EFFECTS OF BLEOMYCIN ON MOUSE TRANSPLANTABLE TUMORS

MIEKO TAKEUCHI and TADASHI YAMAMOTO

The Institute of Medical Science, University of Tokyo,
Shiba-shirokane, Minato-ku, Tokyo, Japan

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The present paper briefly considers the effect of a new antitumor antibiotic, bleomycin[1], on several strains of transplantable mouse ascites tumors, all of which were originally established as syngeneic in tumor-host relationship. The antibiotic showed moderate to marked effects upon ROUS sarcoma virus-induced mouse ascites sarcoma at fairly wide dose level, particularly when F_1 hybrid mice were employed as host animals. It gave, however, almost no effect upon virus-producing FRIEND ascites tumor and mouse ascites plasmacytoma.

Materials and Methods

1. Tumor cells: (a) Mouse ascites sarcomas, SR-C3H/He Th62[2,3] and SR-C57BL/6 2008B[4], previously established in our laboratory, contain ROUS sarcoma virus, SCHMIDT-RUPPIN strain (SR-RSV), in the masked form in every free cell level and continuously possess histocompatible antigens in C3H/He and C57BL/6 mice respectively.

(b) Mouse ascites sarcoma, SR-C57BL/6 2008A[4], is originally derived from an identical SR-RSV-induced sarcoma in a C57BL/6 mouse as SR-C57BL/6 2008B ascites sarcoma, but has lost its carrier state of viral genome and histocompatible antigens soon after its ascitic conversion.

(c) FRIEND ascites tumor SFAT-5 (IKAWA)[5], established in DDD mice and kindly supplied by Dr. ODAKA in our laboratory, produces continuously active FRIEND disease viruses and has specific histocompatible antigens.

(d) Mouse ascites mammary carcinoma, MM2, was previously converted into ascitic form by YAMAMOTO and OSHIMA in 1957 from a spontaneous mammary carcinoma in a C3H/He mouse[6], having soon lost its strain specificity in tumor take, but after more than 10 years' passages in C3H/He mice proved to have tumor specific transplantation antigens related to mouse mammary tumor virus by NISHIOKA's group[7].

(e) Mouse ascites hepatoma MH134, was converted into ascitic form by SATO *et al.*[8] from a C3H/HeN mouse hepatoma induced after treatment with carbon tetrachloride by ANDERVONT *et al.*[9] It has been maintained in our laboratory in C3H/He mice since 1959 with distinct strain specificity in tumor take.

(f) Mouse ascites plasmacytoma X5563, was recently converted into ascitic form in our laboratory (ISHII and SHIN[10]) from C3H plasmacytoma X5563 kindly supplied by Dr. T. H. YOSHIDA of National Genetics Institute, Mishima, Japan. It was originally discovered in 1957 as a γG myeloma protein producer[11].

2. Inoculation of tumor cells: In case of SR-C3H/He Th62 tumor, 2×10^5 cells were inoculated intraperitoneally to form ascites tumor and 2×10^6 cells were inoculated subcutaneously to yield a solid tumor. With other ascites tumors each animal received intraperitoneally 2×10^6 cells. All the tumor cells were previously taken at the vigorous growth stage, washed and diluted with ice cold Eagle medium after counting the number of cells with Türk stain.

3. Mice: As host animals C3H/He mice for the inoculation of SR-C3H/He Th62, MM2 and X5563 tumor cells, C57BL/6 mice for SR-C57BL/6 2008B, and DDD mice for FRIEND ascites tumor were employed. CCF_1 (BALB/c×C3H/He) hybrid mice were also employed along with C3H/He mice. All the animals were of both sexes and over 5 weeks of age, weighing 20±1 g at the time of inoculation. They were given water and cubed chow (Oriental Co.) *ad libitum* in an air-conditioned animal room.

4. Bleomycin: Serial 2 fold dilutions of copper-free bleomycin complex (Lot CP_3 #28), kindly prepared at Prof. H. UMEZAWA's laboratory, were made every day before injection with physiological saline from dose level 500 to 1.9 mcg per 0.25 ml and injected intraperitoneally at 0.25 ml per mouse. Those doses correspond to 25~0.09 mg/kg. The initial injections were made 1 to 2 hours after the tumor cell inoculation and followed by injections every 24 hours for 10 days.

5. Observation: Measurement of body weight was carried out before each injection at day 0, 5, 7, 10, 15, 20 and 25. Weight loss of 4 to 1 g could be observed at groups treated with 500, 250, 125 and 62.5 mcg of bleomycin per mouse per day within 10-day treatment, followed by gradual recovery. Gross pathological observations were also made on dead animals and survivors which were killed 50~60 days after the tumor cell inoculation.

Results

1. SR-C3H/He Th 62 sarcoma:

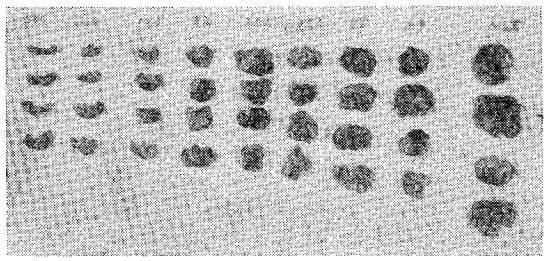
(a) In case of ascites tumors where syngeneic C3H/He mice were inoculated intraperitoneally with tumor cells and treated with varied doses of bleomycin by the same route, the accumulation of the ascites was inhibited or retarded during the

Table 1. Effect of bleomycin complex(-Cu) on SR-C3H/He ascites sarcoma in C3H/He mice.

Initial body wt. mean (g)	Body wt. after 10 days (% change)	Daily dose mg/kg	50 days survivors	Mean survival time (days)	Survival time (treated/control)
18.7	14.3 (76)	25.0	0/5	23.0±2.4	166
20.5	16.9 (82)	12.5	0/5	22.4±4.4	162
21.3	17.4 (81)	6.25	0/5	26.4±4.8	191
19.4	19.1 (98)	3.12	0/5	19.0±2.8	137
19.9	19.9 (100)	1.56	0/5	18.4±2.9	133
20.5	22.8 (111)	0.78	0/5	19.6±0.3	142
20.5	25.6 (124)	0.39	0/5	17.4±1.1	126
21.1	26.4 (125)	0.19	0/5	13.8±2.3	100
20.9	28.4 (135)	0.09	0/5	14.8±1.6	107
18.6	26.0 (139)	0	0/5	13.8±1.5	100

period of treatment at dose levels above 31.25 mcg per mouse per day, *i.e.* 1.56 mg/kg per day, but all the mice finally died with invasive tumor growth. The highest percentage increase in survival time was observed in the group treated with 125 mcg per mouse per day *i.e.* 6.25 mg/kg per day showing a survival time 191.3 % of the control, followed by the groups treated

Plate 1. Effect of bleomycin complex(-Cu) on solid form of SR-C3/He ascites sarcoma in C3H/He mice.



with 25 mg/kg per day, and 12.5 mg/kg per day. Even at dose levels over 0.78 mg/kg per day survival time greater than 130 % were obtained (Table 1).

(b) In case of solid tumor where C3H/He mice were inoculated subcutaneously with tumor cells and treated intraperitoneally, results were more impressive than the ascites tumor experiment when the weight of 14 days' tumors was compared with the control group (Table 2 and Fig. 1). More than 75 % inhibition of tumor was obtained above the dose level of 6.25 mg/kg per day and about 50 % inhibition was observable at a dose level of 0.78 mg/kg per day.

Table 2. Antitumor effect of bleomycin complex(-Cu) on SR-C3H/He sarcoma (solid form) in C3H/He mice.
Treatment started 2 hours after the inoculation.

	Daily dose* (mg/kg)								Control
	25.0	12.5	6.25	3.12	1.56	0.78	0.39	0.19	
Tumor weight (mg)	450 510 550 670	410 450 650 720	580 700 880 970	1,150 1,250 1,450 1,500	1,750 1,800 1,850 1,950	1,720 1,810 2,140 2,450	2,500 2,550 2,700 3,200	2,000 2,200 2,450 2,850	2,400 2,550 2,550 3,450 3,450 4,500 6,650
Average (mg)	545	557	625	1,337	1,838	2,030	2,737	2,625	3,968
Inhibition %	86	86	84	66	53	48	31	33	—
Evaluation	++	++	++	+	+	±	±	±	

* Daily injected intraperitoneally for 10 days.

Table 3. Effect of bleomycin complex(-Cu) on SR-C3H/He ascites sarcoma in CCF_1 mice.

Initial body wt. mean (g)	Body wt. after 10 days (% change)	Daily dose mg/kg	50 days survivors	Mean survival time (days)	Survival time (treated/control)	Toxic deaths
21.4	15.6 (72)	25.0	1/3			3/3 (40)*
21.2	17.2 (81)	12.5	2/4	28.0±8.0	146	2/4 (55)*
19.2	15.5 (80)	6.25	2/4	39.5±8.5	206	
19.3	16.6 (86)	3.12	2/4	34.5±1.5	180	
18.9	18.4 (97)	1.56	3/3	—		
19.3	18.4 (95)	0.78	2/4	44.0±4.0	230	
17.8	19.4 (108)	0.39	1/3	34.5±4.5	180	
17.2	20.4 (118)	0.19	2/4	15.0±1.0	78	
15.7	17.0 (108)	0.09	2/4	22.5±4.5	117	
19.6	23.5 (119)	0	0/6	19.1±4.7	100	

* Mean survival time (days) of toxic death mice.

Table 4. Effect of bleomycin complex(-Cu) on SR-C57 BL/6 2008-B in C57 BL/6 mice.

Initial body wt. mean (g)	Body wt. after 10 days (% change)	Daily dose mg/kg	20 days survivors	Mean survival time (days)	Survival time (treated/control)
23.3	18.1 (77)	25.0	0/4	14.5±3.3	145
21.9	25.2 (115)	12.5	0/4	12.0±2.9	120
26.8	27.0 (100)	6.25	0/4	12.2±4.6	122
27.5	33.3 (121)	3.12	0/4	9.0±2.2	90
31.5	37.3 (135)	1.56	0/5	9.4±1.5	94
31.6	37.9 (119)	0	0/5	10.0±0.0	100

(c) When CCF_1 (BALB/c×C3H/He) hybrid mice were inoculated intraperitoneally with the tumor cells and treated in the same way as the experiment (a), complete cure was observed at the dose level of 1.56 mg/kg per day and survival time >180% was obtained even at dose level of 0.39 mg/kg per day. However, subacute toxic death could be observed with adhesion of intestines and involuted liver among the mice treated at dose levels above 12.5 mg/kg per day 30~40 days after the completion of treatment. Typical tumor death was compatibly observed after prolonged survival time among mice treated at dose levels above 3.12 mg/kg per day, although some of them were completely cured (Table 3).

Table 5. Effect of bleomycin complex(-Cu) on SR-C57 BL/6 2008-A in CCF_1 Mice.

Initial body wt. mean (g)	Body wt. after 10 days (% change)	Daily dose mg/kg	50 days survivors	Mean survival time (days)	Survival time (treated/control)	Toxic deaths
20.7	15.8 (76)	25.0	1/2			1/2 (38.0)*
21.3	18.3 (85)	12.5	0/3			3/3 (41.0*±0)
18.6	18.0 (96)	6.25	3/4			1/4 (36.0)*
18.7	18.5 (98)	3.12	4/4			
18.1	18.7 (103)	1.56	4/4			
17.9	19.2 (107)	0.78	2/4	26.5	149	
17.0	18.5 (108)	0.39	1/4	28.7	162	
17.1	22.2 (130)	0.19	0/4	22.0	124	
18.7	28.3 (151)	0	0/6	17.7	100	

* Mean survival time (days) of toxic death mice.

Table 6. Effect of bleomycin complex(-Cu) on MM2 in C3H/He mice.

Initial body wt. mean (g)	Body wt. after 10 days (% change)	Daily dose mg/kg	50 days survivors	Mean survival time (days)	Survival time (treated/control)	Toxic deaths
17.5	14.7 (84)	25.0	2/5			3/5 (43.7)*
17.2	14.0 (81)	12.5	2/5			3/5 (45.7)*
17.7	15.3 (86)	6.25	3/4			1/4 (42.0)*
17.5	16.0 (91)	3.12	2/4	35.0±10.0	182	
16.6	16.1 (96)	1.56	4/5	28.0	145	
16.5	18.6 (112)	0.78	0/5	23.2± 1.6	120	
15.4	19.9 (129)	0.39	0/5	23.4± 2.1	121	
15.6	20.0 (128)	0.19	0/5	20.6± 2.5	107	
15.3	20.7 (135)	0.09	0/5	19.4± 1.5	101	
15.0	18.4 (122)	0	0/5	19.2± 2.3	100	

* Mean survival time (days) of toxic death mice.

Table 7. Effect of bleomycin complex(-Cu) on MM2 in CCF_1 mice.

Initial body wt. mean (g)	Body wt. after 10 days (% change)	Daily dose mg/kg	50 days survivors	Mean survival time (days)	Survival time (treated/control)	Toxic deaths
19.8	15.1 (76)	25.0	3/4			1/4 (49.0)*
19.7	16.6 (84)	12.5	2/4			2/4 (39.5)*
19.7	17.8 (90)	6.25	4/4			
19.6	18.4 (93)	3.12	3/4	46.0	321	
19.7	19.1 (96)	1.56	3/4	45.0		1/4 ?
19.8	22.4 (113)	0.78	1/4	27.3	190	
21.2	24.9 (117)	0.39	1/4	18.7	130	
20.8	29.5 (141)	0.19	0/4	15.5	108	
21.3	29.0 (136)	0.09	0/3	15.3	107	
20.2	28.6 (141)	0	0/6	14.3	100	

* Mean survival day of toxic death mice.

2. SR-C 57 BL/6 ascites sarcoma 2008 A and 2008 B:

(a) When C 57 BL/6 mice were inoculated intraperitoneally with 2×10^6 cells of SR-C 57 BL/6 2008 B ascites sarcoma and treated with varied dose levels of bleomycin, prolonged survival times were observed at dose levels above 12.5 mg/kg per day (Table 4).

(b) When CCF_1 hybrid mice were inoculated intraperitoneally with SR-C 57 BL/6 2008 A because of its lost strain specificity in tumor take and treated in the same way as above mentioned, completely cured mice were obtained in all groups at dose levels above 1.56 mg/kg per day, although dead mice with subacute toxicity were also observable at dose levels above 6.25 mg/kg per day (Table 5).

3. Mouse ascites mammary carcinoma MM 2:

When C 3 H/He mice were inoculated with non-specific MM 2 tumor cells and treated with bleomycin, some completely cured mice were obtained with all dose levels above 6.25 mg/kg per day, although some died later with subacute toxicity (Table 6). CCF_1 mice were also employed as host animals and a similar but a little better result was obtained as shown in Table 7.

4. FRIEND ascites tumor SFAT-5:

DDD mice were inoculated intraperitoneally with FRIEND ascites tumor cells and likewise treated with bleomycin. The antibiotic gave almost no effect on this tumor, as shown in Table 8. Neither accumulation of the ascites tumor nor splenomegaly, a

Table 8. Effect of bleomycin complex(-Cu) on FRIEND disease tumor SFAT-5 in DDD mice.

Initial body wt. mean (g)	Body wt. after 10 days (% change)	Daily dose mg/kg	50 days survivors	Mean survival time (days)	Survival time (treated/control)
21.3	18.3 (85)	25.5	0/4	20.0±5.2	113
21.4	21.7 (101)	12.5	0/4	22.0±5.5	124
21.5	20.8 (96)	6.25	0/4	16.7±3.1	94
21.9	23.5 (107)	3.12	0/4	20.5±4.8	115
20.4	29.5 (144)	1.56	0/4	16.5±3.7	93
20.6	30.5 (148)	0.78	0/4	13.5±2.5	76
21.5	32.6 (151)	0.39	0/4	12.7±2.2	71
20.5	31.1 (151)	0.19	0/4	13.3±3.0	75
21.5	30.8 (143)	0.09	0/4	18.5±3.9	104
21.2	29.5 (139)	0	0/6	17.7±3.4	100

Table 9. Effect of bleomycin complex(-Cu) on ascites hepatoma MH134 in CCF_1 mice.

Initial body wt. mean (g)	Body wt. after 10 days (% change)	Daily dose mg/kg	40 days survivors	Mean survival time (days)	Survival time (treated/control)
19.2	14.3 (74)	25.0	0/3	26.5±7.5	273
19.8	16.7 (84)	12.5	0/4	20.5±7.8	211
17.6	16.3 (92)	6.25	0/4	14.2±6.0	146
17.3	19.5 (112)	3.12	0/4	11.7±2.3	120
16.4	18.6 (113)	1.56	0/3	12.0±3.4	123
16.1	20.9 (129)	0.78	0/4	11.7±3.4	120
14.9	20.1 (134)	0.39	0/4	11.7±2.3	120
15.5	19.3* (124)	0.19	0/4	8.2±1.7	84
16.5	25.8 (156)	0	0/7	9.7±3.9	100

* In the 7 days.

Table 10. Effect of bleomycin complex(-Cu) on X5563 plasmacytoma in C3H/He mice.

Initial body wt. mean (g)	Body wt. after 10 days (% change)	Daily dose mg/kg	30 days survivors	Mean survival time (days)	Survival time (treated/control)
18.1	13.2 (72)	25.0	0/2	17.5±0.7	126
18.2	16.0 (87)	12.5	0/4	17.0±0.8	123
18.4	19.9 (108)	6.25	0/4	14.7±1.9	106
18.5	20.2 (109)	3.12	0/4	13.7±1.7	99
17.9	19.9 (111)	1.56	0/4	14.0±2.1	101
18.2	21.2 (116)	0.78	0/4	15.2±5.9	110
20.7	23.1 (111)	0.39	0/4	10.5±1.0	76
21.0	22.7 (108)	0.19	0/4	10.7±0.5	77
19.4	23.2 (119)	0.09	0/4	12.0±0.8	86
18.3	22.6 (123)	0	0/6	13.8±0.9	100

unique symptom of FRIEND virus disease, probably caused by the virus produced by the tumor cells, was suppressed.

5. Mouse ascites hepatoma MH 134:

CCF_1 hybrid mice were employed in this experiment. As shown in Table 9, prolonged % survivals were obtained at dose levels of 25 mg/kg per day (273 %) and of 12.5 mg/kg per day (146 %). No complete cure was observable among the treated mice.

6. Mouse ascites plasmacytoma X 5563:

After 30 th passage in C 3 H/He mice as an ascitic form this particular tumor showed 100 % take with mean survival times of 13.8±0.98 and 17.5±1.73 days at the inoculum size of 2×10^6 and 10^5 cells respectively, but no take at less than 10^4 cells as inoculum. After the inoculation of 2×10^6 cells C 3 H/He mice were treated with bleomycin in the same way as above. Only survival times of 120 % were obtained at dose levels of 25 mg and 12.5 mg/kg per day (Table 10).

Discussion

A newly developed antitumor antibiotic, bleomycin, has been reported to show remarkable effects on human cases of squamous carcinoma type. The main purpose of the present study is to search for a possible correlation of this antibiotic effect among our transplantable mouse tumor systems which are syngeneic in tumor-host relationship. The results obtained showed moderate to marked effects of the antibiotic upon some of our SR-RSV-induced ascites tumors, particularly with F_1 hybrid mice as host animals. As shown by the laws of transplantation generally accepted, indicating tumor cells of homozygous animal strains are freely transplantable to syngeneic hosts as well as to semi-syngeneic F_1 hybrids and cancer chemotherapeutists usually employ highly available F_1 hybrids as host animals such as CDF_1 (BALB/c×DBA/2) mice for L1210 leukemia test in place of syngeneic DBA/2 mice. So we employed CCF_1 (BALB/c×C3H/He) mice in place of C3H/He mice for C3H/He mouse compatible tumor systems. However, according to a recent concept of HELLSTRÖM's "allogeneic inhibition" which might have some correlation to "contact inhibition", the laws of transplantation are not always applicable particularly when decreased inoculum size of tumor cells are given to F_1 hybrid mice. The present data are also compatible with an effect of the antibiotic on the tumor cells which would make them susceptible to allogeneic inhibition of F_1 hybrid host. Further investigations must be carried out to test these suggestions.

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