STUDIES ON THE BIOLOGICAL ACTIVITY OF STUBOMYCIN

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Stubomycin showed direct cytotoxic activity on mammalian cells, yeast, and fungi, and rapid hemolytic activity on mouse erythrocytes. The rate and extent of the cytotoxic and hemolytic activities decreased at lower temperatures. Studies with radioactive precursors revealed that a marginal cytocidal concentration of the antibiotic inhibited synthesis of DNA, RNA, and protein of leukemic cells at almost the same rate. Stubomycin did not show any mutagenicity on mammalian cells and bacteria *i.e.* the induction of revertants on six bacterial strains, and chromosomal aberrations, sister chromatid exchanges, and the induction of cells resistant to 6-thioguanine on Chinese hamster cells (DON D-6). The antagonistic effect of various kinds of lipids including phospholipids, cholesterol, olive oil and squalene was studied. Significant antagonism of stubomycin against anti-*Saccharomyces cerevisiae* activity was observed with phospholipids except for egg lecithin and with cholesterol. The primary action of the antibiotic seems to be to change the cell surface and ultimately the lysis and death of the cells.

Stubomycin was isolated from the culture broth and mycelia of *Streptomyces* strain No. KG-2245 by UMEZAWA *et al.* in 1981.[1] Stubomycin has been shown to have marked *in vivo* antitumor activity on Ehrlich ascites carcinoma, P 388 leukemia, Meth A fibrosarcoma, *etc.* The antibiotic is also active against Gram-positive bacteria, some fungi, and HeLa cells *in vitro*.[2] Recently, ŌMURA *et al.* have elucidated the structure of stubomycin as a macrocyclic lactam involving β-phenylalanine.[3] This report describes preliminary studies on the cytocidal activity of stubomycin mainly using mammalian cells, yeast, and fungi.

Materials and Methods

Chemicals

N-Ethyl-*N'*-nitro-*N*-nitrosoguanidine (ENNG, Nakarai Chemicals Ltd., Kyoto), 2-nitrofluorene (Aldrich Chemical Co., USA), and 9-aminoacridine (Tokyo Kasei Kogyo Co., Tokyo) were dissolved in dimethyl sulfoxide. Ethyl methanesulfone and 6-thioguanine (EMS and 6-TG, Sigma Chemical, USA), mitomycin C (Kyowa Hakko Kogyo Co., Tokyo), and benzylpenicillin (Toyo Jozo Co., Shizuoka) were dissolved in phosphate buffer or water.

[^{3}H]Thymidine ([^{3}H]TdR, 16.2 Ci/mmole), [^{3}H]uridine ([^{3}H]UR, 38.3 Ci/mmole), and [^{3}H]leucine (158.0 Ci/mmole) were obtained from the Radiochemical Center, Amersham, UK. Cardiolipin, phosphatidylserine, and egg lecithin (Kitasato Institute, Tokyo), olive oil and cholesterol (Wako Pure Chemical Industries, Ltd., Osaka), and squalene (Kuraray Co., Osaka) were dissolved in ether or methanol.

Cells and Media

Salmonella typhimurium strain numbers TA98, 100, 1535, 1537, and 1538, and *Escherichia coli* WP 2uvrA were provided by Dr. T. KADA, National Institute of Genetics. DON D-6 cells, derived from Chinese hamster lung cells, were maintained in EAGLE's minimum essential medium (MEM) supplemented with fetal calf serum (10%), sodium pyruvate (1 mM), L-serine (0.2 mM), penicillin (100 u/ml), and streptomycin (100 μg/ml) at 37°C in an atmosphere of 5% CO_2 in air. Mouse leukemic EL-4 cells

were maintained in MEM supplemented with 10% calf serum, and cells in the logarithmic growth phase were used for the experiment.

Saccharomyces cerevisiae and *Pyricularia oryzae* have been maintained in our laboratory on potato agar and rice straw agar respectively. To determine the effect of stubomycin on these microorganisms, two media were used: (1) 0.3% yeast extract, 0.3% peptone, 3% glucose, and 0.3% Trypticase soy broth for *S. cerevisiae*; and (2) 0.5% peptone, 0.3% yeast extract, and 3% glucose-potato extract solution (200 g of potato was boiled in 1 liter of water for 30 minutes, and the solution was used as potato extract) for *P. oryzae*.

Measurement of Cytotoxicity

To determine the cytotoxicity of stubomycin on mammalian cells, EL-4 cells (2×10^5) in 2 ml of medium were placed in 30-mm Petri dishes and incubated for 48 hours. Then, 0.01 ml of different concentrations of stubomycin solution were added to each culture dish, and thereafter, the number of trypan blue excluded cells was counted periodically using a hemocytometer. In case of antibacterial activity, the antibiotic was added to suspensions of *S. cerevisiae* (optical density: 0.092) and *P. oryzae* (optical density: 0.043) in 4 ml of medium, and the mixtures were shaken at 27°C.

The growth of *S. cerevisiae* was followed by determining the absorbancy of cultures at 660 nm. In case of *P. oryzae*, the culture was gently dispersed by a homogenizer (Biotron), and the growth of *P. oryzae* was followed by determining the absorbancy of cultures at 660 nm. Values are the mean of three samples.

Measurement of Viability

EL-4 cells (7×10^5) in 7 ml of MEM were exposed to 0.05 ml of different concentrations of stubomycin solution for 5 to 60 minutes. Then at the end of each incubation time, cells were rinsed three times with fresh MEM, and were suspended in 6 ml of culture media. Two ml of each cell suspension were plated on 30-mm Petri dishes. Viable cells were counted after incubation at 37°C for 72 hours. For viable count determination on *S. cerevisiae*, the microorganism suspended in medium was exposed to different concentrations of stubomycin for 10, 30, or 60 minutes. After incubation, the cells were washed three times with 5 ml of ice-cold medium by centrifugation, diluted with the medium, and plated on potato dextrose agar. After 48 hours of incubation at 37°C, the colonies were counted.

Effect of Temperature on Anti-*S. cerevisiae* Activity of Stubomycin

S. cerevisiae (optical density: 0.24) was exposed to 1.25 μg/ml of stubomycin for 30 minutes at different temperatures. At the end of incubation, the cells were centrifuged at 0°C ($600\times g$, 2 minutes). The cells were then resuspended in the growth medium. After incubation for 6.5 hours at 27°C, the absorbancy of the cultures was determined at 660 nm.

Measurement of Macromolecular Synthesis

EL-4 cells (1×10^5 in 2 ml of MEM) were mixed with stubomycin and various precursors, and incubated at 37°C for 60 minutes. The cells were then collected on a Millipore filter and rinsed 3 times with ice-cold 5% trichloroacetic acid. The radioactivity of acid-precipitable material on the filter was determined by a Packard Tri-Carb liquid scintillation spectrometer.

Mutagenicity of Stubomycin

Effect of Stubomycin on Induction of Revertants in Six Bacterial Strains: The method for detection of mutagenicity as described by AMES *et al.*[4] and YAHAGI *et al.*[5] was used. Briefly, stubomycin was dissolved in DMSO and diluted with sterilized distilled water. The antibiotic solution (0.1 ml) was mixed with or without 0.5 ml of S-9 solution, and these mixtures were combined with 0.1 ml of bacterial suspension (1×10^9), then incubated at 37°C for 20 minutes. After incubation, 2 ml of soft agar were added to the mixture and the mixture was plated on 90-mm Petri dishes. After 2 days of incubation at 37°C, the number of revertant colonies were counted. As positive mutagen controls, 2-aminoanthracene for the metabolic study and ENNG, 2-nitrofluorene, and 9-aminoanthracene for the non-metabolic study were used according to the bacteria applied as test organism.

DNA Damaging Effect of Stubomycin Determined by the *rec*-Assay: The *rec*-assay with *Bacillus subtilis* was performed by the method described by KADA *et al.*[6,7] Briefly, strains of M 45 or H 17 were

cultured overnight in brain heart infusion broth. Each culture was streaked radially on the surface of broth agar, and a paper disk (diameter: 8 mm) containing the drug test solution was placed over the starting point of the streaks. The plates were kept at 4°C for 30 minutes, then incubated overnight at 37°C. The lengths of the inhibitory zones were measured.

Effect of Stubomycin on Chromosomal Aberrations and Sister-chromatid Exchanges in DON Cells: DON cells (1×10^5) in 5 ml of medium were plated on 60-mm Petri dishes and incubated at 37°C for 15 hours. After plating, the cells were exposed to stubomycin or EMS for 31~50 hours in medium with or without 5'-bromodeoxyuridine (10^{-5} M). Three hours prior to harvest, Colcemide was added to give a final concentration in the culture of 0.05 μg/ml. At the time of harvest, the trypsinized cells were gently centrifuged, resuspended in hypotonic saline (0.075 M KCl) and allowed to stand for 10 minutes. After fixation with a mixture of methanol - acetic acid (3: 1), the cell suspensions were dropped onto slides, allowed to air dry and stained with Giemsa.

Effect of Stubomycin on Induction of Mutation Resistant to 6-TG in DON Cells: DON cells (2×10^5) in 4.5 ml of medium were plated in 25-cm^2 plastic flasks (Corning, USA). After plating, 0.5 ml of the antibiotic or EMS solution was added to the culture which was then reincubated for 15.5 hours. The resulting culture was washed with phosphate buffered saline, resuspended in fresh MEM, and further incubated for 8.5 hours (day 0). To express mutation, the cells were subcultivated every other day, and the cells were trypsinized to select the mutants on days 13 or 15. The resulting cells (5×10^4) in 10 ml of medium containing 6-TG (10^{-5} M) were plated on 100-mm Petri dishes. In addition, 2×10^2 intact DON cells in 5 ml of medium were plated on 60-mm Petri dishes to determine the viability of the cells. After cultivation of drug exposed and intact cells for 8 days, the colonies formed on Petri dishes were fixed, stained, and counted.

Measurement of Hemolysis

Red blood cells were obtained from normal adult mice (strain *dd*Y). After being washed three times in physiological saline, the cell suspension was diluted with saline to produce an absorbancy of about 1.0 at 550 nm when complete hemolysis occurred in water. The mixtures of erythrocytes and different concentrations of stubomycin were incubated at 37°C for 60 minutes and then centrifuged (1,000 rpm for 5 minutes). The absorbancy of supernatants was measured at 550 nm, and the percent hemolysis was calculated as follows:

$$\text{Percent hemolysis}=\frac{\text{Absorbance of hemolysis by stubomycin}}{\text{Absorbance of complete hemolysis by water}}\times100$$

Effect of Calf Serum on Cytotoxic Activity of Stubomycin

EL-4 cells (1×10^5 in 2 ml MEM) were suspended in MEM supplemented with different concentrations of calf serum. Then, stubomycin (0.2 μg/ml) was added to the cell suspensions. After this mixture was incubated for 30 minutes at 37°C, the cells were washed three times with MEM and resuspended in MEM supplemented with 10% calf serum. The cells were counted after 4 days of incubation.

Effect of Lipids on Antimicrobial Activity of Stubomycin

Four cultures of *S. cerevisiae* were mixed with 0.01 ml of lipid and stubomycin solutions to produce final concentrations of 10 μg/ml and 0.63 μg/ml respectively. After incubation at 27°C for 7 hours, the cultures were washed three times with a methanol - ether (1: 1) solution by centrifugation to remove the lipids. The resulting precipitate was resuspended in saline, and the absorbancy of the suspension was determined at 660 nm.

Results

Growth Inhibitory Effect of Stubomycin on Various Cells

Cells growing in the logarithmic phase *in vitro* were mixed with different concentrations of stubomycin, and their growth was observed periodically. As shown in Figs. 1~3, the antibiotic remarkably inhibited the growth of EL-4 cells, *P. oryzae* and *S. cerevisiae*.

Fig. 1. Effect of stubomycin on the growth of EL-4 leukemic cells.

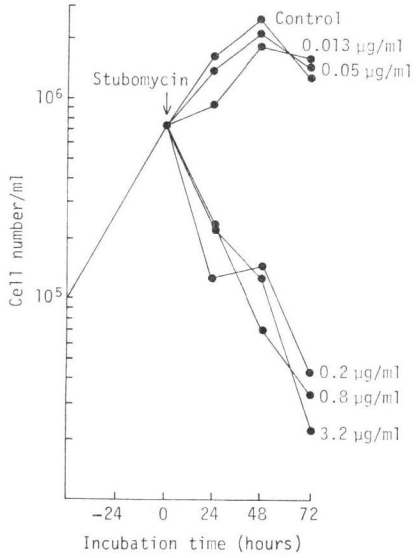


Fig. 2. Effect of stubomycin on the growth of *P. oryzae*.

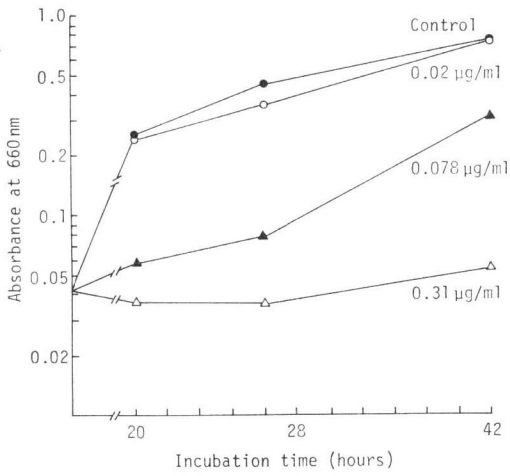


Fig. 3. Effect of stubomycin on the growth of *S. cerevisiae*.

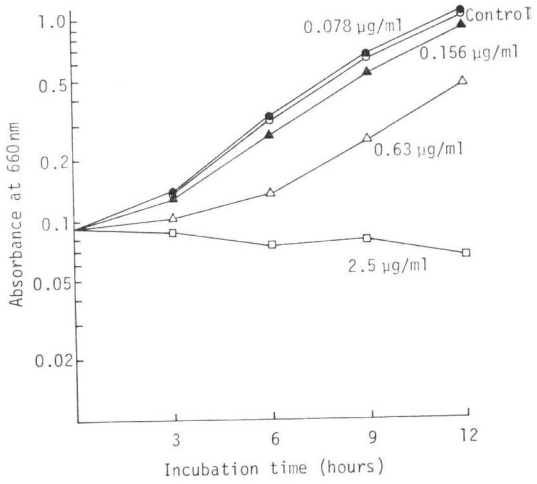
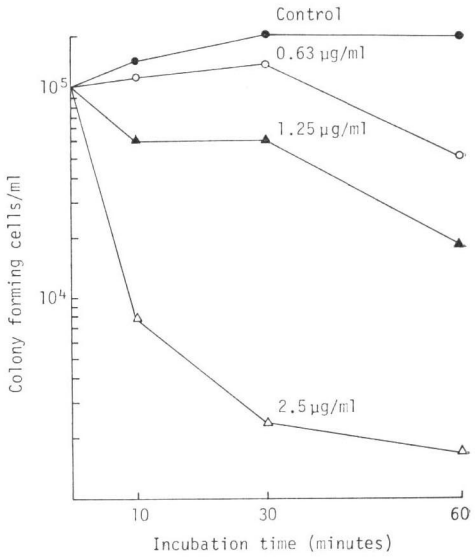


Fig. 4. Effect of stubomycin on the viability of *S. cerevisiae*.



Next, to examine cytocidal activities of stubomycin on EL-4 cells and *S. cerevisiae*, the cells were mixed with stubomycin and incubated for 10, 30, or 60 minutes. At the end of each incubation time the cells were washed and incubated again to determine their growth. As shown in Figs. 4 and 5, remarkable cytocidal activity was observed on *S. cerevisiae* and EL-4 cells at concentrations of 2.5 and 0.8 μg/ml respectively.

Effect of Temperature on the Activity of Stubomycin

The effect of temperature on the growth inhibitory effect of stubomycin on cells was examined. When *S. cerevisiae* was incubated with 1.25 μg/ml of stubomycin at 0 or 10°C for 30 minutes, no growth

Fig. 5. Effect of stubomycin on the viability of EL-4 leukemic cells.

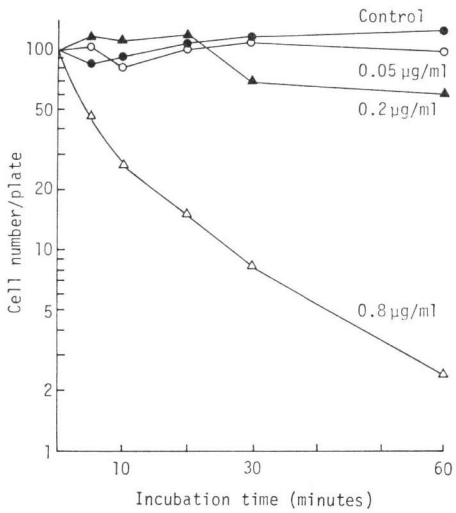
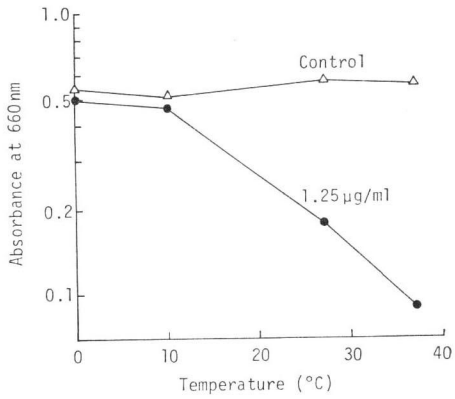


Fig. 6. Effect of temperature on the anti-*S. cerevisiae* activity of stubomycin.

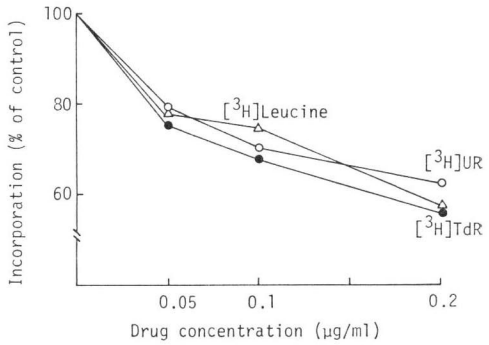


inhibition was observed. However, cells treated with stubomycin at 27°C or 37°C for 30 minutes suffered a considerable loss in growth (Fig. 6).

Effect of Stubomycin on Macromolecular Synthesis in EL-4 Cells

To determine the effect of stubomycin on the synthesis of DNA, RNA and protein, the uptake of various precursors into EL-4 cells was observed.

Fig. 7. Effect of stubomycin on the synthesis of macromolecules in EL-4 cells.



As shown in Fig. 7, when cells were exposed to various concentrations of stubomycin for 60 minutes, the uptakes of [^{3}H]TdR, [^{3}H]UR, and [^{3}H]leucine were equally inhibited at all drug concentrations.

Mutagenicity of Stubomycin

The effects of stubomycin on induction of revertants on six bacterial strains were studied. There were different sensitivities to stubomycin among bacterial strains, but no increases in the number of revertant colonies were observed in any of the strains. In contrast, positive control agents such as ENNG increased the number of revertant colonies (Table 1).

The DNA damaging effect of stubomycin was determed by the *rec*-assay method. As shown in Table 2, there was no difference in growth inhibitory zones between strains of *B. subtilis* M 45 and H 17. In contrast, mitomycin C used as positive control showed a remarkable inhibition of the *rec*$^-$ strain.

The effects of stubomycin on chromosomal aberrations, sister-chromatid exchanges, and the induction of cells resistant to 6-TG were studied using Chinese hamster cells (DON D-6). The antibiotic slightly increased numerical aberrations of chromosomes at a concentration of 0.24 μg/ml, but no increases in structural aberrations and rate of sister-chromatid exchanges were observed (Table 3). Almost no increase in the induction of mutation resistant to 6-TG in Chinese hamster cells was observed even at a nearly lethal dose of the antibiotic, whereas EMS used as positive control induced 30 ~ 40 fold more mutations than the vehicle control (Table 4).

Table 1. Effect of stubomycin on induction of revertants in six bacterial strains.

Agents	Concentrations (μg/plate)	With or without S-9 mixture	Number of revertants/plate					
			Base change type			Frame shift type		
			TA100	TA1535	WP 2uvrA	TA98	TA1537	TA1538
Vehicle control	—	−	283	63	48	51	13	33
Stubomycin	5,000	−	—*	57	25	30	—	—
	1,000	−	—	54	33	42	—	—
	500	−	—	61	42	48	—	—
	100	−	1	61	32	39	0	1
	50	−	60	61	46	31	0	2
	10	−	37	70	33	44	7	14
	5	−	88	—	—	—	15	15
	1	−	204	—	—	—	11	25
	0.5	−	173	—	—	—	16	28
Vehicle control	—	+	266	18	114	109	26	61
Stubomycin	5,000	+	—	12	38	63	—	—
	1,000	+	—	18	41	79	—	—
	500	+	—	14	51	81	—	—
	100	+	0	19	37	92	0	4
	50	+	9	22	60	74	0	18
	10	+	95	17	51	61	22	39
	5	+	107	—	—	—	26	50
	1	+	218	—	—	—	31	62
	0.5	+	247	—	—	—	28	50
Positive control ENNG**	10	−	—	4,712	—	—	—	—
	5	−	—	—	2,124	—	—	—
	2	−	680	—	—	—	—	—
2-Nitrofluorene	5	−	—	—	—	—	—	874
	2	−	—	—	—	149	—	—
9-Aminoacridine	10	−	—	—	—	—	33	—
2-Aminoanthracene	40	+	—	—	366	—	—	271
	1	+	—	61	—	—	85	—
	0.5	+	528	—	—	300	—	—

*: Not tested.
**: *N*-Ethyl-*N'*-nitro-*N*-nitrosoguanidine.

Table 2. Mutagenicity of stubomycin determined by *rec*-assay.

	Concentration (μg/disc)	Inhibitory zone (mm)		Difference (mm)
		M 45	H 17	
Vehicle control	0	0	0	0
Stubomycin	500	4.7	5.4	−0.7
	100	4.7	4.4	0.3
	50	5.7	5.0	0.7
	10	4.0	4.3	−0.3
	5	4.0	3.7	0.3
	1	2.0	1.7	0.3
	0.5	2.0	2.0	0
Negative control Benzylpenicillin	100	13.0	13.7	−0.7
Positive control Mitomycin C	7	12.0	5.0	7.0

Fig. 8. Hemolytic activity of stubomycin on mouse red blood cells.

Mouse red blood cells were mixed with the indicated amounts of antibiotics, and incubated at 37°C for 1 hour.

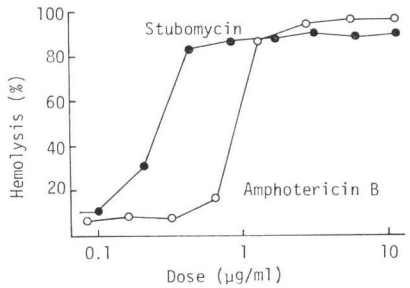


Table 3. Effect of stubomycin on chromosomal aberrations and sister-chromatid exchanges in Chinese hamster cells.

Agents	Concentrations (M)	Mitotic index (per hour)	Chromosomal aberrations/cell						SCEs*/cell (mean±S.D.)
			Structural aberrations				Others	Numerical aberrations	
			Chromatid		Chromosome				
			Gaps	Breaks	Gaps	Breaks			
Vehicle control	—	3.02	0.04	0 02	0	0	0.02	0.10	4.54±1.99
Stubomycin	5×10^{-7}	0.12	0.04	0.02	0	0	0.06	0.28	ND
	2.5×10^{-7}	2.95	0.04	0	0	0	0.06	0.08	4.64±2.01
	1.3×10^{-7}	3.07	0.02	0	0	0	0.06	0.08	4.92±1.70
Ethyl methanesulfonate	10^{-3}	2.42	0.06	0.06	0.08	0.02	0.02	0.10	25.74±6.64

* Sister-chromatid exchanges.

Table 4. Effect of stubomycin on induction of mutation resistant to 6-thioguanine in Chinese hamster cells.

Treatment (M)	Expression time (days)	Cell survival (%)	Number of mutants per dish	Mutation frequencies (mutants/10^5 survivors)
Vehicle control	13	98.9±2.7	0.44±0.73	0.89
	15	86.5±5.3	0.33±0.41	0.76
Stubomycin				
5×10^{-7}	15	92.0±6.9	0	0
2.5×10^{-7}	13	110.7±5.1	0.89±1.27	1.61
	15	90.4±6.2	1.50±1.64	3.32
1.3×10^{-7}	13	97.1±2.5	0.80±0.92	1.65
	15	97.3±7.1	0.66±0.52	1.36
EMS				
2×10^{-3}	13	95.9±3.8	12.50±5.45	26.08
	15	75.9±5.2	11.00±1.52	28.99

Fig. 9. Effect of incubation time on hemolytic activity of stubomycin.

Mouse red blood cells were mixed with the indicated amounts of stubomycin, and incubated at 37°C. The hemolysis was observed periodically.

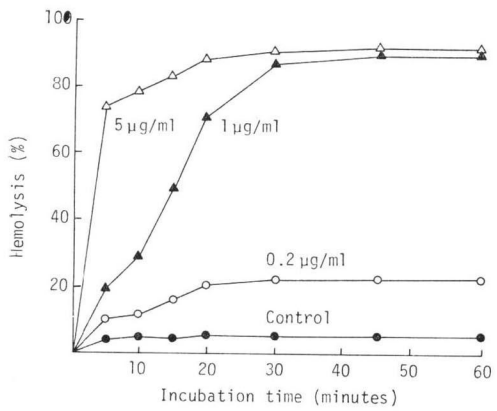
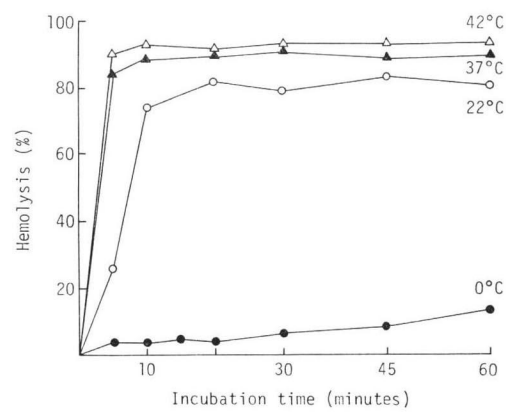


Fig. 10. Effect of temperature on hemolytic activity of stubomycin.

Mouse red blood cells were mixed with a concentration of 1 μg/ml of stubomycin, and incubated at different temperatures.



Hemolytic Activity of Stubomycin on Mouse Erythrocytes

A constant number of mouse erythrocytes was incubated with varying amounts of stubomycin at 37°C for 1 hour. As shown in Fig. 8, stubomycin induced almost complete hemolysis at a concentration of 0.3 μg/ml. Amphotericin B used as positive control also induced significant hemolysis. Next, the hemolytic activity was determined periodically at different concentrations of the antibiotic, and the results are shown in Fig. 9. Rapid hemolysis was observed within 5 minutes at 5 μg/ml, and this hemolysis at each dose reached a maximum after 20~30 minutes. The effect of temperature on hemolytic activity of stubomycin was examined periodically. When mouse erythrocytes were incubated with 1 μg/ml of the antibiotic at 0°C, no marked hemolysis occurred. However, remarkable hemolysis was noted as the temperature increased (Fig. 10).

Effect of Calf Serum on Cytocidal Activity

To determine the effect of calf serum on cytocidal activity of the antibiotic against EL-4 cells, the cells were suspended in MEM supplemented with various concentrations of calf serum and 0.2 μg/ml of stubomycin for 30 minutes at 37°C. As shown in Fig. 11, stubomycin applied to EL-4 cells in the medium not containing any serum markedly inhibited their growth at a concentration of 0.2 μg/ml. In contrast, the anti-EL-4 cell activity of the antibiotic tended to decrease with increasing amounts of serum.

Effect of Lipids on Antibacterial Activity of Stubomycin

The effect of lipids on the antibacterial activity of stubomycin was studied using *S. cerevisiae*. The activity was significantly reduced by phosphatidylserine, olive oil, and cardiolipin. However, egg lecithin and cholesterol did not reduce the activity of stubomycin (Fig. 12).

Fig. 11. Effect of calf serum on the anti-EL-4 activity of stubomycin.

EL-4 cells were incubated with (●) or without (○) stubomycin in the medium supplemented with various concentration of calf serum.

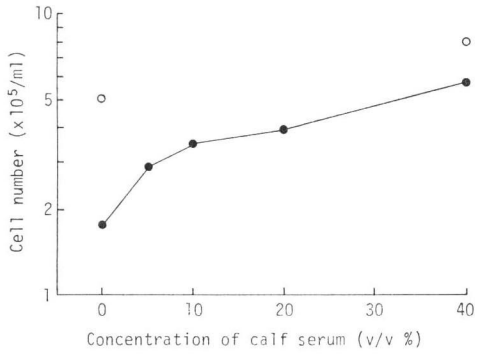
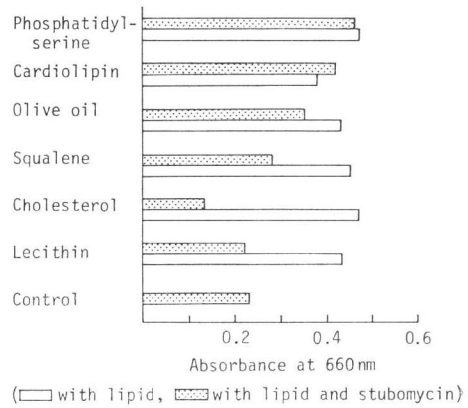


Fig. 12. Effect of several lipids on anti-*S. cerevisiae* activity of stubomycin.

S. cerevisiae were incubated with lipid or lipid and stubomycin. Control culture was added only with the antibiotic. Values are the mean of 3 samples.



Discussion

The antitumor antibiotic, stubomycin, possesses marked cytotoxic activity against mammalian cells, yeast, and fungi *in vitro*. Although the cells were exposed only once to stubomycin for a short

period of time, cell growth was inhibited. The hemolytic activity of stubomycin on mouse erythrocytes was also rapid. These results suggest that stubomycin is quickly incorporated into the cells. The biological activity of the antibiotic described above depended on various conditions, *i.e.* marked activities were observed at higher temperatures than at lower ones, and were inhibited by phosphatidylserine, cardiolipin, cholesterol, *etc.*

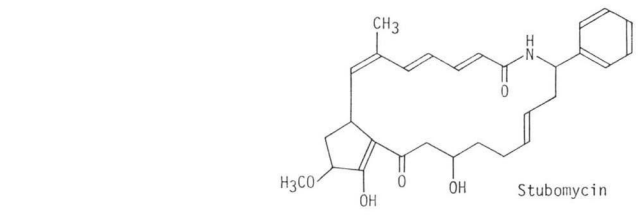
It is well known that polyene antibiotics mediate a change in the cellular permeability of a number of organisms, thus promoting leakage of important cellular constituents.[8~12] Previously, it has been demonstrated by many researchers[13~17] that exogenously added sterols can protect sensitive organisms from the action of polyenes, and polyene antibiotics induce hemolysis of rat and human erythrocytes.[13~22] Therefore, it is suggested that the biological activities of stubomycin are similar to those of polyenes.

The effects of stubomycin on hemolysis are similar to those of filipin *i.e.* rapid hemolysis induced at lower concentrations ($1 \sim 2$ μg/ml) without at time lag for $10 \sim 15$ minutes.[22] Furthermore, the drug concentration of stubomycin that causes cell death and hemolysis is almost the same, and a similar phenomenon was observed in a study of polyene antibiotics.[21]

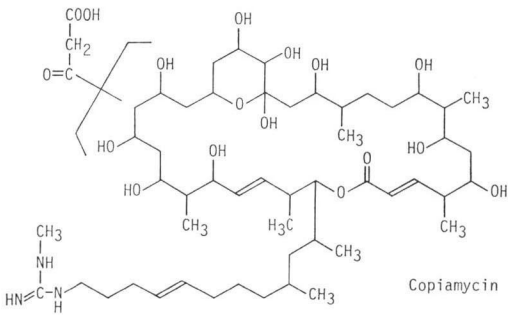
While the antimicrobial activity of polyene antibiotics is generally reduced by sterols, that of stubomycin was not reduced by cholesterol. On the other hand, the antimicrobial activities of macrocyclic lactam antibiotics azalomycin F[24,25] and copiamycin,[26,27] were more strongly reduced by phospholipids with unsaturated fatty acid and basic hydrophilic groups, and were moderately reduced by egg lecithin.[28] In the present study, some phospholipids reduced the anti-*Saccharomyces cerevisiae* activity of stubomycin but there was no reduction by egg lecithin. Stubomycin is also a macrocyclic lactam antibiotic although the mechanism of action seems to differ from that of copiamycin and azalomycin F.

The anti-EL-4 cell activity of the antibiotic tended to decrease with increasing amounts of calf serum. Because calf serum contains different and relatively large amounts of lipid,[29] it is considered that these lipids inhibited the cytocidal activity of stubomycin.

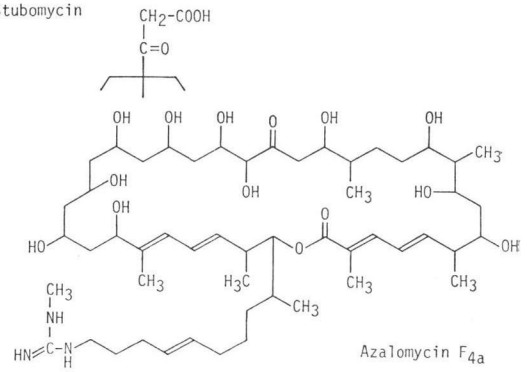
Most antitumor antibiotics with direct cytotoxic activity used clinically at present, primarily inhibit DNA synthesis,[30] and most of them cause mutation.[4,31] Stubomycin showed DNA, RNA, and protein synthesis inhibition at almost the same time and to the same degree. On the other hand, stubomycin did not show any mutagenesis in mammalian cells and bacteria. The main mechanism of mutation is considered to be DNA damage and errors in replication of DNA. From the data obtained in the present experiment, it seems that stubomycin can mediate a change in the cell surface ultimately leading to lysis and death of the cells.



Stubomycin



Copiamycin



Azalomycin F_{4a}

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