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RESEARCH ARTICLE

Determination of lethal feeding period of bromadiolone anticoagulant for screening individual rats (Rattus rattus) for development of resistance

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Abstract

The second generation anticoagulant, bromadiolone, is presently being used worldwide for control of rodent pests, however, its intensive use has led to the development of resistant rat populations in many parts of the world. In India, there is no report on the development of resistance against bromadiolone. Present study was conducted to determine lethal feeding period of bromadiolone against house rat, Rattus rattus, one of the most common commensal rodent pest worldwide in order to further use it for screening of rats for development of resistance. A total of eight groups of both the sexes (n=5 per group) were fed on cereal based 0.005% bromadiolone bait for variable periods i.e. 0, 2, 4, 8, 16, 24, 48 and 72 hrs in no-choice feeding tests. Lethal feeding period (LFP_{50}) was found to be 4.69 and 5.63 hrs for male and female rats, respectively. Different LFP_{50} values for two sexes indicate sex specific variations. None of the rat of either sex survived after 16, 24, 48 and 72 hrs of feeding test while 60% mortality was achieved in both the sexes after 8 hrs of feeding test. Feeding durations equivalent to LFP_{50} and double the LFP_{50} can be used for screening populations of R. rattus for bromadiolone resistance.

Introduction

The house rat, Rattus rattus Linnaeus, 1758 (Rodentia: Muridae), is one of the most commonly encountered and economically important commensal rodent pest (Parshad, 1999). It not only inflicts heavy damage to stored food but also has nuisance value being a disease carrier or vector (Singla et al., 2008). It is purely an indoor pest (Roberts, 1977; Brooks et al., 1987). Among all the available methods, chemical control by rodenticides is the most widely used and efficient method for the control of rodent pests both under agricultural and commensal situations (Prakash and Mathur, 1987; Chopra et al., 1996). Zinc phosphide, an acute rodenticide has been used predominantly over several years. However, its repeated use leads to rapidly recovering and bait shy rodent populations (Parshad, 1989; El-Deeb et al., 2011). With the development of first generation chronic anticoagulant rodenticides, it was possible to control bait shy rodent populations. However, within a few years of their continuous use, resistance to almost all of the anticoagulants was detected in most of the countries including India (Boyle, 1960; Deoras, 1966; Jackson and Kaukeinen, 1972; Mukthabai et al., 1981). The second generation anticoagulants such as bromadiolone, were developed to combat resistance to first generation anticoagulants. But after a very short time, the initial success of these compounds was shaken by the reports indicating rodent populations showing cross resistance to them (Redfern and Gill, 1980; Rowe et al., 1981; Lund, 1984). The resistance to second generation anticoagulants has not become as wide spread as to the first generation anticoagulants (Buckle, 1994). Today, resistance to both first and second generation anticoagulants has been reported in the United Kingdom, Germany, Denmark, Belgium and the Netherlands (Pelz, 2001; Lodal, 2001; Pelz and Klemann, 2004; Pelz et al., 2005; Diaz et al., 2010; Baert et al., 2012). No case of resistance to bromadiolone has yet been reported from India.

In India, bromadiolone is the only second generation anticoagulant being used commonly since its introduction in 1988 for the control of agricultural and commensal rodents. Resistance to anticoagulants can develop in a population after 5-10 years of their sustained use. The resistant rats if not managed can spread resistance to next generations very quickly due to their high breeding potential thus affecting the distribution and spread of resistant individuals in the wild (Jacob et al., 2012). Detection of early onset of resistance is therefore necessary to implement measures to interrupt its spread to next generations. Tests are needed to identify resistant rodent populations. Guidelines propose lethal feeding period (LFP) tests and blood clotting response tests (EPPO, 1995) as screening tools for anticoagulant resistance (Drummond, 1966; Drummond and Wilson, 1968; WHO, 1970). LFP is defined as the number of days of continuous, no-choice feeding required to kill a given percentage of the rats tested (WHO, 1982). Individuals that survived the lethal feeding period required to kill 99% of susceptible animals (i.e. the LFP_{99}) were considered resistant (Buckle and Prescott, 2012). Early anticoagulant resistance test methods relied on laboratory no-choice feeding tests (WHO, 1982). A resistance test was developed in which survival after 21 days of continuous feeding on 0.025% warfarin bait was considered to be the indicative of resistance (EPPO, 1995). Using this test, the presence of warfarin resistance was confirmed in mouse infestations from many parts of the United Kingdom (UK). Bentley (1969) has defined resistant rats in UK as those that survived a standard feeding period of 6 days on 0.005 % warfarin in the laboratory. No systematic study on lethal feeding periods of bromadiolone against R. rattus has so far been conducted in India. Present study was hence carried out to determine lethal feeding periods of bromadiolone against this species in order to further use it for screening of rats for development of resistance.

Material and Methods

The present work was carried out in Animal House Laboratory, Department of Zoology, Punjab Agricultural University, Ludhiana, India. Bromadiolone (0.25% concentrate) used as a rodenticide during the present study was kindly supplied by M/s Ultima Search Pvt. Ltd., Mumbai.

Collection and maintenance of animals

The house rat, R. rattus of both sexes were captured live from poultry farms in and around Ludhiana and kept individually in laboratory cages for acclimatization. Food and water were provided ad libitum. Food consisted of cereal based plain bait consisting of mixture of broken wheat, powdered sugar and groundnut oil at a ratio of 96: 2: 2. Proper hygienic conditions were maintained throughout the experimental period.

Treatment

After acclimatization, mature and healthy rats of both sexes were selected, weighed and divided into groups. During treatment, a total of eight groups of rats of both the sexes (n=5 per group) were fed on cereal based 0.005% bromadiolone bait for variable periods i.e. 0, 2, 4, 8, 16, 24, 48 and 72 hrs in no-choice feeding tests. 20-40 g of fresh bromadiolone bait was offered to each rat and after the specified period of exposure, unconsumed and spilled bait was collected and weighed to record the consumption (g/100g bw) of bait. From the total consumption of bromadiolone bait after a specified period, dose of active ingredient ingested (mg/kg bw) was also calculated. After the end of treatment, rats were again fed on cereal based plain bait and observed regularly for mortality and other clinical signs. Per cent mortality was recorded using following formula:

$$\text{Mortality (\%)} = \frac{\text{Number of the rats died in a group}}{\text{Total number of rats treated in a group}} \times 100$$

Based on mortality data, different lethal feeding period values i.e. LFP_{10}, LFP_{50} and LFP_{99} were determined using probit analysis (Finney, 1971) through polo software.

Statistical analysis

Values are expressed as mean ± SD. All pair wise treatment comparisons were made using analysis of variance and Students' t-test at 5% level of significance.

Results and Discussion

Data on toxicity of 0.005% bromadiolone bait to male and female R. rattus after different feeding durations has been presented in Tables 1 and 2. None of the rats of either sex survived after 16, 24, 48 and 72 hrs of feeding period while 60% mortality was achieved in both the sexes after 8 hrs of feeding. In male rats (Table 1), per cent mortality ranged from 20% after feeding 0.005% bromadiolone bait for 2 hrs to 100% after feeding for 16 hrs and

above. Death of the rats occurred within 3-14 days. The average consumption of 0.005% bromadiolone bait ranged from 0.80 g/100g bw after feeding for 2 hrs to 33.40 g/100g bw after feeding for 72 hrs. There was no dose dependent effect of bromadiolone on days to death of the rats. The average active ingredient ingested after feeding for 16 hrs causing 100% mortality was 7.20 mg/kg bw.

In female rats (Table 2), per cent mortality ranged from 40% after feeding for 4 hrs to 100% after feeding for 16 hrs and above. The average consumption of 0.005% bromadiolone bait ranged from 1.24 g/100g bw after feeding for 4 hrs to 48.20 g/100g bw after feeding for 72 hrs. Death of the rats occurred within 1-15 days. There was no dose dependent effect of bromadiolone on days to death of the rats. The average active ingredient ingested after feeding for 16 hrs causing 100% mortality was 8.20 mg/kg bw.

A significant ($P<0.05$) difference in consumption of 0.005% bromadiolone bait between male and female rats was observed only after 72 hrs of feeding (Fig. 1). At all the other feeding durations, the difference in bromadiolone consumption between male and female rats was found to be non-significant. The consumption was higher by female rats, but the death of the rats occurred within 7-15 days. In male rats, despite of low consumption of bromadiolone bait, the death occurred within 5-9 days. Different lethal feeding periods (LFP_{10}, LFP_{50} and LFP_{99}**)** of 0.005% bromadiolone bait and their respective 95% fiducial limits are given in Table 3. LFP_{50} values were calculated to be 4.69 and 5.63 hrs for male and female rats, respectively.

During present study, there seemed to be great individual variation in the response of the R. rattus towards bromadiolone toxicity as indicated by the large range of days to death in a particular group and higher standard deviation values for consumption of bromadiolone bait (Tables 1 and 2). Similar individual variation in response of R. rattus towards different compounds has also been reported earlier (Brooks et al., 1990; Singla et al., 2013a and b; Garg and Singla, 2014).

Our study reports that R. rattus has to be fed for 4-6 hrs for 50% mortality. Parshad (1986), however, observed 60% mortality of R. rattus fed on bromadione bait for 3 hrs in no-choice feeding test. Drummond and Wilson (1968) proposed the use of warfarin at 0.005% in bait for susceptibility testing, using a 6-day feeding period as a screening test. A 25-day no-choice feeding test using 0.025% warfarin bait for R. rattus and a 30 day no-choice feeding test for Rattus argentiventer were established as a method of screening warfarin resistant rats (Melanda, 1983). Krishnamurthy et al., (1968) carried out susceptibility studies of R. rattus in Hapur, India, using 0.025 % warfarin in semolina. The house rat, being considerably more tolerant of warfarin than Norway rats (Rattus norvegicus), required a 13-day feeding period for 100% mortality. These results can be compared favourably with those obtained by Bentley and Larthe (1959) using the same warfarin concentration against R. rattus.

Earlier studies have revealed LFP_{50} and LFP_{99} values of 0.025% warfarin bait for house mice to be 4.8 and 29.5 days, respectively. Similarly studies on R. norvegicus revealed LFP_{50} and LFP_{99} values of 0.025% warfarin bait to be 1.7 and 5.8 days, respectively thus showing that house mice possess a remarkable degree of tolerance to warfarin (Rowe and Redfern, 1965). In the laboratory, complete mortality of resistant house mice was achieved with 0.005% difenacoum and bromadiolone bait after one and two day's period of no-choice feeding (Buckle and Prescott, 2012).

Table 1: Mortality of male Rattus rattus fed on 0.005% bromadiolone bait for different durations of time

Body weight (g) (n = 5 each)	Treatment duration (hrs)	Dose of 0.005% bromadiolone bait ingested (g/100g bw)	Dose of active ingredient ingested (mg/kg bw)	Number of animals died/treated	Per cent mortality	Days to death (Range)
169.80±15.40	0	-	0	0/5	0	-
143.20±21.53	2	0.80±0.84	0.4±0.42	1/5	20	6.00±0.00 (6)
153.00±54.79	4	2.20±1.64	1.1±0.82	2/5	40	10.50±4.95 (7-14)
146.40±27.69	8	3.20±1.64	1.6±0.82	3/5	60	6.00±2.83 (4-12)
172.80±43.72	16	14.40±0.89	7.2±0.45	5/5	100	5.50±0.71 (5-8)
142.60±15.76	24	15.60±2.61	7.8±1.30	5/5	100	4.50±0.71 (4-9)
143.80±21.90	48	24.20±10.64	12.1±5.32	5/5	100	3.00±0.00 (3-12)
144.80±19.51	72	33.40±7.89	16.7±3.95	5/5	100	5.50±0.71 (5-9)

Values are Mean±SD

Table 2: Mortality of female Rattus rattus fed on 0.005% bromadiolone bait for different durations of time

Body weight (g) (n = 5 each)	Treatment duration (hrs)	Dose of 0.005% bromadiolone bait ingested (g/100g bw)	Dose of active ingredient ingested (mg/kg bw)	Number of animals died/treated	Per cent mortality	Days to death (Range)
169.80±15.40	0	0	0	0/5	0	0
145.20±30.08	2	0.22±0.33	0.11±0.17	0/5	0	0
104.00±4.90	4	1.24±1.64	0.62±0.82	2/5	40	2.00±1.41 (1-3)
141.40±35.80	8	3.20±2.39	1.6±1.19	3/5	60	4.50±0.71 (4 - 15)
115.00±12.17	16	16.40±4.04	8.2±2.02	5/5	100	4.50±3.54 (2-14)
141.00±36.37	24	14.60±2.19	7.3±1.10	5/5	100	7.00±1.41 (6-12)
140.40±25.66	48	28.20±3.19	15.1±2.92	5/5	100	7.00±1.41 (6-11)
122.60±16.94	72	48.20±9.98	24.1±4.99	5/5	100	7.50±0.71 (7-15)

Values are Mean±SD

Table 3: Different lethal feeding periods of bromadiolone in male and female Rattus rattus

Sex	Lethal feeding period (hrs)		95% Fiducial limit	
			Lower	Upper
Male	LFP_{10}	1.73	0.19	3.16
	LFP_{50}	4.69	2.19	8.15
	LFP_{99}	28.63	13.41	643.57
Female	LFP_{10}	2.79	0.50	4.35
	LFP_{50}	5.63	3.18	9.39
	LFP_{99}	20.15	11.14	307.26

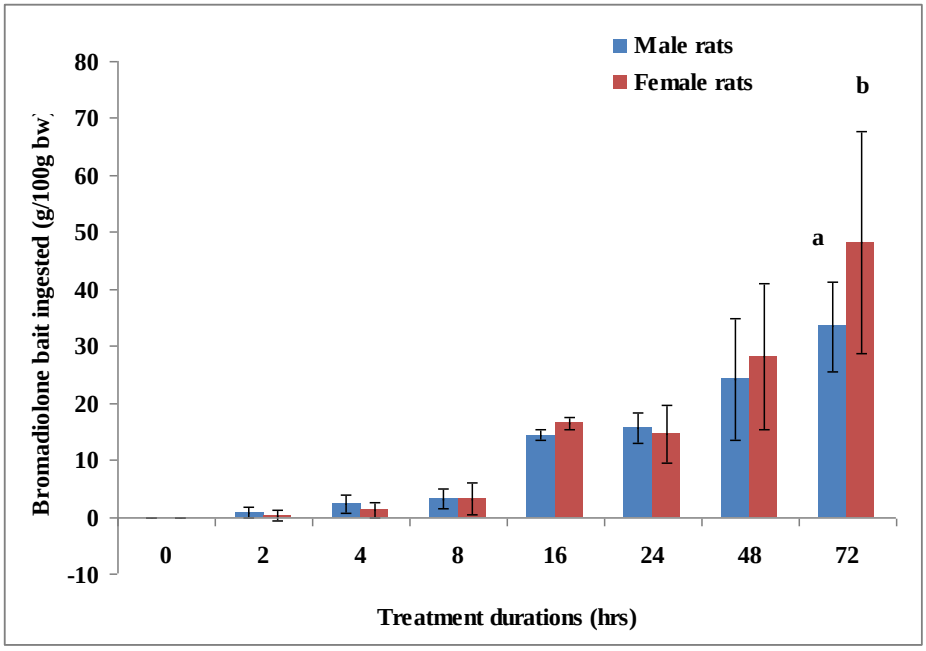

Fig. 1: Comparison of 0.005% bromadiolone bait ingested by male and female Rattus rattus at different treatment durations. Bars with different superscripts differ significantly at $P \leq 0.05$.

Conclusions

Present study reveals that house rat, R. rattus can ingest lethal dose of bromadiolone in a short feeding period of less than one day. Different lethal feeding periods for two sexes indicate male rats to be more susceptible to bromadiolone toxicity than female rats. Feeding durations equivalent to LFP_{50} and double the LFP_{50} determined during present studies can be further used for screening populations of R. rattus for development of bromadiolone resistance.

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