MARDI Res. Bull., (1983) 11, 3: (264—271)

WARFARIN RESISTANCE IN *RATTUS TIOMANICUS* (MILLER)

LEE C.H.*, MUSTAFA MD. D.*, SOH K.G.** AND MOHAN E.*

Keywords: Warfarin resistance, *Rattus tiomanicus.*

RINGKASAN

Rattus tiomanicus telah diperangkap hidup dari kawasan-kawasan koko-kelapa Hilir Perak, di mana jadual kawalan tikus dengan warfarin telah gagal. Mereka telah ditempatkan di makmal dan diberi makanan dan air *ad libitum*. Dua kumpulan (10 jantan dan 10 betina setiap kumpulan) diberi makan umpan beracun 0.025% warfarin tanpa pilihan selama 8 dan 14 hari manakala dua kumpulan lagi diberi makan umpan beracun 0.025% bromethalin dan 0.025% scilliroside selama sehari. Sebanyak lima daripada 20 ekor tikus hidup dalam ujian 8 hari manakala seekor hidup dalam ujian 14 hari. Dalam ujian pemakanan bromethalin dan scilliroside semua tikus didapati mati.

Tikus-tikus yang mati dalam ujian warfarin didapati memerlukan takaran yang lebih tinggi dan masa yang lebih lama untuk membawa maut berbanding dengan tikus yang lebih peka pada warfarin (LEE dan MUSTAFA, 1982). Cara pemakanan yang tidak seragam dan tidak menentu ke atas umpan warfarin diperhatikan di antara tikus yang mati dan yang hidup dalam ujian-ujian warfarin yang dijalankan. Tikus yang hidup dalam ujian warfarin telah memakan umpan beracun sebanyak setengah hingga sejumlah sama berat badannya, dan kebolehannya untuk menahan takaran racun yang begitu tinggi menunjukkan bahawa mereka akan hidup dalam mana-mana program kawalan tikus dengan warfarin. Ini menunjukkan ketahanan tikus ke atas warfarin.

Bromethalin dan Scilliroside pada kadar 0.025% dapat memberi 100% maut ke atas tikus yang diuji dengan pemakanan umpan beracun yang rendah. Bromethalin didapati membawa maut dalam masa yang singkat manakala kematian kronik diperhati dalam peracunan scilliroside. Sifat bromethalin ini boleh diperbaiki untuk memberi pemakanan umpan beracun yang lebih tinggi dan juga memanjangkan masa maut ke satu atau dua hari dengan menggunakan kepekatan racun yang lebih rendah. Dengan keupayaan untuk membawa maut 100%, bromethalin bersama-sama racun-racun rodensia 'generasi kedua' dan/atau scilliroside bersama-sama racun 'acute' adalah pilihan untuk kawalan rodensia terutamanya di kawasan-kawasan di mana ketahanan ke atas antikoagulant didapati.

INTRODUCTION

Warfarin was the first anticoagulant to be used extensively as a rodenticide in Europe and the United States in the early 1950s. In most countries where it is used, resistant rats have been reported. BOYLE (1960) and DRUMMOND and BENTLEY (1967) reported warfarin resistant rats in Great Britain; LUND (1967) in Denmark; OPHOF and LANGEVELD (1968) in Netherlands and TELLE (1972) in Germany. In the United States the excessive and indiscriminate use of anticoagulants have resulted in selected resistant *Rattus norvegicus, Rattus rattus* and house mice.

The advent of warfarin in Peninsular Malaysia began in the 1960s and since then it has been widely used as the 'standard' for rodent control especially in cocoa and oil palm plantations. The occurrence of warfarin resistant rats in oil palm plantations was speculated as early as 1977 (WOOD and LIAU). It was only recently that a case of warfarin resistance in *Rattus rattus diardii* was recorded from a cocoa area in Kuala Bernam Estate (LAM *et al.*, 1982). In early 1982 unsatisfactory rodent control with warfarin was found in some cocoa-coconut fields with severe rodent damage in Hilir Perak that has had successful baiting programmes for about 10 years prior to this.

*Cocoa-Coconut Branch, MARDI, Teluk Intan, Perak.
**Eli Lilly (M) Sdn. Bhd., Kuala Lumpur.
*Kuril Research, Bagan Pasir, Perak.

This paper presents laboratory findings of warfarin resistance in a population of *Rattus tiomanicus*, which was the only species live-trapped, from the affected areas. Two other alternative rodenticides (i.e. bromethalin, a second generation rodenticide and scilliroside, an acute poison) were evaluated against these rats.

MATERIALS AND METHODS

Rattus tiomanicus were live-trapped from cocoa-coconut fields in Hilir Perak where unsatisfactory control was noted in the warfarin application programme after about 10 years of effective control. The test conditions were similar to that reported by LEE and MUSTAFA (1982). The animals were divided into four groups (each comprising of 10 males and 10 females).

The bait formulation of 0.025% warfarin in maize base wax cube was prepared by adding 5% of mastermix (0.5% warfarin in rice bran) to 50% mesh grind maize, 5% refine sugar, 15% grind fish fry heads and prawn dust, 5% coconut oil (used as a sticker) and 20% melted paraffin wax. A similar bait concentration of 0.025% bromethalin was commercially prepared from 0.1% technical concentrate while 0.025% scilliroside was prepared from 1% technical concentrate.

No choice feeding tests were undertaken after the animals were prebaited on non-poison baits for two days. Two groups of the animals were allowed unrestricted feeding on the 0.025% warfarin wax maize baits for eight and 14 days while the third and fourth groups were given 0.025% bromethalin and scilliroside poison baits for one day respectively. Daily bait consumption (to the nearest 0.1g) for each animal and mortality was recorded. Dead rats were autopsied for poisoning symptoms. Rats were considered to have survived a test if they were alive 28 days after the beginning of the test.

RESULTS

In the warfarin treatment, five (three males and two females) out of 20 rats survived the 8-day no-choice test *(Table 1)*. The mean lethal dose for males and females were 90.7 mg/kg (range 63.1 – 144.2) and 148.7 mg/kg (range 90 – 161.3) respectively. The duration to death for males was 6.7 days (range 5 – 9) with one male dying on the 9th day after a dose of 144.2 mg/kg. In the females, the days to death was 8.8 (range 5 – 17) with two females succumbing on the 10th and 17th day after having consumed a dose of 148.8 and 150 mg/kg respectively. Those that survived had taken a mean lethal dose of 132.4 mg/kg (range 123.0 – 147.6) for males and 137.7 mg/kg (range 77.3 – 198.2) for females.

In the 14-day feeding period one female survived out of 20. The mean lethal dose for males were 113.6 mg/kg (range 51.5 – 149.2) and 144.7 mg/kg (83.1 – 242.3) for females. Most of the animals died within 5 – 12 days except for three males and a female which died on the 16th, 17th, 18th and 15th day respectively. The four animals also had a lethal dose of 149.2, 135.8, 137.7 and 242.2 mg/kg respectively. The only female that survived had consumed 231.4 mg/kg of warfarin.

The animals that survived the warfarin feeding tests have consumed baits ranging from half to almost its body weight *(Table 2)*. The feeding pattern and behaviour of both alive and succumb rats were inconsistent and more of erratic bait consumption *(Table 3)*.

The third group of animals subjected to bromethalin feeding for one day had no survivors. The mean lethal dose for the males and females were 13.4 mg/kg (range 7.7 – 15.3) and 14.2 mg/kg (range 6.9 – 16.2) respectively. All the animals died within 16–24 hours.

The fourth group of animals subjected to scilliroside feeding also had no survivors.

TABLE 1: RESULTS OF NO-CHOICE FEEDING TEST IN WHICH *RATTUS TIOMANICUS* WERE GIVEN 0.025% RODENTICIDE IN WAX CUBE MAIZE BASE FOR VARIOUS NUMBER OF DAYS

Rodenticide	Number of feeding days	Sex	Mean body weight (g)	Mortality (dead/treated)	Mean bait intake		Lethal dose (mg/kg)		Survival dose (mg/kg)		Days to death	
					last day (plain) (g)	1st day (poison) (g)	Mean	Range	Mean	Range	Mean	Range
Warfarin	8	M	108.5	7/10	3.0	6.3	90.7	(63.1 – 144.2)	132.4	(123.0 – 147.6)	6.7	(5– 9)
		F	87.4	8/10	4.1	7.3	148.7	(90.0 – 161.3)	137.7	(77.3 – 198.2)	8.8	(5–17)
Warfarin	14	M	113.7	10/10	1.6	6.7	113.6	(51.5 – 149.2)		–	10.0	(5–18)
		F	87.5	9/10	1.7	7.1	144.7	(83.1 – 242.3)	231.4		8.3	(5–15)
Bromethalin	1	M	102.5	10/10	6.8	5.1	13.4	(7.7 – 15.3)		–	1.0	
		F	96.2	10/10	7.2	5.6	14.2	(6.9 – 16.2)		–	1.0	
Scillirocide	1	M	113.8	10/10	9.4	3.9	8.5	(3.5 – 13.6)		–	7.5	(2–17)
		F	86.6	10/10	7.6	3.5	6.8	(4.8 – 12.4)		–	6.9	(2–15)

TABLE 2: TOTAL BAIT CONSUMED BY RATS THAT SURVIVED THE 8 AND 14 DAYS OF 0.025% WARFARIN BAIT TREATMENT

Treatment (Day of no-choice feeding)	Sex	Body wt. (g)	Bait consumed (g)	Warfarin consumed (mg/kg)
8	M	113.5	57.5	126.7
8	M	88.0	43.3	123.0
8	M	78.4	46.3	147.6
8	F	113.0	35.1	77.3
8	F	76.2	60.4	198.16
14	F	83.5	77.3	231.44

TABLE 3: SUMMARY OF DAILY POISON BAIT (0.025% WARFARIN) CONSUMED BY RATS (5 ALIVE AND 5 DEAD) SUBJECTED TO AN 8–DAY CONTINUOUS FEEDING

Rats	Amount of bait (g) consumed on day							
	1	2	3	4	5	6	7	8
A_1	1.5	9.8	8.5	7.1	11.8	5.1	6.7	7.0
A_2	8.7	6.9	6.4	5.3	7.9	0.6	8.5	5.0
A_3	6.3	8.5	6.4	3.8	6.2	5.1	7.9	2.3
A_4	5.0	6.0	7.8	8.1	11.2	9.7	5.3	7.3
A_5	8.3	6.3	3.6	8.3	2.1	2.8	0.5	3.2
D_1	8.4	6.9	6.2	7.9	2.9	9.0	2.4	*
D_2	6.7	8.1	7.2	7.8	10.2	2.0	3.2	4.7
D_3	6.3	5.5	4.8	9.0	5.5	1.2	8.7	*
D_4	9.4	9.1	9.0	6.3	11.8	8.5	1.7	6.5
D_5	6.0	6.3	6.7	4.7	7.2	8.0	1.7	*

A – alive 1,2,3, – males
D – dead 4,5 – females
* – dead on this day

The mean lethal dose for the males and females were 8.5 mg/kg (range 3.5 – 13.6) and 6.8 mg/kg (range 4.8 – 12.4) respectively. The mean duration to death was 7.5 days (2 – 17) for males and 6.9 days (2 – 15) for females. Fifty-five per cent of the animals died by the 2nd day and a male and female died on the 17th and 15th day respectively.

DISCUSSION

Warfarin resistance in rats is under genetical control involving single genes which may lie very close to each other or at the same locus (GREAVES and AYRES, 1967). In nature, the genes for warfarin resistance is not selected for (LUND, 1967) and as such it is unlikely for any warfarin resistant rats to occur when there are no control measures using warfarin (BENTLEY, 1970). Resistance to warfarin occurs when a failure or unsuccessful control is noted in areas where selection effect of warfarin has been applied over a period of time. It would be unwise to prospect for resistant rats in

areas where good control with anti-coagulants were achieved even though they have been applied for quite some time. It is only reasonable to screen for warfarin resistant rats following a failure in control programmes.

In most warfarin resistance screening studies, a 6-day continuous feeding on 0.025% warfarin bait (by weight) has been used as the deciding factor (WHO, 1975; DRUMMOND and BENTLEY, 1967; JACKSON and KAUKEINEN, 1972). In the present study, an 8-day continuous feeding used would not only meant a higher level imposed but also that survival indicates the possible occurrence of resistant rats. LEE and MUSTAFA (1982) obtained 95% mortality of susceptible *R. tiomanicus* over the 8-day feeding duration whereas in the present screening only 75% mortality was obtained. The duration to death and the mean lethal dose found in this study was far higher than that established by LEE and MUSTAFA (1982). These factors are evidences of increase tolerance to warfarin.

The normal susceptibility level of most rodent species to anticoagulants differs from each other (DUBOCK and KAUKEINEN, 1978) and for each species this has to be measured to enable the determination/ monitoring of resistance (DRUMMOND, 1971). For *R. tiomanicus* this has been done (LEE and MUSTAFA, 1982), and a 14-day continuous feeding period on 0.025% warfarin bait has been suggested as a suitable test for detecting warfarin resistance at LFP 95 within the upper 95% fiducial limit. At this feeding test a female survived a dosage of 231.4 mg/kg, a level requiring the animal weighing 83.5 g to consume 77.3 g of poison bait, an amount almost equivalent to its body weight.

The animals that survived the 8- and 14-day feeding tests had consumed more than half to equivalent of their body weight of baits. They would definitely survived the ordinary control practice undertaken in any warfarin baiting programme. These showed the built up of warfarin resistant rats. In the early 1960s when warfarin resistant rats were found in Wales and Scotland, Coumatetralyl, which is closely related to warfarin was offered as an alternative since it was more effective than the other anticoagulants against resistant rats (ANON., 1968). Preliminary results in Denmark showed similar findings (LUND, 1969). Subsequently, resistance to coumatetralyl was also noted (LUND, 1972). More recently it has been established that resistance to warfarin will confer resistance to other anti-coagulants like coumachlor, chloro-phacinone, pindone and other 'first generation' rodenticides (JACKSON, 1979). Baiting with any one of them in warfarin resistant areas would be unpromising.

Intermittent and irregular feeding upon poison baits were noted in both rats that survived and succumbed to the feeding tests. This was also noted in resistant *Rattus rattus diardii* by LAM *et al.*, (1982) and in coumatetralyl resistant rats in Denmark (LUND, 1972).

Total mortality was achieved with both 0.025% bromethalin and scilliroside baits. At this concentration, although consumption of bromethalin baits was low (3–7 g), total mortality was obtained as the dosage taken (mean 14.2, range 6.9 – 16.2 mg/kg) was beyond that required to achieve 100% mortality (KAMARUDIN and MAULUD, 1981). A further reduction in poison bait uptake was seen in scilliroside.

The animals succumbed to bromethalin within 16 – 24 hours whereas animals died from scilliroside by the second day. The animals after having consumed scilliroside baits were slightly weaker and feeble whereas poor co-ordination and continuous muscular twitching was seen with bromethalin poisoning. The short duration to death seen in bromethalin poisoning shows its acute nature. Scilliroside, a cardiac glycoside exhibits possible chronic poisoning if a small dose is taken.

Both bromethalin and scilliroside are single dose toxicants with the former characterised by enhanced bait consumption and delayed death (JACKSON, 1979). The latter acts as an emetic to other mammals and would be safe for use. As such, bromethalin and other members of the second generation rodenticide and/or scilliroside with other members of acute poisons would be alternatives for rodent control especially in areas with warfarin resistance.

CONCLUSION

Rats from areas under unsuccessful warfarin control programmes required a higher dose of warfarin and a longer duration to bring about death in screening tests under laboratory conditions. Only 75% mortality was attained in contrast to 95% obtained with susceptible rats (LEE and MUSTAFA 1982) for an 8-day feeding period. Screening with 14-day feeding period as proposed by LEE and MUSTAFA (1982) for detecting warfarin resistance gave one survival out of 20. Intermittent and irregular feeding was noted in both rats that succumbed and survived the feeding tests. The survived rats consumed large amounts of the baits indicating that they would survive any warfarin control programmes. These showed that resistance to warfarin has developed. Warfarin resistance is cross-resistance to other 'first generation' rodenticides and hence it is pointless to bait with them.

Total mortality of the resistant rats was obtained with 0.025% bromethalin and scilliroside baits. A lower bait consumption and duration to death was noted. Cases of chronic death was exhibited in scilliroside poisoning. Bromethalin baits can be improved by using a lower concentration of the poison in the bait. Bromethalin with other 'second generation' rodenticides and/or scilliroside with other acute poisons would be alternatives towards the control of rats in resistant areas.

ACKNOWLEDGEMENT

The authors would like to thank the Director-General of MARDI for the facilities and assistance provided. Thanks are also extended to fellow officers of the Cocoa and Coconut Branch, MARDI for their constructive suggestions and criticisms in the preparation of this manuscript. Thanks are also due to Sandoz Ltd. for the supply of scilliroside.

SUMMARY

Rattus tiomanicus from cocoa-coconut fields in Hilir Perak under unsuccessful warfarin control programmes were live-trapped and brought back to the laboratory. They were conditioned and provided with food and water *ad libitum*. Subsequently two batches (10 males and 10 females each) were provided with 0.025% warfarin in wax maize base for 8- and 14-days' no-choice feeding while two other batches were given 0.025% bromethalin and scilliroside baits respectively for 1-day. Five out of 20 rats survived the 8-days test while one survived the 14-day feeding period and none survived the bromethalin and scilliroside feeding tests.

Animals that succumbed to the warfarin tests required a higher dose and a longer duration to death in contrast to that reported by LEE and MUSTAFA (1982) for susceptible rats. Intermittent and irregular feeding on warfarin baits were noted in both rats that succumbed and survived the feeding tests. Survived rats consumed baits from half to almost the equivalent to their body weights. To tolerate such high doses, they would definitely survived any warfarin control programmes in the field. This showed that the animals have built up resistance to warfarin.

Bromethalin and scilliroside at 0.025% were able to bring about 100% mortality of the rats with low bait consumption and in the case of bromethalin a short duration to death. Bait consumption for bromethalin could be enhanced with delayed death of 1–2 days by having a lower bait concentration. Being able to bring about total mortality, bromethalin with other 'second generation' rodenticides and/or scilliroside with other acute poisons would be alternatives towards the control of rodents especially in areas with warfarin resistant rats.

REFERENCES

ANON (1968). Coumatetralyl and warfarin resistant in rats and mice. Infestation Control Lab. Techn. Circular No: 19, Min. of Agric. Fisheries and Food, Hook Rise, Tolworth, Surbiton, Surrey.

BENTLEY, E.W. (1970). Development in methods for the control of rodents. R.H.S. 3 : 129–130.

BOYLE, C.M. (1960). Case of apparent resistance of *Rattus norvegicus* Berkenhout to anticoagulant poisons. *Nature London* 188 : 517.

DRUMMOND, D.C. (1971). Warfarin resistant rats – some practical aspects. PANS 17(1) : 5–8.

DRUMMOND, D.C. and BENTLEY, E.W. (1967). The resistance of rodents to warfarin in England and Wales. In Report of the Int. Conf. on rodents and rodenticides, Paris, European and Mediterranean Plant Protect. Orgn. p. 57–67.

DUBOCK, A.C. and KAUKEINEN, D.E. (1978). Brodifacoum (Talon™ rodenticide). A novel concept. 8th Vertebrate Pest Conf. Sacramento, California, 25 pp.

GREAVES, J.H. and AYRES, P. (1967). Heritable resistance to warfarin in rats. *Nature London* 215 : 877.

GREAVES, J.H. and AYRES, P. (1969). Some rodenticidal properties of coumatetralyl. *J. Hyg. Camb.* 67 : 315–322.

JACKSON, W.B. (1979). New developments in rodenticides. In Great Plains Wildlife Damage Control Workshop, Manhattan, Kansas, U.S.A.

JACKSON, W.B. and ASHTON, A.D. (1979). Present distribution of anticoagulant resistance in the United States. 8th Steenbook Symposium, vitamin K metabolism and vit. K-dependent proteins, Univ. of Wisconsin, Maddison. In Suttie, J.W. ed. Vit. K metabolism and vit. K-dependent proteins. Univ. Park Press, Baltimore. pp. 392–397.

JACKSON, W.B. and KAUKEINEN, D.E. (1972). Resistance of wild Norway rats in North Carolina to warfarin rodenticides. *Science* 176 : 1343–1344.

KAMARUDIN, K.A. and MAULUD, A.R. (1981). Prestasi bromethalin, racun yang mempunyai ciri-ciri baru untuk mengawal tikus. *Tek. Pert.* 2(2) : 140–148.

LAM, Y.M., LEE, A.K., MOHAN, E. and TAN, Y.P. (1982). A case of warfarin resistance in *Rattus ratts diardii* (Jentink). *MARDI Res. Bull.* 10 : 378–383.

LEE, C.H. and MUSTAFA, MD. D. (1983). Laboratory evaluation of 0.02% warfarin against *Rattus rattus tiomanicus*. *MARDI Res. Bull.* 11(2) *(in press)*.

LUND, M. (1967). Warfarin resistance in Denmark. In Rept. Intl. Conf. Rodents and Rodenticides, Paris EPPO Publ. Ser. A. 41 : 49–57.

LUND, M. (1969). SchrReihe Ver. Wass-Boden-u. Luft-hyg. p. 32–37.

LUND, M. (1972). Rodent resistance to the anticoagulant rodenticides with particular reference to Denmark. Bull. Wld. Hlth. Org. 47 : 611–618.

OPHOF, A.J. and LANGEVELD, D.W. (1968). Warfarin resistance in the Netherlands. WHO/VC/68.109, 5pp.

TELLE, H.J. (1972). Resistance of the Norway rats to Warfarin in the Federal German Republic. (In German). ANZ, Schaedlings – Kunde 45 (2) : 17–20.

WHO, (1975). Instructions for determining susceptibility or resistance of rodents to anticoagulant rodenticides. WHO/-VBC/75.595, 7pp.

WOOD, B.J. and LIAU, S.S. (1977). Preliminary studies on the toxicity of anticoagulants to rats of oil palms, with special reference to the prospect of resistance. In Int. Dev. in oil palms. E.A. Earp and W. Newall ed. Incorp. Soc. Plrs. Kuala Lumpur, p. 641–659.

Accepted for publication on 11th May, 1983.