following 5 mM of cypermethrin administration. GGT enzyme rate constant significantly elevated in brain, heart, kidney and serum at 12 h ($p<0.05$, $p<0.001$) whereas reduced in testis. GGT enzyme rate constant significantly increased in heart, liver, kidney and serum at 24 h following dose administration ($p<0.01$, $p<0.001$) in male albino rats (Table 3). ALP enzyme rate constant reduced in brain, heart, testis and serum ($p<0.05$, $p<0.001$) at 6 h and increased in brain, liver and kidney at 12 h following cypermethrin administration. GGT enzyme rate constant significantly increased in brain and reduced in heart, liver and kidney ($p<0.05$, $p<0.01$, $p<0.001$) at 24 h following cypermethrin administration (Table 4).

Table 1: Changes in rat ALT (x 10^{-2} mM/min) rate constant (k) at 6, 12 and 24 hr in brain, heart, liver, kidney, testis and serum following 5 mM alpha-cypermethrin administration in male albino rat.

Sample	6 h	12 h	24 h
Brain control	25.52 ± 2.1	36.44 ± 2.7	32.45 ± 1.8
Brain treated	22.27 ± 1.7	50.43 ± 3.1**	42.33 ± 2.1*
Heart control	36.69 ± 2.4	31.74 ± 1.5	28.83 ± 1.2
Heart treated	29.39 ± 2.0	33.74 ± 2.1	31.96 ± 2.3
Liver control	0.71 ± 0.02	1.02 ± 0.03	0.74 ± 0.03
Liver treated	0.93 ± 0.03*	1.17 ± 0.01*	0.88 ± 0.02*
Kidney control	23.80 ± 1.2	32.82 ± 2.3	18.40 ± 0.9
Kidney treated	25.03 ± 1.5	49.08 ± 3.3**	36.26 ± 2.7**
Testis control	27.48 ± 1.6	36.81 ± 2.4	23.80 ± 1.3
Testis treated	25.34 ± 1.1	30.06 ± 1.9	7.12 ± 0.08**
Serum control	0.94 ± 0.03	2.15 ± 0.04	1.00 ± 0.07
Serum treated	0.97 ± 0. 01	2.15 ± 0.02	1.18 ± 0.04

*p<0.05 and **p<0.01

Table 2: Changes in rat AST (x 10^{-2} mM/min) rate constant (k) at 6, 12 and 24 hr in brain, heart, liver, kidney, testis and serum following 5 mM alpha-cypermethrin administration in male albino rat.

Sample	6 h	12 h	24 h
Brain control	7.55 ± 0.09	7.18 ± 0.13	9.08 ± 0.31
Brain treated	18.77 ± 0.12***	17.24 ± 1.1***	8.83 ± 0.22
Heart control	6.46 ± 0.08	5.05 ± 0.14	2.06 ± 0.15
Heart treated	3.31 ± 0.05**	5.64 ± 0.27	2.61 ± 0.18*
Liver control	3.95 ± 0.07	3.44 ± 0.07	4.63 ± 0.23
Liver treated	3.81 ± 0.05	3.26 ± 0.06*	4.56 ± 0.27
Kidney control	4.61 ± 0.08	4.10 ± 0.12	4.37 ± 0.25
Kidney treated	4.99 ± 0.12	6.39 ± 0.19*	4.44 ± 0.22
Testis control	105.40 ± 3.8	107.85 ± 6.1	74.48 ± 3.6
Testis treated	73.44 ± 2.4**	77.06 ± 3.7*	58.22 ± 3.3*
Serum control	2.07 ± 0.03	3.19 ± 0.21	2.14 ± 0. 19
Serum treated	2.79 ± 0.05	3.46 ± 0.13	2.71 ± 0.11*

*p<0.05, **p<0.01 and ***p<0.001

Table 3: Changes in rat GGT (x 10^{-4} µM/min) rate constant (k) at 6, 12 and 24 hr in brain, heart, liver, kidney, testis and serum following 5 mM alpha-cypermethrin administration in male albino rat.

Sample	6hr	12hr	24hr
Brain control	12.38 ±0.52	9.80 ± 0.6	9.16 ± 0.42
Brain treated	7.14 ± 0.08*	12.32 ± 0.7*	9.80 ± 0.45
Heart control	7.00 ± 0.07	7.28 ± 0.32	4.48 ± 0.091
Heart treated	5.83 ± 0.08*	10.08 ±0.56*	33.33 ± 1.3***
Liver control	1.12 ± 0.01	1.68 ± 0.07	1.62 ± 0.009

Liver treated	1.40 ± 0.03*	1.90 ± 0.09	4.40 ± 0.03***
Kidney control	235.29 ± 8.5	462.18 ± 12.7	336.13 ± 10.2
Kidney treated	369.75 ± 7.4***	757.51 ± 14.1***	845.94 ± 16.2***
Testis control	49.02 ± 2.3	81.23 ± 3.5	68.35 ± 2.6
Testis treated	45.10 ± 3.1	63.03 ± 2.6*	62.75 ± 3.4
Serum control	0.39 ± 0.007	0.45 ± 0.006	0.37 ± 0.005
Serum treated	0.20 ±0.009*	0.60 ± 0.007*	0.52 ± 0.002**

*p<0.05, **p<0.01 and ***p<0.001

Table 4: Changes in rat ALP (x 10^{-3} µM/min) rate constant (k) at 6, 12 and 24 hr in brain, heart, liver, kidney, testis and serum following 5 mM alpha-cypermethrin administration in male albino rat.

Sample	6 h	12 h	24 h
Brain control	4.76 ± 0.12	2.93 ± 0.03	2.80 ± 0.05
Brain treated	3.19 ± 0.15*	3.21 ± 0.04*	3.27 ± 0.07*
Heart control	7.61 ± 0.22	5.86 ± 0.04	6.78 ± 0.04
Heart treated	3.56 ± 0.11***	5.52 ± 0.07	3.97 ± 0.05***
Liver control	1.40 ± 0.04	1.14 ± 0.03	3.02 ± 0.03
Liver treated	1.17 ± 0. 02	0.88 ± 0.009*	1.86 ± 0.008**
Kidney control	6.22 ± 0.15	6.30 ± 0.06	3.21 ± 0.03
Kidney treated	6.93 ± 0.13	8.99 ± 0.07**	2.45 ± 0.03*
Testis control	23.10 ± 1.2	24.89 ± 1.2	12.68 ± 0.06
Testis treated	19.14 ± 0.92*	22.98 ± 1.5	12.38 ± 0.05
Serum control	0.74 ± 0. 01	1.13 ± 0.08	0.82 ± 0. 02
Serum treated	0.49 ± 0. 02*	1.23 ± 0.06	0.97 ± 0.01

*p<0.05, **p<0.01 and ***p<0.001

Table 5: Changes in rat ACP (x 10^{-3} µM/min) rate constant (k) at 6, 12 and 24 hr in brain, heart, liver, kidney, testis and serum following 5 mM alpha-cypermethrin administration in male albino rat.

Sample	6 h	12 h	24 h
Brain control	3.54 ± 0.05	4.85 ± 0.1	5.84 ±
Brain treated	6.28 ± 0.12***	10.56 ± 0.14***	9.01 ± 0.11**
Heart control	3.77 ± 0.19	2.92 ± 0.04	3.98 ± 0.06
Heart treated	3.65 ± 0.13	11.22 ± 0.11***	4.58 ± 0.07*
Liver control	7.19 ± 0.15	6.26 ± 0.09	6.38 ± 0.05
Liver treated	10.73 ± 0.21*	11.19 ± 0.13***	2.38 ± 0.06***
Kidney control	4.47 ± 0. 22	5.02 ± 0.05	6.47 ± 0.09
Kidney treated	13.66 ± 0.35***	15.42 ± 0.08***	4.25 ± 0.04*
Testis control	7.51 ± 0.27	7.83 ± 0.09	8.36 ± 0.06
Testis treated	4.55 ± 0. 16**	5.16 ± 0.06*	4.70 ± 0.08**
Serum control	2.1 ± 0.07	2.8 ± 0.05	3.3 ± 0.03
Serum treated	3.4 ± 0.06*	3.3 ± 0.04*	3.9 ± 0.02

*p<0.05, **p<0.01 and ***p<0.001

Table 6: Changes in rat HK (x 10^{-4} µM/min) rate constant (k) at 6, 12 and 24 hr in brain, heart, liver, kidney, testis and serum following 5 mM alpha-cypermethrin administration in male albino rat.

Sample	6 h	12 h	24 h
Brain control	3.81 ± 0.07	4.32 ± 0.06	4.03 ± 0.07
Brain treated	9.82 ±0. 21***	11.68 ± 0.13***	9.18 ± 0.14***
Heart control	2.28 ± 0.04	2.96 ± 0.02	2.51 ± 0.03
Heart treated	3.58 ± 0.05*	4.58 ± 0.08*	4.03 ± 0.06**
Liver control	4.99 ± 0.09	6.24 ± 0.13	5.82 ± 0.08
Liver treated	9.76 ± 0.15***	11.10 ± 0.17***	9.47 ± 0.12**
Kidney control	3.87 ± 0.08	4.67 ± 0.05	4.10 ± 0.05
Kidney treated	8.26 ± 0.17***	9.79 ± 0.13***	8.70 ± 0.14***
Testis control	4.90 ± 0.06	5.28 ± 0.07	4.74 ± 0.06
Testis treated	6.34 ± 0.24**	7.52 ± 0.11*	6.37 ± 0.11*
Serum control	11.3 ± 0.14	11.7 ± 0.22	11.1 ± 0.21
Serum treated	13.5 ± 0.19*	14.4 ± 0.16*	15.5 ± 0.22*

*p<0.05, **p<0.01 and ***p<0.001

ACP enzyme rate constant increased significantly in brain, liver, kidney and serum ($p<0.05$, $p<0.001$) whereas reduced in testis at 6 h following dose administration. ACP enzyme rate constant increased significantly increased in brain, heart, liver, kidney and serum ($p<0.05$, $p<0.001$) whereas reduced in testis at 12 h and increased in brain, heart whereas reduced in liver, kidney and testis ($p<0.01$, $p<0.01$, $p<0.001$) at 24 h following 5 mM cypermehtrin administration (Table 5). Hexokinase enzyme rate constant increased significantly in brain, heart, liver, kidney, testis and serum ($p<0.05$, $p<0.01$, $p<0.001$) at 6 h following dose administration. Hexokinase enzyme rate constant increased in brain, heart, liver kidney, testis and serum ($p<0.05$, $p<0.01$, $p<0.001$) at 12 and 24 h following cypermethrin administration in male albino rats (Table 6).

The order of magnitude difference in the enzyme rate constants suggested that transamination process occurred on a quantitatively greater scale than of dephosphorylation, transpeptidation or even phosphorylation. Just as much as differences in enzyme activities and enzyme specific activities were noted between control and α-cypermethrin (5 mM) treated samples, the enzyme rate constant between control and pesticide treated rat tissues samples (Table 1-6) registered significant differences in several instances. Since 5mM α-cypermethrin displayed the observed variations in rate constant of each catalytic activity studied of the K value for other concentration of α-cypermethrin or carbendazim is not reported. This was indicative of the fact that catalytic processes within the animal cell were significantly influenced through elevation or depression of specific catalytic activities, and consequently the metabolic pathways under their regulatory control. Such perturbation of cellular metabolism resulted in inimical cellular homeostasis,

causing degenerative disorders as a cumulative effect. It is also noticeable that though considerable difference between control and treated enzyme activities were recognized at 6 h, such differences tend to multiply over 24 h duration suggestive of active detoxifying mechanism at play within the host tissue. However, initiation of tissue destruction has been noted that seem to establish an irreversible phenotypical change in the cells of the various and in specific locations. Tissue specific change in the rate constant of the marker enzymes was suggestive of the lack of tissue resistance to pyrethroids. The 5 mM of cypermethrin administration produced significant changes at different time intervals on selected enzyme rate constant.

CONCLUSION

Experimental results indicated that simultaneous with changes in enzyme activity and rate constant of selected enzymes in selected tissues and serum. Tissue specific changes in the enzyme rate constant were suggestive of the lack of tissue resistance to pyrethroids.

REFERENCES

1. Lakkawar AW, Chattopadhyay SK, Somvanshi R, Sharma AK. Experimental cypermethrin toxicity in young rabbits-a hematological and biochemical study. Praxis veterinaria 2006; 54 (3):195-202.
2. Aldana L, Tsutsumi V, Carigmill A, Silveria MI, De Mejia EJ. Tecopherol modulates liver toxicity of the pyrethroid cypermethrin. Toxicol. Lett 2001; 125:107-116.
3. Selmanoglu G, Barlas N, Songur S, KocSkaya EA. Carbendazim induced haematological, biochemical and histopathological changes to the liver and kidney of male rats. Hum. Exp. Toxicol 2001; 20: 625-630.
4. Onikienko EA. Enzymatic changes from early stages of intoxication with small doses of chloroorganic insecticides. Gigienari. Fiziol. Truda. Taksikol. Klinikackiev Gos. IZ. Med. Git. Ukr. USSR 1963; 77.
5. Meister A, Tate SS, Ross LL. Membrane Bound Gamma Glutamyl Transpeptidase: In Martonosi, a (Ed). The Enzyme of Biological Membranes. Plenum Press., New York. 1973; 315-347.
6. Shukla Y, Antonym M, Mehrota NK. Studies on γ-Glutamyl transpeptidase in rodents exposed to benomyl. Bull. Environ. Contam. Toxicol 1989; 42:301-306.
7. Reitman S, Frenkel S. A Colorimetric method for the determination of serum glutamic oxaloacetate and glutamic pyruvic transaminases. Am J Clin Path 1957; 28:5663.

8. Bessey OA, Lowry OH, Brock J. A Method for the determination of alkaline phosphatase. J. Biol. Chem 1946; 164:321-329.
9. Volohonsky G, Tuby CNYH, Porat N. A Spectrophotometric assay of gamma-glutamylcysteine synthetase and glutathione synthetase in crude extracts from tissues and cultured mammalian cells. Chem. Biol. Interact 2002; 140:49-65.
10. Tennis Wood M, Biri CE, Clark AF. Acid phosphatase androgen dependant markers of rat prostate. Can. J. Biochem 1976; 54:350.
11. Daniel MC, Moser VC. Utility of a neurobehavioral screening buttery for differentiating the effects of two pyrethroids, permethrin and cypermethrin. Neurotoxicol. Teratol 1993; 15:71-83.
12. Lowry OH, Rosebrough NJ, Farr AL. Protein measurement with Folin phenol reagent. J. Biol. Chem 1951; 193:265-275.