Additive Effects of Tetrocarcin-A and Diminazene Aceturate Concurrently Administered against *Babesia rodhaini*-Inoculated Mice

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Several compounds have been reported to be effective against piroplasmosis [4], and two of them, 6-methoxy-8-aminoquinoline (pamaquine) and diminazene aceturate, are commercially available in Japan. In recent years, tetrocarcin-A (TC-A) was studied about the efficacy against *Theileria sergenti* [8] and *Babesia rodhaini* infections [2], but it has not yet been developed due to narrow safety margin [7].

Additive effects or offsetted toxicity have been experimentally proved in the study of concurrent administration of two kinds of drugs [1]. In the present study, the toxicity and the effect against *B. rodhaini* infections were examined when TC-A was concurrently administered with diminazene.

In the present study, 130 female ICR-Jcl mice about 4 weeks (27-29 days) old weighing 16-24 (mean 20.2)g were purchased from Nihon Kurea Co. Ltd., and they were divided into 13 dose groups of 10 animals each.

Mice were raised in alminum cages (30×20×12cm in size) at 22±2°C and supplied with solid feed and tap water *ad libitum*. Parasitized blood was collected on day 3 after the inoculation from mice inoculated with the *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 ml and was once injected intraperitoneally to mice. TC-A and diminazene dissolved in 0.05 ml sterile distilled water were concurrently administered to mice per 10g of body weight, in intraperitoneal and subcutaneous routes respectively, on day 3 after the inoculation with *B. rodhaini*.

Based on the ED₅₀ of each drug, three standard doses were determined for concurrent administration: 1/2 ED₅₀ of TC-A (1.10mg/kg)+1/2 ED₅₀ of diminazene (4.75 mg/kg), 1/4 ED₅₀ of TC-A (0.55 mg/kg)+3/4 ED₅₀ of dimi-

nazene (7.13 mg/kg), and 3/4 ED₅₀ of TC-A (1.64 mg/kg)+1/4 ED₅₀ of diminazene (2.38 mg/kg). Additionally 3 or 4 descending and ascending doses were set up from each standard dose. The results of these concurrent administrations were evaluated by Ozawa's method [9]: if the ratio of B (each ED₅₀ newly obtained in these concurrent administrations) to A (each standard dose above mentioned) is 1, the efficacy is additive, if the ratio is larger than 1, it is antagonistic, and, if the ratio is less than 1, it is synergistic.

LD₅₀ of the concurrent administration was evaluated for confirming if the toxicity can be depressed only in the synergistic effect dose of ED₅₀.

ED₅₀ and LD₅₀ of each drug have been obtained already by Litchfield-Wilcoxon's method [5]: ED₅₀ of TC-A and diminazene are 2.19 mg/kg [7] and 9.5 mg/kg [6] respectively. LD₅₀ of TC-A and diminazene in mice are 45.7 mg/kg [7] and 347 mg/kg [3] respectively. The effects of the concurrent administration of TC-A and diminazene were additive, antagonistic, and synergistic respectively in the doses of 1/2 ED₅₀ (of TC-A)+1/2 ED₅₀ (diminazene), 1/4 ED₅₀+3/4 ED₅₀, and 3/4 ED₅₀+1/4 ED₅₀. Since B/A ratios were 0.95, 1.98, and 0.83 respectively (Table 1). From these results, more favourable anti-piropasmodic effect was obtained by the concurrent administration of TC-A and diminazene at a ED₅₀ ratio of 3:1 (Table 1), while toxicity was offsetted when TC-A and diminazene were concurrently administered at a LD₅₀ rate of 3:1 since the B/A ratio was 1.20 (Table 2).

Consequently, the efficacy was synergistic when TC-A and diminazene were concurrently administered against *B. rodhaini*-inoculated mice in the ED₅₀ ratio of 3:1 (3/4 ED₅₀ and 1/4 ED₅₀ respectively). While toxicity was antagonistic when TC-A and diminazene were administered in the LD₅₀ ratio of 3:1 (3/4 ED₅₀ and 1/4 ED₅₀ respectively).

Table 1. Effects of concurrent administration of TC-A and diminazene against *B. rodhaini*-inoculated mice

ED ₅₀ ratio of TC-A and diminazene	Dose (mg/kg)	Total number of mice used	Survival rate of mice (%)	ED ₅₀ (mg/kg) (B)	B/A ratio
1:1	4.87	10	20	5.53	0.95 (additional)
	5.85 ^{b)}	10	70		
	7.01	10	80		
1:3	6.40	10	0(1.0) ^{d)}	15.24	1.98 (antagonistic)
	7.68 ^{c)}	10	10		
	9.22	10	10		
	11.06	10	30		
3:1	3.35	10	50	3.35	0.83 (synergistic)
	4.02 ^{d)}	10	60		
	4.83	10	100(91.7) ^{a)}		

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

b) TC-A (1/2ED₅₀, 1.10 mg/kg)+diminazene (1/2ED₅₀, 4.75 mg/kg)=5.85 mg/kg (A), Standard dose.

c) TC-A (1/4ED₅₀, 0.55 mg/kg)+diminazene (3/4ED₅₀, 7.13 mg/kg)=7.68 mg/kg (A), Standard dose.

d) TC-A (3/4ED₅₀, 1.64 mg/kg)+diminazene (1/4ED₅₀, 2.38 mg/kg)=4.02 mg/kg (A), Standard dose.

Table 2. Acute toxicity of concurrent administration with TC-A and diminazene to mice

LD ₅₀ ratio of TC-A and diminazene	Dose (mg/kg) ^{a)}	Total number of mice used	Mortality (%)	LD ₅₀ (mg/kg) (B)	B/A ratio
3:1	174.28	10	70	145.55	1.20 (antagonistic)
	209.13	10	70		
	250.96	10	90		

a) TC-A (3/4LD₅₀=34.28 mg/kg)+diminazene (1/4LD₅₀=86.75 mg/kg)=121.03 mg/kg (A), Standard dose. Doses were determined based on the standard dose calculated.

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要 約

Babesia rodhaini 感染マウスに対する Tetrocarcin-A とジミナゼン製剤の併用効果（短報）：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）——抗ピロプラズマ剤の tetrocarcin-A とジミナゼン製剤を *Babesia rodhaini* 感染マウスに併用し，両剤の種々の用量の組み合わせにおける抗ピロプラズマ効果の増強および毒性の軽減について検討した。その結果 TC-A の $3/4ED_{50}$ とジミナゼンの $1/4ED_{50}$ を併用した場合に抗原虫作用は相乗的に，その割合での毒性は拮抗的に作用し，毒性が強く安全域の狭い TC-A がジミナゼンとの併用により抗ピロプラズマ剤として有効であることがわかった。