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The toxicity of brodifacoum to susceptible and warfarin-resistant *Rattus tiomanicus* (Miller)

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Key words: brodifacoum, toxicity, efficacy, warfarin-resistance, Malaysian wood rat *(Rattus tiomanicus)*, second-generation anticoagulant

Abstrak

Kebisaan racun brodifakum pada tikus belukar, *Rattus tiomanicus* telah dinilai secara 'oral intubation'. Kaedah pemberian makanan tanpa pilihan dijalankan pada spesies yang sama (pada tikus yang tahan warfarin dan yang peka). Dos kematian (LD_{50}) yang didapati ialah 0.31 (0.26–0.36) mg/kg manakala jangka masa pemberian makanan yang berkesan untuk membawa maut (LFP_{50}) ialah 0.68 (0.05–0.98) hari. LFP_{50} bagi tikus yang tahan warfarin dua kali lebih lama daripada tikus yang peka. Semua tikus mati antara 5 hari hingga 9 hari. Walaupun tikus yang tahan warfarin menunjukkan toleransi terhadap brodifakum, kesemua tikus mati selepas 4 hari diberi makan racun tersebut. Jangka masa pemberian makanan (LFP_{99}) bagi tikus yang peka ialah 3 hari. Jangka masa tersebut boleh digunakan sebagai asas untuk menentu/mengesan ketahanan tikus terhadap racun brodifakum. Brodifakum adalah racun rodensia yang berkesan untuk mengawal tikus yang tahan warfarin dan yang tidak. Antara ciri-ciri brodifakum ialah penerimaan umpan yang baik, boleh membawa maut selepas sekali makan dan mengambil masa yang singkat.

Abstract

The toxicity of brodifacoum was evaluated by oral intubation with wild (susceptible) *R. tiomanicus*. No-choice feeding studies with warfarin-resistant and susceptible rats of the same species were undertaken to determine the efficacy of the compound. The single oral dose (LD_{50}) for brodifacoum was 0.31 (0.26–0.36) mg/kg and the mean lethal feeding period (LFP_{50}) was 0.68 (0.05–0.98) day. The LFP_{50} for the warfarin-resistant rats was twice more than that of susceptible rats. All the rats died between a mean of 5 days and 9 days. Total death of warfarin-resistant rats was achieved in a 4-day feeding period. The LFP_{99} determined with susceptible rats gave a 3-day feeding period and this was used as the baseline for monitoring rat resistance to brodifacoum. The warfarin-resistant rats exhibited tolerance to brodifacoum with a certain degree of cross resistance. Brodifacoum was found to be an effective rodenticide against warfarin-resistant and susceptible rats. Its favourable properties include good bait acceptance, a single dose poison and short duration to death.

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Introduction

Brodifacoum, 3 – [3 – (4'-bromobiphenyl-4-yl) –1, 2, 3, 4, – tetrahydronaphth-1-y] – 4 – hydroxycoumarin is one of the more potent anticoagulants available for rat control (Dubock and Kaukeinen 1978). Redfern et al. (1976) established the single-dose oral LD_{50} for rats to be 0.26 mg/kg and for mice 0.4 mg/kg. They achieved complete mortality of warfarin-resistant and non-resistant Norway rats with 0.005% brodifacoum baits following a 2–day feeding or 1–day feeding with 0.01% brodifacoum baits. Brodifacoum was introduced into Malaysia in the late 1970s and Lam (1980) reported its LD_{50} for the rice-field rat, *Rattus argentiventer* (Robinson and Kloss), to be 0.17 mg/kg. He also noted that total mortality of *R. argentiventer* could be achieved with 1–day feeding upon 0.002% or 0.005% brodifacoum baits in the laboratory. With *R. tiomanicus* (Miller), the predominant plantation rodent pest, Khoo and Dubock (1981) estimated the LD_{50} of brodifacoum to be approximately 0.33 mg/kg. The present study on the toxicity of brodifacoum to warfarin-resistant and susceptible *R. tiomanicus* is an attempt to provide more information pertaining to its use for the control of the species, in addition to evaluating the performance of the chemical against warfarin-resistant rats.

Materials and methods

R. tiomanicus, live-trapped from Hilir Perak cocoa-coconut fields that are free of rodenticides (not known to have been baited before), were used to determine the LD_{50} (lethal dose) and the LFP_{50} (lethal feeding period) of brodifacoum. These would represent wild population of *R. tiomanicus* and would be susceptible to any rodenticide. They are referred to as susceptible *R. tiomanicus*.

The efficacy of brodifacoum against warfarin-resistant *R. tiomanicus* was carried out with laboratory-bred warfarin-resistant rats. These animals were bred using rats from cocoa-coconut fields with unsatisfactory control in the warfarin baiting programme and had survived a 14day feeding period on 0.025% warfarin baits (Lee et al. 1983). The population obtained has an LFP_{50} of 6 days. This is higher than the 4 days established for susceptible *R. tiomanicus* by Lee and Mustafa (1983). Resistance is applied to a population when the susceptibility of the majority of the population to a poison decreases following selection with that poison (Oppenoorth and Welling 1976). The above population is thus a warfarin-resistant population.

All test-animals were conditioned in the laboratory in individual cages (45 cm x 45 cm x 30 cm) for 2 weeks before the study. The test procedure was similar to that reported by Lee and Mustafa (1983) and guidelines provided by WHO (Anon. 1982).

Determination of LD_{50} oral toxicity

Brodifacoum liquid concentrate was administered orally by gavage to non-starved animals. The amount required for each test animal was calculated based on its body weight and the dosage of the treatment. Brodifacoum liquid concentrate (0.25% a.i.) was then diluted and the amount required was prepared in volumes ranging from 0.05 mL to 0.50 mL, and then administered to the animals. For each dosage, 20 live-trapped and laboratory-conditioned animals (10 males and 10 females) were used. The animals were maintained on laboratory pellets, fresh copra and ripe bananas with water ad lib. throughout a 30–day observation period. Days to death were recorded and dead animals were autopsied for poisoning symptoms.

Feeding tests

No-choice feeding tests were carried out. The brodifacoum bait was prepared in commercial maize-base wax-cubes at 0.003% concentration. Three groups of animals (10 males and 10 females in each

Table 1. Oral toxicity of brodifacoum to susceptible *Rattus tiomanicus*

Dose (mg/kg)	Sex	Body wt. (g)	Mortality	Days to death
0.2	M	97.3	2/10	6.5(6– 7)*
	F	87.3	3/10	7.0(6– 8)
0.3	M	82.0	4/10	6.3(4– 8)
	F	86.0	4/10	6.3(4– 8)
0.4	M	92.0	6/10	5.5(3–10)
	F	96.4	7/10	5.7(3–10)
0.6	M	113.7	9/10	5.4(4– 9)
	F	80.5	10/10	5.8(4– 9)
0.8	M	91.0	10/10	6.6(5–11)
	F	88.6	10/10	7.2(5–11)

*The first values are means while those in brackets are ranges

group) that had been live-trapped earlier from the field and laboratory-conditioned were prebaited for 2 days with plain baits. They were then allowed unrestricted feeding upon the poison baits for 1, 2, and 3 days respectively. At the end of the prescribed feeding period, the animals were maintained on laboratory pellets, fresh copra and ripe bananas for a 30–day observation period. The amount of baits consumed was measured (to the nearest 0.1 g) daily and mortality recorded. Dead rats were autopsied for symptoms of poisoning. The above test protocol was similarly followed for feeding tests with laboratory-bred warfarin-resistant *R. tiomanicus*, and terminated when 100% mortality was achieved.

The results obtained in the oral intubations and feeding tests were used to determine the LD_{50} and LFP_{50} by probit analysis (Finney 1971).

Results

In the oral intubation studies on susceptible *R. tiomanicus*, there was a linear relationship between brodifacoum concentration and mortality. The highest mortality was achieved when the animals were treated at the dosage of 0.8 mg/kg body weight *(Table 1)*. The duration to death ranged from 3 days to 11 days for all treatments. The mean days for both males and females to succumb to brodifacoum ranged from 5.4 days to 7.2 days. The single dose oral LD_{50} for males and females were 0.33 and 0.29 mg/kg respectively *(Table 2)*. Although the females were more susceptible, the difference between the sexes was not statistically significant. The pooled LD_{50} calculated was 0.31 mg/kg. The LD_{95} and LD_{99} values were 0.71 and 1.00 mg/kg respectively.

In the no-choice feeding test with susceptible *R. tiomanicus*, 100% mortality was obtained in the 3–day feeding period *(Table 3)*. The amount of poison baits consumed daily ranged from 6.4 g to 9.6 g. The amount consumed was comparable to the amount of plain baits consumed (7.6 g to 10.4 g). Death of susceptible rats occurred between 3 days and 16 days after feeding, and a slight decrease in duration to death was noted with increased feeding time.

Variation in susceptibility of susceptible *R. tiomanicus* to brodifacoum was noticeable in the feeding tests. The minimum dose fatal to the animal was 1.30 mg/kg, and the animal was a female weighing 90 g which ate 3.9 g of the bait and died on the eighth day. In contrast, the highest dose survived was 2.36 mg/kg by a male weighing 126 g and ate 9.9 g of the bait in the 1–day feeding period. The LFP_{50} (probit analysis) was 0.89 days for males and 0.50 days for females *(Table 2)*. Although the females were more susceptible than the males, the difference was not significant. The LFP_{50} of the pooled data was 0.68 days with 95% fiducial limits of 0.05–0.98 days. The LFP_{95} and LFP_{99} were 1.81 days and 2.76 days respectively.

In the no-choice feeding tests with warfarin-resistant *R. tiomanicus*, poison bait uptake was comparable to that of plain baits *(Table 3)*. Total mortality of the animals was achieved following a 4–day feeding period. The duration to death ranged from 3 days to 14 days and an increased feeding period did not lead to a decrease in the time to death. The susceptibility of animals to brodifacoum

Table 2. Probit analysis of data from oral intubation and no-choice feeding test with brodifacoum against *Rattus tiomanicus*

Treatment	Sex	Regression equation	Chi-sq.	d.f.	LD_{50} (mg/kg)	LD_{95} (mg/kg)	LD_{99} (mg/kg)
Oral	M	$y = 4.84x + 7.36$	0.72	3	0.33 (0.24–0.40)*	0.77	1.09
intubation	F	$y = 5.21x + 7.80$	2.18	3	0.29 (0.22–0.36)	0.64	0.89
	Pooled	$y = 4.96x + 7.54$	2.21	3	0.31 (0.26–0.36)	0.71	1.00
					LFP_{50} (days)	LFP_{95} (days)	LFP_{99} (days)
No-choice feeding	M	$y = 10.02x + 5.52$	0.002	1	0.89 (0–1000)*	1.29	1.52
	F	$y = 2.59x + 5.79$	0.53	1	0.50 (0–1000)	2.16	3.96
(Susceptible *R.t.*)	Pooled	$y = 3.79x + 5.66$	0.25	1	0.68 (0.05–0.98)	1.81	2.76
No-choice feeding	M	$y = 4.08x + 4.32$	1.10	2	1.47 (0.80–1.93)	3.70	5.46
(Warfarin-resistant *R.t.*)	F	$y = 3.10x + 4.67$	1.24	2	1.28 (0.48–1.81)	4.32	7.21
	Pooled	$y = 3.56x + 4.50$	1.89	2	1.38 (0.97–1.72)	3.99	6.24

*Values in brackets are values of 95% fiducial limits.

Table 3. Results of no-choice feeding tests in which susceptible and warfarin-resistant *R. tiomanicus* was given 0.003% brodifacoum baits in maize-base wax cubes for various number of days

Feeding (day)	Sex	Body wt. (g)	Mortality	Mean bait intake (g)		Lethal dose (mg/kg)	Survival dose (mg/kg)	Day to death
				last day plain	1st day poison			
Susceptible *R. tiomanicus*								
1	M	120.0	7/10	9.9	9.4	3.05(2.36– 4.68)*	1.25(0.24–2.36)*	9.0(5–16)*
	F	94.5	8/10	8.6	8.0	3.00(1.30– 5.00)	1.19(0.33–2.05)	7.5(3–11)
2	M	115.4	10/10	10.4	9.6	5.15(3.72– 6.11)	–	5.0(3–9)
	F	108.0	9/10	9.5	9.8	4.79(2.31– 5.86)	2.25	6.3(3–11)
3	M	110.0	10/10	10.2	9.2	7.21(5.89– 9.08)	–	7.0(4–9)
	F	108.6	10/10	7.6	6.4	6.12(2.10– 9.34)	–	7.2(4–9)
Warfarin–resistant *R. tiomanicus*								
1	M	105.0	3/10	10.3	9.8	4.65(3.20– 5.82)	2.78(0.24–4.36)	7.0(4–12)
	F	98.0	4/10	9.5	9.0	4.12(2.91– 5.76)	2.35(0.91–4.70)	6.0(3–11)
2	M	112.4	6/10	11.0	10.0	6.23(3.70– 8.45)	3.95(2.40–5.30)	8.0(4–14)
	F	100.6	7/10	8.6	9.2	6.10(4.20– 8.30)	3.20(2.30–5.41)	9.0(5–11)
3	M	114.4	9/10	9.8	10.2	7.9 (6.2 – 9.72)	4.85	9.5(4–14)
	F	95.8	8/10	8.4	8.8	7.2 (6.54–10.35)	5.2 (3.79–6.60)	8.6(4–14)
4	M	120.0	10/10	11.5	10.4	8.43(5.42–10.65)	–	7.7(4–11)
	F	106.2	10/10	8.9	9.5	7.65(4.50–11.68)	–	8.2(5–12)

*The first values are means while those in brackets are ranges

varied. The minimum fatal dose was 2.91 mg/kg (a female weighing 96.0 g ate 9.3 g of the poison bait and died on the 10th day) and the maximum dose survived was 6.61 mg/kg (a female weighing 100 g ate 22.0 g of the bait in a 3–day feeding period). The LFP_{50} derived from probit analysis for males was 1.47 days while for females it was 1.28 days *(Table 2)*. These differences in susceptibility were not statistically significant. The pooled data showed an LFP_{50} of 1.38 days and ranged from 0.97 day to 1.72 days at 95% fiducial limits. The LFP_{95} and LFP_{99} determined were 3.99 days and 6.24 days respectively.

In the studies, all the dead rats were autopsied and found to have died because of excessive bleeding. This and the interval of several days before death is indicative of anticoagulant poisoning.

Discussion

Brodifacoum with an LD_{50} of 0.31 mg/kg (0.33 for males and 0.29 for famales) is highly toxic to *R. tiomanicus*. It is several times more toxic than bromadiolone (another second generation anticoagulant that has an LD_{50} of 2.27 mg/kg to the same species; Lee and Mastor 1984). High toxicity and with good bait acceptance, brodifacoum clearly has potential for controlling *R. tiomanicus* populations. A single feeding ad lib. on low bait concentrations (0.003%) by a majority of the animals is probably sufficient to bring about mortality.

Feeding studies with susceptible rats gave an LFP_{50} of 0.68 day. This further substantiates the high potency of brodifacoum. The variation in susceptibility was noted when a male rat survived a poison dose equivalent to 2.36 mg/kg, about three times the estimated LD_{99} of 0.91 mg/kg. This points to the inherent high variation in susceptibility of the species. Prolonged and continuous use of brodifacoum is likely to lead to the development of brodiacoum-resistant rats.

Anon. (1982) suggested that the LFP_{99} of a rodenticide established against a wild (susceptible) population of rat species be used as a guideline to monitor the resistance. In conforming to this suggestion, the LFP_{99} of 2.76 days determined from the feeding studies with susceptible rats indicated that a 3–day feeding period could be used as a guideline to monitor the resistance to brodifacoum. In the present study 100% mortality of susceptible rats noted following a 3–day feeding period on 0.003% brodifacoum baits supports its use as a guideline.

Feeding studies with warfarin-resistant rats showed that some of the rats, (3/20), were able to survive a 3–day feeding period. The lowest fatal dose of 2.91 mg/kg was higher than the highest survival dose (2.36 mg/kg) of susceptible rats. This implies that warfarin-resistant animals require a higher amount of brodifacoum to bring about death.

The highest survival dose for warfarin-resistant rat (6.61 mg/kg) was more than twice that of susceptible rat (2.36 mg/kg). Similarly the LFP_{50}, LFP_{95} and LFP_{99} for the former (1.38, 3.99 and 6.24 days respectively) were more than twice that of the latter (0.68, 1.81 and 2.76 days respectively). This showed that warfarin-resistant rat populations would require more brodifacoum for effective control. Siddiqi et al. (1983) reported that areas with warfarin-resistant house mice required more brodifacoum baits for effective control.

The findings in this study showed that warfarin-resistant rats exhibited tolerance to brodifacoum with a certain degree of cross-resistance. Linowsky (1983) similarly reported that warfarin-resistant mice (*Mus musculus* L) showed a certain degree of cross-resistance to bromadiolone. It can perhaps be inferred that warfarin resistance in rats and mice not only confers resistance to other first-generation anticoagulants but also a certain degree of cross-resistance to the second-generation anticoagulants as

speculated by Lund (1984).

Assuming that cross-resistance exists, total mortality of warfarin-resistant rats was achieved following a 4–day feeding period on 0.003% brodifacoum baits. Similarly, 100% mortality of warfarin-resistant *R. norvegicus* was noted following a 6–day feeding period on brodifacoum (Dubock and Kaukeinen 1978). As for the house mice, *M. musculus* and the roof rat, *R. rattus* warfarin-resistant animals were completely killed following a 2–day feeding on 0.005% brodifacoum baits (Redfern *et al.* 1976). In a choice-feeding study, Rowe and Bradfield (1976) reported that 0.01% brodifacoum baits were required to completely kill warfarin-resistant mice. These findings implied that the second-generation anticoagulants, in this case brodifacoum, is efficient in controlling susceptible and warfarin-resistant rats as suggested earlier by Marsh (1984).

Conclusion

Brodifacoum is found to be a highly potent single-dose anticoagulant poison for *R. tiomanicus*. The LD_{50} and LFP_{50} (0.003% brodifacoum baits) for susceptible rats were 0.31 mg/kg and 0.68 day respectively. The LFP_{99} of 2.76 days indicates that a 3–day feeding period can be used as a baseline for the monitoring of resistance to brodifacoum. Warfarin-resistant rats were found to require a higher dose to cause death and the LFP_{50} value of 1.38 days was twice that of susceptible ones. The findings also indicated a certain degree of warfarin cross resistance to brodifacoum. Despite this disadvantage, brodifacoum with its good bait acceptance and single-feeding properties has the potential to be an effective rodenticide.

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