ACTIVITY OF PHENOMYCIN AGAINST TRANSPLANTABLE ANIMAL TUMORS

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Phenomycin, a new polypeptide antibiotic, was observed to exhibit significant inhibitory activity against EHRLICH carcinoma and sarcoma 180 in *ddD* mice, both ascitic and subcutaneous solid forms. It also showed a marked activity against the growth of adenocarcinoma 755 in C57BL/6 mice. The LD_{50} for mice was 8 mg/kg both by intraperitoneal and subcutaneous injections.

Phenomycin, a new polypeptide antibiotic, was isolated from a strain of *Streptomyces fervens*. It yields lysine, histidine, ammonia, arginine, aspartic acid, threonine, serine, glutamic acid, proline, glycine, alanine, valine, methionine, isoleucine, leucine, tyrosine, and phenylalanine by STEIN-MOORE analysis. It moves to the cathode and is shown to be a basic peptide by cellulose acetate film electrophoresis[1].

The chemotherapeutic activity of phenomycin was investigated by tests against several transplantable mouse tumors and the results are presented in this publication.

Materials and Methods

The phenomycin sample used in this experiment was highly purified and was prepared from the culture filtrate by repeated column chromatography with cation exchange resin (Amberlite IRC-50), CM-cellulose and Sephadex G-25. It gave a single spot and moved to the cathode by cellulose acetate film electrophoresis[1].

Transplantation of animal tumors and treatment with phenomycin followed the protocols recommended by the Cancer Chemotherapy National Service Center in U.S.A.[2]

Adenocarcinoma 755 was maintained in C57BL/6 mice, weighing approximately 22 g and transferred at 2-week intervals. EHRLICH carcinoma and sarcoma 180 ascitic form were carried in *ddD* male mice by transferring at a week intervals. The *ddD* male mice were inoculated intraperitoneally with 2×10^6 EHRLICH carcinoma or sarcoma 180 cells, and treated once daily for 7 days by intraperitoneal administration of phenomycin starting 24 hours after the inoculation. Survival time and body weight were observed as a criteria of tumor growth.

The ascitic tumor cells of EHRLICH carcinoma (8×10^6 cells in the experiments shown in Table 1 and 4×10^6 cells in those in Table 2) or sarcoma 180 (10^7 cells) were implanted subcutaneously in the inguinal region of *ddD* mice. Treatment with phenomycin began 24 hours after implant: one dose daily for 7 days. Animals were sacrificed on the 8th day. Tumors were excised and the weights were measured. The change of body weight was observed as an indicator of toxicity of phenomycin.

Tumors of 2 mm^3 of adenocarcinoma 755 were implanted in the back region of C57BL/6 mice. Treatment was begun 24 hours after the implant: one dose daily for 10 days. The animals were sacrificed on the 11th day, tumors were excised and weighed. The change of body weight was observed as a criteria of toxicity of the antibiotic.

Results

Phenomycin significantly inhibited the ascitic form of EHRLICH carcinoma when it was injected intraperitoneally for 7 days starting a day after the inoculation of tumor cells. It prevented ascitic increase and prolonged the survival period at doses of more than 32 μg/kg×7. All the animals survived at the dose of 125 μg/kg×7 in the period of 62 days observation. Control animals were observed to die on 11.3±1.3 day. The survival periods of phenomycin-treated animals were more than 62 days (phenomycin 125 μg/kg), >39.5±8.8 days (63 μg/kg×7), and >32.6±6.4 days (32 μg/kg×7).

A similar activity of phenomycin was demonstrated against the ascitic type of sarcoma 180. It inhibited ascites increase and prolonged the survival of tumor-bearing mice at doses of more than 32 μg/kg×7. All the animals survived at doses of 125 and 250 μg/kg. Control mice died on 20.9±2.3 day. The survival time of phenomycin-treated animals were more than 63 days (phenomycin 250 and 125 μg/kg×7), >58.5±5.0 days (63 μg/kg×7), and >31.8 ±12.4 days (32 μg/kg×7). The results are illustrated in Fig. 2.

Fig. 1. Activity of phenomycin on the ascitic form of EHRLICH carcinoma in mice

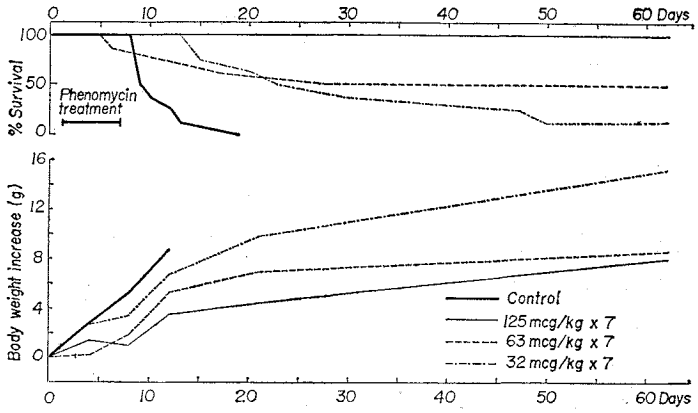
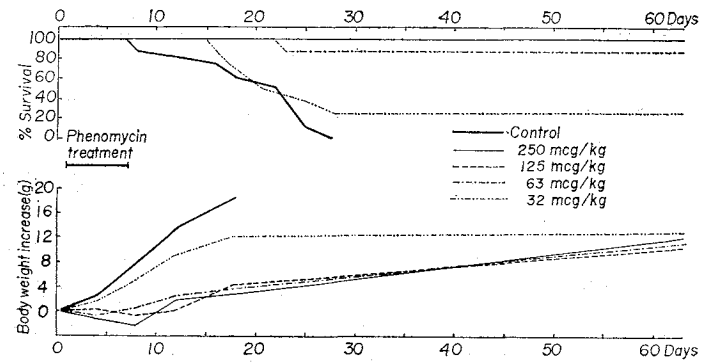


Fig. 2. Activity of phenomycin on the ascitic form of sarcoma 180 in mice



Phenomycin was demonstrated to exhibit an inhibitory activity against subcutaneous solid form of EHRLICH carcinoma. It was effective at the dose of more than 125 μg/kg daily for 7 days, when the intraperitoneal injection of phenomycin started a day after the tumor inoculation and tumor size was observed a day after termination of the treatment. Approximately 88

Table 1. Activity of phenomycin on the solid form of EHRLICH mouse carcinoma by intraperitoneal administration

Daily dose μg/kg	Period of treatment days	Observation day	Average body weight		Tumor weight	
			0 day g	Gain g	Average mg	Inhibition %
500	1~7	8	21.0	−4.6	61±13	88
250	1~7	8	22.5	−3.0	178±15	64
125	1~7	8	23.4	−0.9	250±35	49
0		8	22.0	+2.9	493±55	

% inhibition of tumor growth was demonstrated with the dose of 500 μg/kg×7, 64 % inhibition with 250 μg/kg×7 and 49 % inhibition with 125 μg/kg×7. A similar but less activity was observed by the subcutaneous administration, when the observation of tumor size was performed a week after finishing the treatment with phenomycin. The results are presented in Tables 1 and 2.

Table 2. Activity of phenomycin on the solid form of EHRLICH mouse carcinoma by subcutaneous administration

Daily dose μg/kg	Period of treatment days	Observation day	Average body weight		Tumor weight	
			O day g	Gain g	Average mg	Inhibition %
500	1~7	14	22.5	+1.2	131±20	69
250	1~7	14	21.4	+2.6	275±69	34
125	1~7	14	20.4	+3.2	356±50	14
0		14	23.0	+4.9	415±49	

Table 3. Activity of phenomycin on the solid form of sarcoma 180 by intraperitoneal administration in mice

Daily dose μg/kg	Period of treatment days	Observation day	Average body weight		Tumor weight	
			O day g	Gain g	Average mg	Inhibition %
500	1~7	8	24.3	−3.1	165±29	65
250	1~7	8	23.6	−2.2	202±30	57
125	1~7	8	21.9	+1.3	356±30	24
0		8	25.5	+3.0	470±57	

Table 4. Activity of phenomycin on adenocarcinoma 755 by intraperitoneal administration in C57BL/6 mice

Daily dose μg/kg	Period of treatment days	Observation day	Average body weight		Tumor weight	
			O day g	Gain g	Average mg	Inhibition %
500	1~10	11	22.2	Toxicity		
250	1~10	11	23.6	−6.6	88±30	96
125	1~10	11	20.9	−1.9	395±47	84
0		11	23.6	+4.2	2,396±297	

Phenomycin exhibited a significant effect on the growth of subcutaneous solid form of sarcoma 180 by the intraperitoneal administration of more than 250 μg/kg daily for 7 days. Approximately 65 % inhibition was demonstrated with the dose of 500 μg/kg×7 and 57 % inhibition with 250 μg/kg×7. The results are summarized in Table 3.

As presented in Table 4, phenomycin was observed to exert a marked activity against adenocarcinoma 755 in C57BL/6 mice. Almost complete inhibition (96 %) was observed with intraperitoneal injection of 250 μg/kg×10 and 84 % inhibition with 125 μg/kg×10.

Judging from the change of body weight of mice, phenomycin was toxic to tumor-bearing mice at a dose of 125 μg/kg daily for 7 or 10 days. It killed C57BL/6 mice bearing adenocarcinoma 755 following intraperitoneal injection of 500 μg/kg×10. Acute toxicity of phenomycin to normal *ddD* mice was: LD_{50}=8 mg/kg both by the intraperitoneal and subcutaneous routes.

Discussion

As report in the previous paper[1], phenomycin is related to enomycin. Chemical and biological characteristics of both antibiotics are similar. Phenomycin exhibits a significant antitumor activity but no antimicrobial activity. The degree of inhibition of the transplantable tumors by phenomycin was observed to be comparable to that by enomycin[3].

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References

1) NAKAMURA, S.; T. YAJIMA, M. HAMADA, T. NISHIMURA, M. ISHIZUKA, T.TAKEUCHI, N. TANAKA & H. UMEZAWA : A new antitumor antibiotic, phenomycin. J. Antibiotics, Ser. A 20(4) : 210~216, 1967
2) Cancer Chemotherapy National Service Center : Protocols for screening chemical agents and natural products against animal tumors and other biological systems. Cancer Chemoth. Reports 25 : 1~22, Dec. 1962.
3) SUHARA, Y.; M. ISHIZUAK, H. NAGANAWA, M. HORI, M. SUZUKI, Y. OKAMI, T. TAKEUCHI & H. UMEZAWA : Studies on enomycin, a new antitumor substance. J. Antibiotics, Ser. A 16 (2) : 107~108, Mar. 1963