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Sub-acute exposure of fenvalerate in buffalo calves: Toxicokinetic evaluation

Rajdeep Kaur*, Daundkar Prashant S., Kamalpreet Kaur Gill, Simerjot Singh and S. P. S. Saini

Department of Veterinary Pharmacology and Toxicology
Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana (India)
*rajksidhu@yahoo.com

Abstract

The effect of sub-acute fenvalerate administration on its toxicokinetics was studied in male buffalo calves. Four healthy male buffalo calves were administered fenvalerate orally (@ 1 $mg.kg^{-1}.day^{-1}$) for 21 consecutive days. On day one, blood samples were collected for upto 48 h post administration for toxicokinetic study. The calves were then drenched at the same dose rate for 21 days and sampling was repeated on 21st day for toxicokinetic analysis. Concentrations of fenvalerate in the serum samples were analysed using gas chromatography (GC) and toxicokinetic parameters were calculated as per the non compartmental analysis. Significantly reduced distribution at steady state (0.11 $L.kg^{-1}$ Vs 0.19 $L.kg^{-1}$) on 21st day of continuous exposure compared to day one, clearly indicated limited distribution of the pesticide in various body fluids and tissues upon repeated administration. There was improved persistence of toxicant in the body upon sub-acute exposure as shown by significant increase in area under curve (AUC=156.4 Vs 69.1 $\mu g.h.ml^{-1}$) after sub-acute administration. This fact was further supported by increased mean residence time (MRT=16.7 h Vs 12.6 h) of the pesticide. There was significant increase in the elimination half life (11.6 h Vs 8.74h) of the toxicant on day 21 compared to that observed on day 1, which was further underlined by a significant decrease in its clearance (0.006 Vs 0.015 $L.kg^{-1}.h^{-1}$). Sub-acute exposure of fenvalerate at the proposed dose rate resulted in limited tissue distribution, augmented persistence and slow elimination from the body of animals. This altered toxicokinetics of fenvalerate upon subacute exposure might be due to its effect on organs of metabolism and/elimination, thereby posing increased risk of toxicity in buffalo calves.

Keywords: Fenvalerate, Sub-acute, Toxicokinetic, Gas Chromatography, Buffalo calves.

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Introduction

Pyrethroids - derivatives of carbamic acid, represent a large variety of compounds which have field applications as insecticides, herbicides and fungicides. Many of these chemicals are potential neurotoxicants, particularly following occupational, accidental or intentional exposure. Fenvalerate [(RS) α-cyano-3-phenoxybenzyl (RS) 2-(4-chlorophenyl)-3-methylbutyrate] is a widely used synthetic pyrethroid insecticide, that is effective against a wide range of pests, including strains resistant to organochlorine, organophosphorus and carbamate insecticides [1]. Fenvalerate is an insecticide of moderate mammalian toxicity. In laboratory animals, central nervous system toxicity is observed after acute or short-term exposure. Fenvalerate is widely used in both agriculture and veterinary practices against a variety of insect pests in low quantities/concentrations. Although it is considered much safer than other insecticides, but still toxicity from exposures to fenvalerate has been reported in human beings and animals. Animals are likely to come in contact with this insecticide from the recently sprayed fields or through its residues [2].

Fenvalerate is rapidly absorbed from the gastrointestinal tract, skin and following inhalation. The most important metabolic degradation pathways for synthetic pyrethroids are the oxidation on phenoxy ring, hydrolysis of ester linkage and conjugation of metabolites.

* *Author for correspondence*

All metabolic degradation products of fenvalerate are pharmacologically inactive or inferior to the parent compound, implying that metabolic modifications lead to detoxification [3]. Fenvalerate upon subacute exposure has been reported to produce biochemical and haematological alterations in buffalo calves [2,4]. Toxicokinetic studies on fenvalerate have been conducted in rats and mice [5], goats [6], fish [7] and rainbrow trout [8]. However, there is a paucity of information regarding the effect of continuous exposure of fenvalerate on its toxicokinetics. The present study was therefore conducted to elucidate the effect of sub-acute fenvalerate administration on its toxicokinetics in buffalo calves.

Materials and Methods

Fenvalerate (Reagent®, 20% EC) insecticide was commercially obtained from Bharat Insecticides Limited, New Delhi. Fenvalerate Accustandard® was obtained from 125 Market St., New Haven, CT06513·USA. All the reagents used for the analysis were of GC grade.

The experiment was performed on four healthy male buffalo calves (6-12 months; 90-130 kg). The animals were acclimatized in the animal shed of department under uniform conditions for 4 weeks before the commencement of experiment. During this period, all animals were dewormed and subjected to regular clinical examination. Animals were fed green fodder, wheat straw and concentrate as per the standard body requirement with free access to clean drinking water. The experimental protocol followed the ethical guidelines on the proper care and use of animals and had been approved by the Institutional Animal Ethics Committee.

The animals were orally administered fenvalerate @ 1.0 mg/kg/day for 21 consecutive days. The daily oral dose of fenvalerate was selected on the basis of the recommended concentrations of fenvalerate used for crop protection, per acre yield of fodder and average daily consumption of fodder by buffalo calves to resemble the levels of the insecticide to which dairy animals are likely to be exposed. All the calves were weighed weekly to make necessary corrections in the dosage of fenvalerate as per body weight.

Blood samples (4-5 ml) were collected in tubes via jugular venipuncture at 0.5, 1, 2, 3, 4, 8, 12, 16, 24, 36 and 48 hours following oral fenvalerate drenching on day 1. Serum was separated and stored at -20^{0}C. Blood samples were again collected in the similar way on 21st day after fenvalerate administration to separate serum for analysis of insecticide on GC. The method developed by Gill et al [9] was followed with some modifications for extraction of fenvalerate in serum. Fenvalerate in the clean up extracts was estimated using Gas Chromatography (GC) through Electron Capture Detector (ECD).

The retention time of fenvalerate was 15.30 min, with a total run time of 30 minute (Fig.1). Calibration curve was prepared by spiking the calf serum sample with different concentrations (0.125, 0.25, 0.5, 1, 5 and 10 ppm) of fenvalerate standard. The correlation coefficient (r^2) was 0.984. Unknown serum concentrations were computed from the equation of the calibration curve. Various toxicokinetic parameters were calculated from the serum concentration-time profile of fenvalerate for each animal according to standard equations given by Gibaldi and Perrier [10]. The differences between two means based on individual observation were determined by Student's *t*-test using Microsoft excel. The significance was assessed at $P < 0.05$ and $P < 0.01$ level.

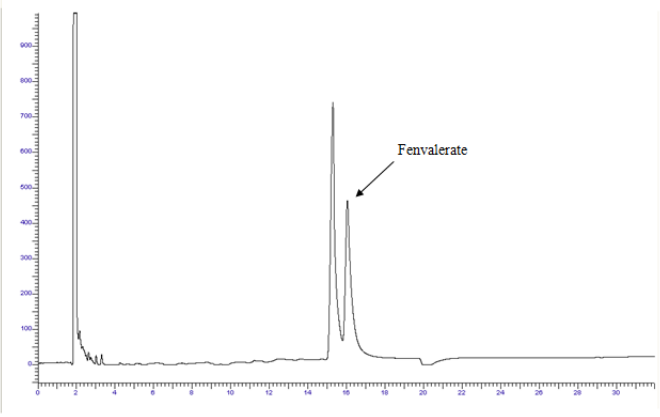


Figure 1. Chromatogram of fenvalerate (1 µg.ml^{-1}).

Results

Serum levels (μg.ml^{-1}) of fenvalerate in buffalo calves after a single oral administration (1 mg.kg^{-1} body weight) on day 1 and on day 21 following daily oral exposure are presented in table 1. It can be observed from a semilogarithmic plot of mean blood level time profile that, serum concentration of insecticide was significantly higher after subacute dosing of fenvalerate at most of the time points compared to day 1 (Fig.2). Serum concentration of 0.67 ± 0.08 μg.ml^{-1} of fenvalerate was detected at 0.5 h, which started to increase rapidly and peaked (C_{max}) to 4.84 ± 0.30 μg.ml^{-1} at 4 h (T_{max}) upon single oral administration of fenvalerate on day 1. Thereafter, the concentration started declining slowly with a minimum serum concentration of 0.16 ± 0.05 μg.ml^{-1} at 48 h. Fenvalerate serum concentration of 1.79 ± 0.06 μg.ml^{-1} was detected at 0.5 h which started increasing thereafter and reached to a peak (C_{max}) of 6.04 ± 0.07 μg.ml^{-1} at 8 h (T_{max}) on 21st day of repeated exposure. Thereafter, the concentration started to decline slowly with a minimum serum concentration of 0.77 ± 0.04 μg.ml^{-1} after 48 h.

Table 1. Serum levels (μg.ml^{-1}) of Fenvalerate in buffalo calves following a single oral administration (1 mg.kg^{-1} body weight) on day 1 and day 21

Time after oral administration (h)	Serum concentration in μg.ml^{-1} (Mean ± SE)	
	Day 1	Day 21
0.5	0.67 ± 0.08	1.79 ± 0.06**
1	1.08 ± 0.13	2.42 ± 0.07**
2	1.98 ± 0.11	2.98 ± 0.14**
3	3.57 ± 0.21	3.88 ± 0.18
4	4.84 ± 0.30	4.81 ± 0.25
8	3.58 ± 0.35	6.04 ± 0.07**
12	2.43 ± 0.24	5.58 ± 0.12**
16	1.65 ± 0.22	4.22 ± 0.13**
24	0.87 ± 0.14	2.83 ± 0.12**
36	0.39 ± 0.09	1.30 ± 0.05**
48	0.16 ± 0.05	0.77 ± 0.04**

The values given are mean ± SE of results obtained from 4 animals.
* Significant difference (P<0.05)
** Significant difference (P<0.01)

The various toxicokinetic parameters after oral fenvalerate administration on day 1 and on day 21 following daily oral dosing are presented in table 2. There was significant difference in maximum plasma concentration (C_{max}) between day 1 (4.84 μg.ml^{-1}) and day 21 (6.04 μg.ml^{-1}). Significantly reduced volume of distribution (0.10 L.kg^{-1} Vs 0.16 L.kg^{-1}) was observed on 21st day of continuous exposure as compared to day 1. Significant increase in area under curve was observed (AUC=156.4 Vs 69.1 μg.h.ml^{-1}) after sub-acute administration. The value of mean residence time (MRT=16.7 h) was significantly longer on 21st day as compared to first day (MRT= 12.6 h). There was significant increase in the elimination half life ($t_{½b=}$11.6 h) of fenvalerate on day 21 compared to that observed on day 1 ($t_{½b=}$ 8.74 h), which was further underlined by significant decrease in its clearance (0.006 Vs 0.015 L.kg^{-1}.h^{-1}).

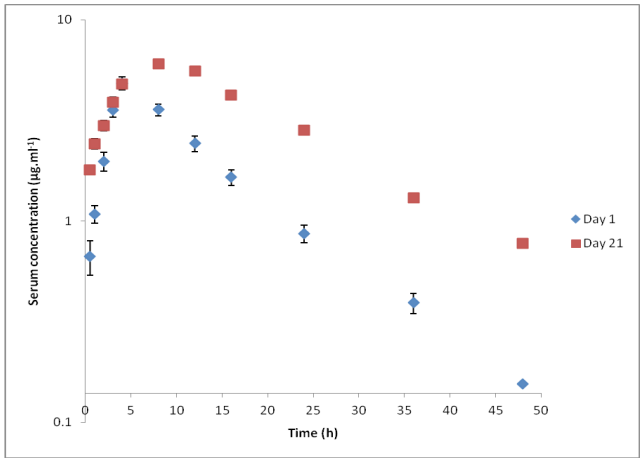


Figure 2. Semilogarithmic plot of mean serum concentration-time profile of fenvalerate following single oral administration at the dose rate of 1mg.kg^{-1} on day 1 and on day 21 after daily oral dosing in healthy buffalo calves.

Table 2. Toxicokinetics of Fenvalerate following repeated oral administration for 21 days (1 mg. kg^{-1}) in healthy buffalo calves

Parameter	Unit	Mean ±SE	
		Day1	Day 21
Cmax	μg.ml^{-1}	4.84 ± 0.30	6.04 ± 0.07*
Tmax	h	4.00 ± 0.00	7.00 ± 1.00
B	μg.ml^{-1}	6.14 ± 0.32	9.97 ± 0.45**
b	h^{-1}	0.08 ± 0.005	0.05 ± 0.002**
$t_{½b}$	h	8.74 ± 0.34	11.6 ± 0.16**
$AUC_{0-α}$	μg.h.ml^{-1}	69.1 ± 7.92	156.4 ± 3.22**
$AUMC_{0-α}$	μg. h^2 .ml^{-1}	882.2 ± 134.8	2611.6 ± 72.1**
V_d	L.kg^{-1}	0.16 ± 0.009	0.10 ± 0.004**
Vdss	L.kg^{-1}	0.19 ± 0.01	0.11 ± 0.002**
Kel	h^{-1}	0.08 ± 0.003	0.06 ± 0.001**
MRT	h	12.6 ± 0.49	16.7 ± 0.23**
CL	L.kg^{-1}.h^{-1}	0.015 ± 0.001	0.006 ± 0.0001**

The values given are mean ± SE of results obtained from 4 animals.
* Significant difference (P<0.05)
** Significant difference (P<0.01)

Discussion

Pyrethroids are potent and effective insecticides which exhibit good insecticidal action and low mammalian toxicity [11]. Studies in rodents and epidemiological data have shown that pyrethroids undergo extensive metabolism by carboxylesterases and cytochrome P450s (CYPs) [12]. In addition, some pyrethroids have also been found to induce the expression of cytochromes. Hydrolysis of pyrethroids is generally considered as a detoxification process [13]. Fenvalerate is used in public health and animal husbandry for the control of flies in cattle sheds. Animals are likely to get exposed to this insecticide from the recently sprayed fields through its residues in feed and fodder. Fenvalerate is rapidly absorbed from the gastrointestinal tract, skin and lungs. The limited information available on the kinetics of fenvalerate after repeated oral administration in animals makes it difficult to interpret toxicological findings and to make risk assessments for fenvalerate. Many toxicodynamic changes appear to have a toxicokinetic basis. The present study was aimed to evaluate the effect of repeated administration of fenvalerate on its toxicokinetics.

Liver is the predominant site of fenvalerate metabolism through hydrolysis in plasma, kidney, stomach and brain tissues by one or more hepatic microsomal esterases. Sub-acute oral exposure of fenvalerate has been shown to produce liver damage as demonstrated by significant elevation in plasma aspartate aminotransferase and gamma glutamyl transpeptidase in buffalo calves [2]. Thus, inhibition of liver specific enzymes might have resulted in reduced metabolism; significant elevation in the blood levels and enhanced fenvalerate toxicity after its repeated oral exposure in buffalo calves. The blood concentrations of fenvalerate were comparatively more on day 21 following daily oral administration than day 1. The fact was further supported by greater value of C_{max} after repeated oral exposure of fenvalerate for 21 days as compared to its single exposure on first day.

The volume of distribution (Vd) is an accurate indicator for diffusion of the drug/xenobiotic into the body tissues [14, 15]. Significantly reduced distribution (0.10 $L.kg^{-1}$) on 21st day after continuous exposure compared to day 1 (0.16 $L.kg^{-1}$), clearly indicates limited distribution of the pesticide in various body fluids and tissues upon repeated administration. The plaucible cause for reduction in distribution of fenvalerate could be the saturation of metabolic pathways after its subacute administration in buffalo calves.

There was significant increase in area under curve (AUC=156.4 $\mu g.h.ml^{-1}$) upon sub-acute administration, which points towards improved persistence of fenvalerate in the body of buffalo calves upon sub-acute exposure. This fact was further supported by increased mean residence time (MRT=16.7 h) of the pesticide on day 21 compared to day 1 (MRT = 12.6 h). This might have occurred due to reduced metabolism and excretion of fenvalerate as organs involved may get damaged due to continuous daily exposure of the insecticide (Gill et al., 2014). The longer residence of the fenvalerate upon subacute exposure might augment its deleterious effects on various body systems i.e. hepatic, renal as well as haemopoietic in buffalo calves [2,4].

Significant increase in the elimination half life (11.6 h) of the toxicant on day 21 compared to that observed on day 1, which was further underlined by significant decrease in its clearance (0.006 $L.kg^{-1}.h^{-1}$) indicates slower elimination of fenvalerate after repeated administration as compared to its single exposure ($t_{½b}$= 11.6 h, CL_B=0.015 $L.kg^{-1}.h^{-1}$). In the case of the pyrethroids, renal elimination of their metabolites is the major pathway of detoxification [16]. Gill et al. [4] reported increase in BUN and creatinine levels in plasma on sub-acute fenvalerate exposure in buffalo calves. This indicates that kidney function is affected following subacute exposure of this insecticide, so this might be the possible reason behind decreased elimination of fenvalerate after sub-acute exposure as compared to its single exposure.

Conclusion

Sub-acute exposure of fenvalerate at the recommended dose rate resulted in limited tissue distribution, augmented persistence and reduced elimination from the body of animals. This altered toxicokinetics of fenvalerate could be due to its effect on organs of metabolism and/elimination, thereby posing increased risk of toxicity in buffalo calves following its long term exposure thereby incurring production and health losses.

Conflict of Interest: None Declared

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