THERAPEUTIC TREATMENTS IN EQUINE INFECTIOUS ANEMIA

V. THE USE OF ACTINOMYCIN, FERMICIDIN AND CHROMOMYCIN FOR NATURALLY AFFECTED WITH CHRONIC INFECTIOUS ANEMIA

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INTRODUCTION

The authors have already reported[1~4] that beneficial results were not obtained at all in the therapeutic treatments using several chemotherapeutics for the cases of naturally affected or artificially inoculated infectious anemia. Recently, 3 new antibiotics (actinomycin, fermicidin and chromomycin) were offered from Takeda Pharmac. Institute. In the basic experiment[5] in the institute, while these antibiotics were found to be highly antibiotic to bacteria and viruses in vitro, the toxicity for mice and rats were severe as indicated in table 1.

The present work was conducted on the basis of the above groundwork, for the purpose of examining their therapeutic effectiveness or toxicity for infectious anemia horses. Clinical, hematological, biochemical and pathological investigations were carried out during the whole course of the experiment.

MATERIALS AND METHODS

The patients were 3 horses naturally affected with typical chronic infectious anemia. They were 5~14 years of age, black in hair color, male or female, 320~500 kg. Adequate feed was provided and there was proper care constantly throughout the whole course of the experiment. The original medicines were fine or minute powder respectively. Medicinal doses for horses were supposed to 10~20 γ/kg in actinomycin, 15~25 γ/kg in fermicidin and to 5~20 γ/kg in chromomycin from the results of the preliminary test concerning the toxicity for mice and rats. The antibiotics were all injected intravenously.

TABLE 1. *Biological Findings in 3 New Antibiotics* (Takeda Institute)

(a) *Antibiotic Values* (γ/cc)

MICROBES	ANTIBIOTICS		
	Actinomycin	Fermicidin	Chromomycin
E. coli	50.00	—	> 100.00
S. typhi	—	—	> 100.00
V. cholerae	—	—	> 100.00
Prot. vulgaris	50.00	—	> 100.00
Pseudom. aeruginosa	—	—	> 100.00
B. subtilis	0.10	—	0.06
Staphyl. aureus (Terajima)	0.05	—	0.07
Yeasts	—	0.04~0.20	> 100.00
Fungi	—	0.20~0.50	> 100.00
Mycobact. tuberculosis (Avian type)	10.00	•	6.00
Trich. vaginalis	•	0.20	•
Sarc. lutea	—	—	0.07
S. dysenteriae (Type I)	—	—	> 100.00
Influenza virus (PR 8)	0.50	2.00	2.00
Vaccina virus	0.10	•	> 0.20
Fibroblast (Chick embryo)	0.10	•	> 0.20

(b) *Toxicity* (LD_{50}, mg/kg)

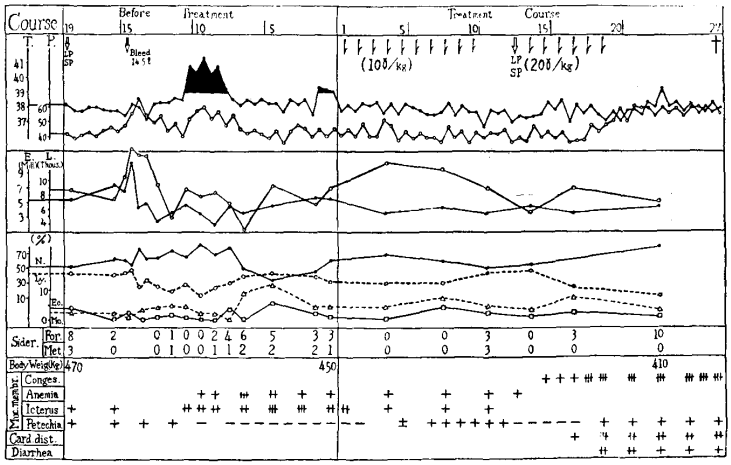
	MOUSE			RAT	
	i. v.	i. p.	i. m.	i. v.	i. p.
Actinomycin	—	1.0	1.0	—	—
Fermicidin	180	—	—	2.0	—
Chromomycin	—	1.0	—	—	0.87

EXPERIMENTAL RESULTS

1. Treatment with Actinomycin in Horse No. 1

The patient was mongrel Percheron, male, 5 years, 470 kg. On inspection, general conditions were recognized as apparently healthy and well nourished. The liver and the spleen biopsies and hematological examinations were conducted on the 19th day before treatment, and the patient was diagnosed as having typical chronic infectious anemia. While 14.5 L of blood was bled on the 15th day prior to treatment for the purpose of provoking the sickness, typical fever attack of infectious anemia appeared on the 5th day after bleeding. Anemia, icterus and the increases in siderocyte numbers, as indicated in chart 1, were evident in accordance with lysis of temperature, but neither petechia

CHART 1. *Treatment with Actinomycin in Horse No. 1*



nor cardiac disturbance were noted. Erythrocyte numbers decreased to 2.76 million in minimum. Then the treatment was commenced on the 16th day after bleeding; temperature and pulse rate were normal; erythrocyte numbers were 3.41 million; siderocyte was found in 3 per 10,000 leucocytes; body weight was 450 kg (20 kg less than before); mucous membranes were dirty anemic and icteric but neither petechia nor cardiac disturbance were found.

Actinomycin was injected intravenously 10 γ/kg of body weight daily for 10 days for the 1st cure. Slight anemia and icterus were found through the course of the 1st cure and petechiae appeared on the buccal and the nasal mucosae from the 7th day, but other symptoms were not obtained. Leucocytosis and disappearance of siderocytes were observed during the treatment, however, no marked alteration of erythrocyte numbers was noticed. After a 3-day interval the injections were recommended at the rate of 20 γ/kg of body weight for the 2nd cure. Congestions and petechiae became apparent on the mucous membranes on the 5th and the 6th days while heavy cardiac disturbance and the severe diarrhea appeared respectively. As the general condition aggravated on the 6th day of the 2nd cure, the injections were discontinued. But the symptoms gradually turned to the worse, and difficulty in standing appeared from the 12th day of the 2nd cure. Finally the patient died on the second day following.

In hematological findings, the slight increase in numbers of erythrocytes and neutrophiles was found as the general conditions were aggravated. Siderocytes reappeared more frequently toward the end of the observations. In biochemical findings of serum, as indicated in table 2, A/G and γ-globulin decreased, whereas β-globulin, glucose, inorganic phosphorus and iron increased respectively in accordance with the continuance of the experiment. In the biopsical findings of the liver and the spleen after the 1st treatment, the activities of reticulo-endothelial system (RES) were reduced as compared with the findings before treatment. On the other hand, the histological findings after the necropsy, showed the increase of reticular cells and "L-cells" in the liver, spleen and lymph nodes. This case was histologically diagnosed as the relapsed type of equine infectious anemia[6,7].

TABLE 2. *Biochemical Findings of Serum*

HORSE NO.	ANTIBIOT.	TIME OF EXAMINATION (day)	PROTEIN (g/dl) Total	Alb.	Glob.	A/G	GLOB.-FRAC. (g/dl) α-	β-	γ-	GLUCOSE (mg/dl)	INORG. SUBST. (mg/dl) Ca	P	Fe
1	Actinomycin	Before	7.0	2.20	4.80	0.46	3.55	0.75	0.50	44	13.8	3.5	0.080
		Injec. 4	6.8	2.35	4.45	0.53	2.95	0.92	0.58	44	14.5	4.2	0.055
		Injec. 5	7.8	1.20	6.60	0.18	2.08	3.52	1.00	65	12.0	4.3	0.065
		Rest	7.8	1.10	6.70	0.16	2.32	3.38	1.00	49	16.3	3.0	0.125
		Injec. 1	8.0	2.55	5.45	0.47	0.92	3.90	0.63	98	11.0	2.6	0.085
		Injec. 4	8.4	3.00	5.40	0.56	0.70	3.85	0.85	81	11.0	4.0	0.115
		After*	9.0	1.80	7.20	0.25	1.50	4.95	0.75	121	11.2	4.6	0.120
2	Fermicidin	Before	7.0	0.85	6.15	0.01	5.43	0.15	0.57	47	13.2	3.1	0.080
		Injec. 3	8.2	0.65	7.55	0.08	2.90	3.74	0.91	19	13.7	2.9	0.055
		Injec. 5	7.3	0.80	6.50	0.01	1.60	4.00	0.90	14	14.9	3.7	0.055
		Rest	8.7	1.20	7.50	0.16	1.80	4.32	1.38	18	11.8	3.3	0.065
		Injec. 5	8.6	1.15	7.45	0.15	1.25	5.15	1.05	25	13.7	4.3	0.075
		After 10	7.8	1.70	6.10	0.28	2.05	3.29	0.76	45	8.5	3.7	0.095
		After 17	8.2	1.20	7.00	0.17	1.75	4.33	0.92	62	10.2	4.2	0.080
		After 25	8.0	1.40	6.60	0.21	2.10	3.65	0.85	39	11.5	3.1	0.075
3	Chromomycin	Before	7.9	1.45	6.45	0.22	3.95	1.58	0.92	67	10.3	3.9	0.075
		Injec. 4	7.8	1.75	6.05	0.29	4.25	1.37	0.43	41	12.5	3.3	0.100
		Injec. 8	7.5	1.40	6.10	0.23	4.19	1.35	0.56	53	12.5	4.8	0.110
		Rest	6.7	1.35	5.35	0.25	3.90	1.18	0.27	44	11.8	2.9	0.090
		Injec. 4	7.6	1.45	6.15	0.24	3.70	1.38	1.07	44	13.7	2.9	0.085
		Injec. 7	7.6	1.40	6.20	0.23	3.93	1.52	0.75	41	13.9	3.3	0.045
		Rest	7.8	1.10	6.70	0.16	4.38	1.37	0.95	35	14.5	2.7	0.055
		Injec. 2	7.7	1.30	6.40	0.20	3.76	1.72	0.92	56	8.7	2.6	0.065
		Injec. 6	7.8	1.35	6.55	0.19	3.90	1.60	1.05	33	10.2	3.5	0.095
		After 11	7.6	1.25	6.25	0.22	3.80	1.75	0.70	42	10.5	3.9	0.050
		After 19	7.9	1.40	6.50	0.22	3.65	1.90	0.95	58	11.3	4.2	0.080
		After 22	7.4	1.65	5.75	0.29	4.15	1.05	0.55	54	13.6	3.0	0.125

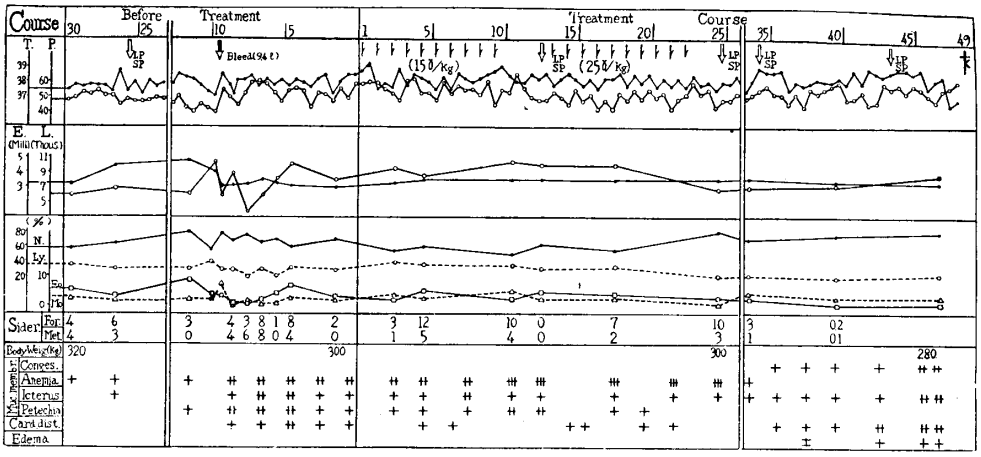
Note: *—General conditions aggravated.

2. Treatment with Fermicidin in Horse No. 2

The patient was mongrel Percheron, female, 5 years, weighing 320 kg. Slight anemia, icterus and petechia on mucous membranes were found clinically, but the temperature was almost normal. Erythrocytes indicated 3.51~5.58 millions in numbers, siderocytes were found to number 3~6 per 10,000 leucocytes. The figures of chronic infectious anemia were noted from the results of the biopsies. Although 9.4 L of blood was bled, on the 10th day before treatment, no provocative fever attack of infectious anemia appeared. However, not only anemia, icterus and petechia on the mucosae became aggravated, but also cardiac disturbance was accelerated.

Fermicidin was injected in the amount of 15 γ/kg of body weight daily for 10 days for

CHART 2. *Treatment with Fermicidin in Horse No. 2*



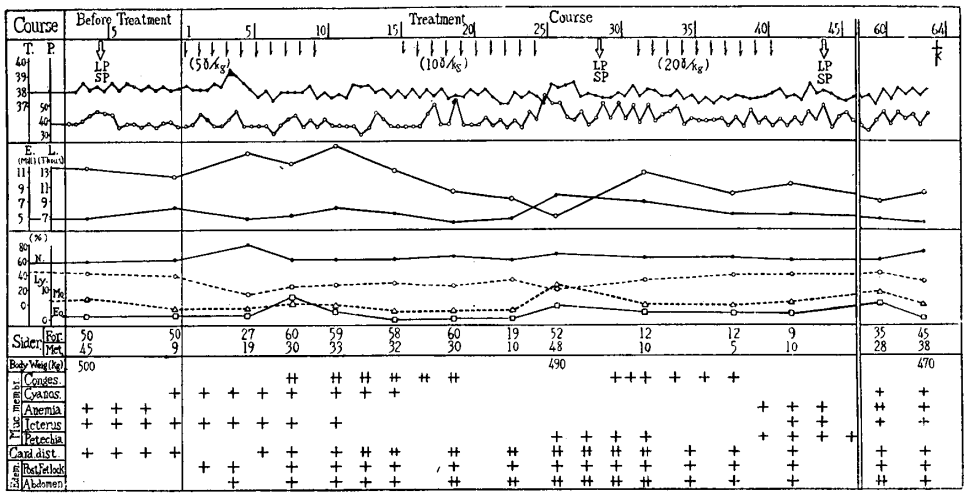
the 1st cure and, after a 3-day interval, the 2nd cure injections were commenced at the rate of 25 γ/kg of body weight daily for 10 days. In the clinical observations, increase in emaciation and anemia on the mucous membranes were found in accordance with repetition of the injections. From the 13th day after the injections, the general conditions gradually worsened and in the terminal period of the experiment, congestion, icterus, cardiac disturbance and edema were more obvious. Erythrocyte numbers ranged from 3.32 to 4.56 million and leucocytes from 6,900 to 10,200 throughout the whole course. Siderocytes appeared in 0.2 per 10,000 leucocytes in every blood examination. In the biochemical findings, increase in A/G and β-globulin was found. According to the histological findings of the biopsy, the activities of RES were slightly reduced against the findings obtained before treatment. On the post-mortem histology, the activities of RES were relapsed in comparison with the before-treatment.

3. Treatment with Chromomycin in Horse No. 3

The patient was Percheron, male, 14 years, weighing 500 kg and well nourished. The diagnosis as typical chronic infectious anemia was obtained with the clinical, hematological and the biopsical examinations on the 6th day before treatment. The temperature indicated 38.2°C while negligible anemia and icterus on mucous membranes and slight cardiac disturbance were found. Erythrocyte numbers indicated 4.8 million and siderocytes were counted in 50 per 10,000 leucocytes by the formalin-fixation-test.

Chromomycin was injected intravenously at the rate of 5 γ/kg of body weight per day for 10 days for the 1st cure. The injections for the 2nd and the 3rd cures were conducted with a 6-day interval and the injection doses were prescribed 10 γ/kg or 20 γ/kg of body weight. From the 4th and the 6th days in the 1st cure, severe congestion and cyanosis on mucosae, edema in the posterior fetlocks and in the lower abdomen appeared respectively. The cardiac disturbance was also noted by the auscultation in the heart. Edema and cardiac disturbance aggravated with the advance of the experiment, but, from the 3rd cure, they became slight and continued toward the end. No fever attacks were found

CHART 3. *Treatment with Chromomycin in Horse No. 3*



throughout the whole course of the experiment, however the patient was slaughtered for the pathological investigations on the 64th day from starting the injections.

In the hematological findings, erythrocyte numbers indicated 6.1 million in the highest and 3.82 million in the lowest, however, no other marked alterations were found. Leucocytes increased in accordance with repetition of the injections in the 1st cure, and they indicated 16,600 at the highest on the 1st day just after the end of the 1st cure. They ranged from 6,800 to 10,600 through the 2nd and 3rd cures. No remarkable changes in differential counts in leucocytes were noted. Siderocytes were found 9~60 by the formalin-fixation-test and 5~48 by the methanol-fixation-test per each 10,000 leucocytes. So, the antibiotic was considered not effective for infectious anemia. In the biochemical findings, as indicated in table 2, no marked alterations were found in comparison with condition of the before-experiment examination. Regarding the biopsy, the histological findings of this case similar to those of the second case.

CONSIDERATIONS

As above described data show, none of the patients indicated typical fever attacks throughout the whole treatment course, but in general, the clinical symptoms worsened and the gradual decrease of body weight was recognized with the repetition of injections. Especially in No. 1, cardiac disturbance was markedly aggravated from the end of the 2nd cure and the horse finally died. These findings seem to be induced by intoxication due to the continuous applications of actinomycin. According to the above clinical reactions, the toxicity for horses is considered to be the highest in actinomycin. Although the temporary disappearance of siderocytes was found in No. 1 during the injection period, they appeared always at every examination in the other 2 cases throughout the course of treatment. In the biopsical findings, particularly in the horses Nos. 1 and 3, the temporary

reduction of activity of RES was recognized during the course of the injections, whereas the figures of typical infectious anemia were obtained in all the cases by the anatomical and histo-pathological examinations. Especially in the case of the horse No. 1, it was diagnosed as to be the so-called relapsed type[6,7] of equine infectious anemia. Considering from these findings, the antibiotics seem to have some effect on the activities of the RES which were caused by the infectious anemia virus. On the basis of the above descriptions, it may be said that the present 3 antibiotics are non-effective for infectious anemia.

Summary

Therapeutic treatments with actinomycin, fermicidin and chromomycin for 3 chronic infectious anemia horses were conducted. The results thus obtained may be summarized as follows:

1. The medicines were all injected intravenously. The dosage schedules of antibiotics per day were; 10~20 γ/kg in actinomycin, 15~25 γ/kg in fermicidin and 5~20 γ/kg in chromomycin.

2. The toxicity of the antibiotics for the 3 horses in this experiment is considered to be the highest in actinomycin and the lowest in fermicidin.

3. In the biopsies, especially in No. 1, the temporary reduction of activity of reticulo-endothelial system and also temporary disappearance of siderocytes in the blood examinations were found respectively during the period of the repetition of the injections.

4. These 3 antibiotics were found to be not effective for infectious anemia horses.

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