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Preliminary Study Of The Genetics Of Resistance In The House Mouse

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PRELIMINARY STUDY OF THE GENETICS OF RESISTANCE IN THE HOUSE MOUSE

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ABSTRACT: A wild house mouse (*Mus domesticus)* population originally trapped near Reading, Berkshire, United Kingdom, and maintained as a colony in the laboratory, was subjected to the discriminating feeding period of the warfarin resistance test, as used by Wallace and MacSwiney (1976) and derived from the work of Rowe and Redfern (1964). Eighty percent of this heterogeneous population survived the resistance test. A similar proportion of the population was found to survive the normally lethal dose of bromadiolone administered by oral gavage. The majority of this population of mice were classified as "warfarin-resistant" and "bromadiolone-resistant." The dose of 10mg.kg^{-1} of bromadiolone administered by oral gavage appeared to give good discrimination between susceptible and resistant individuals. The results of breeding tests indicate a single dominant gene that confers both "warfarin-resistance" and "bromadiolone-resistance," with complete expression of the resistance genotype in both males and females. Individual mice were classified as to genotype by back-crossing to a homozygous-susceptible strain, and resistance-testing the F1 generation. Separate strains of homozygous-resistant and homozygous-susceptible house mice are now being established.

KEY WORDS: rodenticide resistance, warfarin, bromadiolone, house mouse

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INTRODUCTION

House mice (*Mus domesticus*) (Marshall and Sage 1981) have a naturally low susceptibility to first generation anticoagulants. For example, the acute oral LD_{50} is reported for warfarin to be 374 mg.kg^{-1} (Hagan and Radomski 1953) and for diphacinone to be 250 to 300 mg.kg^{-1} (Itoh et al. 1973).

Warfarin resistance in the house mouse was first described by Dodsworth (1961), although at that time it was not considered to be widespread. Rowe and Redfern (1964) acknowledged that some house mouse populations were extremely difficult to kill with warfarin and, therefore, investigated its toxicity to mice in the laboratory. The test animals were obtained from breeding pens which were stocked from 13 different localities where no previous warfarin treatment had been carried out. In the test, individually caged mice were offered an unrestricted amount of 250 ppm warfarin for a fixed number of days, weighing food consumption, and recording survival or mortality. Thirteen mice were tested over a 28-day feeding period resulting in complete mortality. However, of 53 mice tested over a 21-day feeding period, five animals survived. Rowe and Redfern (1965) suggested that these survivors are indicative of the probable presence of mice "resistant" to 250 ppm warfarin in any sizable mouse population.

Apart from the work of Rowe and Redfern (1964), which was concerned specifically with the toxicity of warfarin to house mice, there is no published laboratory test to identify anticoagulant-resistance in the house mouse. On the basis of the results of Rowe and Redfern (1964), Wallace and MacSwiney (1976) used a 21-day feeding period on a 250 ppm bait as a warfarin-resistance test to discriminate between warfarin-resistant and warfarin-susceptible individuals. In this way, they were able to demonstrate that warfarin resistance in the house mouse was controlled by a major resistance gene, which they designated "War;" whose expression was very variable and strongly influenced by modifiers (MacSwiney and Wallace 1978).

This paper deals with a stock of wild house mice (*Mus domesticus*) trapped near Reading, Berkshire, United Kingdom (UK). The mice were initially shown to be resistant to warfarin by the 21-day warfarin feeding test (Rowe and Redfern 1964). Subsequently, it was found that the warfarin resistant stock were relatively resistant to bromadiolone, and a dose of bromadiolone was established that would distinguish between the susceptible and resistant phenotypes. This was then used to demonstrate single factor inheritance of the resistance.

METHODS

Wild anticoagulant-resistant stock were derived from mice trapped from a warfarin-resistant infestation near Reading (Berkshire, UK), and subsequently maintained in population pens. The anticoagulant-susceptible stock were albino Swiss mice (CD-1) obtained from Charles River UK Ltd.

Except where stated, all animals were maintained on laboratory diet (PCD MOD [C] FG; Special Diet Services; Wiltham, Essex, UK). This diet contains a supplement of vitamin K_3 at 10 ppm.

Warfarin was obtained as a proprietary 250 ppm bait formulation. Bromadiolone was obtained as a proprietary 0.1% liquid concentrate and as a 0.23% liquid concentrate. The concentrations of warfarin and bromadiolone were verified by HPLC.

Lethal Feeding-Period Test

Five male and five female wild mice were weighed, individually caged in stainless steel test cages with mesh floors, and maintained on ground laboratory diet in food bowls which were held in place by metal clips. Food consumption was measured daily for each mouse, taking spillage into account. The test animals were then presented with the 250 ppm warfarin diet for a no-choice feeding period of 21 days. Again food consumption was measured daily for each mouse, taking spillage into

account. The animals were then maintained on the ground laboratory diet for an observation period of 28 days. Throughout the experiment, the test animals were inspected for signs of toxicosis. At death, or at the end of the observation period, the animals were weighed again.

Intubation

Groups of animals were either received from Charles River UK Ltd., or removed from the population pen, and maintained in single sexed groups for an acclimatization period. They were then weighed, and dosed by gavage with the required concentration of bromadiolone at a rate of 1 ml per 100 g body weight; to achieve the highest dosage of 34.5 $mg.kg^{-1}$, the 0.23% concentrate was dosed at a rate of 1.5 ml per 100 g body weight. Animals were not starved prior to dosing. They were then maintained in single sex groups for an observation period of 21 days. Throughout the experiment, the test animals were inspected for signs of toxicosis.

RESULTS

Feeding Test With Warfarin

The results of the 21-day warfarin feeding test are presented in Table 1. Only two out of the ten animals died. They had consumed less than 30% of the warfarin formulation consumed by the survivors. The eight survivors showed no reduction in daily food consumption during the test, demonstrating a high level of resistance to warfarin. The contrast in the amount of active ingredient consumed by the survivors and those that died, suggests two distinct levels of susceptibility to the anticoagulant. The results indicate that 80% of the house mouse stock were warfarin-resistant and 20% were warfarin-susceptible.

Table 1. Wild house mice—mortality and warfarin dose consumed during 21-day no-choice feed on warfarin bait.

		Dose of warfarin consumed ($mg.kg^{-1}$)	
		Survived	Died
Sex	Mortality	mean (range)	mean (range)
Male	0/5	808 (657-1084)	-- --
Female	2/5	973 (842-1077)	195 (149-241)

Bromadiolone Toxicity By Gavage

The results of the initial bromadiolone gavage tests with the "Reading" house mice are presented in Table 2. Of the 30 mice that received a potentially lethal dose of bromadiolone, three animals died (10%). The proportion of animals that survived bromadiolone was comparable with the 80% of animals that survived the 21-day warfarin feeding test, suggesting a high level of cross-resistance between the two anticoagulants.

The results of the bromadiolone gavage tests with the anticoagulant-susceptible Swiss mice are presented in Table 3. A bromadiolone dose of 1.8 $mg.kg^{-1}$ body weight achieved incomplete mortality in both males and females. A dose of 0.9 $mg.kg^{-1}$ gave no mortality, and a dose of 3.6 $mg.kg^{-1}$ and above, gave complete mortality.

Table 2. Wild house mice—mortality following intubation with bromadiolone over a range of doses.

Dosed ($mg.kg^{-1}$)	Sex	Mortality
2.9	male	1/3
2.9	female	0/3
5.8	male	1/3
5.8	female	1/3
11.5	male	0/3
11.5	female	0/3
34.5	male	0/6
34.5	female	0/6

Table 3. Anticoagulant-susceptible Swiss mice—mortality following intubation with bromadiolone.

Dosed ($mg.kg^{-1}$)	Sex	Mortality
0.9	male	0/10
0.9	female	0/10
1.8	male	7/10
1.8	female	7/10
3.6	male	10/10
3.6	female	10/10
7.2	male	10/10
7.2	female	10/10

Breeding Experiments

The inheritance of the bromadiolone resistance was examined by setting up a series of breeding experiments in which the putative resistance gene is represented by *R*, and its allele for susceptibility is represented by *r*. The principal type of design employed was the Test-Cross, in which the wild mouse of unknown genotype (*RR*, *Rr* or *rr*) is crossed with a mouse of the anticoagulant-susceptible Swiss strain, which is of known genotype *rr*. In a Test-Cross, assuming unifactorial dominant inheritance, the expected proportions of resistant offspring of homozygous resistant (*RR*), heterozygous (*Rr*) and homozygous susceptible (*rr*) parents are 100%, 50%, and 0%, respectively. If the Test-Cross offspring were given a dose of bromadiolone that would selectively kill susceptible animals, then assuming a dominant gene, mortality for offspring of homozygous resistant (*RR*), heterozygous (*Rr*) and homozygous susceptible (*rr*) parent would be 0%, 50%, and 100%, respectively.

As a discriminating "Gavage Test" bromadiolone was administered at 10 $mg.kg^{-1}$ body weight, a dose between 5.7 and 10.1 times greater than published LD_{50} values, and which would be lethal to susceptible Swiss mice (Table 3), but that was much less than the maximum dose survived by the putative "resistant" Campus Main Water Distribution System Upgrade - Phase I house mice (Table 2).

The results of Test-Crosses for 29 untreated wild mice are shown in Table 4. Significantly more females (10/14) than males (5/15) were homozygous resistant (χ^2 = 5.32; d.f. = 1; p = 0.02), suggesting a sex-related effect on the fitness of the resistance genotype.

Mortality using the male Test-Cross offspring was 19.8% (39/197), slightly but not significantly greater than the 18.4% using the female Test-Cross offspring; 29/158 ($0\chi^2$ = 0.049; d.f. = 1; p = 0.8-0.9).

Mortality among the offspring of putative heterozygotes was 44% (68/153), slightly less than the theoretical 50% (χ^2 = 1.67; d.f. = 1; p = 0.1 to 0.05). Litter size was substantially greater where the female parent was a Swiss mouse (mean litter size of 15.7; compared with 8.6 for wild females), presumably owing to the superior mothering ability of the domesticated strain.

If we define resistance as 100% survival, and susceptibility as 100% mortality in the Gavage Test, then the results are consistent with the hypothesis that the bromadiolone resistance is due to a single dominant autosomal gene. On this basis, assuming no selection within the population pen, the frequency of the resistance gene can be calculated to be 0.74, and the theoretical frequencies of the *RR*, *Rr*, and *rr* genotypes as 0.55, 0.38, and 0.07, respectively.

Development of Wild Homozygous Strains

The breeding nucleus of a putatively homozygous resistant strain of pure wild ancestry was formed from the eight mice (two males and six females) indicated by asterisk in Table 3. To date all 40 of their offspring have received and survived a Gavage Test.

The female mouse 94/16 (Table 3) was shown by the Test-Cross to be of the susceptible (*rr*) genotype. It was crossed with a wild heterozygote and the offspring were Test-Crossed. By this means, two homozygous susceptible (*rr*) offspring were identified, and were mated together to form the breeding nucleus of a bromadiolone-susceptible strain of pure wild ancestry. The Gavage Test has given complete mortality in all eight of their offspring tested to date.

DISCUSSION

Published LD_{50} values for bromadiolone are 0.99 $mg.kg^{-1}$ (Meehan 1978) and 1.75 $mg.kg^{-1}$ (Grand 1976). Other published bromadiolone toxicity data against the house mouse refer to no-choice feeding tests, where the animals are fed bromadiolone bait, normally containing 50 ppm of active ingredient for a pre-determined period of whole days. Since a mouse would normally consume between 10 to 20 $mg.kg^{-1}$ body weight of active ingredient per day, feeding on a 50 ppm formulation in a no-choice situation, this type of toxicity data is of little value in determining the toxicity of bromadiolone to a susceptible house mouse.

The bromadiolone toxicity data presented for anticoagulant-susceptible Swiss mice in Table 3 correspond well with the published acute oral LD_{50} value of 1.75 $mg.kg^{-1}$ (Grand 1976), and provide support for the Gavage Test, that dosing at 10 $mg.kg^{-1}$ is lethal to anticoagulant-susceptible mice.

Although the risk that the test misidentified a few animals cannot be excluded at this stage, the results of the breeding study strongly indicate a genetic basis for bromadiolone resistance in the wild "Reading" mice, which is consistent with a single dominant autosomal gene controlling the resistance phenotype.

There are numerous reports in the literature of house mice suspected to be resistance to the second generation anticoagulant bromadiolone, based on their survival of a 21-day feeding test on the field strength (50 ppm) formulation.

Rowe et al. (1981) investigated the efficacy of 50 ppm bromadiolone against groups of house mice in a pen environment with alternative food available, and in six field treatments. Survivors from both the pen and the field were subjected to either a 50 ppm or a 100 ppm bromadiolone formulation for a no-choice feeding period of 21 days. Ten individuals survived after consuming between 118 and 410 $mg.kg^{-1}$ body weight of bromadiolone.

MacNicoll and Gill (1987) considered 11 out of 30 wild house mice were resistant to bromadiolone following their survival of a 21-day feeding test on a 50 ppm bromadiolone formulation.

Lund (1984) investigated the effect of bromadiolone against the *Mus musculus* species of house mice trapped from Denmark and southern Sweden, using no-choice feeding tests of up to ten days duration, and formulation strengths of 50 ppm and 100 ppm. He reported mice surviving following consumption of up to 115.8 $mg.kg^{-1}$ body weight of bromadiolone, and considered that this was resistance of practical importance.

Unlike the major gene controlling warfarin-resistance in the house mouse (Wallace and MacSwiney 1976), the putative gene controlling bromadiolone resistance in the "Reading" mice would appear to be fully expressed in both males and females. This may be an artifact of the test doses chosen, since in resistance studies the expression of a gene always varies with the dose administered.

Rowe and Redfern (1967) in a study with LAC Grey mice, noted that male mice were more susceptible to warfarin than female mice. The incomplete dominance of the warfarin resistance gene, as reported by Wallace and MacSwiney (1976) may indicate that the 21-day feeding test on a 250 ppm warfarin bait was too severe to effectively separate resistant from susceptible male individuals. Although this discriminating feeding period distinguished resistant and susceptible female mice effectively, a number of male warfarin-resistant mice must have died as a result of the feeding test, and have been misclassified. The original toxicity data of Rowe and Redfern (1964) made no attempt to establish a resistance baseline, and gave no mention of the increased susceptibility of male mice, which they were to report in their later publication.

Although practical bromadiolone resistance occurs in field populations of house mice, the authors have as yet no evidence that the levels detected in the "Reading" stock are of significance. This will require further investigation.

Table 4. Genotype determination of wild house mice, by performing a Test Cross with an anticoagulant-susceptible mouse, and testing the litter with a Gavage Test (dosing bromadiolone at 10 $mg.kg^{-1}$ body weight).

Animal No.	Sex	Test Cross Litter		Mortality following Intubation of Bromadiolone at 10 $mg.kg^{-1}$		Suspected Resistance Status of Wild Parent
		Males	Females	Males	Females	
93/1*	male	4	7	0/4	0/7	*RR*
93/2*	male	5	9	3/5	3/9	*Rr*
93/3*	female	6	4	0/6	0/4	*RR*
93/5*	female	3	7	0/3	0/7	*RR*
93/6*	female	11	0	0/11	0/0	*RR*
93/7*	female	7	2	0/7	0/2	*RR*
93/8*	female	1	9	0/1	0/9	*RR*
93/9*	female	1	7	0/1	0/7	*RR*
93/10	male	6	8	4/6	6/8	*Rr*
93/11*	male	6	6	0/6	0/6	*RR*
93/12	male	11	8	9/11	3/8	*Rr*
93/13	male	6	8	1/6	1/8	*Rr*
93/14	male	6	5	2/6	2/5	*Rr*
94/1	male	8	6	4/8	1/6	*Rr*
94/2	male	8	5	6/8	3/5	*Rr*
94/3	female	4	4	2/4	1/4	*Rr*
94/4	male	7	5	5/7	1/5	*Rr*
94/5	male	7	7	2/7	2/7	*Rr*
94/6	male	3	4	1/3	0/4	*Rr*
94/7	male	7	5	0/7	0/5	*RR*
94/8	male	5	10	0/5	0/10	*RR*
94/12	female	5	2	2/5	0/2	*Rr*
94/13	female	5	3	0/5	0/3	*RR*
94/15	female	4	3	0/4	0/3	*RR*
94/16	female	4	5	4/4	5/5	*rr*
94/17	male	6	8	0/6	0/8	*RR*
94/18	female	5	3	0/5	0/3	*RR*
94/20	female	4	5	0/4	0/5	*RR*
94/21	female	3	3	3/3	1/3	*Rr*

RR = homozygous resistant
Rr = heterozygous resistant
rr = homozygous susceptible

*This was referred to earlier in text.

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