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Kinetics of Distribution of Cypermethrin in Blood, Brain, and Spinal Cord after a Single Administration to Rabbits

R. N. Khanna, G. S. D. Gupta, M. Anand

Industrial Toxicology Research Centre, Post Box Number 80, M. G. Marg, Lucknow 226 001, India

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The synthetic pyrethroids constitute an unique group of insecticides having pyrethrin-like structures with better performance characteristics and account for over 30% of insecticide use globally (Vijverberg and Bercken 1982; Soderlund and Bloomquist 1989; Giray et al. 2001). The pyrethroids are potent neurotoxicants in both vertebrates and invertebrates, but acute toxicity in mammals is low (Elliot and Janes 1978; Vijverberg and Bercken, 1990; Gupta et al, 1999). Cypermethrin is one of the pyrethroid α-cyano-3-phenoxy benzyl esters which combine high insecticidal activity with a degree of photostability suitable for use in cotton, vegetables and other crops and is also used in veterinary products (Elliott 1976). Although the mechanism of toxicity of cypermethrin is not completely classified it is regarded as nerve poison (Narahashi, 1985, 1996). It has been shown to cause neurotoxicity in experimental animals characterized by ataxia, excessive salivation, choreoathetosis, tremors and clonic convulsions (Eadsforth and Baldwin 1983; WHO 1992; Desi et al. 1986; Patel et al. 1998; Varshneya and Kanwar 1995; Edward and Pamela 1981).

The correlation of clinical symptoms of acute cypermethrin poisoning with the concentration of cypermethrin in brain, spinal cord or blood is not available in the literature. However, an investigation of the distribution of DDT in the brain showed that the relative concentration varied both with the brain areas and with the time following intravenous administration (Schwabe, 1965). The present investigation was undertaken in an attempt to determine the kinetics of distribution of cypermethrin in different brain areas, at various times, after intravenous administration of a single dose of cypermethrin in rabbits.

MATERIALS AND METHODS

Adult healthy rabbits (1.5-2.5 kg) of either sex from the ITRC animal house colony were divided into six groups of four rabbits each and were anaesthetized with ip injection of sodium pentobarbitol (25-30 mg/kg). The tracheas were cannulated and the animals were ventilated with respiratory pump. One group served as controls. The rabbits of the other groups were injected a single dose

Correspondence to: R. N. Khanna

(5 mg/kg, iv) of cypermethrin (99.5% pure, Rallis India Ltd.), dissolved in peanut oil, through a cannula inserted into a femoral vein and were sacrificed at an interval of 15 min, 30 min, 1,2,4,8 and 12 hr by giving air directly into the heart. One rabbit was daily cannulated and sacrificed after a fixed time interval.

At the end of each interval, blood was drawn from the femoral vein in a heparinized tube. Cerebral cortex, cerebellum, brain stem (medula and pons) and spinal cord were also removed. All the blood and brain samples were immediately frozen for subsequent analysis. The analysis of the compound and its metabolites was carried out by modifying the method of croucher et al (1985). The tissues were homogenized with 5 ml of 0.15 M KCl. Both the tissues and blood samples were extracted successively with 10, 10, 5, 5 and 5 ml of hexane-acetone (1+1 by volume). The combined solvent extract was cleaned up on a florisil column by using small volumes of hexane to complete the transfer. Solvents were concentrated under reduced pressure, dried with anhydrous Na_2SO_4 and transfer to 10 ml volumetric flask with hexane and made to volume.

Analysis was carried out by using a Varian Vista 6000 GC equipped with an electron capture detector (Ni^{63}). A 6'x1/4" glass column packed with 3% SE-30 on 100/120 mesh Gas-chrome Q was employed. Carrier gas was nitrogen at 60 ml/min. The injector, detector and column temperature were maintained at 250°, 250° and 230 °C respectively. The chart speed was 0.5 cm/min and the attenuation was fixed at $2x10^{-9}$. The retention times for compounds analysed were cypermethrin 2.55 minutes, 3-phenoxy benzoic acid 4.5 minutes. The recorder response of elution of every compound was a single sharp peak and the height was found to be proportional to the concentration. The identity of the peak for compounds in blood and tissue was based on its retention time and the absence of these peaks in samples of control animals. Recovery experiments with cypermethrin and 3-phenoxybenzoic acid added to different tissues and following a procedure outlined above are compiled in Table 1. The tissue lipid was determined by using the method of Folch et al (1957).

Table 1: Recoveries* of cypermethrin and 3-phenoxybenzoic acid from different tissues.

Tissues	Recovery (%)	
	Cypermethrin	**3-phenoxybenzoic acid**
Blood	91±5	82±4
Cerebral cortex	92±4	80±3
Cerebellum	88±5	77±5
Brain stem	90±8	79±4
Spinal cord	89±3	81±6

*Mean ±S.E. of 6 replicates in each tissue.

RESULTS AND DISCUSSIONS

The present results emphasize that after intravenous administration, cypermethrin distributes differentially in the CNS and that this pattern changes with time. The symptoms of cypermethrin poisoning were excessive salivation, irregular jerking movements of the limbs and convulsions. These were more intense from 30 minutes to 2 hr and had subsided after 4 hour of cypermethrin treatment. In the whole body autoradiographic analysis, Kurihara et al (1970) also reported the similar results of the penetration of neurotoxic pesticide lindane into all parts of CNS within 15 minutes of administration.

The concentration of cypermethrin per gram of CNS lipid were higher in the brain stem and spinal cord than in the cerebral cortex and cerebellum (Fig. 1). Generally, the concentration of cypermethrin was increased in the lipids of all the brain areas upto 30 minutes and then gradually decreased with time. Thus, two patterns of cypermethrin turnover in the CNS lipids were apparent, a relatively fast uptake and release with the maximum concentration at 30 minutes and a slower uptake and release with a relatively lower maximum concentration. The first pattern was characteristic of areas with a high percentage of lipid i.e. in white matter (brain stem and spinal cord) and the second was evident in areas with a low percentage of lipid i.e. in gray matter (cerebral cortex and cerebellum). The cypermethrin levels in the whole blood lipids were also relatively increased upto 30 minutes and showed a decline similar to that exhibited by different areas of brain.

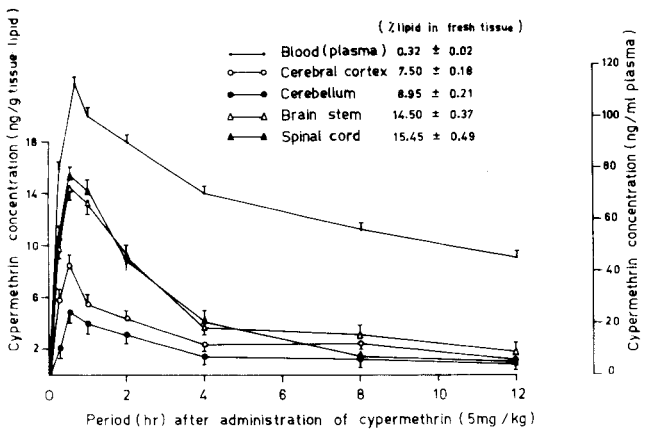

Fig. 1. Distribution of cypermethrin in lipids of the central nervous system and in blood of the rabbit. Mean of 4 rabbits ±S.E.

When cypermethrin concentrations were expressed in terms of wet tissue weight (Fig. 2) instead of the lipid fraction, the differences between various CNS areas appeared to be much more. For example, at 15 minutes, the cypermethrin per unit fresh tissue in the spinal cord was about five times greater than in the cerebral cortex whereas cypermethrin expressed as concentration in the spinal cord lipid was less than two times higher than in the cerebral cortex lipid.

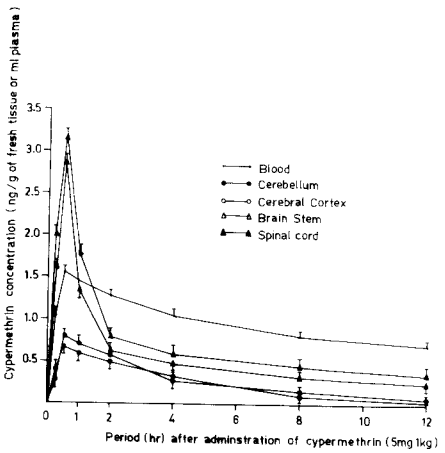

Fig. 2. Cypermethrin concentration per unit fresh tissue weight in the central nervous system and blood of the rabbit. Mean of 4 rabbits ±S.E.

The pattern of distribution in fresh tissue shows that although cypermethrin concentration is higher in white matter initially, it disappears more rapidly from gray matter than from areas with a high myelin content (compare cerebral cortex and spinal cord, Fig. 2). Thus twelve hours after administration, cypermethrin fresh tissue concentrations are higher in the spinal cord and brain stem than in the cerebral cortex and cerebellum. The lower maximum cypermethrin concentration in gray than in white matter results from the fact that blood levels may fall before equilibration is complete in gray matter. The regional differences in the quantity of myelin present may account for the differences in the distribution of cypermethrin in the various regions of the brain. It is possible that cypermethrin may be highly soluble in myelin and hence reaches a higher maximum concentration therein.

The ratio of the distribution of cypermethrin in brain areas to blood (Fig. 3) was maximum at 30 minutes compared to other intervals studied and this could be because of no accumulation of the compound in the brain during the experimental period. The rate of formation of 3-phenoxybenzoic acid appears to be maximum in the first four hours (Fig. 4) and a parallelism existed between

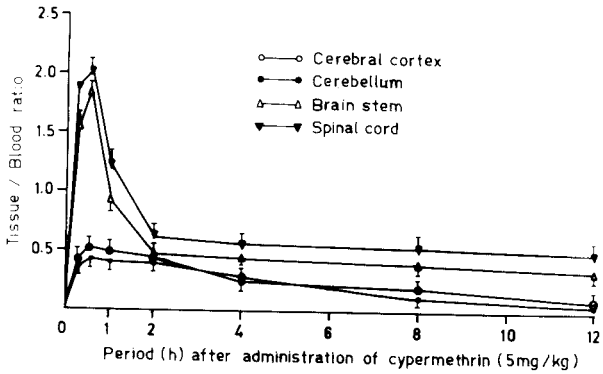

Fig. 3. Distribution pattern of cypermethrin in the central nervous system in relation to blood of the rabbit. Mean of 4 rabbits ±S.E.

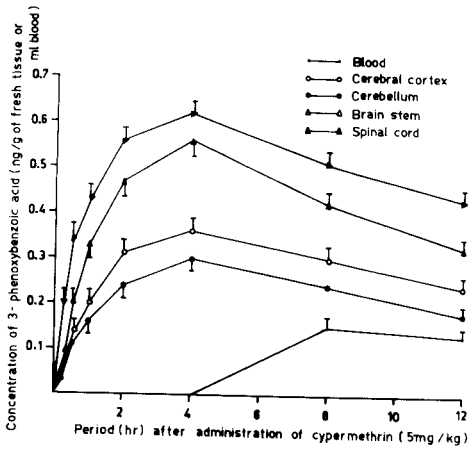

Fig. 4. Concentration of 3-phenoxy benzoic acid per unit fresh weight in the central nervous system and blood of the rabbit. Mean of 4 rabbits ±S.E.

decrease in cypermethrin concentration and increase in the concentration of 3-phenoxybenzoic acid (Fig. 2 & 4). The convulsions produced by the dose of cypermethrin used suggest that the early symptoms of intoxication may be due to the combined effect of the compound and its metabolite in CNS areas with a high myelin content so appeared with the peak concentration of both the compounds

in the spinal cord and brain stem. In the later stages, effects on CNS areas with gray matter may predominate.

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