Rodenticidal effects of a mixture of warfarin and vitamin D₃ as poison baits

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(Received: 27 May 1999; Accepted: 6 November 1999)

Key words: warfarin, vitamin D₃, poison bait, laboratory rat, black rat, rodenticidal effect

Abstract: A mixture of baits containing 0.025% warfarin and 0.05% vitamin D₃ showed high toxicity to laboratory rats (Wistar strain) and black rats, Rattus rattus (probably warfarin-resistant). In laboratory rats, the acute LD₅₀ value of the mixture baits was 1.42 g/100 g b.w. and the active ingredients ingested were warfarin 3.6 mg/kg b.w. and vitamin D₃ 7.1 mg/kg b.w. Mean lethal days in the mixture baits decreased to about 1/124 of that of the warfarin baits when it was compared with the previous results using warfarin-resistant rats (Tanikawa 1991) and mean active ingredient intake (dose of warfarin and vitamin D₃) also decreased to about 1/29. The results suggest that the mixture baits are more effective than poison baits containing only 0.025% warfarin.

Introduction

There are some reports of the rodenticidal effect of a mixture of warfarin and vitamin D₂ (Lund, 1974; Greaves et al., 1974; Bai et al., 1978). Recently, the synergistic effect of warfarin and vitamin D₃ was clearly observed in acute oral toxicity to laboratory rats, Rattus norvegicus (Tanikawa and Kusano, 1993).

The present report has confirmed the toxic effect of a mixture of warfarin and vitamin D₃ as poison baits to laboratory rats and black rats, R. rattus.

Materials and Methods

Preparation of poison baits

Technical grade warfarin purchased from Aldrich Chem. Co. Inc. and vitamin D₃ from Riken Vitamin Co., Ltd. were mixed thoroughly with standard bait flour. The concentrations of warfarin and vitamin D₃ were 0.025% and 0.05%, respectively.

Test rats

Male laboratory rats of Wistar strain were employed at 10 weeks from birth. Male and female black rats were trapped in the Shinjuku district in Tokyo, where high warfarin resistance in the species has
Table 1. Mortality and survival time of male laboratory rats (Wistar strain) by baits intake of 0.025% warfarin and 0.05% vitamin D₃.

<table>
<thead>
<tr>
<th>Poison baits given (g/100 g b.w.)</th>
<th>Poison baits ingested (g/100 g b.w.)</th>
<th>Body weight (mean, g)</th>
<th>Mortality</th>
<th>Days to death (mean days ±SD)</th>
<th>Intake (a.i.* mg/kg)</th>
<th>Warfarin</th>
<th>Vitamin D₃</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.00</td>
<td>1.00</td>
<td>289.4</td>
<td>1/10</td>
<td>3 ±0</td>
<td>2.5</td>
<td>5.0</td>
<td></td>
</tr>
<tr>
<td>1.40</td>
<td>1.36</td>
<td>292.4</td>
<td>3/5</td>
<td>4-6 5.0±0.81</td>
<td>3.4</td>
<td>6.8</td>
<td></td>
</tr>
<tr>
<td>1.96</td>
<td>1.87</td>
<td>286.0</td>
<td>4/5</td>
<td>4-5 4.5±0.50</td>
<td>4.7</td>
<td>9.4</td>
<td></td>
</tr>
<tr>
<td>2.25</td>
<td>2.22</td>
<td>290.0</td>
<td>5/5</td>
<td>4-6 4.8±0.75</td>
<td>5.6</td>
<td>11.1</td>
<td></td>
</tr>
<tr>
<td>3.84</td>
<td>3.84</td>
<td>308.4</td>
<td>5/5</td>
<td>3 ±0</td>
<td>9.7</td>
<td>19.3</td>
<td></td>
</tr>
</tbody>
</table>

* a.i., active ingredient.

Table 2. Rodenticidal effect of a mixture of 0.025% warfarin and 0.05% vitamin D₃ and 0.025% warfarin baits against black rats, *Rattus rattus*.

<table>
<thead>
<tr>
<th>No. of rats tested (♀ : ♂)</th>
<th>Body weight (mean, g)</th>
<th>Days to death (mean days ±SD)</th>
<th>Total poison baits ingested (mean, g/100 g b.w.)</th>
<th>Intake (mean, a.i. mg/kg)</th>
<th>Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>9 (4 : 5)</td>
<td>117.3 (79-170)*</td>
<td>5.6±1.2</td>
<td>11.9 (6.5-21.4)*</td>
<td>29.8</td>
<td>59.5</td>
</tr>
<tr>
<td>21 (9 : 12)**</td>
<td>96.8 (56-155)</td>
<td>160.0±138.3 (8-441)</td>
<td>1,481.5 (34.1-3,481.5)</td>
<td>3,703.8</td>
<td>21/21</td>
</tr>
</tbody>
</table>

* Range.

** Only 0.025% warfarin baits against warfarin-resistant black rats (Tanikawa, 1991).

been confirmed (Tanikawa, 1991). Therefore, the black rats tested were probably warfarin-resistant. After capture, these black rats were reared on plain baits (CLEA Japan Inc., Tokyo, trade name CE-2) for four weeks in the laboratory and employed as test animals.

**Test procedure**

Individually caged laboratory rats were given poison baits for a certain time in five dosages of 1.0, 1.4, 1.96, 2.25 and 3.84 g/100 g b.w. (body weight). After more than 95% of the poison baits was consumed in 4 hours, plain baits (CE-2) were given. When the percentage of poison baits consumed was less than 95%, feeding time was prolonged to 20 hours. After 24 hours from the start of the feeding plain baits (CE-2) were given. Survival or death in laboratory rats was observed for 10 days. Dead rats were dissected and pathological changes in various tissues were examined by the naked eye. The black rats were continuously reared with poison baits till death. Drinking water was provided *ad lib* to each test rat.

**Results**

The relationship between the amounts of poison baits given in five dosages and the mortality in male laboratory rats is shown in Table 1. The mortality increased with increase of the amounts of poison baits given, and the days to death varied from 3 days to 6 days (the 4th day to the 7th days). Feeding 2.25 g or more of poison baits completely killed the laboratory rats. The LD₅₀ value and its 95% confidence limits calculated after the method of Litchfield and Wilcoxon (1949) were 1.42 g/100 g b.w. and 1.09 to 1.85 g/100 g b.w., respectively. The doses of active ingredients ingested were as follows: warfarin 3.6 mg/kg b.w. and vitamin D₃ 7.1 mg/kg b.w.

In the pathological dissection of the dead rats, the following results were obtained. External haemorrhages occurred around the mouth (1 individual), nose (2 individuals) and eyes (one eye-1 individual, two eyes-3 individuals). Significant internal haemorrhages occurred around the
kidneys and the dorso-lateral wall in the under parts of kidneys (7 individuals),
around testicles (one testicle 6 individuals, two testicles-2 individuals), inside of both
swollen stomach (5 individuals) and small intestine (10 individuals), and lungs (8 indi-
viduals). In some individuals, haemorrhages occurred in the thoracic cavity (1 individual),
brain (3 individuals), mesenteries (2 individuals) and subcutaneous part of the jaw (1 individual).

As shown in Table 2, continuous feeding of poison baits to black rats resulted in
100% mortality. Days to death varied from the 4th day to the 9th day and the mean value was 5.6 days. The amount of
poison baits ingested varied from 6.5 g to 21.4 g/100 g b.w. and the mean value was
11.9 g/100 g b.w. The amounts of active ingredients were as follows: warfarin 29.8
mg/kg b.w. and vitamin D₃ 59.5 mg/kg b.w.

**Discussion**

In the present experiments with laboratory rats (Wistar strain), the acute LD₅₀ value of the poison containing 0.025%
warfarin and 0.05% vitamin D₃ was 1.42 g/100 g b.w. and the active ingredients ingested were warfarin 3.6 mg/kg b.w.
and vitamin D₃ 7.1 mg/kg b.w. In the previous report with laboratory rats of SD
strain (Tanikawa and Kusano 1993), the acute oral LD₅₀ in a mixture of 0.025%
warfarin and 0.05% vitamin D₃ (mixture rate-1:2) in oil suspension was as follows:
warfarin 9.7 mg/kg b.w. and vitamin D₃ 19.5 mg/kg b.w. Therefore, the acute toxic-
ity of the mixture is clearly stronger in the case of the ingestion of poison baits
than in the case of oral administration of oil suspension although the difference in the
strain of test rats used must be taken into consideration. The toxicity of the
former is about 2.7 times as high as that of the latter. Furthermore, variation of the
mean survival days to death in the former was 3.0 to 5.0 days and that in the latter
was 2.7 to 4.0 days. Thus, the speed of

Toxic action in the mixture is slightly slower in the former than in the latter.

Massive doses of vitamin D to rats damaged the circulatory system, digestive
system, excretory system and calcium metabolism, and multiple doses of warfarin
resulted in marked depression of blood coagulation activity and abnormality of
blood vessel structure (Kahn et al., 1971; Bai et al., 1978; Okawa et al., 1979; Shimoto-
suji, 1980; Kusano, 1982). Also, massive dosage of vitamin D₃ is acutely toxic, and
the speed of the toxic action is slow, although that of warfarin is slower than
that of vitamin D₃ (Tanikawa and Kusano, 1993). Generally, warfarin has been known
to be an anticoagulant that is chronically toxic by repeated ingestion (Kusano,
1982). The synergistic toxicity of the mixture to rats (SD strain) has been reported
(Tanikawa and Kusano 1993). In the present experiments, massive bleeding occurred
in several tissues in rats poisoned by the feeding of the mixture of poison baits.
These findings suggest that the toxic action by both warfarin and vitamin D₃ to
the blood vessel system and blood coagulation activity of rats is synergistic.

Tanikawa (1991) reported that in no-choice feeding tests of 0.025% warfarin
baits to warfarin-resistant black rats, mean lethal days, mean intake amount,
and mean active ingredient intake were 160 days, 1481.5 g/100 g b.w., and 3703.8
mg/kg b.w., respectively (Table 2). Mean lethal days in the present mixture baits
were reduced about 1/124 (29.8/3703.8
mg/kg) of that of the warfarin baits, and
the mean active ingredient intake (dose of
warfarin and vitamin D₃) of the present results decreased to about 1/29 (5.6/160
days) that of warfarin. Therefore, the
present results suggest that the toxicity of the poison baits containing 0.025% warfa-
rin and 0.05% vitamin D₃ to the test black rats, that are probably warfarin-resistant,
is higher than the poison baits containing
only 0.025% warfarin.
REFERENCES


実験用ラットとクマネズミに対するワルファリンとビタミンD3混合薬剤の殺鼠効力

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実験用ラットとクマネズミに対するワルファリンとビタミンD3混合薬剤の殺鼠効力

ラットでは、225 g 以上の投与群で 100% の死亡率で死亡は 3～7 日目に認められた。LD50 値はワルファリン 3.6 mg/kg + ビタミン D3 7.1 mg/kg が得られた。一方、ワルファリン抵抗性と思われるクマネズミを用い本薬剤を自由に摂取させたところ、4～8 日目に全個体が死亡し、平均致死薬量はワルファリン 29.8 mg/kg + ビタミン D3 59.5 mg/kg となった。

ラットの病理学的所見では、腎臓背側部に多量の出血が認められ、混合薬剤の血管系に対する共効果がみられた。また、強制的に経口投与のえて、毒剤として自由摂取させた方が、混合薬剤の中毒作用が低薬量で速効的に現れた。一方、クマネズミでは、ワルファリンの影響よりビタミンD3による影響で死亡した可能性が高かった。