抗ピロプラズマ剤TC-Aの特性

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著者	並河, 和彦 佐久間, 佳子 須永, 藤子
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Characteristics of Tetrocarcin-A Compared with Other Anti-Piroplasmotic Drugs

Kazuhiko NAMIKAWA, Yoshiko SAKUMA, Fujiko SUNAGA, and Yasunori KANNO

Department of Infectious Diseases, School of Veterinary Medicine, Azabu University, Fuchinobe, Sagamihara 229, Japan

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ABSTRACT. LD_{50}, ED_{50}, and the safety margin of tetrocarcin-A (TC-A) against *Babesia rodhaini* (*B.rodhaini*) were evaluated by our method, each value obtained showing 45.7 mg/kg, 2.19 mg/kg and 20.9, respectively. Its characteristics appeared inferior to diminazene, but almost equal to quinuronium with respect to safety.—KEY WORDS: *Babesia rodhaini*, safety margin, tetrocarcin-A.

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TC-A, a substance produced by fungi, inhibits the synthesis of nucleic acid and protein. TC-A is effective against sarcoma 180 and leukemia P388 in mice and also shows antibacterial activity against Gram-positive bacteria such as *Bacillus subtilis* [7]. TC-A is also effective against *B. rodhaini, Plasmodium berghei* (*P.berghei*) [2], and *Theileria sergenti* (*T.sergenti*) [6]. LD_{50} and ED_{50} of TC-A, however, have not been determined to evaluate its safety and efficacy against mouse piroplasmosis. In this study, TC-A was examined to determine the toxicity (LD_{50}) against mouse piroplasmosis and the safety margin (LD_{50}/ED_{50}), and the results were compared with those of other anti-piroplasmotic drugs.

MATERIALS AND METHODS

Procedure of study: LD_{50} of TC-A in mice was calculated by Litchfield-Wilcoxon's method [4] to compare with other drugs and to calculate safety margin, and LD_{10} was obtained from the linear dose response chart. Several doses descending from LD_{10} were administered to mice for 15 consecutive days, to determine the maximum tolerable and the minimum toxic doses. These two doses were settled as standard in the case of administration of TC-A to mice weakened by piroplasmosis. A dose, 10 mg/kg, close to the maximum tolerable dose was determined at will and was once administered to mice infected with *B.rodhaini*. From the changes of parasitemia in the medicated mice, the observation period necessary for the determination of ED_{50} was estimated. The maximum safety dose was settled by taking a geometric mean of the minimum toxic and the maximum non-toxic doses, and then several doses descending from the maximum safety dose were administered to *B.rodhaini* inoculated mice to determine ED_{50}. ED_{50} was calculated with the survival rates of mice injected with TC-A. Safety margin was expressed as the rate of LD_{50} to ED_{50}.

Animals and their raising conditions: Female ICR-Jcl mice, about 4 weeks (27–29 days) old and weighing 16–25 (mean 19.5) g, were purchased from Nihon Kurea Co., Ltd. Mice were kept in alminum cages, 30×20×12 cm in size, at 22±2°C and supplied with solid feed and tap water ad libitum.

Preparation of B.rodhaini inocula: Infected blood was collected, on day 3 after inoculation, from mice inoculated with *B.rodhaini* strain maintained in our laboratory. The

blood was diluted with Alsever's solution into a concentration of 2×10^7 parasitized RBC per 0.05 m*l*, and 0.05 m*l* of the diluted blood was intraperitoneally inoculated to mice.

Preparation and administration of TC-A solution: One dose of TC-A was dissolved in 0.1 m*l* of sterile distilled water and the solution was injected intraperitoneally to mice at rate of 0.1 m*l* per 10 g of body weight.

Determination of LD_{50} and LD_{10}: Forty mice were divided into 4 groups of 10 animals each. TC-A was intraperitoneally administered once to these groups in doses of 60.0, 50.0, 41.7 and 34.7 mg/kg respectively. LD_{50} and LD_{10} were calculated by Litchfield-Wilcoxon's method from the mortality rates of mice on day 7 after administration.

Determination of the minimum toxic dose and the maximum tolerable dose: One hundred and twenty mice were divided into 8 groups of 15 animals each, 7 treated and 1 control groups. The treated groups were intraperitoneally injected with the several doses descending from LD_{10}, 35.5, 17.8, 8.9, 4.45, 2.2, 1.1 and 0.55 mg/kg, for 15 consecutive days, and the control group was given only sterile distilled water. Mortality, body weight gains, and feed and water consumptions of the mice were checked throughout the experimental period. Cardiac blood was sampled from 3 mice of each group to count the number of WBC and RBC. Heparin was used as an anticoagulant.

Determination of the period to judge the efficacy: Three mice were used in this experiment. A single dose of 10 mg/kg of TC-a, close to the maximum tolerable dose, was intraperitoneally injected to mice on day 3 after the inoculation of *B.rodhaini*. To count the parasitized RBC, blood smears were prepared with peripheral blood and stained with Giemsa. Parasitized rates for 80 consecutive days following the inoculation were indicated as permillage (‰).

Determination of ED_{50} of TC-A against B.rodhaini infections: Eighty mice were divided into 4 groups of 20 animals. Four different doses, 3.13, 2.50, 2.00 and 1.60 mg/kg of TC-A, were administered to the group of mice respectively on day 3 after the inoculation of *B.rodhaini*. The highest dose of 3.13 mg/kg, the maximum safety dose, was determined by taking a geometric mean of the minimum toxic dose (4.45 mg/kg) and the maximum non-toxic dose (2.2 mg/kg) which were obtained by the administration for 15 consecutive days. ED_{50} was calculated by Litchfield-Wilcoxon's method from survival rates of mice on day 18 after the administration.

RESULTS

No mice died in 34.7 mg/kg dose group, and mortality was 30% in 41.7 mg/kg dose group, 70% in 50.0 mg/kg dose group, and 90% in 60.0 mg/kg dose group (Table 1). The results were plotted to make a probability chart (Fig. 1), and an Ld_{50} of 45.7 mg/kg and an LD_{10} of 35.5 mg/kg were obtained from the chart. The 95% confidence limit of LD_{50} was 40.4–51.7 mg/kg.

Table 2 and Figs. 2–4 show hematological findings, body weight gains, and feed and water consumptions of mice injected intraperitoneally with TC-A for 15 consecutive days to obtain the maximum tolerable dose and the minimum toxic dose. Thirteen mice died by day 4 in the maximum dose (35.5 mg/kg) group. In 17.8 mg/kg dose group, 4 mice died by day 10. No mice died in 8.9 mg/kg or less dose groups. From these results, a dose of 8.9 mg/kg seemed to be the maximum tolerable dose. Hematological examinations showed that no marked changes were observed in RBC counts by day 15 in any dose group (Table 2). WBC counts slightly increased in 4.45 mg/kg and

Table 1. Acute toxicity of TC-A in mice

Dose (mg/kg)[a]	Total number of mice used	Total number of dead mice on day 7 after administration	Mortality (%)	Contribution to χ^2
34.7	10	0	0 (0.93)[b]	0.0122
41.7	10	3	30	0.0028
50.0	10	7	70	0.0040
60.0	10	9	90	0.0010

a) by intraperitoneal administration
b) Values converted by Litchfield-Wilcoxon's method are shown in parentheses.

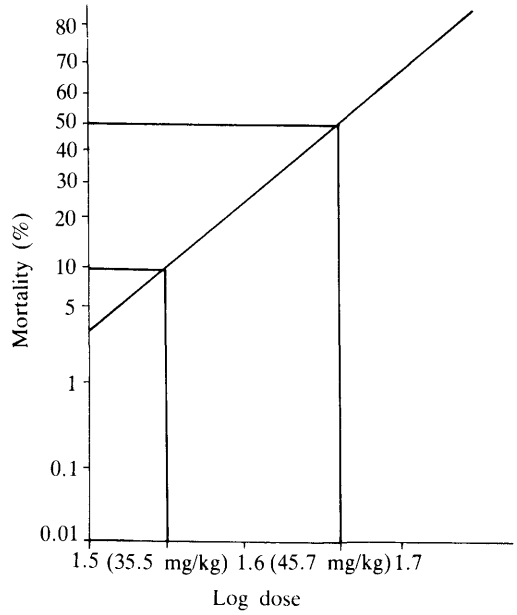

Fig. 1. Relation between log doses and mortality of mice in acute toxicity trials of TC-A, showing LD_{50} and LD_{10}.

8.9 mg/kg dose groups, whereas they increased markedly in both 17.8 and 35.5 mg/kg dose groups. Feed consumptions slightly decreased in both 4.45 mg/kg and 8.9 mg/kg dose groups, and markedly in both 17.8 mg/kg and 35.5 mg/kg dose groups (Fig. 2). Water consumption slightly decreased in 4.45 mg/kg dose group and markedly decreased in 8.9 mg/kg or more dose group (Fig. 3). Body weight gain was depressed in 4.45 mg/kg dose group, slightly decreased in 8.9 mg/kg dose group and markedly in both 17.8 mg/kg and 35.5 mg/kg

Table 2. Mean RBC and WBC counts in mice injected with TC-A for 15 consecutive days

Dose (mg/kg)	Hematology	Days of administration 5	10	15
	Number of mice	2	0	0
35.5	RBC×$10^4/\mu l$	778.5		
	WBC×$10^2/\mu l$	185.0		
	Number of mice	3	3	3
17.8	RBC×$10^4/\mu l$	693.7	706.0	739.3
	WBC×$10^2/\mu l$	191.3	143.0	121.3
	Number of mice	3	3	3
8.9	RBC×$10^4/\mu l$	716.7	852.7	724.7
	WBC×$10^2/\mu l$	86.0	41.3	70.3
	Number of mice	3	3	3
4.45	RBC×$10^4/\mu l$	800.3	823.7	960.7
	WBC×$10^2/\mu l$	89.7	51.3	49.3
	Number of mice	3	3	3
2.2	RBC×$10^4/\mu l$	773.7	804.3	872.7
	WBC×$10^2/\mu l$	47.3	49.3	52.3
	Number of mice	3	3	3
1.1	RBC×$10^4/\mu l$	623.3	807.3	859.3
	WBC×$10^2/\mu l$	29.0	53.7	32.3
	Number of mice	3	3	3
0.55	RBC×$10^4/\mu l$	748.7	971.7	884.3
	WBC×$10^2/\mu l$	34.4	43.0	35.7
	Number of mice	3	3	3
Control	RBC×$10^4/\mu l$	710.7	891.7	834.7
	WBC×$10^2/\mu l$	28.7	51.0	40.0

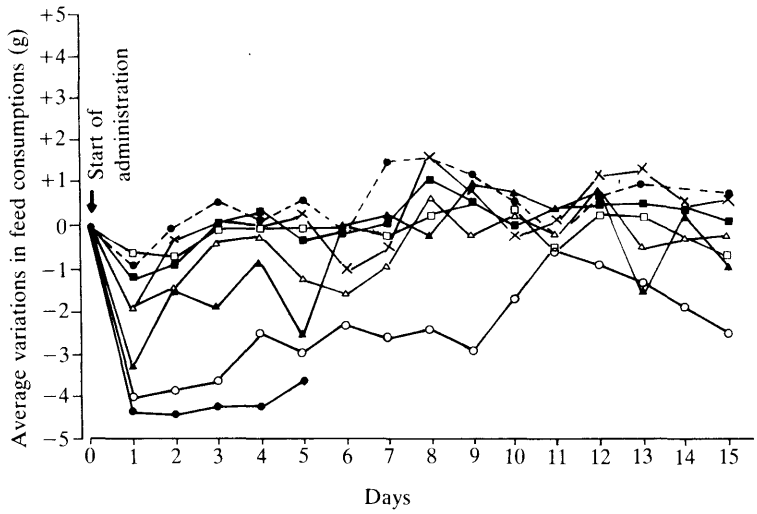

Fig. 2. Average daily variations of feed consumption in mice injected with TC-A in different doses for 15 consecutive days. –●– 35.5 mg/kg, –○– 17.8 mg/kg, –▲– 8.9 mg/kg, –△– 4.45 mg/kg, –■– 2.2 mg/kg, –□– 1.1 mg/kg, –×– 0.55 mg/kg, ..●124 Control.

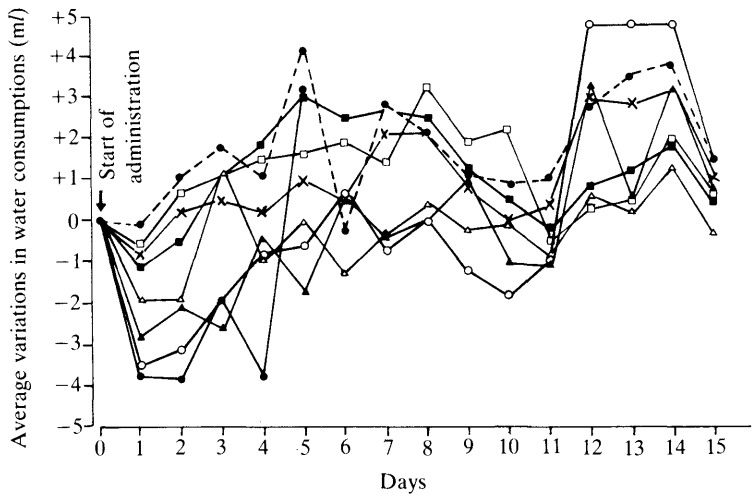

Fig. 3. Average daily variations of water consumption in mice injected with TC-A in different doses for 15 consecutive days. –●– 35.5 mg/kg, –○– 17.8 mg/kg, –▲– 8.9 mg/kg, –△– 4.45 mg/kg, –■– 2.2 mg/kg, –□– 1.1 mg/kg, –×– 0.55 mg/kg, ..●124 Control.

dose groups (Fig. 4). From these results, a dose of 4.45 mg/kg seemed to be the minimum toxic dose. The maximum safety dose for the evaluation of chemotherapeutic effect of TC-A was determined to be 3.13 mg/kg by taking a geometrical mean of 4.45 mg/kg (the minimum toxic dose) and 2.2 mg/kg (the maximum non-toxic dose).

Fig. 5 shows the proliferation of *B.rodhaini* and prolongation periods of survival mice injected intraperitoneally with 10 mg/kg of TC-A on day 3 after the inocula-

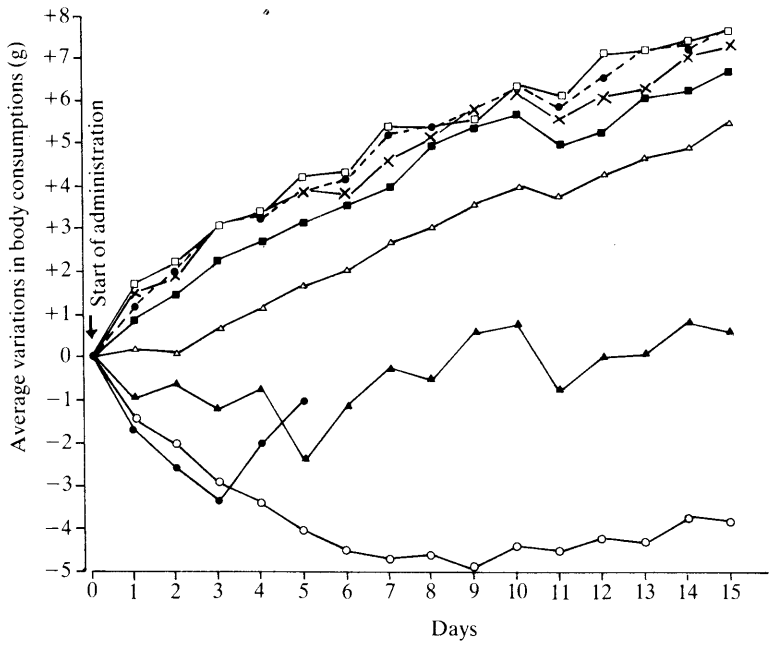

Fig. 4. Average daily variations of body weight gains in mice injected with TC-A in different doses for 15 consecutive days. -●- 35.5 mg/kg, -○- 17.8 mg/kg, -▲- 8.9 mg/kg, -△- 4.45 mg/kg, -■- 2.2 mg/kg, -□- 1.1 mg/kg, -×- 0.55 mg/kg, ..●124 Control.

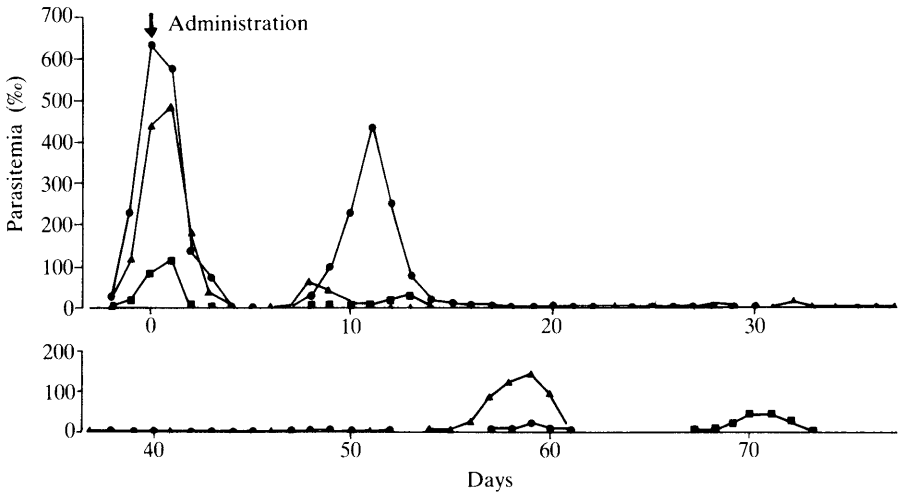

Fig. 5. Changes of parasitemia in 3 mice inoculated with *B. rodhaini* injected with TC-A in a dose of 10 mg/kg. -●- Mouse No. 1, -▲- Mouse No. 2, -■- Mouse No. 3.

tion. Parasitemia level lowered rapidly on day 1 or day 2 after the administration and remained negative from days 4 to 7, but it changed positive again and reached the second peak level on days 8 to 13. In all the three cases, parasitemia was sporadically observed and never completely eradicated, but no mice died throughout the experiment

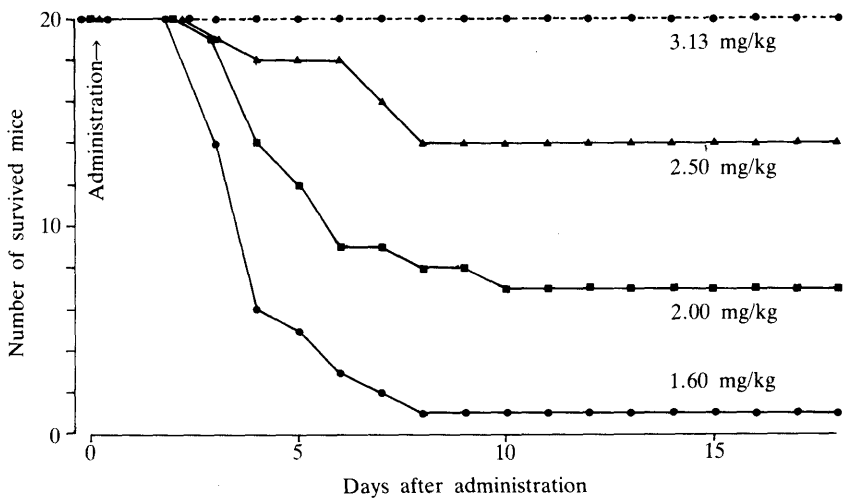

Fig. 6. The number of survived mice inoculated with *B. rodhaini* after administration of TC-A.

Table 3. Efficacy of TC-A in different doses against *B. rodhaini* inoculated mice

Dose (mg/kg)	Number of mice inoculated	Total number of survived mice on day 18	Survival rate (%)	Contribution to χ^2
3.13	20	20	100 (98.4)[a)]	0.00210
2.50	20	14	70	0.0046
2.00	20	7	35	0.0031
1.60	20	1	5	0.0032

a) Values converted by Litchfield-Wilcoxon's method are shown in parentheses.

period. From these results, it was concluded, based on the changes of parasitemia and the survival of mice, that the efficacy of TC-A should be judged after day 13 when the second peak of parasitemia occurred. Consequently, the day for judgement was set on day 18 after the administration of TC-A.

All mice survived in the maximum dose group of 3.13 mg/kg by day 18 after the administration of TC-A. In both 2.50 mg/kg and 2.00 mg/kg dose groups 19 mice each survived by day 3, and in 1.60 mg/kg dose group 15 mice survived by day 3. The number of survival mice successively decreased in 2.50–1.60 mg/kg dose groups: 14 in 2.50 mg/kg dose group, 7 in 2.00 mg/kg dose group, and 1 in 1.60 mg/kg dose group on day 18 after the administration (Fig. 6 and Table 3). Fig. 7 shows the probability chart plotted with dose levels and survival rates of mice. From this chart, ED_{50} of 2.19 mg/kg was obtained, and the confidence limits of 1.99–2.41 mg/kg were calculated.

DISCUSSION

When a new drug is developed, it is evaluated by comparing its LD_{50}, ED_{50} and safety margin (LD_{50}/ED_{50}) with those of the drugs already placed on the market.

Imidocarb, amicarbalide, diminazene and quinuronium are commercially available at present as effective drugs against *B.rodhaini*. LD_{50}, ED_{50} and safety margin of these drugs have been reported: 107,

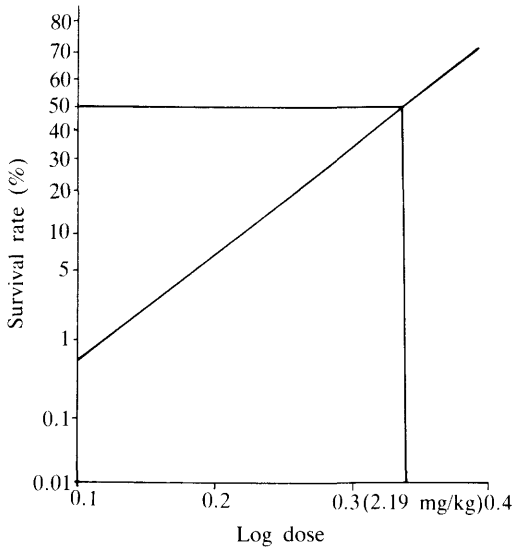

Fig. 7. Relation between log doses and survival rates of mice in the examination of ED_{50}.

mg/kg, 0.22 mg/kg and 485 in imidocarb, 8 mg/kg, 0.32 mg/kg and 25 in quinuronium, 168 mg/kg, 0.93 mg/kg and 172 in amicarbalide and 539 mg/kg, 7.37 mg/kg and 70 in diminazene, respectively [1]. When these drugs are evaluated for safety, imidocarb, amicarbalide, diminazene and quinuronium can be arranged in this order.

Itoh *et al.* [2] recently reported that TC-A is effective against *B.rodhaini* and *P.berghei* in mice. Ohtomo *et al.* [6] also reported that TC-A is effective against bovine piroplasmosis caused by *T.sergenti*. These reports, however, referred to the therapeutic efficacy of TC-A against these protozoal agents, but not to the properties evaluated by the relation between safety and therapeutic effects. We tried to quantitatively compare LD_{50}, ED_{50} and safety margin of TC-A thus obtained were 45.7 mg/kg, 2.19 mg/kg and 20.9, respectively. LD_{50}, ED_{50} and safety margin of diminazene have been reported to be 347 mg/kg, 9.5 mg/kg and 36.5 respectively by the same methods [3, 5]. Based on these data, TC-A is about four times as effective as, but about 1.7 times as risky as diminazene. And then compared with quinuronium in the data reported by Beveridge [1], TC-A is situated between imidocarb and quinuronium in LD_{50} and between amicarbalide and diminazene in ED_{50} and is almost equal to quinuronium with respect to safety margin, although these agents can not be simply compared with each other on their characteristics because of Beveridge calculated ED_{50} of each drug based on the parasitemia of the dosed group of animals relative to that of the undosed controls. Therefore, TC-A can be clinically used as an anti-piroplasmotic drug when the dosage level, the number and period of dosing and the formulation are precisely examined in addition to accurate investigations of its residue.

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要　約

抗ピロプラズマ剤 TC-A の特性：並河和彦・佐久間佳子・須永藤子・菅野康則（麻布大学獣医学部伝染病学教室）――さきに開発した効力試験法を Tetrocarcin-A に適用したところ LD_{50} は 45.7 mg/kg，*Babesia rodhaini* 感染マウスに対する ED_{50} は 2.19 mg/kg で，安全域は 20.9 となり quinuronium とほぼ同じ特性であった．