ISOLATION AND CHARACTERIZATION OF LYMPHOMYCIN

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A new antitumor antibiotic is produced in fermentation broths by *Streptomyces* sp. S-66. This material, named lymphomycin, was isolated on the basis of inhibition of mouse leukemia SN-36 in mice, and toxicity to BURKITT lymphoma cells in tissue culture. It was precipitated from filtered broth with ammonium sulfate and purified by chromatography on columns of Sephadex and carboxymethyl cellulose. Lymphomycin is a black-colored, acidic protein with a molecular weight of about 11,000 and valine as an N-terminal amino acid. It contains no carbohydrates or nucleic acids. It is soluble in water, insoluble in most organic solvents, and is relatively stable in water over the pH range, 5 to 7. It has no distinct absorption maximum in its spectrum. Lymphomycin has no inhibitory activity against bacteria, yeast, fungi and mycoplasmas tested so far. It is cytotoxic to Burkitt lymphoma and human lymphoblastoid cells and peritoneal macrophages, but not inhibitory to human epidermoid carcinoma HeLa, mouse fibroblast L, chick embryo, calf kidney and mouse adenocarcinoma FM3A cells in tissue culture. Lymphomycin is lethal to tumor-bearing mice at 80 mg/kg of body weight per day when given intraperitoneally once daily for 6 days, but not toxic at 40 mg/kg/day. Tests with transplanted rodent tumors indicate that the antibiotic is inhibitory to the growth of both solid and ascitic forms of Sarcoma 180 and lymphatic leukemia SN-36 in mice. The growth of solid tumor of a fibrosarcoma in hamsters was also inhibited at optimal daily doses of 0.8 and 2 mg/kg. Lymphomycin has no effect on the growth of the solid forms of EHRLICH and BASHFORD carcinoma in mice.

In our antibiotic screening program, a black-colored protein exhibiting antitumor activity against transplanted rodent tumors was isolated from the culture broths of a *Streptomyces* designated as S-66. This protein did not exhibit any antimicrobial activity but was active against certain tumor cells both *in vivo* and *in vitro*. As will be described in this paper, one of the mammalian cells most sensitive to this antibiotic *in vitro* was the lymphoblastoid tumor cell. Thus, the antibiotic was given the name of lymphomycin. The present paper describes the production, isolation, chemical characterization and biological activity of lymphomycin.

Materials and Methods

Producing organism:

The organism, *Streptomyces* sp., S-66, was isolated from a soil sample collected in

Towada, Aomori Prefecture, Japan. The organism was maintained on KRAINSKY's agar slant. The taxonomy of the organism will be reported elsewhere.

Purification and characterization of lymphomycin:

CM-cellulose column chromatography and Sephadex gel filtration were performed according to the method described previously for neocarzinostatin[1]. Amino acid analysis and N-terminal amino acid determination were carried out following the methods described previously[1].

Tested tumors:

Lymphomycin was tested for its antitumor effects in several transplanted tumors in mice and hamsters. The tumors used were as follows: EHRLICH ascites carcinoma, CROCKER sarcoma 180 (ascites form), mouse lymphatic leukemia SN-36 (ascites form) and BASHFORD carcinoma (ascites form) in *dd* mice, and NQT-1 tumor in golden hamster.

The ascites form of BASHFORD carcinoma was transformed from the subcutaneous solid tumor by KUMAGAI and TANNO in 1961 and has been maintained in this laboratory[2].

For the test of the tumors in mice, a suspension of 5~10 million tumor cells in ascitic form was inoculated either intraperitoneally or intramuscularly in the thigh.

NQT-1 tumor is the fibrosarcoma induced by repeated injections of 4-nitroquinoline-1-oxide in a golden hamster by KUROKI[3]. For assay of activity against this tumor, a tumor piece was inoculated subcutaneously in the back of golden hamsters weighing 130~150 g by using the usual trocar method.

For all therapeutic tests, the antibiotic was dissolved in saline, and intraperitoneal injection was started 24 hours after tumor implantation. The therapy interval, injection volume and evaluation criteria are shown in the Assay and Results section.

Tested tissue-culture cells:

The tissue-culture cells used were as follows: (A) four systems of cells maintained in monolayer culture; (1) S3 clone of HeLa cells, (2) L, an established line of mouse fibroblast cells, (3) a fibroblast-like strain originated from a calf kidney but not established as a cell line, (4) primary cultures of mouse peritoneal macrophage, and (B) three systems of cells maintained in floating culture; (5) FM3A, an epithelium-like cell strain originated from a mouse mammary adenocarcinoma[4], (6) P3HR-1, a clone of BURKITT lymphoma cells[5], (7) 64-10, a lymphoblastoid cell strain derived from a human leukemia patient[6].

For toxicity tests, lymphomycin was dissolved in the respective maintenance medium in serial two-fold dilutions and inoculated into each tube culture. The culture media of cells and the toxicity profiles of cells to lymphomycin are shown in the Assay and Results section.

Assay of lymphomycin during preparation:

The potency of lymphomycin was chiefly estimated by inhibition of the growth of SN-36 ascitic tumor in mice. Cytotoxic tests on the P3HR-1 clone of BURKITT lymphoma cells were run in parallel.

For the ascites tumor assay, the mice were inoculated intraperitoneally with $1\sim2\times10^6$ leukemic cells. Lymphomycin in fermented beers or from further stages of purification was given intraperitoneally to mice starting 24 hours after cell inoculation. The injections were continued once daily for 6 days. The mice treated with lymphomycin and the controls were killed 3 days after the final treatment. Evaluation was based upon the ascites volume.

For the activity assay with BURKITT lymphoma, the cells were tested in a floating condition. For the growth inhibition test, 2×10^5/ml of P3HR-1 cells were transferred to small bottles containing EAGLE's minimum essential medium (MEM) supplemented with 10 % bovine serum. The cultures were treated with lymphomycin at desired concentrations and incubated at 37°C for 2 days. Activity of the test sample was determined by comparing the number of viable cells with that of control, by means of trypan blue staining.

Experimental Results

Fermentation

For the production of lymphomycin, the following fermentation procedure was employed. Spore stocks of *Streptomyces* sp. S-66, obtained from KRAINSKY's asparagine glucose agar slant, were used to inoculate a seed medium, consisting of 2 % starch, 1.5 % yeast, 2 % soybean meal, 0.25 % NaCl, 0.2 % $CaCO_3$, 0.005 % $MnCl_2 \cdot 4H_2O$, 0.005 % $CuSO_4 \cdot 5H_2O$ and 0.005 % $MgSO_4 \cdot 7H_2O$ in water. After 24-hour incubation, this seed culture was used at 3 % to inoculate a production medium, consisting of 2 % glycerin, 2 % starch, 1 % peptone, 0.5 % meat extract, 0.5 % NaCl and 0.2 % $CaCO_3$. All fermentations were carried out on a rotary shaker at 27°C. The peak fermentation yields of 200 mcg/ml of lymphomycin were obtained in the production medium after 4 days. When 0.2 ml of five times diluted solution of such culture filtrate was injected intraperitoneally once daily for 6 days into SN-36 bearing mice, it consistently inhibited tumor growth.

Isolation and Purification

The fermented broth was filtered at harvested pH 6.8~7.0, and 10 liters filtrate was concentrated to one quarter of the original volume in a flash evaporator below 28°C. The concentrated filtrate was put in a cellophane tube and placed in solid ammonium sulfate to precipitate the antibiotic. The precipitate was collected by centrifugation and then dissolved in a 200 ml water using a magnetic stirrer at 4°C. This suspension was centrifuged to obtain the supernatant which contained the active principle. The supernatant was dialyzed against water for 24 hours. The dialyzed material was freeze-dried and 3.3 g brown-black powder was obtained from 10 liters of broth. This was called crude lymphomycin. The yield was 70 % of the total original activity.

Gel filtration of the crude powder was then conducted using Sephadex G-50 (column 4×92 cm for 1 g of sample) as shown in Fig. 1. The first peak in the figure was found to be an active principle as revealed by inhibition test on the growth of SN-36 and BURKITT lymphoma cells. The peak was pooled, dialyzed against water and lyophilized. Further purification was carried out by means of CM-cellulose column chromatography. This and rechromatographic results are shown in Figs. 2 and 3. In the first run (Fig. 2), five fractions were obtained and the fourth major peak was found to contain almost all of the activity. This active peak was freeze-dried and rechromatographed. In this second chromatograph, peak II (Fig. 3) contained almost all of the activity and the other two minor

Fig. 1. Gel filtration of crude lymphomycin on Sephadex G-50 column.

One gram of crude lymphomycin was filtrated on Sephadex G-50 column, 4.0 ×92 (cm/cm). Elution was made with 0.17 M phosphate buffer pH 6.8 and each tube contained 7 ml of eluate. Reading of O. D. at 280 mμ gave three peaks, I 402 mg, II 345 mg, and III 10 mg by dry weight.

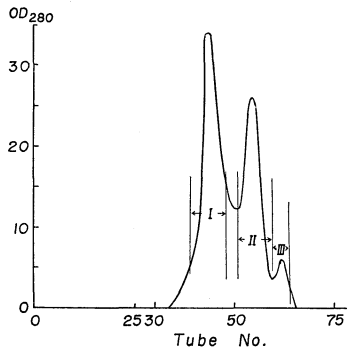


Fig. 2. First chromatography of lymphomycin on CM-cellulose column.

Five handred mg of the fraction I obtained by the gel filtration of Sephadex G-50 were applied on a CMC column, 3.1×60 (cm/cm). The elution was initially made with 0.1 M acetic acid, followed by 0.1 M acetic acid-0.1 M sodium acetate in linear pH gradient fashion. The elution was completed with a mixture of 0.5 M NaCl and 0.2 M sodium acetate. Each tube contained 5 ml of fraction. Reading of O. D. at 280 mμ gave five peaks; I 8 mg, II 22 mg, III 55 mg, IV 120 mg and V 85 mg by dry weight.

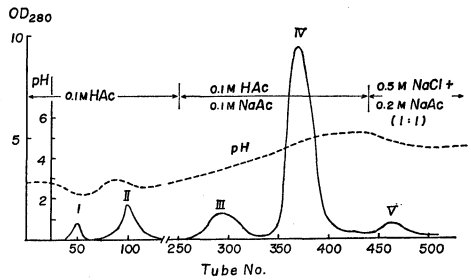


Fig. 3. Rechromatograpy of lymphomycin on CM-cellulose column.

About 200 mg of the active fraction, peak IV on first CMC chromatograpy was applied on a CMC column, 2.4×50 (cm/cm). Elution was made with 0.1 M acetic acid-0.1 M sodium acetate in linear pH gradient fashion. Each tube contained 4 ml fraction. Reading of O. D. at 280 mμ gave three peaks; I 9 mg, II 113 mg and III 15 mg, by dry weight.

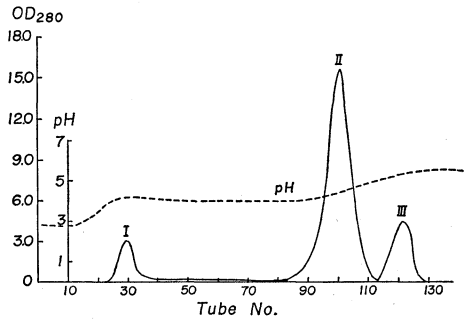
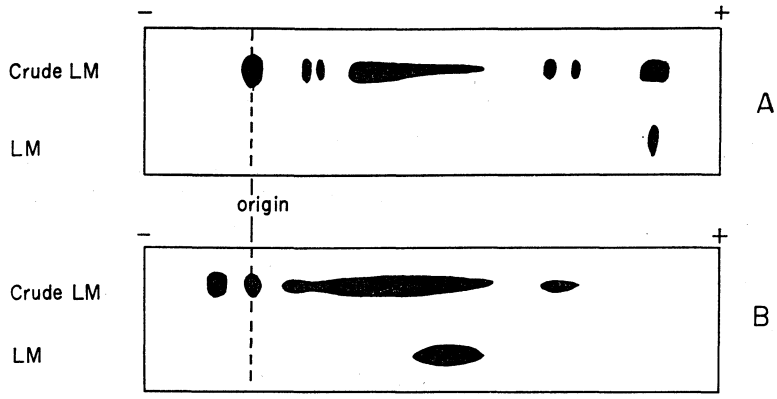


Fig. 4. Polyacrylamide gel electrophoresis of crude and purified lymphomycin.

Fig. A shows the patterns of crude (crude LM) and purified lymphomycin (LM) on electrophoresis in the pH 8.2, 0.075 M tris-citrate buffer in gel and bridge solution. 0.58 % NaCl solution was used for electrode vessels in both A and B. Fig. B. shows the patterns in the pH 5.8, 0.1 M phosphate buffer. The gel was stained with 0.1 % solution of Amideblack in a mixture of methanol, water and acetic acid in a ratio of 5 : 5 : 1.



peaks did not contain any activity. After rechromatography, the lyophilized active fraction was a fluffy black powder, and this was called a purified preparation. This material was obtained at a rate of 180 mg per 10 liters of culture filtrates, corresponding to 9 % in potency from culture filtrates.

Fig. 4 shows polyacrylamide gel electrophoresis[1,7] of both crude and purified lymphomycin. A single spot was found in the purified preparation.

Chemical Characterization

Elementary anlysis of lymphomycin gave: C 44.98, H 5.97, N 11.00. Lymphomycin was found negative for MOLISCH, anthrone-H_2SO_4, $FeCl_3$ and *p*-dimethylaminobenzaldehyde-EHRLICH reagent. However it gave positive biuret, ninhydrin, FOLIN and SAKAGUCHI reactions. It has no definite melting point. It is easily soluble in water above pH 6.0 but not in organic solvents. The elution pattern of gel filtration experiments using Sephadex G-75, G-50 and G-25 revealed that the final preparation is a single and symmetrical moiety.

When the gel filtration experiment of lymphomycin was carried out on Sephadex G-50 column using two proteins, neocarzinostatin and lysozyme, as standards of known molecular weight, lymphomycin was eluted between the two standards (Fig. 5). From this result, the molecular weight was estimated to be between 14,500 and 9,000.

Amino acid composition was also deter-

Table 1. Amino acid composition of lymphomycin

Animo acid	moles/12,000 g*			Assumed no. of residues
	a†	b†	c†	
Lysine	5.39	6.08	7.76	5~7
Histidine	3.94	4.30	4.55	4
Ammonia	13.68	17.99	10.99	10
Arginine	5.56	5.32	5.62	4~5
Asparatic acid	8.12	8.62	10.45	7~10
Threonine	5.39	7.09	4.28	4~6
Serine	6.48	5.83	4.55	4~6
Glutamic acid	15.76	13.68	13.66	11~14
Proline	11.87	12.41	12.06	10~11
Glycine	14.14	16.41	12.33	11~12
Alanine	8.35	8.60	7.76	7
Cystine	none	none	none	0
Valine	8.33	8.62	7.76	7
Methionine	1.16	1.26	1.07	1
Isoleucine	2.78	3.29	2.94	3~5
Leucine	1.39	3.03	3.75	1~3
Tyrosine	trace	trace	trace	0
Phenylalanine	4.12	4.20	4.28	3~4
Tryptophan	none	none	none	0

Total 82~100 amino acid residues were assumed in the lymphomycin molecule, and the molecular weight obtained from this amino acid composition is 10,057~12,804 g.

† Condition of the protein hydrolysis in three time redistilled 6 N HCl at 110°C±5°C, (a) for 20 hours, (b) for 40 hours, (c) for 72 hours.

†† Data form chemical analysis by dimethylaminobenzaldehyde method.

* This value was obtained from Sephadex experiment.

Amino acid analyser: Yanagimoto LC-5.

Fig. 5. Gel filration of lymphomycin, neocarzinostatin and lysozyme by Sephadex G-50.

This experiment was conducted in order to determine the approximate molecular size of lymphomycin using two known proteins as the references. Seven mg lymphomycin, 4 mg neocarzinostatin and 3 mg egg white lysozyme were mixed in a small quantity of water and the mixture was applied on a Sephadex column of 1.25×71 cm/cm. Elution was made with 0.1 M phosphate buffer, pH 7.0. Each tube contained 3 ml of the fraction. Reading of O. D. gave three peaks; I, lysozyme (from enzyme activity against *M. lysodeicticus*, read at O. $D._{540}$), peak II, lymphomycin (O. D. at 280 mμ together with black color), and peak III, neocarzinostatin (from antibacterial activity against *Sarcina lutea*).

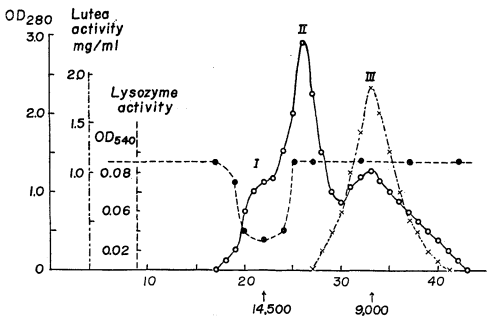


Fig. 6. Ultraviolet absorption spectra of lymphomycin.

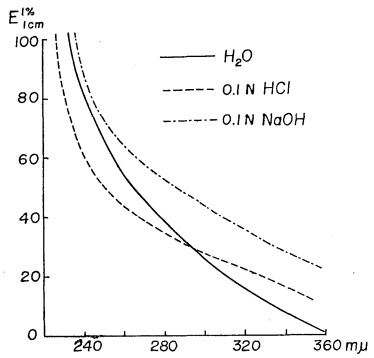
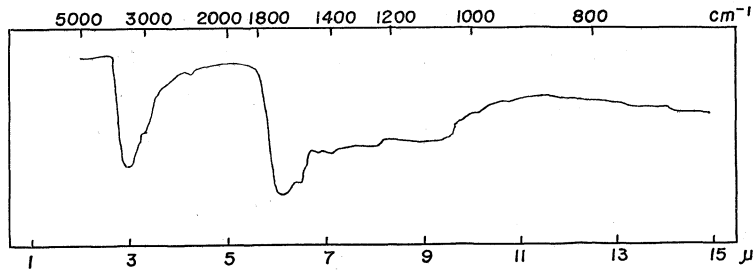


Fig. 7. Infrared absorption spectrum of lymphomycin in KBr.



mined according to the method of MOORE and STEIN[8]. The results obtained are shown in Table 1. When the results were integrated for the number of residues of each amino acid, a molecular weight of about 11,000 was obtained. A high content of glutamic acid, proline and glycine was noted but neither cystine nor tryptophan was found.

N-terminus determination of the antibiotic was made according to the method described previously using 2, 4-dinitrofluorobenzene[1]. Three ether-soluble DNP-amino acids were obtained from the partially purified lymphomycin after the first CM-cellulose chromatography. These were identified as aspartic acid, valine and leucine using paper and silicagel thin-layer chromatography.

After rechromatography by CM-cellulose only one ether-soluble DNP-amino acid was obtained from the purified lymphomycin. This was identified as valine.

Ultraviolet absorption spectra of the protein are given in Fig. 6. No distinct peak was seen. The infrared absorption spectrum was that of the usual protein (Fig. 7).

Antimicrobial Tests

Agar dilution tests were conducted to determine the minimal inhibitory concentration of lymphomycin for a number of microorganisms. The antibiotic at 1,000 mcg/ml was found to have no inhibitory effect against any of the microorganisms tested so far: *Bacillus subtilis* PCI 219, *Staphylococcus aureus* 209-P, *Staphylococcus aureus* Terashima, *Sarcina lutea* PCI 1001, *Klebsiella pneumoniae*, *Shigella flexneri* 2b, *Shigella flexneri* D2, *Xan-*

Fig. 8. Growth inhibition of BURKITT lymphoma P3HR-1 cells by lymphomycin.

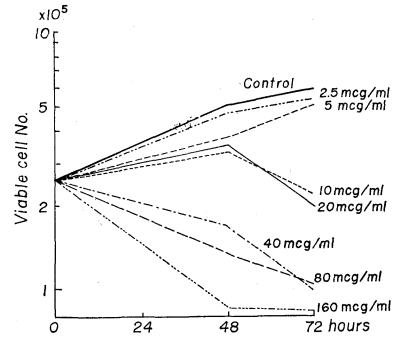


Table 2. Toxicity of lymphomycin on mammalian cells

Cell	Medium*	Minimum toxic concentrations mcg/ml
HeLa S3	YLE	150**
L	YLE	150**
Chick embryo	YLE	>150 †
Calf kidney	MEM	>150 †
FM3A	YLE	150**
P3HR-1	MEM	10**
64-10	MEM	5**
Mouse peritoneal macrophage	YLE	5 †

FM3A, P3HR-1 and 64-10 cell were grown in a floating culture condition and others in a monolayer culture.

YLE : 0.5% lactalbumin hydrolysate and 0.1 % yeast extract in EARLE's balanced salt solution.

* Each of medium was supplemented with 10 % bovine serum.

** Minimum inhibitory concentration of cell growth on the 2nd day of drug treatment.

† Minimum concentrations producing microscopically a degenerative change onto cells on the 2nd day of drug treatment.

Table 3. Effect of lymphomycin on SN-36 and Sarcoma 180 (ascites form) in mice

Tumor	Dose mg/kg	No. of mice	Dead/Treated	Ascites volume (g)	% inhibition
SN-36	80	4	4/4	—	—
	40	4	0/4	0.0	100
	20	4	0/4	0.0	100
	10	4	0/4	0.0	100
	5	4	0/4	0.0	100
	2.5	4	0/4	0.3	51
	0	8	0/8	6.1	—
S-180	40	4	0/4	0.0	100
	25	4	0/4	0.0	100
	5	4	0/4	0.0	100
	1	4	0/4	2.1	48
	0	12	0/12	4.0	—

The treatment began intraperitoneally 24 hours after implantation of tumor cells and continued once daily for 6 days. On the 10th day, the mice were sacrificed and the ascites volume were weighed.

Table 4. Effect of lymphomycin on SN-36 and Sarcoma 180 (solid form) in mice

Tumor	Dose mg/kg	No. of mice	Dead/Treated	Tumor weight (g)	% inhibition
SN-36	80	6	6/6	—	—
	40	6	0/6	0.13	89
	20	6	0/6	0.18	85
	10	6	0/6	0.44	61
	0	10	0/10	1.22	—
S-180	80	6	4/6	—	—
	40	6	0/6	0.53	58
	20	6	0/6	0.51	60
	10	6	0/6	0.53	58
	0	10	0/10	1.28	—

The treatment began intraperitoneally 24 hours after implantation of tumor cells and continued once daily for 6 days. On the 10th day, the tumors in the thigh were excised and weighed.

Table 5. Effect of lymyhomycin on solid tumors in mice

Tumor	Dose mg/kg	No. of mice	Dead / Treated	Tumor wt. (g)	% inhibition
Leukemia SN-36	40	10	0/10	0.25	73
	0	10	0/10	1.05	
Sarcoma 180	40	10	0/10	0.52	50
	0	10	0/10	1.03	
BASHFORD carcinoma	40	10	0/10	1.17	2
	0	10	0/10	1.19	
EHRLICH carcinoma	40	10	0/10	2.15	18
	0	10	0/10	2.61	

The treatment began intraperitoneally 24 hours after implantation of tumor cells and continued once daily for 6 days. On the 10th day the subcutaneous tumors were excised and weighed.

thomonas oryzae, *Xanthomonas citri*, *Candida albicans* M-9, *Candida tropicalis*, *Candia pseudotropicalis*, *Saccharomyces rouxii* Boutroux, *Willia anomala*, *Trichophyton mentagrophytes*, and *Trichophyton cutaneus*.

Two tested mycoplasmas, *Mycoplasma pneumoniae* and *M. pulmonis*, were also insensitive to 200 mcg/ml of the antibiotic in PPLO Broth (Difco) supplemented with 20 % horse serum.

Effect on Mammalian Cells *in vitro*

As shown in Fig. 8, the minimum concentration for growth inhibition of BURKITT lymphoma cells was found to be 10 mcg/ml.

Results of cytotoxicity tests with several mammalian cells in tissue culture are illustrated in Table 2. Lymphomycin is toxic to BURKITT lymphoma cells, 64-10 leukemic cells and mouse macrophage at 5~10 mcg/ml. However, it is toxic only at high doses or not toxic to five other established cell lines or primary culture cells.

Effect on Transplanted Tumors

The effect of lymphomycin on the growth of ascitic sarcoma 180 and SN-36 in mice are given in Table 3. Lymphomycin at 80 mg/kg is lethal to mice bearing the ascites tumors. However the antibiotic at daily doses ranging from 40 to 2.5 or 1.0 mg/kg/day is not toxic and inhibited tumor growth. No tumor growth is seen at 40 to 5 mg/kg/day.

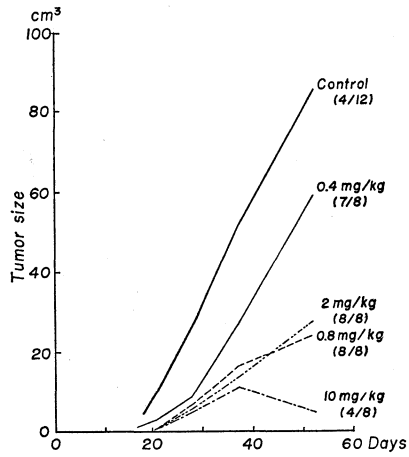
When tested against the same

tumors transplanted intramuscularly in the thigh of mice, lymphomycin at doses of 10~40 mg/kg/day significantly inhibited tumor growth (Table 4). In another experiment, the effect of lymphomycin at a dose of 40 mg/kg/day was tested on four tumors transplanted intramuscularly as mentioned above. The results are shown in Table 5. Sarcoma 180 and SN-36 were significantly inhibited, but no effect was seen in the EHRLICH and BASHFORD carcinomas.

Fig. 9. Effect of lymphomycin on NQT-1 tumor in golden hamster.

Tumor volume is represented by an average number of the three dimensions in cm^3 of tumors.

In parentheses : No. of survived/No. of treated (on 54th day)



NQT-1 tumor was grown as a solid tumor in adult golden hamsters, and was transplanted subcutaneously. Hamsters with solid NQT-1 tumor received intraperitoneal injections of lymphomycin, starting from 24 hours after tumor inoculation and continuing once every other day for 7 weeks. Such a long term treatment was required since the tumor growth in hamsters was very slow. The tumors were measured four times at an appropriate interval and the proportion of tumor-bearing animals surviving was determined on 54th day after tumor inoculation. From preliminary experiments, it has been found that no severe toxic signs were seen in animals administered less than 10 mg/kg/day of lymphomycin by this schedule.

Results of treatments with doses of 0.4 to 10 mg/kg/day are shown in Fig. 9. The dose of 0.8, 2 and 10 mg/kg/day significantly inhibited the tumor growth in hamsters but that of 0.4 mg/kg/day did slightly. As for the proportion of survivors, an increase was observed in the groups treated with 0.4, 0.8 and 2 mg/kg/day, but not with 10 mg/kg/day. From the results, lymphomycin proved to inhibit the growth of NQT-1 tumor in hamsters and result in an increased survival tumor-bearing animals, when the drug was given intraperitoneally at the doses of 0.8 and 2 mg/kg/day once every other day for 7 weeks.

Discussion

The antitumor substance designated as lymphomycin was isolated, purified and characterized. Lymphomycin is a black-colored protein, exhibiting positive biuret and FOLIN reactions. The antibiotic is a protein and negative in MOLISCH, anthrone and $FeCl_3$ reactions, suggesting the absence of either carbohydrates or nucleic acid. As shown in Fig. 6, the absorption spectra of the antibiotic exhibited end absorption. The nature of the black color and the nitrogen concentration of 11 % of the antibiotic was left for further characterization, although the possibility of metalloprotein or chromoprotein can be considered.

Using Sephadex gel filtration, the molecular weight was estimated to be between 14,500 (lysozyme) and 9,000 (neocarzinostatin). The molecular weight determined on the basis of amino acid composition was again 11,000. The antibiotic contained a high concentration of glutamic acid followed by glycine and proline. These three amino acids

residues make up approximately 38 % of total amino acid residues. The amino acid composition looks similar to that of collagen[10], in which glutamic acid, glycine and proline are the three richest amino acids in the molecule. Some of the plant proteins, such as zein[11], are also known to have high contents of both glutamic acid and proline. All of these proteins as well as lymphomycin have a very low content of cystine (almost nil).

The N-terminal amino acid was examined for both partially purified and purified lymphomycin preparations. The former possessed valine, alanine, and aspartic acid, while the latter had only valine. This result is in accord with the analyses obtained by means of polyacrylamide gel electrophoresis, where 3 boundaries were obtained with the partially purified preparation and only one spot for the final preparation.

The purity of lymphomycin was verified by disc and starch gel electrophoresis, cellulose polyacetate electrophoresis, gel filtration on Sephadex G-25, G-50, and G-75 (not shown) and chromatography on CM-cellulose column (Fig. 3).

The several known high molecular-weight antitumor substances isolated from *Streptomyces* culture filtrates, carzinomycin[12], carcinocidin[13], melanomycin[14], and A-280 substance[15,16] seem to have some resemblances to lymphomycin. All of them are neutral or acidic, brown or brown-black proteins which inhibit rodent tumors.

Carzinomycin is reported to be negative in biuret, ninhydrin and $FeCl_3$ reactions and positive in MOLISCH reaction, and to be adsorbed to and eluted from IRC-50 resin. Lymphomycin differs from carzinomycin in that it gives positive biuret, ninhydrin and $FeCl_3$ reactions and negative MOLISCH reaction, and it is adsorbed to IRC-50 but not eluted from the resin as an active form. Carzinocidin was also reported to be negative in biuret, ninhydrin and FOLIN reactions and can easily be differentiated from lymphomycin. Melanomycin was also a brown black protein which can be purified by adsorption and elution on IRC-50 column and gives negative biuret and ninhydrin reactions, thus differentiating it from lymphomycin. The substance A-280 is an acidic brown protein but has a molecular weight of 15,000~25,000. It inhibits HeLa cells at a dose below 1 mcg/ml. These comparative data indicate that lymphomycin is a new antitumor antibiotic.

As described before, lymphomycin was not inhibitory on the growth of any microorganism tested so far including bacteria, yeasts, fungi and mycoplasmas. However, it revealed cytotoxicity on mammalian cells in tissue culture and antitumor activity in animals. Thus, the effect is specifically directed to mammalian cells. In addition, as shown in the results of tissue culture experiments, lymphomycin was found to have more pronounced inhibitory effects against lymphoblastoid cells such as BURKITT lymphoma cell, human leukemia cell and mouse macrophage than against other cells which are epithelial or fibroblastic in nature. *In vivo*, lymphomycin also showed more pronounced inhibitory effects against SN-36 cells of lymphoblastoid nature and S-180 and NQT-1 tumors of sarcoma type than against two other tumors of epithelial nature. This result suggests that lymphomycin has a selective action against lymphoblastoid and sarcoma cells among various mammalian cells.

One of the other characteristics of lymphomycin activity was that it has a relatively strong effect against tumor growth in mice, even against tumors transplanted intramuscularly, inspite of the weak activity against tissue culture cells. These results suggest that the drug is quite stable and circulated well throughout the body, if the effects of the antibiotic are assumed to be due to the direct cytotoxic action on tumor cells.

These preliminary data on the biological activity of lymphomycin, such as the selective toxicity to lymphoblastoid and sarcoma cells, the stability and the good distribution in the body make this drug of sufficient interest to warrant further consideration in studies of its antitumor activity.

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