The comparative rodenticidal effectiveness of several anticoagulants to laboratory rats and mice

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The development and use of anticoagulant rodenticides within recent years has provided an effective countermeasure to the ever increasing rat population. At present, several anticoagulant rodenticides have been used advantageusly in Japan, but it must be pointed out that little cosideration has been paid regarding their individual characteristics.

Bentley et al.[1] as well as other investigators[2,3,4] have shown differences in rodenticidal activities of anticoagulant chemicals when evaluation was made by the bait method. This report presents the results of comparative experiments aimed at discernment of differential effctiveness among some anticoagulants including two recently introduced chemicals, diphacinone and chlorophacinone, by an oral administration method.

Materials and Methods

The laboratory animals used in this test were male dd-strain mice and Wister-strain rats, weighing 17 to 22 g and 95 to 150 g, respectively. In both tests, one group of ten animals was used at each dosage level of test compounds which were prepared as suspensions in 1.5% carboxymethylcellulose (CMC). Acute toxicities were determined following a single oral administration employing a stomach catheter, and mortality was observed over the next 10 days.

In subacute toxicity tests, similar administration of a smaller dose was repeated once a day for 10 days or until all animals died, and the daily mortality was recorded for 20 days after administration of the initial dose.

Each rodenticide was tested at dosages of 2.5, 10 and 25 mg/kg/day in mice, and 0.25, 1.0, 2.5 and 10.0 mg/kg/day in rats. The chemicals under evaluation were the following:

warfarin	3-α-acetonylbenzyl-4-hydroxycoumarin
coumatetralyl	3-(α-tetralyl)-4-hydroxycoumarin
fumarin	3-(α-furyl-β-acetyl) ethyl-4-hydroxycoumarin
diphacinone	2-diphenylacetyl-1, 3-indandione
chlorophacinone	[(chlora-4-phenyl) 1 phenyl 1] acetyl 2 dioxo-1, 3-indandione

Results

Acute toxicity following a single dose. The dosage-mortality relationships for the 5 compounds in mice are presented in Fig. 1. Among the chemicals tested, the 2 indandiones were more effective than the 3 coumarin derivatives. Lethal dose fifty (LD_{50}) values were about 250 mg/kg for chlorophacinone and approximately 300 mg/kg for diphacinone, while those for warfarin, fumarin and coumatetralyl were estimated to be about 700, 700 and 950 mg/kg, respectively.

Fig. 2 shows the mortality in rats. None of the 5 chemicals showed typical dosage-mortality curves. Mortalities observed at the tested dosages were greater than 50% for the indandiones, while warfarin, fumarin

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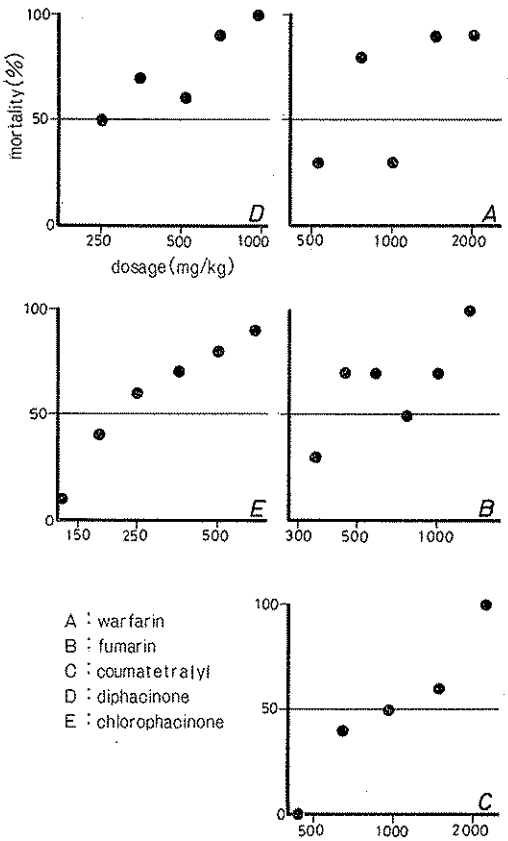

Fig. 1 Acute toxicities of 5 anticoagulants to male mice.

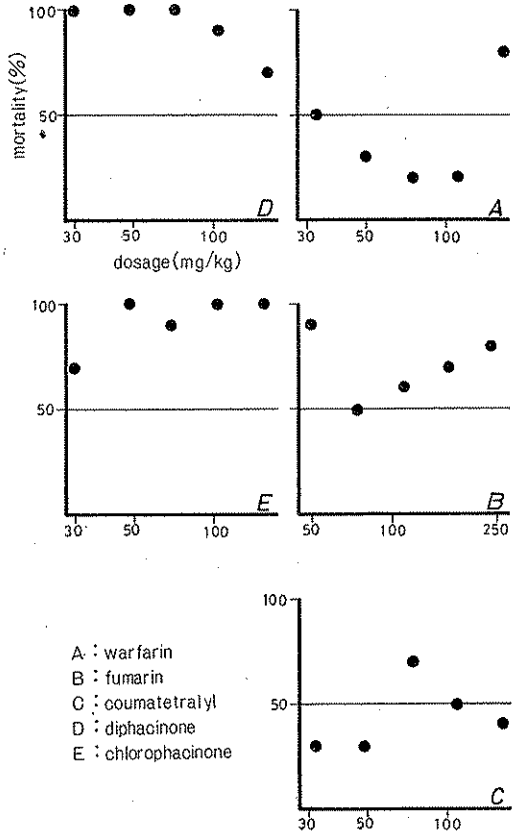

Fig. 2 Acute toxicities of 5 anticoagulants to male rats.

and coumatetralyl did not display a clear correlation with administered dosages.

Subacute toxicity following daily administration. Experimental results seen for mice are presented in Table 1 and those for rats are given in Table 2. When administrations were conducted daily over a 10 day test period, high mortality ranging from 90 to 100% were observed for all 5 chemicals at dosages of 10 and 25 mg/kg. However, when the the dosage was reduced to 2.5 mg/kg, mortality declined to 50% for warfarin and fumarin, and 30% for coumatetralyl; in contrast, mortality was 70% for chlorophacinone and 100% for diphacinone.

Initial mortality in mice was observed on the 3rd or 4th day following the first administration at a dosage of 25 mg/kg, and a similar tendency was observed even for lower dosages.

In comparison to the results shown for mice, dosages exceeding 1 mg/kg produced 100% mortality in rats for all 5 chemicals, while 0.25 mg/kg had the same effect for the 2 indandiones and coumatetralyl. On the other hand, only 40% mortality was observed for warfarin and no deaths were observed for fumarin. Similar to the case in mice, the first mortality in rats was observed on the 4th day at several dosages, but delayed mortality was observed in a few cases. These results are summarized in Table 3 and the time (day) of 50% lethality is shown in parenthesis.

Discussion

The acute toxicities obtained in the present study were rather low in mice for both coumarin derivatives and the indandiones, whereas those seen for rats were remarkably high, although these values may greatly

Table 1 Cumulative mortality in mice following daily administration of small doses of 5 anticoagulants

rodenticide	dosage mg/kg/day	Day following initial anmministration											mortality (%)
		1	2	3	4	5	6	7	8	9	10	11–20	
warfarin	2.5	0	0	0	0	1	1	3	4	4	4	5	50
	10.0	0	0	0	1	3	6	8	9	10			100
	25.0	0	0	0	1	5	7	7	8	9	9	9	90
fumarin	2.5	0	0	0	0	1	1	2	2	3	5	5	50
	10.0	0	0	0	0	4	7	7	8	8	9	9	90
	25.0	0	0	0	2	3	7	8	9	10			100
coumatetralyl	2.5	0	0	0	0	0	0	1	1	1	2	3	30
	10.0	0	0	0	0	0	4	5	7	8	8	8	80
	25.0	0	0	0	0	3	5	7	7	8	10		100
diphacinone	2.5	0	0	0	2	4	4	4	5	5	5	7	70
	10.0	0	0	0	4	7	8	9	10				100
	25.0	0	0	1	2	5	6	7	9	9	9	10	100
chlorophacinone	2.5	0	0	0	0	3	6	7	7	8	10		100
	10.0	0	0	0	0	5	8	8	8	9	9	9	90
	25.0	0	0	0	0	3	6	8	8	9	10		100

Table 2 Cumulative mortality in rats following daily administration of small doses of 5 anticoagulants

rodenticides	dosage mg/kg/day	Day following initial administration											mortality (%)
		1	2	3	4	5	6	7	8	9	10	11–20	
warfarin	0.25	0	0	0	0	0	0	1	1	2	2	4	40
	1.0	0	0	0	0	3	8	10					100
	2.5	0	0	0	1	7	9	10					100
	10.0	0	0	0	2	6	8	9	10				100
fumarin	0.25	0	0	0	0	0	0	0	0	0	0	0	0
	1.0	0	0	0	0	1	5	8	9	9	9	10	100
	2.5	0	0	0	0	4	5	8	10				100
	10.0	0	0	0	2	6	8	10					100
coumatetralyl	0.25	0	0	0	0	4	7	8	9	9	9	10	100
	1.0	0	0	0	0	6	9	9	10				100
	2.5	0	0	0	1	6	7	10					100
	10.0	0	0	0	6	7	9	10					100
diphacinone	0.25	0	0	0	1	1	5	6	6	8	9	10	100
	1.0	0	0	0	1	5	7	9	10				100
	2.5	0	0	0	1	4	6	8	10				100
	10.0	0	0	0	1	6	9	10					100
chlorophacinone	0.25	0	0	0	0	1	7	10					100
	1.0	0	0	0	1	7	9	9	10				100
	2.5	0	0	0	0	6	8	10					100
	10.0	0	0	0	4	7	10						100

Table 3 The LD50 values*1 for five anticoagulants

rodenticides	*2	single dose (mg/kg)	daily dose (mg/kg/day) 25.0	10.0	2.5	1.0	0.25
warfarin	M	≒750	100(4)*3	45(4.5)	25(11)		
	R	10−170		35(3.5)	8.75(3.5)	4.5(4.5)	2.5<(20<)
fumarin	M	≒700	112.5(4.5)	45(4.5)	22.5(9)		
	R	10−170		35(3.5)	12.5(5)	5.0(5)	2.5<(20<)
coumatetralyl	M	≒950	125(5)	60(6)	25<(20<)		
	R	10−170		25(2.5)	8.75(3.5)	3.5(3.5)	1.125(4.5)
diphacinone	M	300	100(4)	35(3.5)	17.5(7)		
	R	10>		35(3.5)	11.25(4.5)	4.0(4)	1.25(5)
chlorophacinone	M	250	112.5(4.5)	40(4)	11.3(4.5)		
	R	10>		35(3.5)	8.75(3.5)	3.5(3.5)	1.125(4.5)

*1 : The values for daily dose indicate the accumulated dosages which gave 50%death in the animals tested.

*2 : M-mouse, R-rat

*3 : The numbers in parenthesis indicate the day on which 50% mortality occurred.

differ according to investigator as cited in Kusano[5]. The high toxicities shown in rats may indicate that even a single intake of bait treated with these chemicals can cause death. However, further testing is required in order to more clearly establish values for the LD_{50}.

In the subacute testing, indandiones were more effective than the coumarin derivatives in both rats and mice. In tests with mice, the LD_{50} did not show sharp delineation since values were 4 to 4.5 days at dosages of 2.5, 10.0 and 25.0 mg/kg/day, while that of diphacinone at the lowest dosage was extended to 7 days. This delay in mortality was also observed for other chemicals showing values as follows: 9 days for fumarin and 11 days for coumatetralyl at dosages of 2.5 mg/kg; 6 days for warfarin at 10 mg/kg (only 40% of the test animals died). Thus, it was shown that for a dosage of 2.5 mg/kg/day, attainment of 50% mortality exceeded 10 days.

In the testing on rats, the time required to produce 50% mortality was shown to be 3.5 to 5 days at each dosage, while the dosages required to produce 50% mortality were one-tenth or one-hundredth less than those required in mice.

Comparison regarding the difference of effectiveness between single and repetitive administration illustrated several points. First, chlorophacinone and diphacinone were shown to be the more potent rodenticides of the chemicals tested irregardless of the administration schedule. Also, coumatetralyl given to mice was the least effective of the 5 chemicals studied; however, when given to rats, it demonstrated nearly the same effectiveness as the indandiones. On the other hand, some of the coumarin derivatives exhibited a delay in lethality in the lower dosage range for the 10 day test. Mortality failed to attain a value of 50% at lower dosages in the same test. These results may indicate a threshold concentration for these chemicals.

Acknowledgement

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数種の抗凝血性殺そ剤のマウスおよびラットに対する効力比較

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抗凝血性殺そ剤ワルファリン，フマリン，クマテトラリル，ダイファシノン，クロロファシノンの基礎効力を比較するため，マウス，ラットを用いて，強制投与法によって，その急性および亜急性（10日間連続投与による）効力を実験室内で調べた．

いずれの薬剤も，ラットに対する効力がマウスに対する効力よりも，急性では数倍から数十倍，また亜急性では10倍以上すぐれた．また，急性・亜急性とも，インダンジオン系のものがクマリン系のものより高い効力を示した．同系統の薬剤を比較した場合，インダンジオン系の二つの間には，差が認められなかったが，クマリン系の薬剤においては，マウスにおいては他の二つより低い効力しか示さなかったクマテトラリルが，ラットに対する亜急性効力では，インダンジオン系のものと同等の効力を示した．

亜急性効力による閾値は，インダンジオン系のものでは示されなかったが，クマリン系のものでは，マウスにおいては，クマテトラリルが1日あたり，2.5～10 mg/kg，またラットにおいてはフマリン，ワルファリンで1日あたり0.25～1.0 mg/kg であるように思われた．